Sustainable Development and Biodiversity 28

Halina Maria Ekiert
Kishan Gopal Ramawat
Jaya Arora *Editors*

Medicinal Plants

Domestication, Biotechnology and Regional Importance

Springer

Sustainable Development and Biodiversity

Volume 28

Series Editor

Kishan Gopal Ramawat,
Botany Department, Mohanlal Sukhadia University, Udaipur, India

Sustainable Development Goals are best achieved by mechanisms such as research, innovation, and knowledge sharing. This book series aims to help researchers by reporting recent progress and providing complete, comprehensive, and broad subject-based reviews about all aspects of sustainable development and ecological biodiversity.

The series explores linkages of biodiversity with delivery of various ecosystem services and it offers a discourse in understanding the biotic and abiotic interactions, ecosystem dynamics, biological invasion, ecological restoration and remediation, diversity of habitats and conservation strategies. It is a broad scoped collection of volumes, addressing relationship between ecosystem processes and biodiversity. It aims to support the global efforts towards achieving sustainable development goals by enriching the scientific literature. The books in the series brings out the latest reading material for botanists, environmentalists, marine biologists, conservationists, policy makers and NGOs working for environment protection.

We welcome volumes on the themes --

Agroecosystems, Agroforestry, Biodiversity, Biodiversity conservation, Conservation of ecosystem, Ecosystem, Endangered species, Forest conservation, Genetic diversity, Global climate change, Hotspots, Impact assessment, Invasive species, Livelihood of people, Plant biotechnology, Plant resource utilization, Sustainability of the environment, Sustainable management of forests, Sustainable use of terrestrial ecosystems and plants, Traditional methods, Urban horticulture.

More information about this series at http://www.springer.com/series/11920

Halina Maria Ekiert · Kishan Gopal Ramawat · Jaya Arora
Editors

Medicinal Plants

Domestication, Biotechnology and Regional Importance

Springer

Editors
Halina Maria Ekiert
Department of Pharmaceutical Botany
Jagiellonian University, Medical College
Kraków, Poland

Jaya Arora
Department of Botany
Mohanlal Sukhadia University
Udaipur, Rajasthan, India

Kishan Gopal Ramawat
Department of Botany
Mohanlal Sukhadia University
Udaipur, Rajasthan, India

ISSN 2352-474X ISSN 2352-4758 (electronic)
Sustainable Development and Biodiversity
ISBN 978-3-030-74778-7 ISBN 978-3-030-74779-4 (eBook)
https://doi.org/10.1007/978-3-030-74779-4

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Herbal drugs and their preparations are part of all civilizations and evolved with time. With the advent of allopathic modern medicine prepared from pure compounds, decline in practice of herbal medicine was felt. However, because of easy availability of herbal medicine over the counter (OTT), low cost, rarely side effects, and traditional belief resulted in surge in demand all over the world, particularly in developing countries. Traditional medicine in East-Asian countries is very old and herbal based. Several important plants from traditional medicinal plant species of East-Asian and North-American origin have been introduced into official European medicine, followed by their successful cultivation and domestication such as *Schisandra* spp., *Lonicera caerulea*, *Aronia* spp. and *Solidago* spp. With world population nearing 7 billion and spending a US dollar per person on herbal medicine in any form (like tea, decoction, poultice, formulation, or raw plant) will have tremendous impact on economy of several countries. Modern healthcare system is still lacking in rural areas of many developing countries or is not fully equipped. This has become evident in recent pandemic of COVID-19. Therefore, medicinal plants and herbal medicines are backbone of rural healthcare system in traditional system of medicine in several countries and provide nutraceutical for population of the developed countries.

This book is a timely compilation of topics related to medicinal plants as surge in demand has consequently resulted in increased research activities. The demand of medicinal plants and their products cannot meet with increased supply. Still most of the medicinal plants are collected from wild, and pressure on resources makes them vulnerable to extinct. Therefore, this book describes research highlights related to conservation, developing agrotechnology toward domestication for biomass production, conservation through biotechnological methods and incorporation of modern tools of genetic engineering and genome editing. How all these activities can be helpful in rural livelihood improvement is also presented.

This book contains wide spectrum of topics on medicinal plants. Whole contents of 27 chapters are divided into three parts: (I) Domestication and cultivation of medicinal plants, (II) Biotechnology in medicinal plants, and (III) Regional importance of medicinal plants. In these parts, development of agrotechnology and cultivation practices for several important medicinal plant species has been described. Details of productions systems and conservation using biotechnological methods are described

in the second part. Rural livelihood improvement with tourism based on medicinal plants cultivation is discussed in the third part. These chapters are written by various research groups working on these plants and selected from different parts of the world.

This book will be useful for all those dealing with medicinal plants, particularly those working with agrotechnology, biotechnology, herbal drugs and formulations, quality control and policy making. This book is source material for traditional medicine systems of India, China, and other countries. The editors are thankful to all the contributors for their cooperation and patience during the process of book publication. The editors are also grateful to the editorial team of Springer, Dr. Markus Spaeth and Dr. Ineke Ravesloot for their continued professional expertise and support during the book production.

Kraków, Poland — Prof. Halina Maria Ekiert
Udaipur, India — Prof. Kishan Gopal Ramawat
Udaipur, India — Dr. Jaya Arora
January 2021

Contents

Chapter 1
Medicinal Plants Domestication, Cultivation, Improvement, and Alternative Technologies for the Production of High Value Therapeutics: An Overview

Kishan Gopal Ramawat and Jaya Arora

Abstract Medicinal plants are source of several valuable drugs known as natural products or secondary metabolites. Only a handful of medicinal plants are cultivated while most of them are still collected from wild. Due to the high demand for these products, over-exploitation resulted in endangering the species, loss of biodiversity, adulteration of plant materials and products, and the effect on ecosystem. Plants and plant products are used in many traditional medicines for several centuries. To meet the demand of raw plant material for direct use or industrial use, agrotechnologies have been developed for several medicinal plants, alternative biotechnologies (micropropagation, production in cell cultures grown in shake flasks and bioreactor, transfer of gene/s in plant and microbes, modification of biosynthetic pathways, etc.) and microbial production system have been attempted. Understanding seed and floral biology, development of agrotechnologies and introduction into new habitat may improve the availability of raw medicinal plant material associated with the improved downstream process can affect high recovery. Similarly, the use of sophisticated detection methods, high throughput screening methods, genomics and proteomics can through light on genes involved, types of biomolecules, and new sources of known drugs. Biotechnological methods (elicitation, immobilization, cloning of selected strains, hairy root cultures, and gene manipulation) including gene editing can help in improvement in the production system. With ever-increasing population and reliability of herbal medicine, demand for medicinal plants continues to increase; hence, domestication of plants along with new technologies is a demand of time to meet the challenge of supply of uniform raw material. This brief overview presents state of research on medicinal plants and their products.

Keywords Medicinal plants · Plant biotechnology · Secondary metabolites · Agrotechnology · Domestication

K. G. Ramawat (✉) · J. Arora
Department of Botany, M. L. Sukhadia University, Udaipur 313001, India

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_1

1.1 Introduction

Plants are called medicinal because of the present of certain secondary metabolites in them which impart profound physiological effect on the mammalian system. These compounds impart curative, additive, or synergistic effects on human health. Though these may be present in the whole plant body, higher concentrations may be present in one or many parts such as stem, root, bark, seeds, or leaves. Though secondary metabolites (not involved in metabolism) are considered as waste products of the plant, many evidences are produced to demonstrate them as plant growth modulators or reused by the producer plant as primary metabolites (involved in primary metabolism). Secondary metabolites are also involved in plant defence (Erb and Kliebenstein 2020). In developing countries, most of the medicinal plants are collected from the wild. In Europe, out of 1300 species used, about 80% medicinal plants are collected from wild (Ramawat 2019a; Ramawat and Goyal 2008; Balunas and Kinghorn 2005). Because cheap labour is involved, most of these materials are collected by illiterate labour resulting in problems of identification, deliberate adulteration, inappropriate part collection, and damaging to the plant and ecosystem. The consequence of uncontrolled collection and commercialization is habitat loss, the encroachment of land by alien species, and unavailability of medicinal plants. This puts pressure on the wild population of medicinal plants and the rate of disappearance has accelerated particularly in developing countries like India, China, Nepal, Kenya, Tanzania, and Uganda (Chen et al. 2016). Therefore, the development of cultivation practices, understanding floral and seed biology, improvement of secondary metabolites content in plants, and developing alternative technologies are ways to save medicinal plants from becoming endangered. It is important to introduce and domesticate wild plants for their sustainable utilization and ensure a continuous supply of uniform material for human welfare (Hua et al. 2018; Tanga et al. 2018; Ramawat 2019b).

In developing countries, herbal medicine is the backbone of the traditional systems of medicine like Ayurveda in India (4500–1600 BC), Chinese traditional medicines (3000 BC), Jamu (Indonesia 800 AD), Kampo (Japan 500 AD), Thai medicine (1200 AD) or Unani medicine (Astutik et al. 2019; He 2015; Ramawat and Goyal 2008; Sheehan and Hussain 2002). Most of these countries, where traditional medicine is used by a large number of people, have invested substantially in herbal research. Traditional systems of medicine, whether Indian Ayurveda or Chinese, are based on plant extract whereas allopathic system works on a pure active molecule. Thus, plants of interest to Ayurveda are different than those required for allopathic system (Ramawat and Goyal 2008). Traditional systems of medicine are safe, time tested, and cheap for the people. Traditional systems contain a wealth of information about use of medicinal plants and scientific study of this information has led to new knowledge about medicinal plants and several drug discoveries (Suntar 2019). With increase in living standards in the recent past, consumption of herbal drugs for wellbeing and longevity has increased tremendously. These countries are also major producers of herbal drugs (Vashist et al. 2016) which are further handled by traders, not trained or

educated in knowledge about medicinal plants (Barata et al. 2011). Because of high bulk is involved, now value addition is done by preparing and selling extract or active ingredients which involve good manufacturing practices (GMP), and many of them either not follow them or ignore. It is estimated that about 0.5 million tons of raw material comprising of about 60,000 medicinal, nutraceutical, and aromatic plants have been traded (WHO 2015). The world market for pharma and related compounds in 2022 will be about $1.12 trillion (Subramani 2018).

In this review, we summarize the importance of medicinal plants, efforts for their cultivation towards availability, alternative technologies for their production, conservation, and improvement. Biotechnological methods (elicitation, immobilization, cloning of selected strains, hairy root cultures, and gene manipulation) including gene editing will not only save the medicinal plants but also improve the quality of human life.

1.2 Historical Use of Medicinal Plants

Some of the landmarks in the description and use of medicinal plants are Ayurvedic, Egyptian descriptions, and Chinese medicine system where the time of collection, preparation of herbal medicine and dosage to be given are described in detail. Particular emphasis is given in Ayurveda about the collection and combination of herbs with minerals which is about 5000 years old. Cultivation and/or use of medicinal plants like *Cannabis sativa*, *Papaver somniferum*, and *Conium maculatum* is as old as human civilization. Modern herbal knowledge includes first pharmacopoeia by Greek physician Galen (129–200 AD), isolation of morphine from *P. somniferum*, quinine from *Cinchona* species, and pilocarpine from *Pilocarpus jaborandi* (Ramawat 2019a; Ramawat and Goyal 2008). Details of historical account and old literature describing medicinal plants are given elsewhere (Khan 2018; Petrovska 2012). Several human diseases in the history of human civilization are well documented, and plants are used as curative agents. Not only humans but animals and insects can recognize the presence of secondary metabolites in plants and their biosynthesis in plants or their neutralization by insects/animals, both have co-evolved with the evolution of these species (Ramawat and Goyal 2019). It is evident that old descriptions are about plant morphology and its usage, whereas (see Chap. 25) plant-based drugs were developed with the development of modern scientific tools and a strong base in chemistry. In the last 2–3 decades, metabolomics, molecular markers, high throughput screening, and genetic manipulations added new dimensions in the medicinal plant research.

1.3 Domestication of Medicinal Plants

Plants are sources for food, cloth and shelter, and humans have learned to select and cultivate plants for their needs. Domestication of plants and animals is an evolutionary process spreading over centuries involving farmers, crop adaptation, and its environment. Domestication involves changes in traits and is a slow co-evolutionary process leading to new species or cultivars. Domestication of plants and animals is described as greatest advance in development of human civilization. Various selection pressures lead to evolution of more desirable and suitable cultivars (Diamond 2002; Purugganan 2019). The process of natural selection and wide seed dispersal contributes significantly in the adaptation and evolution of crop plants. Out of 452,000 plant species, currently <500 is in cultivation. Therefore, domestication has given rise to a lesser germplasm pool and contributes towards loss of diversity.

The various centres of crop origin and plant domestication are MesoAmerica, the Southern Andes, the Near East, Africa (probably the Sahel and the Ethiopian highlands), Southeast Asia, and China, from where they spread to other regions. The probable centres of various present-day crop plants are presented in Fig. 1.1 from which it is evident that important crops were domesticated all over the world and related plant species were selected the man for their use.

It takes several years or decades to develop agrotechnologies for the cultivation of plants and understand seed and floral biology. Cultivation practices for various species evolved with human being's civilization and about 38% of the total land is used for cultivation purposes. Gepts (2014) defined the process of domestication

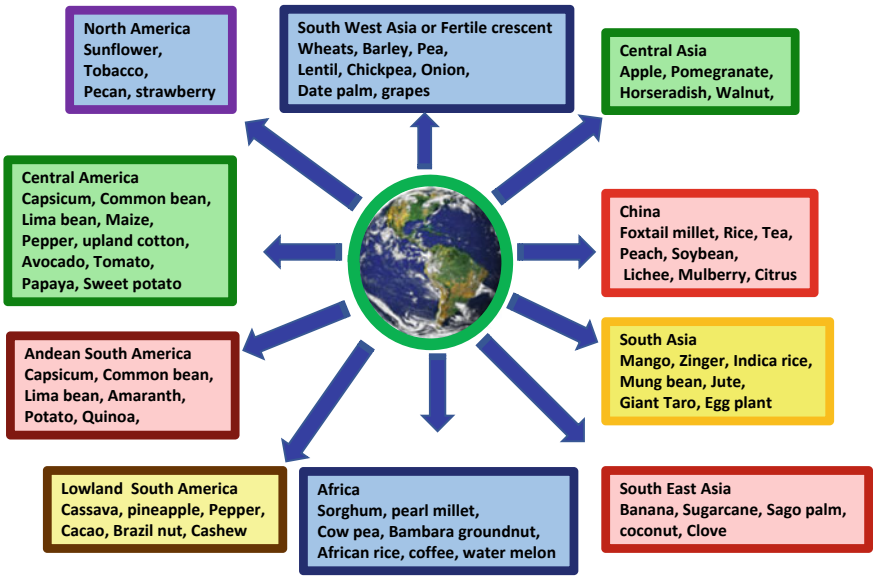


Fig. 1.1 Centres of agricultural crop origin and examples of plant species

as an "evolutionary process driven by natural and human (whether conscious or unconscious) selection applied to wild plants or animals and leading to adaptation to cultivation and consumption or utilization. Domestication can be complete, whereby organisms become entirely dependent on humans for their continued existence or can be partial or incipient, whereby they still reproduce independently of human intervention".

The domestication of plants followed by agriculture leads to the settlement of humans around such places. Therefore, the process of gathering, selecting, and cultivating started with the start of civilization at the end of recent ice-age during 10–12,000 years ago (Fuller et al. 2014). How, when, and where plants have been domesticated in time to time is a point of discussion in various disciplines. Inputs by archaeological and new molecular tools have helped in developing understanding about the relationship between wild and cultivated plants and their domestication (Larson et al. 2014). Man has always an attraction for better fruits and grains, colour, or size of fruits, and all these factors contributed to the slow process of selection and evolution leading to better-cultivated plants. Therefore, early breeding was based on simply visible traits. These characters provided improved fruit and seeds and enable use of technology for harvest, storage, and cultivation. Still very large variation in fruit size, taste, and colour exists in plants like tomato and brinjal demonstrating wide genetic variation within the species and model to trace wild relatives. Next-generation sequencing techniques are being employed to identify these conserved characters (alleles) in some crop plants for their exploitation (Li and Olsen 2016). Similarly, the uniform size of plants, branching, and non-seed shattering habits paved the way for mechanization in wheat and rice crops. During this, domestication, development, and selection of specific plant parts like cabbage (leaf), kale (leaf), cauliflower (unripe flower), and broccoli (unripe flower and stem) took place from a lean and thin mustard plant (*Brassica oleracea*) during the evolution process (Ramawat 2019a; Gepts 2014; Larson et al. 2014). Some examples of domestication of crop plants during last 12,000 years are: wheat, rice, pearl millet, sweet potato, and cotton (Fig. 1.2). Medicinal plants come after realization of primary need and man learned to use the plants for diseases and health. Cytogenetic and molecular markers are useful tools to compare and determine the wild relatives and genetic structure of the plants. At the beginning of domestication, wild germplasm is rich in diversity but development of homogeneous crops for cultivation may result in loss of valuable biodiversity (Chinthiya and Bhavyasree 2019).

India being a major producer and exporter of medicinal herb, a separate medicinal plant board has been established under the Ministry of Health and Family Welfare (a central government initiative). The worldwide demand for medicinal herbs and related products is estimated to be ~US $60 billion (Shepherd 2007). This board funds research and takes measure for the availability of medicinal herbs and provides 30, 50, and 70% subsidy for the development of agrotechnology (Table 1.1) and cultivation practices of medicinal plants (https://www.nmpb.nic.in/content/prioritised-list-medicinal-plants-cultivation). Similarly, other countries have prioritized plants for their local demand and domestication efforts for medicinal plants (Katumba et al. 2004). Selected examples of medicinal plants promoted by the Indian National

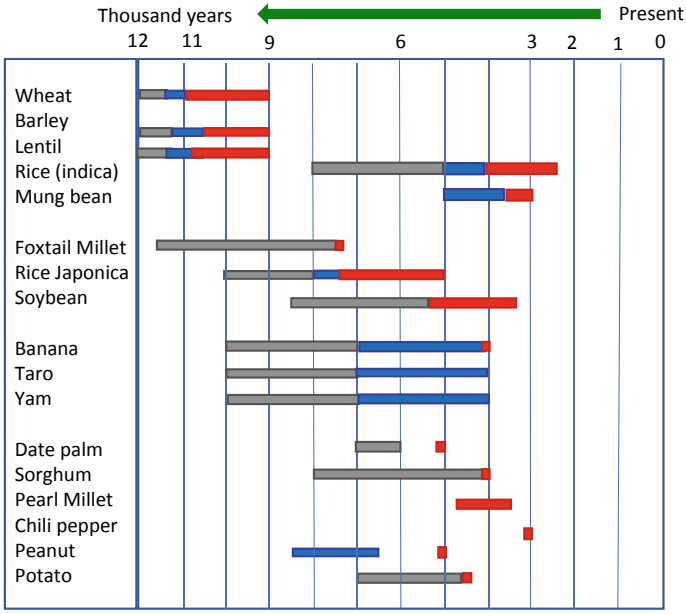


Fig. 1.2 Chronological chart listing the time frames over which a few selected examples of plants were domesticated. Grey bars ( ) represent documented exploitation before domestication, blue bars ( ) represent pre-domestication cultivation of plants, red bars ( ) represent the period over which morphological changes associated with domestication. Simplified and developed from Larson et al. (2014)

Medicinal plants board for domestication in India and worked out by the authors are presented in Table 1.2. For these plants, in vitro techniques were used to study seed biology, micropropagation, and production of secondary metabolites towards conservation. Similarly, the medicinal plants promoted in Pakistan are *Asparagus recemosus, Carum carvi, Rauvolfia serpentina, Atropa accumonicata, Valeriana jatamansi,* and *Linum usitatissimum*, (Sher et al. 2010), in Poland are roseroot (*Rhodiola rosea*, Weglarz et al. 2008), in Mexico are cultivars of *Agastache mexicana* (Carrillo-Galván et al. 2020), and in Israel are basil, caraway, chamomile, and dill (Dudai 2012). These plants were put for domestication based on size of vegetative or reproductive parts, their useful metabolites contents, and growth and yield parameters (Carrillo-Galván et al. 2020). Therefore, it is of utmost importance to develop agrotechnologies towards domestication of medicinal plants collected from wild. However, uniform cultivation of selected germplasm may result in permanent loss of biodiversity for some species (Ramawat 2019a). Improvement of germplasm of the opium poppy through hybridization is also a continuous process to develop varieties suitable for factory-based extraction to avoid illegal drug trafficking (Fig. 1.3). Note the differences in wild and cultivated plants.

Before supporting the cultivation practices, there is need to prioritize the medicinal plants based on several parameters of demand and supply as well as their biology,

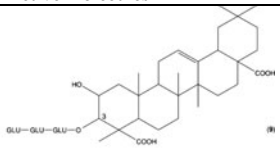
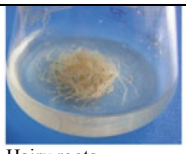
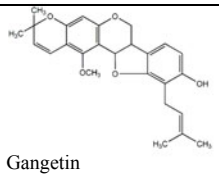
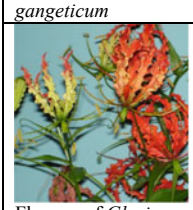
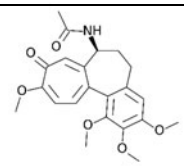
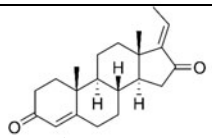
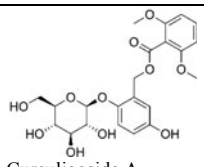
Table 1.1 Selected examples of medicinal plants promoted by the Indian medicinal plants board for development of agrotechnology towards domestication and cultivation

% Subsidy for cultivation cost	Selected examples of plant species promoted for cultivation and development of agrotechnologies towards domestication
30%	*Abrus precatorius* Linn., *Acorus calamus* Linn., *Andrographis paniculata* (Linn.) Burn, *Artemisia annua* Linn, *Asparagus racemosus* Willd., *Celastrus paniculatus* Willd., *Chlorophytum borivillianum* Sant., *Embelia ribes* Burm. f., *Emblica officinalis* Gaertn, *Gymnema sylvestre* R. Br., *Hemidesmus indicus* R.Br., *Plantago ovata* Forssk., *Psoralea corylifolia* Linn, *Rubia cordifolia* Linn., *Terminalia bellirica* Gaertn., *Terminalia chebula* Retz, *Vitex negundo* Linn., *Withania somnifera* (Linn.) Dunal
50%	*Acacia catechu* Willd., *Atropa belledona* Linn., *Desmodium gangeticum* (Linn.) DC., *Gloriosa superba* Linn, *Glycyrrhiza glabra* Linn, *Mesua ferrea* Linn., *Pueraria tuberosa* DC, *Pterocarpus marsupium* Roxb., *Rheum emodi* Wall., *Smilax* china Linn., *Valeriana wallichi* DC
70%	*Aconitum heterophyllum* Wall. ex Royle, *Berberis aristata* DC., *Commiphora wightii* (Arn.) Bhandari, *Nardostachys jatamansi* DC., *Oroxylum indicum* Vent, *Picrorhiza kurroa* Benth. ex Royle, *Podophyllum hexandrum* (Royle) T.S. Ying, *Pterocarpus santalinus* Linn. f., *Saussurea costus* C.B. Clarke, *Swertia chirata* Buch-Ham

phytochemistry, and traditional usage. Work related to the collection, storage, marker compounds associated with quality control and microbial load, etc. need to be investigated. Vast data generated in the last two decades about ancestry, relationship, and possible origin of crop plants using next-generation sequencing and molecular markers will through light after in-depth analysis about domestication and selection of characters (Chinthiya and Bhavyasree 2019; Larson et al. 2014). The details of domestication of plants are beyond the scope of this article and can be found elsewhere (Gepts 2014; Larson et al. 2014).

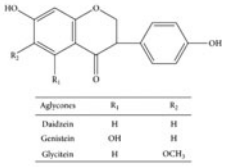
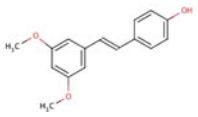
It is certain that to meet the demand of medicinal plants for ever-increasing population, more plants should be collected from wild resulting in endangering the species and loss of habitat. This can be avoided by the cultivation of medicinal plants but a uniform crop may be detrimental for the biodiversity of that species (Ramawat 2019a) and we may lose it forever if in situ conservation is not practised. Therefore, conservation and cultivation should be practised hand-in-hand. Most of the medicinal plants have been completely vanished from localities nearby to human habitat as witnessed by the eradication of *Taxus baccata* from Jageswar locality (Almorah district), *Chlorophytum borivilianum*, *Curculigo orchioides,* and *Gloriosa superba* from all nearby forests to Udaipur, and *Commiphora wightii* from the wild near Jodhpur. Therefore, there is a need to conserve them in situ as well as develop methods of agrotechnology and cultivation (Ramawat 2019a; Goyal et al. 2014). In India about 20% out of 400, in China 5–20% out of 5000 species, in Europe ~130

Table 1.2 Plant species for domestication by developing seed biology, agrotechnology and cultivation/micropropagation methods at author's laboratory

Plant species	Plant part	Active molecules	Use
 Flowers of *Chlorophytum borivilianum*	 Tuberous roots	 Several saponins	Adaptogenic activity
 *Desmodium gangeticum*	 Hairy roots	 Gangetin	Gastroprotective
 Flowers of *Gloriosa superba*	 tubers	 Colchicine alkaloid	Cell division inhibitor (anti-microtubulin polymerization agent)
 *Commiphora wightii*	 Plantlet in vitro	 Guggulsterone	Antihyper-cholesterolemia
 Germinating tubers of *Curculigo*	 Tubers from leaf explants	 Curculigoside A	active on β-amyloid aggregation

(continued)

Table 1.2 (continued)

	 Seeds		Green energy
 *Pueraria tuberosa*	 Tuber		cardiovascular disease, osteoporosis, hormone-dependent cancer and loss of cognitive function
 *Pterocarpus marsupium*	 Wood	 Pterostilbene (also marsupsin, pterosupin)	Antihyperglycemic activity

Aglycones	R_1	R_2
Daidzein	H	H
Genistein	OH	H
Glycitein	H	OCH_3

After Goyal et al. (2014, 2015)

Fig. 1.3 Breeding the opium poppy (*P. somniferum*) towards domestication and improvement. Wild (inset) has small flower and capsule with almost no production of alkaloids while cultivated variety has large leaves, flowers and capsules with high production of the opium latex. Photo by KGR

species, in Hungary 40 species, and in Germany 3–6% plants are obtained from cultivation (Schippmann et al. 2002). In general, people prefer medicinal plants collected from wild (as slow-growing wild plants accumulate more secondary metabolites as compared to cultivated, e.g. in ginseng) whereas pharmaceutical companies prefer uniform certified biomass for processing. There are certain advantages of cultivation and domestication over wild collections, such as uniform, certified material without adulteration, taxonomically identified, producers, and pharmaceutical companies (buyers) can enter in an agreement for long-term supply of material with price, post-harvest processing, quality control at each point, product standard can be adjusted for price and consumer preference, etc. However, all medicinal plants cannot be cultivated due to inherent biological problems like seed set, germination, and edaphic factors, and wild plant population will serve the requirement. Therefore, management of sustainable utilization is the need of the hour and proper protection of wild species/relatives and exploitation of available genetic resources (Chinthiya and Bhavyasree 2019; Yuan et al. 2010) (see Chaps. 2, 3, 5, 7, 23 in this book).

1.4 High Value Metabolites from Plants

There are several hundred species of recognized medicinal plants and remaining all are not explored completely. Still, new bioactive molecules are discovered and their biosynthetic pathways are determined. Drug discovery from the medicinal plant is a very long, tedious, time-consuming, and highly expensive research (Suntar 2019; Li et al. 2019). There are many challenges in isolation and determination of biological properties like correct identification of the plant, collection of large quantities of biomass from the wild, presence of low amount of bioactive molecule in plant, and this may be frustrating if desired success in finding novel drug is not achieved at the end (Atanasov et al. 2015). This is the main reason for the decline of research on drug discovery from plants. The details of the pros and cons of drug discovery from plants are discussed in detail elsewhere (Suntar 2019; Li et al. 2019; Atanasov et al. 2015). A few examples of selected long-standing drugs of plant origin are presented in Table 1.3.

Medicinal plants such as *Catharanthus roseus* (>200 alkaloids), *Podophyllum* species (several podophyllotoxins), *P. somniferum* (several benzoisoquinoline alkaloids), and *Cannabis sativa* (cannabinoids, terpenes, phenolics), are rich sources of many bioactive molecules. Most of the high-value therapeutics are produced in very minute quantities, for example, taxol in *Taxus* (500 mg out of 12 kg bark), vincristine and vinblastine in *C. roseus* (1 g vinblastine out of 500 kg dried leaves), and camptothecine in *Camptotheca acuminata* (0.042% in shoots) (Oberlies et al. 2009). Only a few compounds are produced in plenty like morphine in latex (minimum 55 kg raw opium/ha should be returned to government in plants cultivated in Rajasthan, India) of *P. somniferum* or oleo-gum (400 g/young plant to 1600 g old plant equals to 3200 kg/ha) yielding plants producing secondary metabolites in large quantities (Anonymous 2007, 2020; Krishna et al. 2014). Yet, there are no alternative synthetic

Table 1.3 Selected examples of long-standing drugs obtained from plants and their biological effect (after Ramawat 2019b; Aslam and Ahmad 2016)

Natural compound	Plant species	Biological effect/use
Atropine	*Atropa belladonna*	Mydriatic, anhidrotic, antispasmodic
Cocaine	*Erythroxylon coca*	Narcotic, local anaesthetic
Curcumin	*Curcuma longa*	Anti-inflammatory, anticancer
Digoxin	*Digitalis purpurea*	Cardiac glycoside
Glycyrrhetic acid	*Glycyrrhiza glabra*	Anti-inflammatory, peptic ulcer treatment
Menthol	*Mentha arvensis*	Local anaesthetic
Morphine	*Papaver somniferum*	Narcotic, analgesic
Quinine	*Cinchona officinalis*	Antimalaria
Reserpine	*Rauwolfia serpentina*	Antihypertensive, tranquilizer
Vincristine, vinblastine	*Catharanthus roseus*	Leukaemia, cancers

drugs for several plant-based drugs. These bioactive molecules are large and complex, contains specific arranged aromatic rings and chiral centres, make them a difficult candidate to obtain by chemical synthesis but may serve as a base for novel synthetic drugs (Ajayi et al. 2019). These plants were known primarily for their medicinal values, and it took several years to identify several of these compounds. Taxol was isolated in 1966 with structure established in 1971 but took several years to establish the drug before it was approved by the Food and Drug Administration, USA (FDA) in 1994 (Weaver 2014). Similar was the case for FDA approval for camptothecine. Plants are sources of several types of drugs and are continuously find their way as FDA approved drug in vast number of diseases (Newman and Cragg 2020).

Still it is not clear to us, why such complex compounds are produced in such a low quantity in plants but are effective against dreaded diseases like cancer. Yet, many minor compounds, biosynthetic intermediates, and biosynthetic pathways are not known clearly. Metabolomics and transcriptomics may through light on these aspects as evident in *Podophyllum hexandrum*, where transcriptome analysis showed six enzymes involved in podophyllotoxin biosynthesis (Lau and Sattely 2015). A new opportunity is the production of bioplastic from Cannabis stem fibre (Andre et al. 2016), which may provide the use of by-products and support cultivation of medicinal plants.

1.5 Improvement and Breeding

1.5.1 Introduction, Selection, and Cloning

The introduction of a plant in a new area requires similar agroclimatic conditions as well as pollinator present in the old habitat. Uniform material in quality and assured supply are basics to run a plant-based industry. In countries like India, temperate to tropical and desertic conditions are available making them suitable for various types of plant introduction, domestication, and development of agrotechnologies. Introduction to a new country is regulated by the law of land and which differ from country to country. Generally, endangered and economically important species are closely monitored, e.g. seeds raised plants of asafoetida (Umbelliferae) were introduced recently while *Cinchona* species, *Artemisia* species, were introduced long back (The Economics times, 27 October 2020).

Because of high income from medicinal plants over cereals and other conventional crops, medicinal plant cultivation is directly related to an increase in the socio-economic level of farmers (see Chaps. 27, 28, 29 in this book). This is very well documented, e.g. growing Isabgol, the opium poppy, ajowan, fennel, Mentha, and several others cereals in India. Until now, rural tourism related to medicinal plants agriculture is not launched very well in most of the developing countries, which can further boost the rural economy.

1.5.2 Cloning by Conventional and Non-conventional Methods

Several medicinal and aromatic plants are propagated by vegetative methods, and in such plants, conventional cloning and selection are best ways to obtained improved plant material, e.g. in *Mentha* species, Vetiver, *Pandanus* species, *Zinger*, *Curcuma*, tea (*Camellia sinensis*), *Taxus brevifolia*, *Ginkgo biloba* (stem cuttings), etc. In vegetatively propagated crops, once cloned and selected, they remain stable for several generations. Clones can be selected based on active molecule production, phenotypic characters, and specific compound production as done in vetiver (*Chrysopogon zizanioides*) for khusinol, khusimol and khusilal (Singh et al. 2019; see Chaps. 15, 16 in this book).

Non-conventional cloning is done by plant tissue culture using micropropagation methods in static and agitated liquid media. Micropropagation methods for several medicinal plants have been developed using static, liquid, and bioreactor systems. Protocols for micropropagation for several medicinal plants were developed in our laboratory. Shoots on the static medium may be slow-growing but stout whereas cultures in the agitated liquid medium are fast-growing with high rate of multiplication as evident in *C. orchioides* cultures grown in liquid medium. Department of Biotechnology, Government of India, and other funding agencies encouraged and

supported research on medicinal plants for the past 3–4 decades. This results in generating large data about medicinal plants and protocols for their micropropagation. A list of selected medicinal plants for which micropropagation protocols have been developed is presented in Table 1.4. However, large-scale production of medicinal plants using these protocols is yet to be practised in most of the cases (Ramawat 2019b; Goyal et al. 2015). In medicinal plants where a regenerative protocol was available and its bioactive molecules are in demand, transgenic plants were obtained by using an Agrobacterium T-DNA-based transgenic system to obtained desirable molecules. Thus, combing genetic engineering with a propagation system to obtain bioactive molecules, modified bioactive pathways and molecules, or used as a production platform (heterologous expression leading to molecular pharming) was attempted using a highly regenerative system of Bacopa (Table 1.4; Yadav et al. 2014).

1.5.3 Improvement by Conventional and Non-Conventional Methods

Medicinal plants though important come after food, and therefore, conventional hybridization methods, which are long and time-consuming, are used to a lesser extent. Increased polyploidy in plants results in higher vigour in plant parts as compared to diploid because of presence of a greater number of chromosome sets. This method was explored in many medicinal plant species (Ajowan, Anis, *Artemisia annua*, *Eclipta alba*, *C. sativa*, Patchouli, etc.) if this also results in higher metabolic rate and production of useful metabolites (Niazian 2019).

Methods of genetic engineering, practised for the last 40 years, insert gene/s at a random site, on one/more sites, may on different chromosomes, one/multiple copies of the gene and hence not suitable for target-specific changes. This type of genetic transformation may not yield desirable qualitative/quantitative changes in the production of secondary metabolites. Recent and advanced tools of molecular biology include synthetic promoters, variable transcription factors, genome editing tools, and site-specific recombinases that can be helpful in accurate and faster plant improvement (Liu et al. 2013).

Besides conventional plant breeding, non-conventional methods of plant improvement using plant biotechnology and tools of genetic/genomic manipulation (CRISPR, clustered regularly interspaced short palindromic repeats; Cas CRISPR, CRISPR associated proteins; GWAS, genome-wide association; MAS, marker-assisted selection; TALENs, transcription activator-like effector nucleases; TILLING, targeting-induced local lesions in genomes; ZFNs, zinc-finger nucleases), help in cloning, conservation and preservation of selected material, understand structure and functioning of genome. Consequently, these methods are beneficial for higher secondary metabolites production or specific compound production and conservation of natural

Table 1.4 Recent examples of micropropagation of medicinal plants of high value as well their genetic transformation to develop altered/high secondary metabolites producing plants

Plant species Common name Family	Principal bioactive molecule	Medicinal use	Method of propagation	References
I. Micropropagation of medicinal plants				
Acorus calamus Sweet flag Acoraceae	Flavonoids, polyphenolics	Antioxidants, Antibacterial	Shoots from rhizome bud explants	Babar et al. (2020)
Andrographis paniculata Nees, 'Kalmegh, Acanthaceae	Diterpenoids, flavonoids, quinic acids, xanthones, nor-iridoids	For common cold, diarrhoea, fever, liver, and cardiovascular	Shoots from stem explant	Pandey et al. (2017)
Cannabis sativa L Marijuana, Cannabaceae	Cannabinoids (THC), terpenes	Narcotic, pain relieving,	Photoautotrophic micropropagation from nodal cuttings	Kodym and Leeb (2019)
Carum copticum L Ajowan Umbellifeae	Thymol	Antibacterial, antiulcer, improve cholesterol,	indirect somatic embryogenesis and indirect shoot regeneration	Niazian et al. (2017)
Celastrus paniculatus Willd Malkangani Celastraceae	Seed oil, lupeol, sesquiterpene polyalcohol,	Improves memory and cognitive functions	Shoots from leaf via organogenesis	Moola and Kumari (2020)
Digitalis purpurea L. Foxglove, Scrophulariaceae	Digoxin and digitoxin	Against heart failure, anticancer,	Plant from leaf segments via organogenesis	Pérez-Alonso et al. (2018)
Hypericum gaitii, goat weed Hypericaceace	Hypericin	Wound healing, bactericidal and antiinflammatory	Multiple shoots were induced from apical and axillary meristems	Swain et al. (2016)

(continued)

Table 1.4 (continued)

Plant species Common name Family	Principal bioactive molecule	Medicinal use	Method of propagation	References
Rauvolfia serpentine *Rauvolfia tetraphylla* *Rauvolfia hookeri* Apocynaceae	Reserpine, serpentine, Ajmalicine,	Snakebites and mental illness, hypertension, and reduces blood pressure	Shoots from nodal explant	Hussain et al. (2018)
Securidaca longipedunculata (Fresen), violet tree Polygalaceae	Methylsalicylate, flavonoids, alkaloids elymoclavine, and dehydroelymoclavine,	As a pesticide, malaria, stomach problems, toothache, headache, sleeping sickness	Shoots formation	Lijalem and Feyissa (2020)
Vaccinium L (berries) Ericaceae	Polyphenolics, anthocyanin, tannins	Antioxidant metabolites	Axillary shoots, organogenesis	Debnath and Goyali (2020)
II. Agrobacterium-mediated genetic transformation of medicinal plants				
Artemisia annua Asteraceae	Artemisinin	Antimalarial drug	Chloroplast genome transformation	Kaushal et al. (2020)
Artemisia aucheri Boiss Asteraceae	Flavonoids, sesquiterpene lactones, lignans, acetylenes, triterpenes	Anti-tumour activity	Shoot organogenesis, Ri based hairy roots,	Sharafi et al. (2014)
Bacopa monnieri, Brahmi, Plantaginaceae	Bacopasides	Memory improvement	*Agrobacterium*-mediated genetic transformation, regeneration	Yadav et al. (2014)
Catharanthus roseus Periwinkle Apocynaceae	Ajmalicine, Several indole alkaloids	Anticancer, antihypertensive	Agrobacterium T-DNA, ovary and shoot apical meristem injection, 12% transformation rate, transgenic lines	Bahari et al. (2020)

(continued)

Table 1.4 (continued)

Plant species Common name Family	Principal bioactive molecule	Medicinal use	Method of propagation	References
Picrorhiza kurroa Royle ex. Benth Kutki Schlophularaceae	Iridoid glycosides known as picrosides (picroside-I–IV, apocynin, androsin, and kutkoside)	Hepato-protective, antioxidative, antiallergic and antiasthmatic, liver anticarcinogenic, and immuno-modulatory	Plant regeneration via direct organogenesis and *Agrobacterium tumefaciens*-mediated genetic transformation	Bhat et al. (2012)
Scutellaria ocmulgee Small Ocmulgee skullcap, Lamiaceae	Flavonoids	Anti-inflammatory, antioxidative, antiviral, anticancer	Leaf and shoot-derived transverse thin cell layer explants, transgenic cultures and plants	Vaidya et al. (2016)
Trachyspermum ammi (L.) Sprague) ajowan Umbellifeae	Thymol	Antibacterial, antiulcer, improve cholesterol	*Agrobacterium*-mediated gene transformation, for drought and salinity tolerance	Niazian et al. (2019)
Veratrum dahuricum L. (Liliaceae)	Cyclopamine, jervine, and veratramine	Anticancer drug	Transgenic plants from embryonic callus	Ma et al. (2020)
Withania somnifera Ashwagantha Solanaceae	Withanolides such as withaferin-A, withanone, withanolide D and withanolide A	Anti-cancer apoptosis, osteo-protective, cardioprotective, stress-reliever	High frequency, efficient and rapid transformation system, transgenic shoots	Mishra et al. (2016)

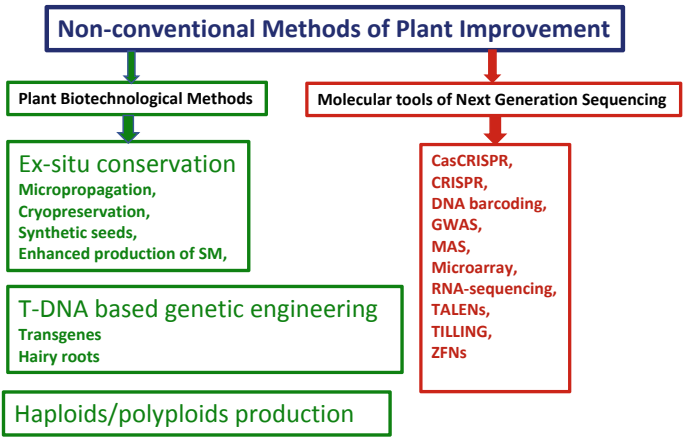


Fig. 1.4 Non-conventional medicinal plants improvement methods include applications of plant biotechnology, Agrobacterium's T-DNA-based genetic engineering and more recent molecular tools (CRISPR, clustered regularly interspaced short palindromic repeats; Cas CRISPR-associated; GWAS, genome-wide association, MAS, marker-assisted selection; TALENs, transcription activator-like effector nucleases; TILLING, targeting-induced local lesions in genomes; ZFNs zinc-finger nucleases

biodiversity. These tools can specifically modify a gene or genetic structure of medicinal plants for higher production of useful metabolites. Details of these methods are beyond the scope of this brief review and can be found elsewhere (Niazian 2019; Sinha et al. 2019; Gandhi et al. 2015) and only summarized here (Fig. 1.4).

CRISPR together with Cas CRISPR acts as a type of adaptive immunity system in prokaryotes and provides sequence-specific protection against foreign DNA or RNA (Klimek-Chodacka et al. 2018). Therefore, CRISPR/Cas9 system works by locating and identifying the foreign DNA sequence by small guide RNA (sgRNA), and then foreign DNA is cleaved by Cas9, an endonuclease (Marchev et al. 2020). Instead of random (as in T-DNA gene-based insertion) targeted regulation of gene expression can change the secondary metabolite production system (Xu et al. 2014). Tools that can create a target-based break in double-stranded DNA are of interest such as ZPNs, TALENs and CRISPR/Cas9 (three types of engineered sequence-specific nucleases). These fusion proteins have two parts; 1, Programmable and sequence-specific DNA binding domain, and 2, A non-domain specific DNA cleavage (Gaj et al. 2013). The sgRNA sequence of CRISPR/Cas can be designed; therefore, its complementary sequence can target any gene of interest in a selected genome and can carry out different functions such as gene mutations, deletion, and insertion, and also transcriptional control is possible (Xu et al. 2014). Some examples of applications of recent molecular tools such as CRISPR/Cas9 are; suppression of rosmarinic acid synthase gene in (*SmRAS*) in *Salvia miltiorrhiza* (Zhou et al. 2018), targeted mutagenesis in Dzfps gene responsible for farnesyl purophosphate synthase enzymes (consequently low squalene) in *Dioscorea zinziberansis* (Feng et al. 2018), GABA shunt pathway

in *Solanum lycopersicum* (Li et al. 2018), glycosyltransferase gene in *Nicotiana benthamiana* affecting the production of recombinant proteins lacking β-1,2-xylose and core α-1,3-fucose (Jansing et al. 2019), in hairy roots of *S. miltiorrhiza* for the production of tansinone by knocking the key gene (Li et al. 2017) and benzylisoquinoline alkaloid biosynthesis in *P. somniferum* by modified reticuline 7-O-methyl transferase and 3'hyoxy-N-methylcoclaurine4'-O-methyltransferase (Alagoz et al. 2016). Therefore, new tools are helpful in obtaining targeted desired changes in the production systems by manipulating the biosynthetic pathways and save precious time and money.

1.5.4 Conservation Using Alternate Technologies

The major challenge in the field of medicinal plant research is to protect, conserve, and propagate rare/endangered medicinal plants and at the same time also meet the demand for plant material. Extensive work has been carried out in different parts of the world to conserve and sustainable use of medicinal plants as well as develop alternative conservation technologies. These include setting up of medicinal plants board or task forces for medicinal plants under the Ministry of Science and Technology (India) and several other countries. Biotechnological studies are supported for conservation in situ and ex situ, development of micropropagation methods, production in cell cultures, and genetic manipulation to alter biosynthetic pathways leading to enhanced/alter production of principal molecule or novel molecules. Plant biotechnological methods are used for the last half-century, whereas molecular tools are widely used only recently. Application of plant biotechnology for the production of secondary metabolites has its production limitation, and only a few products reached at industrial level production such as taxol while micropropagation are used more widely on industrial level for the production of a wide range of species of medicinal and ornamentals plants (Haldar and Jha 2020; Ramawat 2019a) (see Chap. 17 in this book).

1.5.5 Production Bioactive Molecules in Cell Cultures

Plant cell culture technology is used to produce bioactive secondary metabolites of high value and interest. Details of production strategies are beyond the scope of this article and discussed elsewhere (Ramawat 2019a; Espinosa-Leal et al. 2018; Wang et al. 2017; Ochoa-Villareal et al. 2016). However, in brief: callus and cell cultures are optimized for growth by empirical approach manipulating medium constituents one by one, specifically nitrogen, sugar, and plant growth regulators, known as optimization (Goyal and Ramawat 2008; Ramawat 2019b). The cultures optimized for growth are then subjected to various effectors like precursors, biotic, and abiotic elicitors along with modified sugar concentration (usually high) and auxin (usually low) to

enhance the production of secondary metabolites (Goyal and Ramawat 2007; Mathur and Ramawat 2008). In this approach, fed-batch culture is another alternative to add production medium at the stationary phase cultures (Suthar and Ramawat 2010). Use of abiotic and biotic elicitors like plant gum (Dass and Ramawat 2009), fungal elicitors (Roat and Ramawat 2009), angiosperm parasite extract (Goyal et al.2011), and combining more than one effector may be a useful strategy to enhance the production of secondary metabolites in cultures (Arora et al. 2010; Suthar and Ramawat 2010). The selected cultures with optimized conditions are grown in a bioreactor to develop scale-up technology towards industrial production (Espinosa-Leal et al. 2018; Wang et al. 2017; Sharma et al. 2009). Some selected examples of production of secondary metabolites are presented in Table fv showing effectors and bioactive molecules in normal cell cultures and hairy roots, genetically modified Ri plasmid cultures. Hairy roots are organized plant tissues induced by the ability of Ri plasmid of *Agrobacterium rhizogenes* to induce roots, which are fast-growing, negatively geotropic, and grow without auxin in the medium (Sarkar et al. 2018). Because of organized tissues and grown in auxin free medium, hairy roots are supposed to produce higher amounts of secondary metabolites. Random insertion of Ri plasmid genes may affect the genes of host plant, thereby affecting the production/new product formation. The details of these methods are not possible here and can be found elsewhere (Haldar and Jha 2020).

Thus, the optimization of nutrients and culture conditions is a prerequisite for the use of scale-up technology in a bioreactor. Refinement of the conditions is further required for industrial-scale production of a bioactive molecule such as taxol, ajmalicine, etc. This technology has a direct impact on the conservation of the species and making available bioactive molecules by alternative technology. From laboratory to industrial-scale production is a long and tedious process and may not be successful for all the molecules investigated because of inherent biological problems associated with the plant species. Some of the successful examples of bioactive molecules production at large scale are given in Table 1.5. In last four decades, plant biotechnology for medicinal plants has transformed from reporting presence of a secondary metabolite in culture to grown in shake flask and bioreactor to targeted manipulation of desired gene for chosen compound. Moreover, all these techniques help in conserving the plants.

1.5.6 Novel Biomolecules from Medicinal Plants

A large number of plants are yet to be investigated phytochemically leaving aside the pharmacological properties (Ramawat 2019a). We know the compounds present in higher amounts in the plants but several compounds present in minor quantities are yet to be known. Large quantities of pure compounds are required for identification and determining biological properties. New tools of chemistry like Flash®, Ultra High-Pressure Liquid Chromatography (UHPLC), Medium Pressure Liquid Chromatography (MPLC), Electrokinetic chromatography, droplet counter-current,

Table 1.5 Few selected examples to demonstrate effectiveness of added effector on bioactive molecules production in plant cell cultures and transformed cultures (hairy roots) using shake flasks and bioreactor

Class	Effector/culture	Secondary metabolite	Plant species	References
PGR	Morphactin +2, iP, cell culture	Isoflavonoids	*Pueraria tuberosa*	Goyal and Ramawat (2008)
	Benzylaminopurine/callus culture	Anthocyanin	*Angelica archangelica* L	Siatka (2019)
Elicitor	*Cuscuta* extract, cell culture	Puerarin	*P. tuberosa*	Goyal et al. (2011)
	Plant gums, cell cultures	Guggulsterone	*Commiphora wightii*	Dass and Ramawat (2009)
	Gamma irradiation, callus culture	Camptothecin	*Nothapodytes foetida*	Fulzele et al. (2015)
	Yeast extract, silver nitrate	Camptothecin	*Ophiorrhiza mungos* Linn	Deepthi and Satheeshkumar (2016)
Precursor	Sugars, precursors, and morphactin	Guggulsterone	*C. wightii*	Mathur and Ramawat (2008)
Inhibitor	ALAR (N,N-dimethylamino-succinamic acid), chlormequat chloride (CCC)	Guggulsterone	*C. wightii*	Suthar and Ramawat (2010)
	Methyl jasmonate, spermidine, salicylic acid, paclobutrazol	Sweetener, phenolics	*Stevia rebaudiana*	Lucho et al. (2019)
	CCC, paclobutrazol, Daminozide	Sweetener, phenolics	*S. rebaudiana*	Karimi et al. (2019)
Fed-batch culture	Fed-batch process	Guggulsterone	*C. wightii*	Suthar and Ramawat (2010)
Bioreactor	Cell culture	Isoflavonoids	*P. tuberosa*	Sharma et al. (2009)
	Shoot cultures, 10-L nutrient sprinkle	Rosmarinic acid	*Dracocephalum forrestii* W. W. Smith	Weremczuk-Jezyna et al. (2019)
	Hairy roots, 1-L airlift	Rosmarinic acid	*Coleus blumei* L	Bauer et al. (2015)
	Cell suspension, 3-L balloon type airlift	Resveratrol	*Vitis amurensis Rupr*	Sun et al. (2016)
	Cell suspension, 5-L stirred tank	Resveratrol	*Vitis labrusca* L	Chastang et al. (2018)
	Methyl jasmonate, stirred tank	Rosmarinic acid	*Ocimum basilicum*	Pandey et al. (2019)

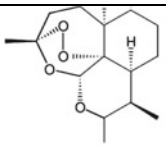
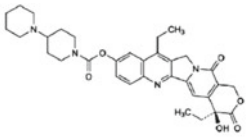
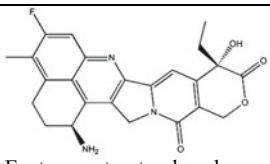
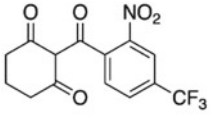
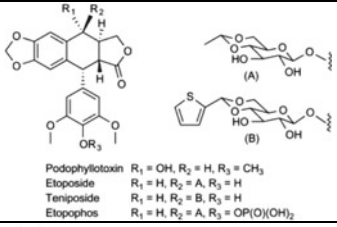
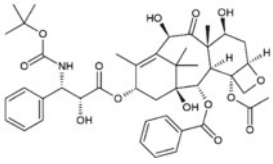
super-critical fluid, and circular chromatographies, Centrifugal partition chromatography (CPC) have revolutionized the separation and purification methods. Coupling of mass spectroscopy with other hyphenated techniques like GC-MS, LC-MS, LC-DAD-TOF-MS, circular dichroism, NMR and NMR-based correlational spectroscopies is precision tools for structure determination of isolated compounds (Khan 2018). Once the pure compounds are available in sufficient quantities, biological properties are determined. Natural compounds are obtained from plants and their derivatives or analogue can be synthesized. Initially, obtained natural products were either not effective or too toxic to use for human consumption. There are several examples that show that derivatives are more effective, less toxic, and safe than basic natural molecules (Table 1.6) (Raafat 2013). Semisynthetic derivatives of taxol (Taxotere), camptothecin (irinotecan) and podophyllotoxin (etoposide and teniposide) are not only more effective but also less toxic to human cells (Ramawat 2019b; Newman and Cragg 2020; Aslam and Ahmad 2016; see Chap. 18 in this book). These all are natural products obtained from plants and effective drugs have been developed from basic molecules present in these plants.

Halogenated secondary metabolites are of rare occurrence in terrestrial plants. It has been observed that the addition/deletion of a halide may change the pharmacological property of the secondary metabolites. Hence, a chlorinated biosynthetic machinery was genetically transferred in *C. roseus* to obtain new halogenated products paving a way to obtain such products in other plants, perhaps with more effective action (Runguphan et al. 2010). Similarly, removal of one or two hydroxyl or acetoxy groups from EGCG (epigallocatechin gallate) has resulted in more effective (antiproliferative, antiangiogenic, and antifibrotic) pro-drug with higher stability and solubility (Ahmed et al. 2019).

Cultivated medicinal plants can be used as bio-factories (known as molecular pharming) to produce molecules of pharmaceutical interest and importance even in developing countries where health infrastructure is not well developed but the population is very high (Singhabahu et al.2016; Shanmugraj et al. 2020). The health system's weaknesses are exposed throughout the world with COVID-19 pandemic. Though molecular pharming is at the initial stage, it can serve a large production system at a low cost for which more investment is required.

Secondary metabolites are generally biosynthesized through long biosynthetic pathways involving several enzymes or genes. It is difficult to manipulate this biosynthetic pathway by conventional breeding programmes but techniques of genetic engineering are involved from time to time to change the expression of genes, add new genes, or block certain genes to obtain novel molecules of interest. Biomass for downstream processing may be obtained from wild, cultivate,d or genetically modified plants or through cell cultures from bioreactors, but the processing remains more, or less the same. The process should be rapid, cost-effective, and produce a molecule of interest in pure form and undamaged (Patra and Srivastava 2017).

Table 1.6 Semisynthetic effective drugs developed from natural molecules of plant (after Ramawat 2019b; Newman and Cragg 2020; Aslam and Ahmad 2016)

Natural molecules	Plant species	New semisynthetic drug	use
Artemisinin	*Artemisia annua*	 Arteether	Antimalarial (resistant strains of Plasmodium falciparum)
Camptothecin	*Camptotheca acuminata*	 Irinotecan	Anticancer
Camptothecin	*Camptotheca acuminata*	 Exatecan, structural analogue	Anticancer
Nitisinone	*Callistemon citrinus*	 Nitisinone	Tyrosinemia
Podophyllotoxins	*Podophyllum* spp.		Anticancer
Taxol	*Taxus brevifolia*	 Docetaxel or Taxotere	Anticancer

1.6 Conclusions

Though plants are sources of many herbal drugs and demand for medicinal plants and their products will continue to increase with increased population, particularly in developing countries. This is well exemplified by the recent coronavirus pandemic. Because of lack of proper antiviral medicine or vaccine, there was a surge in immunostimulant herbs in Asian countries as the population was given a dose of herbal extract containing giloy (*Tinospora cordyfolia*), tulsi (*Ocimum sanctum*), zinger (*Zinziber officinalis*), pepper (*Piper nigra*), etc., and tons of herbs were consumed in this process, and boosting immune system is the only alternative. Therefore, dependency on herbal medicine is continued with search for bioactive molecules, analogues, and derivatives. The process of obtaining herbal drugs or even formulations is not only long, time-consuming, and tedious processes, but they are further hampered by development of formulation, poor bioavailability, solubility and stability related problems, scaling up and intellectual property problems (Anwar et al. 2019). Modern tools are helpful in the screening of plants and molecules based on their biological activity and enhancing the production in plants and cell cultures. Molecular pharming is promising technology not only to produce the desired molecule in crop plants but also anywhere in the world for human welfare. Thus, developing agrotechnologies towards domestication and cultivation along with application of modern tools to conserve and improve the medicinal plants will be helpful in conservation and meeting the demand of herbal medicine. New molecular tools have come to an edge to obtained desired targeted products.

References

Ahmed RSI, Soave C, Edbauer TG et al (2019) Discovery of green tea polyphenol-based antitumor drugs: mechanisms of action and clinical implications. In: Joshee N, Dhekney S, Parajuli P (eds) Medicinal plants from farm to pharmacy. Springer, Cham, pp 313–332

Ajayi O, Aderogba M, Obuotor E et al (2019) Acetylcholinesterase inhibitor from *Anthocleista vogelii* leaf extracts. J Ethnopharmacol 231:503–506

Alagoz Y, Gurkok T, Zhang B, Unver T (2016) Manipulating the biosynthesis of bioactive compound alkaloids for next-generation metabolic engineering in opium poppy using CRISPR-Cas 9 genome editing technology. Sci Rep 6:30910. https://doi.org/10.1038/srep30910

Andre CM, Hausman JF, Guirriero G (2016) *Cannabis sativa*: the plant of the thousand and one molecules. Front Plant Sci 7:19. https://doi.org/10.3389/fpls.2016.00019

Anonymous (2007) The ayurvedic pharmacopoeia of India (formulations), 1st edn. Department of Indian Systems of Medicine and Homeopathy, Ministry of Health and Family Welfare, Government of India, New Delhi, India

Anonymous (2020) https://dor.gov.in/narcoticdrugspsychotropic/licensed-cultivation-opium

Anwar N, Teo YK, Tan JBL (2019) The role of plant metabolites in drug discovery: current challenges and future perspectives. In: Swamy MK, Akhtar MS (eds) Natural bio-active compounds chemistry, pharmacology and health care practices, vol 2. Springer, Singapore, pp 25–51

Arora J, Goyal S, Ramawat KG (2010) Enhanced stilbene production in cell cultures of *Cayratia trifolia* through co-treatment with abiotic and biotic elicitors and sucrose. In Vitro Cell Dev Biol Plant 46:430–436. https://doi.org/10.1007/s11627-010-9308-5

Aslam MS, Ahmed MS (2016) Worldwide importance of medicinal plants: current and historical perspectives. Recent Adv Biol Med 2(2016):88–93. https://doi.org/10.18639/RABM.2016.02.338811

Astutik S, Pretzsch J, Kimengsi N (2019) Asian medicinal plants' production and utilization potentials: a review. Sustainability 11:5483. https://doi.org/10.3390/su11195483

Atanasov AG, Waltenberger B, Pferschy-Wenzig EM et al (2015) Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 33:1582–1614

Babar PS, Deshmukh AV, Salunkhe SS et al (2020) Micropropagation, polyphenol content and biological properties of Sweet Flag (*Acorus calamus*): a potent medicinal and aromatic herb. Vegetos. https://doi.org/10.1007/s42535-020-00107-8

Bahari Z, Sazegiri S, Niazi A, Afshrifar A (2020) The application of an Agrobacterium-mediated in planta transformation system in a *Catharanthus roseus* medicinal plant. Czech J Genet Plant Breed 56(1):34–41

Balunas MJ, Kinghorn AD (2005) Drug discovery from medicinal plants. Life Sci 78:431–441

Barata AM, Rocha F, Lopes V, Bettencourt E, Figueiredo AC (2011) Medicinal and aromatic plants Portugal. In: Munir O, Ameenah B, Fakima G (eds) Medicinal and aromatic plants of the world, Encyclopaedia of Life-support Systems (EOLSS), developed under the auspices of the UNESCO. Eolss Publishers, Oxford, UK

Bauer N, Vukovic R, Likic S et al (2015) Potential of different *Coleus blumei* tissues for rosmarinic acid production. Food Technol Biotechnol 53(1):3–10

Bhat WW, Lattoo SK, Rana S et al (2012) Efficient plant regeneration via direct organogenesis and *Agrobacterium tumefaciens*-mediated genetic transformation of *Picrorhiza kurroa*: an endangered medicinal herb of the alpine Himalayas. Vitro Cell Dev Biol-Plant 48:295–303. https://doi.org/10.1007/s11627-012-9434-3

Carrillo-Galván G et al (2020) Domestication of aromatic medicinal plants in Mexico: Agastache (Lamiaceae)—an ethnobotanical, morpho-physiological, and phytochemical analysis. J Ethnobiol Ethnomed 16(1). Accessed 26 Nov 2020

Chastang T, Pozzobon V, Taidi B et al (2018) Resveratrol production by grapevine cells in fed-batch bioreactor: experiments and modelling. Biochem Eng J 131:9–16

Chen SL, Yu H, Luo HM, Wu Q, Li CF, Steinmetz A (2016) Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin Med 11:37. https://doi.org/10.1186/s13020-016-0108-7

Chinthiya A, Bhavyasree RK (2019) Domestication in plants: a key to unexplored variability. Int J Curr Microbiol App Sci 8(1):133–138

Dass S, Ramawat KG (2009) Elicitation of guggulsterone production in cell cultures of *Commiphora wightii* by plant gums. Plant Cell Tiss Organ Cult 96:349–353. https://doi.org/10.1007/s11240-008-9493-7

Debnath SC, Goyali JC (2020) In vitro propagation and variation of antioxidant properties in micropropagated *Vaccinium* berry plants—a review. Molecules 25(4):788. https://doi.org/10.3390/molecules25040788

Deepthi S, Satheeshkumar K (2016) Enhanced camptothecin production induced by elicitors in the cell suspension cultures of *Ophiorrhiza mungos* Linn. Plant Cell Tiss Org Cult 124:483–493. https://doi.org/10.1007/s11240-015-0908-y

Diamond J (2002) Evolution, consequences and future of plant and animal domestication. Nature 418:700–707

Dudai (2012) Domestication and breeding of wild medicinal and aromatic plants-thirty years of experience in Israel. Acta Hortic 955(955):175–183

Erb M, Kliebenstein DJ (2020) Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. Plant Physiol 184(1):39–52. https://doi.org/10.1104/pp.20.00433

Espinosa-Leal CA, Puente-Garza CA, García-Lara S (2018) In vitro plant tissue culture: means for production of biological active compounds. Planta 248:1–18. https://doi.org/10.1007/s00425-018-2910-1

Feng S, Song W, Fu R, Zhang H, Xu A, Li J (2018) Application of the CRISPR/Cas9 system in *Dioscorea zingiberensis*. Plant Cell Tissue Organ Cult. https://doi.org/10.1007/s11240-018-1450-5

Fuller DQ, Denham T, Arroyo-Kalin M et al (2014) Convergent evolution and parallelism in plant domestication revealed by an expanding archaeological record. Proc Nat Acad Sci USA 111:6147–6152

Fulzele DP, Satdive R, Kamble S, Singh S, Singh S (2015) Improvement of anticancer drug camptothecin production by gamma irradiation on callus cultures of *Nothapodytes foetida*. Int J Pharl Res Allied Sci 4:19–27

Gaj T, Gersbach CA, Barbas IIICF (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends Biotechnol 31:397–405. https://doi.org/10.1016/j.tibtech.2013.04.004

Gandhi SG, Mahajan V, Bedi YS (2015) Changing trends in biotechnology of secondary metabolism in medicinal and aromatic plants. Planta 241:303–317. https://doi.org/10.1007/s00425-014-2232-x

Gepts P (2014) Domestication of plants. In: Alfen NV (ed) Encyclopedia of agriculture and food systems, vol 2. Elsevier, San Diego, pp 474–486

Goyal S, Ramawat KG (2007) Effect of chemical factors on production of isoflavonoids in *Pueraria tuberosa* (Roxb.ex.Willd.) DC suspension culture. Indian J Exp Biol 45:1063–1067

Goyal S, Ramawat KG (2008) Synergistic effect of morphactin on cytokinin-induced production of isoflavonoids in cell cultures of *Pueraria tuberosa* (Roxb. ex. Willd.) DC. Plant Growth Regul 55:175–181. https://doi.org/10.1007/s10725-008-9271-x

Goyal S, Sharma V, Ramawat KG (2011) Marked effect of *Cuscuta* on puerarin accumulation in cell cultures of *Pueraria tuberosa* grown in shake flasks and a bioreactor. Plant Biotechnol Rep 5:121–126. https://doi.org/10.1007/s11816-011-0167-2

Goyal S, Arora J, Ramawat KG (2014) Biotechnological approaches to medicinal plants of Aravalli hills: conservation and scientific validation of biological activities. In: Ahuja MR, Ramawat KG (eds) Biotechnology and biodiversity. Sustainable development and biodiversity, vol 4. Springer, Cham. https://doi.org/10.1007/978-3-319-09381-9_11

Goyal S, Sharma V, Ramawat KG (2015) A review of biotechnological approaches to conservation and sustainable utilization of medicinal lianas in India. In: Parthasarathy N (ed) Biodiversity of lianas, sustainable development and biodiversity. Springer, Switzerland, pp 179–210. https://doi.org/10.1007/978-3-319-14592-1_11

Halder M, Jha S (2020) Morphogenesis, genetic stability, and secondary metabolite production in untransformed and transformed cultures. In: Ramawat KG et al (eds) Plant cell and tissue differentiation and secondary metabolites, reference series in phytochemistry. Springer, Switzerland AG. https://doi.org/10.1007/978-3-030-30185-9_15

He K (2015) Traditional Chinese and Thai medicine in a comparative perspective. Complement Ther Med 23:821–826. https://doi.org/10.1002/elsc.200800114

Hua MJ, Zhu Q, Dong X et al (2018) Principles of medicinal plant introduction and domestication. Guangxi Zhiwu/guihaia 38(8):973–983

Hussain SA, Ahmad N, Anis M (2018) Synergetic effect of TDZ and BA on minimizing the post-exposure effects on axillary shoot proliferation and assessment of genetic fidelity in *Rauvolfia tetraphylla* (L.). Rend Fis Acc Lincei 29:109–115. https://doi.org/10.1007/s12210-018-0667-x

https://economictimes.indiatimes.com/industry/cons-products/food/india-starts-cultivation-of-hing-which-it-imports-in-its-raw-form-for-usd-100-million/hing-cultivation/slideshow/78889714.cms (27 Oct 2020)

Jansing J, Sack M, Augustine SM, Fischer R, Bortesi L (2019) CRISPR/Cas9-mediated knockout of six glycosyltransferase genes in *Nicotiana benthamiana* for the production of recombinant proteins lacking β-1,2-xylose and core α-1,3-fucose. Plant Biotechnol J 17(2):350–361

Karimi M, Ahmadi A, Hashemi J, Abbasi A et al (2019) Plant growth retardants (PGRs) affect growth and secondary metabolite biosynthesis in *Stevia rebaudiana* Bertoni under drought stress. South African J Bot 121:394–401

Katumba BM, Boffa JM, Abigaba G, Okorio J (2004) Domestication of medicinal tree species in the *Victoria* lakeshore region. Uganda J Agric Sci 9:84–88
Kaushal C, Abdin MZ, Kumar S (2020) Chloroplast genome transformation of medicinal plant *Artemisia annua*. Plant Biotechnol J. https://doi.org/10.1111/pbi.13379
Khan RA (2018) Natural products chemistry: the emerging trends and prospective goals. Saudi Pharm J 26(5):739–775
Klimek-Chodacka M, Oleszkiewicz T, Lowder L et al (2018) Efficient CRSPR/Cas9-based genome editing in carrot cells. Plant Cell Rep 37(4):575–586
Kodym A, Leeb CJ (2019) Back to the roots: protocol for the photoautotrophic micropropagation of medicinal *Cannabis*. Plant Cell Tiss Organ Cult 138:399–402. https://doi.org/10.1007/s11240-019-01635-1
Krishna A, Kumar V, Yadav RP (2014) Production and trade related issues of opium poppy cultivation with special reference to Barabanki district, UP, India. Acta Hort 1036:111–118 https://doi.org/10.17660/ActaHortic.2014.1036.12
Larson G, Piperno DR, Allaby RG et al (2014) Current perspectives and the future of domestication studies. Proc Natl Acad Sci USA 111(17):6139–6146. https://doi.org/10.1073/pnas.1323964111
Lau W, Sattely ES (2015) Six enzymes from may apple that complete the biosynthetic pathway to the etoposide aglycone. Science 349(6253):1224–1228. https://doi.org/10.1126/science.aac7202
Li LF, Olsen KM (2016) To have and to hold: selection for seed and fruit retention during crop domestication. Curr Top Dev Biol 119:63–109
Li B, Cui G, Shen G, Zhan Z, Huang L, Chen J, Qi X (2017) Targeted mutagenesis in the medicinal plant *Salvia miltiorrhiza*. Sci Rep 7:43320. https://doi.org/10.1038/srep43320
Li R, Li X, Fu D, Zhu B, Tian H et al (2018) Multiplexed CRISPR/Cas9-mediated metabolic engineering of γ-aminobutyric acid levels in *Solanum lycopersicum*. Plant Biotechnol J 16(2):415–427
Li F, Wang Y, Li D, Chen Y, Dou QP (2019) Are we seeing a resurgence in the use of natural products for new drug discovery? Expert Opin Drug Discov 1–4. https://doi.org/10.1080/17460441.2019.1582639
Lijalem T (2020) Feyissa T (2020) In vitro propagation of *Securidaca longipedunculata* (Fresen) from shoot tip: an endangered medicinal plant. J Genet Eng Biotechnol 18:3. https://doi.org/10.1186/s43141-019-0017-0
Liu W, Yuan JS, Stewart CN Jr (2013) Advanced genetic tools for plant biotechnology. Nat Rev Genet 14:781–793. https://doi.org/10.1038/nrg3583
Lucho SR, do Amaral MN, López-Orenes A et al (2019) Plant growth regulators as potential elicitors to increase the contents of phenolic compounds and antioxidant capacity in *Stevia* Plants. Sugar Tech 21:696–702. https://doi.org/10.1007/s12355-018-0673-4
Ma R, Yu Z, Cai Q et al (2020) *Agrobacterium*-mediated genetic transformation of the medicinal plant *Veratrum dahuricum*. Plants 9:191. https://doi.org/10.3390/plants9020191
Marchev AS, Zhenya PY, Milen IG (2020) Green (cell) factories for advanced production of plant secondary metabolites. Crit Rev Biotechnol 40(4):443–458. https://doi.org/10.1080/07388551.2020.1731414
Mathur M, Ramawat KG (2008) Improved guggulsterone production from sugars, precursors, and morphactin in cell cultures of *Commiphora wightii* grown in shake flasks and a bioreactor. Plant Biotechnol Rep 2:133–136. https://doi.org/10.1007/s11816-008-0051-x
Mishra S, Bansal S, Sangwan RS et al (2016) Genotype independent and efficient agrobacterium-mediated genetic transformation of the medicinal plant *Withania somnifera* Dunal. J Plant Biochem Biotechnol 25:191–198. https://doi.org/10.1007/s13562-015-0324-8
Moola AK, Kumari BDR (2020) Rapid propagation of *Celastrus paniculatus* Willd.: an endangered medicinal plant through indirect organogenesis. Vegetos. https://doi.org/10.1007/s42535-020-00105-w
Newman DJ, Cragg JM (2020) Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod 83:770–803

Niazian M (2019) (2019) Application of genetics and biotechnology for improving medicinal plants. Planta 249:953–973. https://doi.org/10.1007/s00425-019-03099-1

Niazian M, Sadat-Noori SA, Galuszka P et al (2017) Genetic stability of regenerated plants *via* indirect somatic embryogenesis and indirect shoot regeneration of *Carum copticum* L. Ind Crops Prod 97:330–337

Niazian M, Sadat-Noori SA, Tohitfar M et al (2019) *Agrobacterium*-mediated genetic transformation of ajowan (*Trachyspermum ammi* (L.) Sprague): an important industrial medicinal plant. Ind Crops Prod 132:29–40

Oberlies NH, Flora S, Weaver AL (2009) Camptothecin and taxol. Chem Int 25(4). https://doi.org/10.1515/ci.2003.25.4.4

Ochoa-Villarreal M, Howat S, Hong S, Jang MO, Jin YW, Lee EK, Loake GJ (2016) Plant cell culture strategies for the production of natural products. BMB Rep 49(3):149–158. https://doi.org/10.5483/bmbrep.2016.49.3.264

Pandey S, Bahadur AN, Kanungo V, Tewari U (2017) Rapid micropropagation of *Androgrphis paniculata* Nees, from nodal explants to study effect of various plant growth regulators. Indian J Sci Res 7(2):149–157

Pandey P, Singh S, Banerjee S (2019) *Ocimum basilicum* suspension culture as resource for bioactive triterpenoids: yield enrichment by elicitation and bioreactor cultivation. Plant Cell Tiss Organ Cult 137(1):65–75

Patra N, Srivastava AK (2017) Mass production of artemisinin using hairy root cultivation of *Artemisia annua* in bioreactor. In: Pavlov A, Bley T (eds) Bioprocessing of plant in vitro systems. Reference series in phytochemistry. Springer, Cham, pp 343–359

Pérez-Alonso N, Martin R, Capote A et al (2018) Efficient direct shoot organogenesis, genetic stability and secondary metabolite production of micropropagated *Digitalis purpurea* L. Ind Crops Prod 116:259–266

Petrovska BB (2012) Historical review of medicinal plants' usage. Pharmacol Rev 6(11):1–5. https://doi.org/10.4103/0973-7847.95849

Purugganan MD (2019) Evolutionary insights into the nature of plant domestication. Curr Biol 29:R705–R714

Raafat KM (2013) Exploration of the protective effects of some natural compounds against neurodegeneration exploiting glycine receptors in vivo model. Nat Prod Chem Res 1(3):1–6

Ramawat KG (2019a) An introduction to biodiversity and chemotaxonomy. In: Ramawat KG (ed) Sustainable development and biodiversity. Springer, Switzerland, AG, pp 1–14. https://doi.org/10.1007/978-3-030-30746-2_1

Ramawat KG (2019b) An introduction to the process of cell, tissue, and organ differentiation, and production of secondary metabolites. In: Ramawat K, Ekiert H, Goyal S (eds) Plant cell and tissue differentiation and secondary metabolites. Reference series in phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-030-11253-0_35-1

Ramawat KG, Goyal S (2008) The Indian herbal drugs scenario in global perspectives. In: Ramawat KG, Merillon JM (eds) Bioactive molecules and medicinal plants. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-74603-4_18

Ramawat KG, Goyal S (2019) Co-evolution of secondary metabolites during biological competition for survival and advantage: an overview. In: Merillon JM, Ramawat K (eds) Co-evolution of secondary metabolites. Reference series in phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-319-76887-8_45-1

Roat C, Ramawat KG (2009) Elicitor-induced accumulation of stilbenes in cell suspension cultures of *Cayratia trifolia* (L.) Domin. Plant Biotechnol Rep 3:135–138. https://doi.org/10.1007/s11816-009-0082-y

Runguphan W, Qu X, O'Connor SE (2010) Integrating carbon–halogen bond formation into medicinal plant metabolism. Nature 468:461–464

Sarkar S, Ghosh I, Roychowdhury D, Jha S (2018) The effects of rol genes of *Agrobacterium rhizogenes* on morphogenesis and secondary metabolite accumulation in medicinal plants. In: Kumar

N (ed) Biotechnological approaches for medicinal and aromatic plants. Springer, Singapore, pp 27–51

Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. Biodiversity and the ecosystem approach in agriculture, forestry and fisheries. In: Satellite event on the occasion of the ninth regular session of the commission on genetic resources for food and agriculture. Rome, 12–13 Oct 2002. Inter-Departmental Working Group on Biological Diversity for Food and Agriculture. Rome, pp 1–21

Shanmugaraj B, Bulaon JC, Phoolcharoen W (2020) Plant molecular farming: a viable platform for recombinant biopharmaceutical production. Plants 9:842. https://doi.org/10.3390/plants9070842

Sharafi A, Sohi HH, Mirzaee H et al (2014) In vitro regeneration and *Agrobacterium* mediated genetic transformation of *Artemisia aucheri* Boiss. Physiol Mol Biol Plants 20:487–494. https://doi.org/10.1007/s12298-014-0248-0

Sharma V, Goyal S, Ramawat KG (2009) Scale up production of isoflavonoids in cell suspension cultures of *Pueraria tuberosa* grown in shake flasks and bioreactor. Eng Life Sci 9(3):267–271

Sheehan HE, Hussain SJ (2002) Unani Tibb: history, theory, and contemporary practice in South Asia. Ann AAPSS 583:122–135

Shepherd AW (2007) A guide marketing cost and how to calculate them. Marketing extension guide. Food and Agricultural Organization, Rome

Sher H, Alyemeni MN, Faridullah (2010) Cultivation and domestication study of high value medicinal plant species (its economic potential and linkages with commercialization). African J Agri Res 5(18):2462–2470

Siatka T (2019) Effects of growth regulators on production of Anthocyanins in callus cultures of *Angelica archangelica.* Nat Prod Commun 14(6) https://doi.org/10.1177/1934578X19857344

Singh N, Singh VR, Lal RK, Verma RS, Mishra A, Yadav R (2019) Quantification of genotypic and chemotypic diversity for elite clone selection with high-quality essential oil traits in Vetiver [*Chrysopogon zizanioides* (L.) Roberty]. J Essent Oil-Bearing Plants 22(4):1150–1162

Singhabahu S, Hefferon K, Makhzoum A (2016) Plant Molecular Pharming. In: Jha S (eds) Transgenesis and secondary metabolism. Reference series in phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-319-27490-4_21-1

Sinha S, Sandhu K, Bisht N, Naliwal T, Saini I, Kaushik P (2019) Ascertaining the paradigm of secondary metabolism enhancement through gene level modification in therapeutic plants. J Young Pharmacists 11(4):337–343

Subramani P (2018) Herbal drug discovery: challenges and perspectives. Curr Pharmacogenomics Personalized Med (Formerly Current Pharmacogenomics) 16(1):63–68(6). https://doi.org/10.2174/1875692116666180419153313

Sun D, Li C, Qin H et al (2016) Somatic embryos cultures of *Vitis amurensis* Rupr. in air-lift bioreactors for the production of biomass and resveratrol. J Plant Biol 59(5):427–434

Suntar I (2019) Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochem Rev. https://doi.org/10.1007/s11101-019-09629-9

Suthar S, Ramawat KG (2010) Growth retardants stimulate guggulsterone production in the presence of fungal elicitor in fed-batch cultures of *Commiphora wightii*. Plant Biotechnol Rep 4:9–13. https://doi.org/10.1007/s11816-009-0110-y

Swain D, Lenka S, Hota T et al (2016) Micro-propagation of *Hypericum gaitii* Haines, an endangered medicinal plants: assessment of genetic fidelity. Nucleus 59:7–13. https://doi.org/10.1007/s13237-015-0146-z

Tanga M, Lewu FB, Oyedeji OA, Oyedeji OO (2018) Cultivation of medicinal plants in South Africa: a solution to quality assurance and consistent availability of medicinal plant materials for commercialization. Acad J Med Plants 6(7):168–177. https://doi.org/10.15413/ajmp.2018.0133

Vaidya BN, Jackson CL, Perry ZD et al (2016) *Agrobacterium*-mediated transformation of thin cell layer explants of *Scutellaria ocmulgee* small: a rare plant with anti-tumor properties. Plant Cell Tiss Organ Cult 127:57–69. https://doi.org/10.1007/s11240-016-1029-y

Vasisht K, Sharma N, Karan M (2016) Current perspective in the international trade of medicinal plants material: an update. Curr Pharm Des 22:4288–4336

Wang J, Li J, Li J et al (2017) Production of active compounds in medicinal plants: from plant tissue culture to biosynthesis. Chin Herbal Med 9(2):115–125

Weaver BA (2014) How taxol/paclitaxel kills cancer cells. Mol Biol Cell, Perspect Cell Biol Hum Health 25(12):2677–2681. https://doi.org/10.1091/mbc.E14-04-0916

Węglarz Z, Przybył J, Geszprych A (2008) Roseroot (*Rhodiola rosea* L.): effect of internal and external factors on accumulation of biologically active compounds. In: Ramawat K, Merillon J (eds) Bioactive molecules and medicinal plants. Springer, Berlin, Heidelberg, pp 297–315

Weremczuk-Jezyna I, Kochan E, Szymczyk P et al (2019) The antioxidant and antimicrobial properties of phenolrich extracts of *Dracocephalum forrestii* W. W. Smith shoot cultures grown in the nutrient sprinkle bioreactor. Phytochem Lett 30:254–260

World Health Organization (2015) Connecting global priorities: biodiversity and human health: a state of knowledge review. ISBN 97892 4 150853 7

Xu T, Li Y, Van Nostrand JD, He Z, Zhou J (2014) Cas9-based tools for targeted genome editing and transcriptional control. Appl Environ Microbiol 80:1544–1552. https://doi.org/10.1128/AEM.03786-13

Yadav S, Sharma P, Srivastava A et al (2014) Strain specific *Agrobacterium*-mediated genetic transformation of *Bacopa monnieri*. J Genet Eng Biotechnol 12(2):89–94

Yuan QJ, Zhang ZY, Hu J et al (2010) Impacts of recent cultivation on genetic diversity pattern of a medicinal plant, *Scutellaria baicalensis* (Lamiaceae). BMC Genet 11:29

Zhou Z, Tan H, Li Q, Chen J, Gao S, Wang Y, Chen W, Zhang L (2018) CRISPR/Cas9-mediated efficient targeted mutagenesis of RAS in *Salvia miltiorrhiza*. Phytochemistry 148:63–70. https://doi.org/10.1016/j.phytochem.2018.01.015

Part I
Domestication and Cultivation of Medicinal Plants

Chapter 2
Introducing Wild-Growing Medicinal Plant into Cultivation: Dropwort (*Filipendula vulgaris* Moench)—A Rich Source of Phenolic Compounds

Katarzyna Bączek, Jarosław L. Przybył, Olga Kosakowska, and Zenon Węglarz

Abstract Dropwort is a wild-growing plant, long used in traditional European medicine. The species is a rich source of phenolic compounds. The plant raw materials are: flowers, herb, and underground organs (rhizomes with tuberous roots). They have been used in the treatment of difficult-to-heal wounds, cold, rheumatism, and kidney problems. Dropwort extracts reveal potential in the prevention of neurodegenerative disorders, as well. Due to dynamic changes in the use of agricultural lands, natural sites of this species gradually disappear. Introduction into the cultivation seems to be a chance to preserve its natural resources and to provide standardized raw materials for the industry. In this chapter, we present the results of our investigations concerning the factors affecting the quality of different raw materials obtained from cultivated dropwort plants.

Keywords Intraspecific variability · Plant development · Propagation · Raw materials · HPLC · Flavonoids · Phenolic acids

Abbreviations

BF	Beginning of flowering
EF	End of flowering

K. Bączek (✉) · J. L. Przybył · O. Kosakowska · Z. Węglarz
Institute of Horticultural Sciences, Warsaw University of Life Sciences—SGGW, Nowoursynowska Street 166, 02-787 Warsaw, Poland
e-mail: katarzyna_baczek@sggw.edu.pl

J. L. Przybył
e-mail: jaroslaw_przybyl@sggw.edu.pl

O. Kosakowska
e-mail: olga_kosakowska@sggw.edu.pl

Z. Węglarz
e-mail: zenon_weglarz@sggw.edu.pl

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_2

FF Full flowering
HPLC High performance liquid chromatography
MPM Malignant pleural mesothelioma
nd Not detected

2.1 Introduction

Dropwort (*Filipendula vulgaris* Moench, syn.: *Filipendula hexapetala* Gilib. or *Spiraea filipendula* L.) is a wild-growing perennial plant, occurring through Europe, western and central Asia and northwestern part of Africa (Meusel et al. 1965). The species is characteristic for dry grasslands or continental steppes and can be found on meadows, pastures, edges of arable lands, along sunny forest roads and railway embankments; it prefers non-acidic soils rich in humus and calcium (Kostarkiewicz-Gierlat and Stachurska-Swakon 2017). The species is well-adapted to drought and low temperatures (Cortan et al. 2019).

The genus *Filipendula* consists of 15 species of flowering plants in the family Rosaceae (Schanzer 1994). Among them dropwort is distinguished by characteristic structure of underground organs. They consist of a short rhizome with thin roots bearing tubers. The plant produces a rosette of leaves with flowering shoots up to 50–80 cm high. The leaves are strongly dissected. The panicle-shaped inflorescence consists of creamy-white or pale-pink fragrant flowers, up to 2 cm in diameter. Blooming usually takes place in June–July, and star-shaped fruits ripe at the end of July (Motyka and Panych 1936; Mowszowicz 1985) (Figs. 2.1, 2.2 and 2.3).

So far, low genetic variability both within and among dropwort populations has been found. Wind pollination along with long-distance insect pollination ensure gene flow between populations what prevent from genetic drift of the species (Weidema et al. 2000). However, the number of dropwort populations has declined. This is connected mainly with the change in the use of agricultural lands observed during the last decades. In Europe, dry grasslands, were it grows, occur mainly at the edges of arable lands or forests. The disappearance of plants specific for such sites is caused by overfertilization, ceased grazing, shrub encroachment or intensive mowing of meadows and roadsides, making impossible to release the seeds and in consequence—offspring of many plant species. Thus, dropwort populations are fragmented and small.

In Europe, dropwort has been used for ages in traditional medicine. The raw materials collected from this plant are: flowers (1); herb, consisting of root leaves and flowering stems (2); and underground organs (3). The biologically active compounds present in these organs are mainly phenolics including flavonoids, phenolic acids, salicylates, tannins, and traces of coumarins. Among these rutoside, hyperoside, luteolin, luteolin-7-glucoside, spireoside, astragalin, kaempferol, quercetin, quercitrin, avicularin, myricetin; some catechin derivatives, namely: (+)-catechin, (−)-epigallocatechin; as well as phenolic acids, i.e., gallic, ellagic, syringic,

Fig. 2.1 Flowers (all photographs in this chapter were prepared by authors of this chapter)

Fig. 2.2 Dropwort leaves

salicylic, chlorogenic, caffeic, and rosmarinic acids have been identified in dropwort leaves and flowers (Smolarz et al. 1999; Bączek et al. 2012; Pukalskienė et al. 2015; Movsumov et al. 2017). The aboveground organs contain also 0.05–0.1% of essential oil, with salicylaldehyde as a dominant compound (Pavlović et al. 2007; Radulović et al. 2007). The information on chemical composition of underground organs are much more scarce. So far, the following flavonoids have been identified in dropwort

Fig. 2.3 Underground organs

rhizomes and roots: rutoside, hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, and spireoside (Smolarz et al. 1999). Capecka et al. (2012) indicate on high content of flavan-3-ols and phenolic acids (ellagic, gallic, and salicylic acids) in these organs, as well.

The application of dropwort in folk medicine is well documented (Radulović et al. 2007). Its flowers have been used in colds and rheumatism, similarly to meadowsweet flowers (*Filipendula ulmaria* L.). They reveal anti-inflammatory, antipyretic, diaphoretic, and diuretic activity. The leaves and flowers are used in the form of infusions to treat difficult-to-heal wounds and eye inflammation, whereas decoctions prepared from underground organs are applied in the treatment of sore throats, kidney diseases, and diarrhea (Radulović et al. 2007; HMPC 2011; Katanić et al. 2018). Nowadays, the abovementioned application finds its grounds in the laboratory studies on dropwort activity (Katanić et al. 2014, 2018; Pukalskienė et al. 2015; Smardžic et al. 2016, 2018). The essential oil obtained from dropwort leaves shows activity against pathogenic bacteria and fungi, such as: *Escherichia*

coli (ATCC 25922 and 95), *Staphylococcus aureus* (ATCC 6538), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella enteritidis* (ATCC 13067), *Phialophora fastigiata* (FSB81), *Aspergillus niger* (ATCC 10031) or *Candida albicans* (ATCC 10231) (Radulović et al. 2007; Katanić et al. 2014). It has been shown that flower preparations prevent the formation of gastric ulcers and reveal anticancer activity against malignant pleural mesothelioma (MPM) management (Smardžic et al. 2018; Pulito et al. 2019). Dropwort preparations may also be useful in the therapy of neurodegenerative disorders such as Parkinson's or Alzheimer's and show nootropic potential (increasing brain activity) at a level similar to piracetam (Shilova and Suslov 2015; Neagu et al. 2015). Their antihyperalgesic and antioxidant activity have also been confirmed (Oszmiański et al. 2007; Smardžic et al. 2016, 2018).

As mentioned above, dropwort has been used in people medicine for ages. Modern analytical tools give the chance to discover its therapeutic potential from scratch, to document the activity and to find new indications or confirmation for its application. Currently, due to limited occurrence (loss of habitats) of dropwort, and thus limited availability of the raw material, the plant is used extremely rarely (Weidema et al. 2000). The only chance to produce significant amount of good quality raw materials is to introduce the plant into cultivation. This is also indispensable for the production of high-quality standardized extracts.

The aim of this work was to present the results of several-years studies on dropwort growing in cultivation conditions, with a special emphasis paid on the value potential of organs originated from this plant. The dynamics of the accumulation of biologically active compounds in aboveground and underground parts of dropwort was also shown. This altogether may indicate on the possibility of obtaining the plant material variable in respect of biological activity, and also easier for standardization.

2.2 Chemical Diversity of Wild-Growing Populations

The information on development and chemical diversity of dropwort wild-growing populations are relatively scarce. In Europe, the species is rare or even extremely endangered (Duda 2009; Weidema et al. 2000). The abundance of individual plants within dropwort population is strictly related with the habitat conditions, i.e., the soil humidity, its pH as well as with the presence of other vascular plants on the site. These factors significantly influence the development and reproductive possibilities of the species (Kostarkiewicz-Gierlat and Stachurska-Swakon 2017).

In our study, six wild-growing populations of dropwort were analyzed in situ in terms of chemical diversity of its herb and underground organs. The populations originated from eastern part of Poland (Table 2.1). The leaves and flowers (herb) were collected at the full-flowering stage whereas underground organs—in the early spring. The raw materials were dried at 40 °C and subjected for chemical analysis using high-performance liquid chromatography (HPLC). The analysis was carried out according to Bączek et al. (2012). The obtained results

Table 2.1 Geographical coordinates of dropwort populations

	Location	Coordinates	
1	Siemiatycze	N 52° 23.705′	E 022° 53.123′
2	Drohiczyn	N 52° 23.825′	E 022° 40.338′
3	Sytki	N 52° 23.620′	E 022° 40.452′
4	Goraj	N 50° 42.855′	E 022° 40.545′
5	Łada	N 50° 43.822′	E 022° 39.155′
6	Kozłowo	N 52° 37.389′	E 022° 44.900′

indicate on high intraspecific chemical diversity. In the herb of these populations, seven flavonoids (quercetin, astragalin, hyperoside, kaempferol, spireoside, (+)-catechin and (−)-epigallocatechin) and seven phenolic acids (gallic, ellagic, syringic, salicylic, caffeic, rosmarinic, and chlorogenic acids), were identified. Hyperoside, spireoside, and astragalin were the dominant compounds. All the abovementioned compounds reveal strong antioxidant activity (Raza et al. 2017; Kohlmünzer 2000). Moreover, some of them exhibit significant pro-health effects, e.g., astragalin reveals hypotensive activity; quercetin reduces the level of lipids in the blood, reveals antiaggregation, anti-inflammatory, hepatoprotective, and hypoglycemic properties, whereas hyperoside has diuretic and anti-inflammatory effects (Kohlmünzer 2000). In turn, dropwort underground organs are rich in flavan-3-ols, namely: (+)-catechin, (+)-epicatechin, (−)-epigallocatechin, (−)-epigallocatechin gallate, as well as gallic and ellagic acids. (+)-Catechin is a dominant here (480.4 mg × 100 g^{-1} DW). It has been introduced into the official medicine as a drug regenerating liver tissues damaged due to infections or intoxications (Kohlmünzer 2000). One of the most important raw material, listed in many pharmacopoeias, rich in this compound is oak bark (*Quercus cortex*). According to Elansary et al. (2019) the content of catechin in the bark of *Quercus robur* reach 44.52 mg × 100 g^{-1} DW. Thus, underground organs of dropwort, containing 10 times more catechin than oak bark, seem to be an interesting source of these substances.

The results of our experiment showed that the content of particular compounds in dropwort raw materials was very variable. This phenomenon was observed especially in the case of flavonols, e.g., spireoside, the content of which, depending on the population, ranged in the herb from 124.0 to 952.3 mg × 100 g^{-1} DW. Similar tendency was observed for hyperoside. Lower differences among analyzed populations were observed when regards flavan-3-ols and phenolic acids, especially in the case of underground organs (Table 2.2).

To sum up, the observed chemical variability of investigated wild-growing populations may be related to various factors, both endo- and exogenic. The most important seems to be genetic diversity followed by plant's age and stage of their development. Environmental conditions could have a crucial meaning here, as well.

Table 2.2 Intraspecific chemical diversity of wild-growing dropwort (mg × 100 g^{-1} DW)

Compounds	Herb			Underground organs		
	Mean	Min.	Max.	Mean	Min.	Max.
Flavonoids						
Quercetin	1.1	0.2	1.9	n.d.		
Astragalin	441.2	153.3	656.1	n.d.		
Hyperoside	536.7	157.0	809.5	n.d.		
Kaempferol	146.0	61.3	187.4	n.d.		
Spireoside	490.1	124.0	952.3	n.d.		
(+)-catechin	227.5	100.1	330.4	480.4	360.4	563.8
(+)-epicatechin	n.d.			281.8	155.4	372.9
(−)-epigallocatechin	176.1	55.7	340.2	202.9	57.0	384.2
(−)-epigallocatechin gallate	n.d.			80.0	53.9	122.5
Phenolic acids						
Gallic acid	79.6	35.6	130.2	104.4	61.4	127.6
Ellagic acid	94.3	13.7	141.3	13.0	4.2	22.6
Syringic acid	192.9	125.8	251.6	n.d.		
Salicylic acid	16.0	6.6	22.6	n.d.		
Caffeic acid	50.3	12.2	90.8	n.d.		
Rosmarinic acid	53.0	18.4	102.4	n.d.		
Chlorogenic acid	151.3	71.1	280.8	n.d.		

ND not detected

2.3 The Quality of Raw Material from Cultivated Plants

2.3.1 The Effect of Plant Propagation Method on the Yield and Quality of Raw Material

One of the most important problems when introducing wild-growing plants into cultivation seems to be production of propagating material. The use of seeds for establishing plantations is often unreliable. One of the most important traits typical for wild-growing plants in uneven, stretched over time seed germination. As a result of evolution, this phenomenon allows the species to survive in unfavorable environmental conditions. Hence, viable seeds can survive in the soil seed bank for up to several dozen years. Only favorable environmental conditions, during which the dormancy of seeds is broken, can induce germination. Developing a seed germination protocol for the purpose of plant cultivation is extremely laborious and time-consuming. In such cases, different methods of vegetative propagation are implemented, including the use of in vitro techniques. Sometimes, however, the use of simple methods related to obtaining cuttings gives satisfactory results.

In our investigation on dropwort, two methods of plant propagation have been used; i.e., traditional generative propagation with seeds and vegetative one via stem–root cuttings (obtained by the division of maternal plants) (Fig. 2.4, 2.5 and 2.6). The natural germination of dropwort seeds is relatively weak and uneven. Sowing seeds directly into the ground seems risky. Thus, the generative propagation which relies on the productions of seedlings used for plantation establishment should be recommended. In this study, we have compared the yield and quality of above- and underground organs of dropwort originated from plantations established by generative and

Fig. 2.4 Seeds

Fig. 2.5 Seedlings

Fig. 2.6 Rooted Cuttings

vegetative way of plant's reproduction. The underground organs were collected in the first (autumn, harvest in October) and in the second year of plant's vegetation (spring, harvest in May). Root leaves were harvested in the second year, in spring. The raw materials were weighted, dried, and subjected to chemical analysis concerning phenolic compounds (HPLC). The results showed that the plants obtained from cuttings provided visibly higher mass of both root leaves and underground organs. Irrespectively of the propagation method, the mass of underground organs increased from the first to the second year of plant's development (Table 2.3).

The method of plant's propagation affected the content and composition of phenolics in dropwort raw materials. Plants cultivated from seedlings contained more hyperoside, (+)-catechin, and syringic acid in the root leaves when compared to these grown from cuttings. Other identified compounds (except for astragalin) were present on the similar level (Table 2.4). More significant differences were noticed in

Table 2.3 Fresh mass of raw material (g × plant^{-1})

Plant organs/term of harvest	Seedlings	Cuttings
Root leaves		
Second year of plants vegetation	34.8 ± 5.6	55.7 ± 8.4*
Underground organs		
First year of plants vegetation (October)	86.0 ± 12.0	127.5 ± 17.9*
Second year of plants vegetation (May)	111.3 ± 17.8	155.2 ± 26.4*

*$p < 0.05$

Table 2.4 Chemical characteristics of root leaves (mg × 100 g^{-1} DW)

Compounds	Seedlings	Cuttings
Flavonoids		
Quercetin	1.6 ± 0.1	1.5 ± 0.1
Astragalin	55.4 ± 5.5	79.4 ± 6.4*
Hyperoside	389.4 ± 21.2*	327.3 ± 22.9
Kaempferol	12.3 ± 1.1	11.3 ± 1.0
Spireoside	17.3 ± 1.2	19.3 ± 1.4
(+)-catechin	230.3 ± 20.7*	201.1 ± 22.1
(-)-epigallocatechin	158.4 ± 12.7	144.3 ± 10.1
Phenolic acids		
Ellagic acid	45.9 ± 4.1	49.2 ± 3.4
Gallic acid	200.7 ± 14.0	188.3 ± 11.8
Syringic acid	236.9 ± 19.0*	187.2 ± 16.8
Salicylic acid	9.4 ± 0.7	6.4 ± 0.6
Caffeic acid	95.2 ± 8.6	89.6 ± 6.3
Rosmarinic acid	131.0 ± 14.4	144.3 ± 11.5
Chlorogenic acid	111.3 ± 12.8	99.3 ± 7.9

*$p < 0.05$

the case of underground organs. Independently from the harvest term, underground organs collected from the plants propagated by seedlings were more abundant with almost all detected phenolics than the plants from cuttings. Underground organs harvested in the first year were characterized by a visibly higher content of flavan-3-ols in comparison to those collected in the second year. This was specially visible when regards (−)-epicatechin. In contrary, the amount of ellagic and gallic acids in underground organs increased from the first to the second year of plant's vegetation (Table 2.5). This may be explained by the fact that phenolic acids are precursors in the biosynthesis of other phenolics (Kohlmunzer 2000). Based on the above observations, especially those concerning the mass of raw materials, it can be concluded that vegetative propagation of dropwort is more promising than the generative one. However, the quality of investigated raw materials, reflected in phenolics content, seems to be better in plants reproduced via seeds.

2.3.2 *Accumulation of Biomass and Biologically Active Compounds*

When collecting raw materials from wild-growing or cultivated plants, the data on their harvest conditions are particularly important. Although there are many rules

Table 2.5 Chemical characteristics of underground organs (mg × 100 g^{-1} DW)

Compounds	First year of plants vegetation			Second year of plants vegetation		
	Seedlings	Cuttings	Mean	Seedlings	Cuttings	Mean
(+)-catechin	317.2 ± 24.9	229.3 ± 13.8	273.3*	226.5 ± 20.4	131.9 ± 14.5	179.2
(−)-epicatechin	453.8 ± 25.8	389.1 ± 17.3	421.5*	232.2 ± 17.2	193.4 ± 15.5	212.8
(−)-epigallocatechin	334.4 ± 30.1	358.3 ± 21.1	346.4*	206.0 ± 16.5	239.3 ± 21.5	222.7
(−)-epigallocatechin gallate	52.2 ± 3.7	38.3 ± 2.7	45.3*	41.9 ± 3.8	25.2 ± 1.8	33.6
Ellagic acid	32.3 ± 2.9	12.7 ± 1.4	22.5	42.6 ± 3.6	28.7 ± 2.4	35.7*
Gallic acid	76.4 ± 8.4	69.3 ± 7.6	72.9	105.2 ± 8.7	94.0 ± 10.3	99.6*
Sum	1266.3*	1097.0		854.4*	712.5	

*$p < 0.05$

regarding this issue, the individual reaction of each species should be taken into consideration. In general, the accumulation of secondary metabolites (including phenolics) in plant's tissues is associated with their physiological function and strongly depends on the stage of ontogenetic development. In dropwort, the content of phenolic compounds fluctuated during ontogenesis and was related to plant's organs. Taking into account that in dropwort almost all organs provide herbal raw materials, the investigations on the above listed relations are meaningful in terms of its cultivation.

The mass and chemical composition of dropwort's above- and underground organs in connection with the age of plants and stage of their development were determined. Following raw materials were evaluated: flowers, root leaves, shoot leaves, as well as underground organs separated into rhizomes, roots, and tubers. Underground organs and root leaves were collected in both years of vegetation, in October. Flowers and shoot leaves were obtained in the second year, three times during plant's development: at the beginning of flowering, at the full-flowering stage, and at the end of flowering (Figs. 2.7 and 2.8). The investigated raw materials were weighted, dried, and analyzed by HPLC in respect of phenolic compounds (Fig. 2.9a, b, c).

The mass of rhizomes, roots, and tubers in the second year of vegetation was significantly higher than in the first year, while the mass of root leaves was comparable in both years (Table 2.6). The mass of flowers increased from the beginning to the end of flowering; opposite tendency was observed for shoot leaves (Table 2.7). The obtained results showed that the stage of plant's development affected not only the weight but also the chemical composition of investigated raw materials. Among dropwort organs, flowers seem to be especially interesting. Recent phytochemical and pharmacological studies confirm traditional usage of this raw material and support its importance in modern medicine. Besides significant antioxidant potential, the flowers reveal dose-related antihyperalgesic activity with a good safety profile. When given its gastroprotective activity, this raw material decreases production of pro-inflammatory eicosanoids ex vivo in human platelets. Such biological properties are associated with the unique chemical composition of dropwort flowers, especially

Fig. 2.7 Plants in the second year of vegetation (vegetative stage)

Fig. 2.8 Plants in the second year of vegetation (flowering stage)

with the presence of spireoside, kaempfeol, and astragalin derivatives as leading compounds (Smardžic et al. 2016, 2018). In our work, the following flavonoids were identified in the flowers: quercetin, astragalin, hyperoside, kaempferol, spireoside, (+)-catechin, and (−)-epigallocatechin, with a domination of spireoside and hyperoside. The highest content of astragalin, hyperoside, kaempferol, and spireoside was found at the beginning of flowering and then decreased significantly reaching its minimum at the end of flowering (Table 2.8). The opposite relation was noticed in the

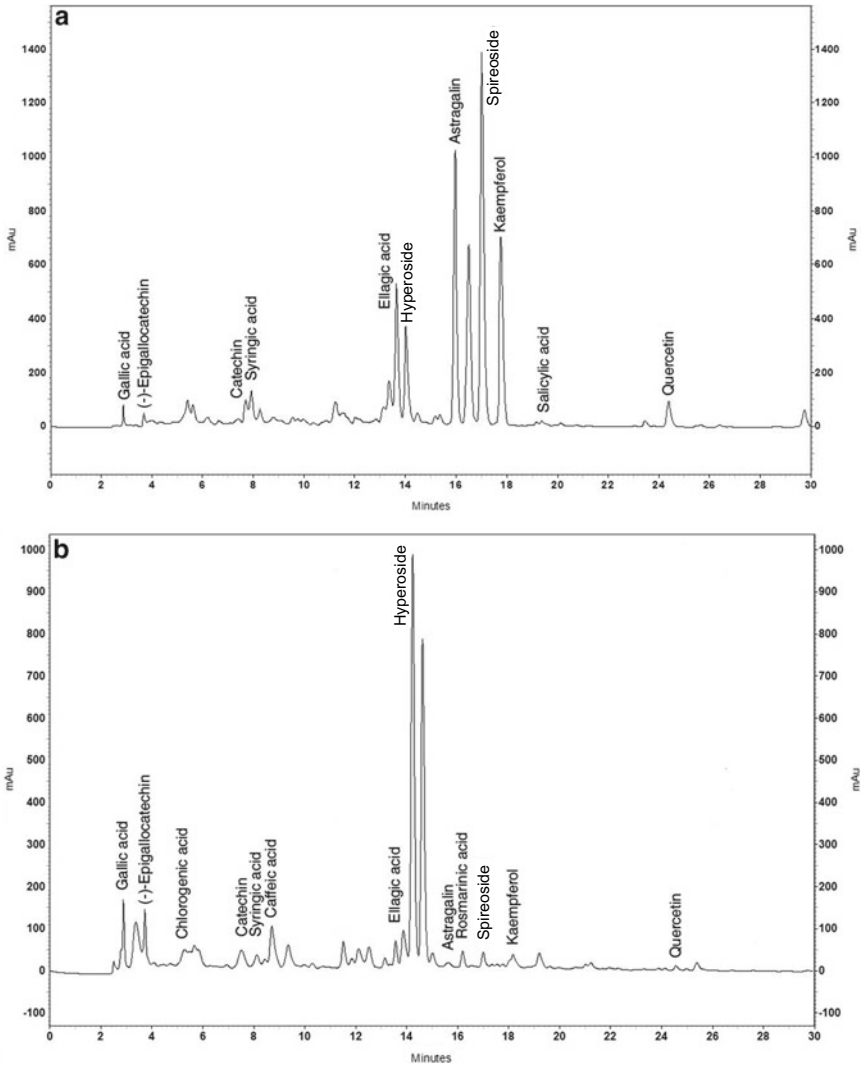


Fig. 2.9 Chromatograms of dropwort flower extracts (**a**), leaves extracts (**b**), and underground organs extracts (**c**)

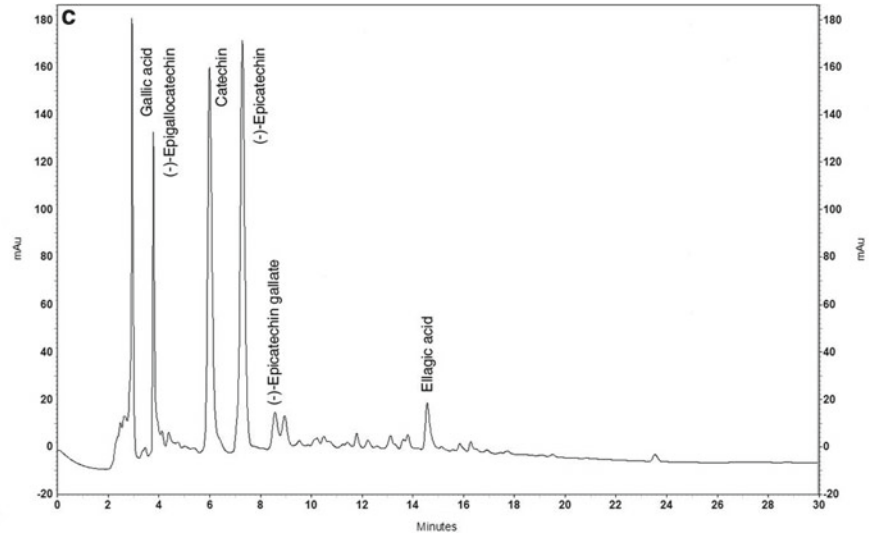


Fig. 2.9 (continued)

Table 2.6 Fresh mass of raw materials in the first and second year of vegetation (g × plant^{-1})

Plant organs	First year of plants vegetation	Second year of plants vegetation
Root leaves	111.19 ± 21.13	103.28 ± 15.49
Rhizomes	10.82 ± 1.51	70.53 ± 11.99*
Roots	5.98 ± 0.96	24.18 ± 4.35*
Tubers	81.25 ± 13.81	194.01 ± 22.98*

*$p < 0.05$

Table 2.7 Fresh mass of raw materials in the second year of vegetation (g × plant^{-1})

Plant organs	Term of harvest		
	BF	FF	EF
Shoot leaves	20.50 ± 2.46a	14.81 ± 2.07b	13.51 ± 2.03c
Flowers	28.89 ± 4.04c	48.21 ± 6.27b	61.87 ± 9.90a

*$p < 0.05$
BF beginning of flowering, *FF* full flowering, *EF* end of flowering

case of (+)-catechin and (−)-epigallocatechin. In turn, the highest level of all detected phenolic acids (ellagic, gallic, syringic, and salicylic acids) was shown during the full flowering stage. Here, syringic and gallic acid were the dominants (up to 485.9 and 450.2 g × 100 g^{-1} DW, respectively). These compounds reveal antioxidant, antimicrobial, anti-inflammatory, antiendotoxic, neuroprotective, hepatoprotective, cardioprotective, and gastroprotective activities (Srinivasulu et al. 2018; Kahkeshani

Table 2.8 Content of phenolic compounds in flowers (mg × 100 g^{-1} DW)

Compounds	Term of harvest		
	BF	FF	EF
Flavonoids			
Quercetin	64.6 ± 5.2a	69.7 ± 6.6a	11.3 ± 1.2b
Astragalin	542.3 ± 41.3a	341.3 ± 33.8ab	84.1 ± 8.7c
Hyperoside	651.8 ± 55.4a	489.5 ± 30.6b	155.4 ± 13.2c
Kaempferol	164.9 ± 15.5a	128.9 ± 12.2a	33.8 ± 3.1b
Spireoside	999.9 ± 73.0a	753.0 ± 55.0ab	126.0 ± 9.2c
(+)-catechin	63.1 ± 5.7c	90.3 ± 7.9b	147.0 ± 12.9a
(−)-epigallocatechin	46.8 ± 3.9bc	50.2 ± 4.7b	68.2 ± 5.9a
Phenolic acids			
Ellagic acid	204.1 ± 18.4b	346.0 ± 29.4a	89.8 ± 8.1c
Gallic acid	271.6 ± 19.8b	450.2 ± 32.4a	289.8 ± 20.6b
Syringic acid	286.1 ± 22.9b	485.9 ± 36.0a	169.4 ± 14.9c
Salicylic acid	68.3 ± 5.1a	69.3 ± 5.1a	26.8 ± 2.0b

*$p < 0.05$
BF beginning of flowering, *FF* full flowering, *EF* end of flowering

et al. 2019). In our work, the presence of salicylic acid in dropwort flowers should be underlined, since it is well known for significant anti-inflammatory properties (Kohlmünzer 2000). The high content of phenolics in dropwort flowers may be attributed to their physiological role in protecting the generative organs from biotic and abiotic stress factors (Figueiredo et al. 2008; Verma and Shukla 2015). Moreover, phenolic compounds effectively control certain steps of cell growth and differentiation and thus play an important role in the reproduction mechanisms (Agati et al. 2012; Ferreyra et al. 2012; Verma and Shukla 2015).

Besides flowers, others aboveground organs namely root leaves and shoot leaves were evaluated in our study. In both examined organs, the same phenolics were detected, and their content was on the similar level. Root leaves collected in the first year of plants vegetation contained slightly more identified phenolics (except for chlorogenic acid and spireoside) than those harvested in the second year. It was shown that the amount of phenolics in the shoot leaves depended on the stage of blooming. Here, the highest content of hyperoside, which was a dominant compound, was marked at the end of this phase (452.7 mg × 100 g^{-1} DW) (Table 2.9). Hyperoside is known for its various, multidirectional pharmacological activities. Besides anti-inflammatory, antioxidant, and diuretic effects, it also reveals antidepressant, neuroprotective, cardioprotective, antidiabetic, anticancer, antifungal, and gastroprotective activity (Raza et al. 2017). Among underground organs, rhizomes appeared to be the most abundant in all the identified phenolics, except for (+)-catechin and (−)-epigallocatechin. Underground organs harvested in the first year were characterized by higher content of phenolics in comparison to these collected in the second

Table 2.9 Content of phenolic compounds in leaves (g × 100 g^{-1} DW)

Compounds	Root leaves		Shoot leaves (Second year of plants vegetation)		
	First year of plants vegetation	Second year of plants vegetation	BF	FF	EF
Flavonoids					
Quercetin	1.0 ± 0.1	0.5 ± 0.0	0.2 ± 0.0b	9.9 ± 0.9a	7.3 ± 0.8a
Astragalin	50.7 ± 5.7	40.4 ± 4.2	61.0 ± 5.8a	62.0 ± 6.4a	48.7 ± 4.7b
Hyperoside	345.5 ± 29.0*	263.2 ± 22.6	384.8 ± 31.2b	370.5 ± 31.5b	452.7 ± 38.0a
Kaempferol	12.3 ± 1.1	9.5 ± 0.9	27.2 ± 2.6ab	36.8 ± 3.5a	28.3 ± 2.7ab
Spireoside	15.0 ± 1.1	19.7 ± 1.5	18.7 ± 1.5b	24.3 ± 1.8a	20.2 ± 1.5ab
(+)-catechin	61.3 ± 5.3	50.8 ± 4.5	41.1 ± 3.4c	55.3 ± 4.3b	72.4 ± 7.4a
(−)-epigallocatechin	91.1 ± 8.4	80.6 ± 7.5	56.0 ± 4.9	67.1 ± 5.7	76.7 ± 6.4
Phenolic acids					
Ellagic acid	82.5 ± 9.1	60.5 ± 6.4	21.7 ± 2.4b	44.6 ± 5.0a	19.3 ± 2.0b
Gallic acid	228.5 ± 24.4	222.8 ± 22.9	184.6 ± 17.6	165.9 ± 14.2	178.9 ± 18.6
Syringic acid	250.1 ± 22.3*	131.5 ± 12.8	192.4 ± 17.9c	234.8 ± 19.5b	305.6 ± 26.0a
Caffeic acid	73.0 ± 6.5*	39.1 ± 3.8	86.6 ± 8.1b	98.1 ± 8.1a	83.1 ± 7.1b
Rosmarinic acid	83.4 ± 7.4*	60.3 ± 5.8	122.2 ± 11.4a	109.7 ± 9.1b	86.0 ± 7.3c
Chlorogenic acid	111.9 ± 10.0	138.8 ± 13.5	67.6 ± 6.3c	128.9 ± 10.7a	96.2 ± 8.2b

*$p < 0.05$

BF beginning of flowering, *FF* full flowering, *EF* end of flowering

year (Table 2.10). This phenomenon may be associated with the need of physiological protection of younger, intensively growing plants against various stressors. It should be underlined that phenolics not only protect cells against free radicals, but also play an important role as antimicrobial and strengthening agents (Andersen and Markham 2006). For instance, phenolic acids as lignins components incrust cell walls making them more resistant against various pathogens (Weng and Chapple 2010). In turn, catechins (classified as tannins) due to their astringed properties indicate a high antibacterial activity (Kohlmünzer 2000).

2.3.3 *Diversity of Plants in Their Cultivation*

The introduction wild-growing plants into cultivation enables to obtain more homogenous raw materials. Dropwort belongs to the plants with an allogamous way of reproduction (Weidema et al. 2000). Thus, when the plantation is established via seeds originating from wild-growing population, the offspring may be highly differentiated, as a result of cross-pollination. A high range of phenotypic variability may create problems with raw material standardization. However, this phenomenon

Table 2.10 Content of phenolic compounds in underground organs (g × 100 g^{-1} DW)

Compounds	First year of plants vegetation				Second year of plants vegetation			
	Rhizomes	Roots	Tubers	Sum	Rhizomes	Roots	Tubers	Sum
(+)-catechin	293.3 ± 33.7b	396.0 ± 35.6a	213.7 ± 23.9c	903.0	280.9 ± 32.0b	321.6 ± 30.2a	215.4 ± 18.3c	817.9
(−)-epicatechin	212.9 ± 20.9a	175.5 ± 16.0b	68.8 ± 6.3c	457.2*	177.1 ± 16.1a	134.0 ± 13.3ab	59.3 ± 5.4b	370.4
(−)-epigallocatechin	222.4 ± 18.7b	268.7 ± 23.1a	294.7 ± 25.6a	785.8*	201.4 ± 15.7c	233.1 ± 14.5b	281.6 ± 18.3a	716.1
(−)-epigallocatechin gallate	98.4 ± 9.4a	80.9 ± 7.8a	28.7 ± 2.8b	208.0	94.1 ± 9.0a	70.7 ± 6.2b	38.6 ± 3.2c	203.4
Ellagic acid	29.1 ± 2.2a	20.9 ± 1.6ab	13.0 ± 1.1b	63.0	37.8 ± 2.8a	20.0 ± 1.4b	14.5 ± 1.4c	72.3
Gallic acid	151.6 ± 15.5a	131.0 ± 14.7ab	72.2 ± 8.3b	354.8*	128.8 ± 14.4a	113.0 ± 10.7b	63.9 ± 6.3c	305.7
Sum	1007.7a	1073.0a	691.1b		920.1a	892.4b	673.3c	

*$p < 0.05$

Table 2.11 Diversity of plant organs in terms of their mass (g × plant^{-1})

Raw materials	Mean	Min.	Max.
Flowers	61.3	35.6	111.8
Shoot leaves	30.50	7.0	60.3
Root leaves	102.6	25.9	176.5
Rhizomes	74.0	41.9	94.6
Roots	26.8	9.3	41.32
Tubers	301.9	192.7	447.9

opens a huge possibilities for breeders, where single plants may become valuable components for breeding (Carlen 2011).

High intraspecific variability of dropwort concerning the mass and chemical composition of raw materials was observed. Individual plants were separately assessed, and both minimum and maximum values of the investigated parameters were determined (Tables 2.11, 2.12 and 2.13). Concerning the mass of raw materials, the leaves were the most diverse (Table 2.11). Among the aboveground organs (flowers, shoot leaves, and root leaves), the richest in flavonoids, especially in astragalin, hyperoside, and spireoside, were flowers. It is worth noting that hyperoside

Table 2.12 Diversity of aboveground organs in terms of phenolic compounds content (mg × 100 g^{-1} DW)

Compounds	Flowers			Shoot leaves			Root leaves		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Flavonoids									
Quercetin	11.2	0.1	26.1	2.1	0.8	3.7	1.2	0.2	2.3
Astragalin	683.1	471.2	1221.5	60.0	29.1	111.9	32.4	26.2	61.2
Hyperoside	765.8	405.1	1654.2	409.8	57.7	637.6	389.7	239.2	508.3
Kaempferol	142.1	136.5	246.1	15.5	11.3	22.6	12.2	10.8	14.3
Spireoside	686.4	205.5	1236.9	15.1	8.8	28.3	10.7	8.1	16.7
(+)-catechin	41.2	21.3	87.4	120.3	64.2	211.3	95.4	35.3	184.9
(−)-epigallocatechin	72.48	43.9	150.8	246.0	141.0	480.3	168.9	51.6	589.7
Phenolic acids									
Gallic acid	382.8	270.3	1096.3	108.8	56.6	242.5	158.1	7.9	383.1
Ellagic acid	205.6	117.4	284.0	14.6	4.1	26.8	19.1	1.4	29.6
Syringic acid	346.7	270.2	598.2	289.4	112.3	450.4	360.4	118.3	546.7
Salicylic acid	26.5	5.8	83.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Caffeic acid	n.d.	n.d.	n.d.	70.1	40.4	114.0	51.6	21.9	101.8
Rosmarinic acid	n.d.	n.d.	n.d.	135.0	78.2	204.4	96.6	24.5	170.5
Chlorogenic acid	n.d.	n.d.	n.d.	173.0	70.1	247.7	106.3	32.2	216.2

ND not detected

Table 2.13 Diversity of underground organs in terms of phenolic compounds content (mg × 100 g^{-1} DW)

Compounds	Rhizomes			Tubers			Roots		
	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Flavonoids									
(+)-catechin	351.5	192.5	655.5	127.9	42.1	205.7	288.9	49.1	569.3
(−)-epicatechin	270.4	116.5	432.3	91.6	24.0	165.6	232.3	23.6	386.5
(−)-epigallocatechin	238.6	119.4	477.3	112.5	38.4	187.1	168.3	8.5	269.9
(−)-epigallocatechin gallate	105.4	26.6	220.6	49.1	27.1	79.3	80.7	32.1	118.9
Phenolic acids									
Ellagic acid	50.7	8.1	41.0	4.8	1.1	11.2	25.7	12.2	45.5
Gallic acid	71.1	31.6	128.4	35.2	13.1	51.5	64.5	17.9	162.0

was also present in significant amounts in the leaves. The most varied in terms of the content of flavonoids was spireoside. The highest content of phenolic acids in the aboveground organs was also found in the flowers. However, syringic acid was present in high quantities in the leaves, as well. Gallic acid was present in all three aboveground organs and clearly differentiated these organs (Table 2.12). Among the underground organs, the highest content of phenolics was detected in the rhizomes. Rhizomes were the most differentiated as to (−)-epigallocatechin gallate content (26.6–220.6 mg × 100 g^{-1} DW), tubers—(−)-epicatechin (24.0–165.6 mg × 100 g^{-1} DW), and roots—(−)-epigallocatechin (8.5–269.9 mg × 100 g^{-1} DW).

2.4 Perspectives

Due to the need for protection of dropwort natural recourses, as well as in order to obtain a considerable amount of standardized raw materials, the species should be introduced into cultivation. The presented work summarizes our efforts concerning this issue. Taking into account that wild-growing dropwort populations are small, scattered and highly diversified as to morphological and chemical traits, they provide heterogeneous raw materials in the amount not sufficient for industrial purposes. Thus, crucial questions concerning agrotechnical problems including, i.e., the range of intraspecific variability between individual plants in cultivation, the way of plantation establishment as well as the raw materials quality depending on the plant's age and developmental phase, have appeared. It was shown that dropwort plantation may be successfully established both by seedlings production and *via* vegetative propagation (by stem–roots cuttings), however, the latter one seems more promising. Further works on effective ways of plants propagation, including in vitro techniques and/or trials on seeds germination improvement, should be undertaken. Our results show that when being cultivated, dropwort produces high and stable yield of both

above- and underground organs. The accumulation of biologically active compounds in these organs is strongly associated with the age of plants and stage of their development. Thus, depending on the harvest term, it is possible to obtain raw materials with variable quality. Taking into consideration medicinal potential of dropwort organs reflected in a high content of phenolics, future works should focus on the production of standardized extracts from this plant followed by the determination of their pharmacological activity.

Acknowledgements The work was supported by Polish Ministry of Science and Higher Education, project NCBiR No. R12 06803.

References

Agati G, Azzarello E, Pollastri S, Tattini M (2012) Flavonoids as antioxidants in plants: location and functional significance. Plant Sci 196:67–76

Andersen RM, Markham KR (2006) Flavonoids: chemistry, biochemistry, and applications. Taylor and Francis, CRC Press, Boca Raton, FL, USA. ISBN 0-8493-2021–6

Bączek K, Cygan M, Przybył J, Kosakowska O, Węglarz Z (2012) Seasonal variations of phenolics content in above- and underground organs of dropwort (*Filipendula vulgaris* Moench). Herba Pol 58(3):24–31

Capecka E, Geszprych A, Przybył JL, Kunicki E, Binder A, Bączek K, Węglarz Z (2012) Accumulation of phenolic compounds in underground organs of dropwort (*Filipendula vulgaris* Moench). Acta Sci Pol Hortorum Cultus 11(4):101–109

Carlen C (2011) Breeding and cultivation of medicinal plants. In: Bagetta G, Cosentino M, Corasaniti MT, Sakurada S (eds) Herbal medicines for human health. Taylor and Francis, London, UK, pp 79–91

Committee on Herbal Medicinal Products (2011) Community herbal monograph on *Filipendula ulmaria* (L.) Maxim., herba. EMA/HMPC/434881/2010

Cortan D, Krak K, Vit P, Mandak B (2019) Development, characterization, and cross-amplification of 17 microsatellite markers for *Filipendula vulgaris.* App Plant Sci 7(12):11307

Duda J (2009) Zielona Arka Śląska. Śląski Ogród Botaniczny, Mikołów. ISBN 978-83-925250-1-1

Elansary HO, Szopa A, Kubica P, Ekiert H, Mattar AM, Al-Yafrasi M, El-Ansary DO, El-Abedin TKZ, Yessoufou K (2019) Polyphenol profile and pharmaceutical potential of *Quercus* spp. bark extracts. Plants 8:486

Ferreyra MLF, Rius SP, Casati P (2012) Flavonoids: biosynthesis, biological functions, and biotechnological applications. Front Plant Sci 3:222

Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC (2008) Factors affecting secondary metabolite production in plants: volatile compounds and essential oils. Flavour Frag J 23:213–226

Kahkeshani N, Farzaei F, Fotouhi M, Alavi SS, Bahramsoltani R, Naseri R, Momtaz S, Abbasabadi Z, Rahimi R, Farzaei MH, Bishayee A (2019) Pharmacological effects of gallic acid in health and diseases: a mechanistic review. Iran J Basic Med Sci 3:225–237

Katanić J, Pferschy-Wenzig EM, Mihailović V, Boroja T, Pan SP, Nikles S, Kretschmer N, Rosić G, Selaković D, Jaksimović J, Bauer R (2018) Phytochemical analysis and anti-inflamatory effects of *Filipendula vulgaris* Moench extracts. Food Chem Toxicol 122:151–162

Katanić J, Mihailović V, Stanković N, Boroja T, Mladenović M, Solujić S, Stanković MS, Vrvić MM (2014) Dropwort (*Filipendula hexapetala* Gilib.): potential role as antioxidant and antimicrobial agent. EXCLI Journal 14:1–20

Kohlműnzer S (2000) Farmakognozja. Podręcznik dla studentów farmacji. PZWiL, Warszawa

Kostarkiewicz-Gierłat K, Stachurska-Swakoń A (2017) The influence of habitat conditions on the abundance and selected traits of the rare medicinal plant species. Ecol Quest 25:9–18

Meusel H, Jager EJ, Weinert E (1965) Vergleichende chorologie der zantraleuropaischen flora. Fischer, Jena, Germany

Motyka J, Panych T (1936) Rośliny lecznicze i przemysłowe w Polsce. Opis. Uprawa. Zbiór. Handel. Książnica—Atlas. S.A. Zjednoczone Zakłady Kartograf. i Wydawn., T.N.S.W. Lwów—Warszawa

Movsumov IS, Garaev EE, Herbette G et al (2017) Flavonoids of *Acacia dealbata* and *Filipendula vulgaris* growing in Azerbaijan. Chem Nat Comp 53:754–755

Mowszowicz J (1985) Przewodnik do oznaczania krajowych roślin zielarskich. PWRiL, Warszawa

Neagu M, Pauna G, Albua C, Radub GL (2015) Assessment of acetylcholinesterase and tyrosinase inhibitory and antioxidant activity of *Alchemilla vulgaris* and *Filipendula ulmaria* extracts. J Taiwan Inst Chem Eng 2015:1–6

Oszmiański J, Wojdyło A, Lamer-Zarawska E, Świąder K (2007) Antioxidant tannins from *Rosaceae* plant roots. Food Chem 100:579–583

Pavlović M, Petrović S, Ristić M, Maksimović Z, Kovacević N (2007) Essential oil of *Filipendula hexapetala*. Chem Nat Comp 43(2):228–229

Pulito C, Korita E, Sacconi A, Valerio M, Casadei L, Lo Sardo F et al (2019) Dropwort-induced metabolic reprogramming restrains YAP/TAZ/TEAD oncogenic axis in mesothelioma. J Exp Clin Cancer Res 38:349

Pukalskienė M, Venskutonis PR, Pukalskas A (2015) Phytochemical composition and antioxidant properties of *Filipendula vulgaris* as a source of healthy functional ingredients. J Funct Foods 15:233–242

Radulović N, Mišić M, Aleksić J, Jaković D, Palić R, Stojanović G (2007) Antimicrobial synergism and antagonism of salicylaldehyde in *Filipendula vulgaris* essential oil. Fitoterapia 78:565–570

Raza X, Xu H, Sun J, Tang Z, Oyang Z (2017) Pharmacological activities and pharmacokinetic study of hyperoside: a short review. Trop J Pharm Res 16(2):483–489

Schanzer I (1994) Taxonomic revision of the genus *Filipendula* Mill. (Rosaceae). Jof Jap Bot 69:290–319

Shilova IV, Suslov NI (2015) Nootropic effect of meadowsweet (*Filipendula vulgaris*) extracts. B Exp Biol Med 158(2):659–663

Smardžic S, Arsenijević J, Božić D, Milenković M, Tešević V, Maksimović Z (2018) Antioxidant, anti-inflamatory and gastroprotective activity of *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench. J Ethnopharmacol 213:132–137

Smardžic S, Tomić M, Pecikoza U, Stepanović-Petrović R, Maksimović Z (2016) Antihyperalgetic activity of *Filipendula ulmaria* (L.) Maxim. and *Filipendula vulgaris* Moench in a rat model of inflammation. J Ethnopharmacol 193:652–656

Smolarz HD, Dzido TH, Sokołowska-Woźniak A (1999) High performace liquid chromatographic determination of flavonoids in *Filipendula hexapetala* Gilib. Acta Pol Pharm 56:169–172

Srinivasulu C, Ramgopal M, Ramanjaneyulu G, Anuradha CM, Suresh Kumar C (2018) Syringic acid (SA) a review of its occurrence, biosynthesis, pharmacological and industrial importance. Biomed Pharmacother 108:547–557

Weidema IR, Magnussen LS, Philipp M (2000) Gene flow and mode of pollination in a dry-grassland species, *Filipendula vulgaris* (Rosaceae). Heredity 84(3):311–320

Weng JK, Chapple C (2010) The origin and evolution of lignin biosynthesis. New Phytol 187:273–285

Verma N, Shukla S (2015) Impact of various factors responsible for fluctuation in plant secondary metabolites. J Appl Res Med Aromat Plants 2:105–113

Chapter 3
Domestication of *Andrographis paniculata* (King of Bitters)

Hosakatte Niranjana Murthy, So Young Park, and Kee Yoeup Paek

Abstract *Andrographis paniculata* (AP) is popularly known as 'King of bitters', and it is an important medicinal plant which is cultivated in Southeast Asia and other tropical parts of the world. AP contains monoterpenoids, ent-labdane diterpenoids, flavonoids, quinic acids and xanthones. Andrographolide and its derivatives isolated from AP are reported to have a wide range of pharmacological effects including anti-inflammatory, antimalarial, antidiabetic, antileukemia, and anticancer activities. In this review, we trace domestication of AP by considering phytogeographical and historical evidences. These lines of evidences suggest that AP was domesticated in South India. Further, AP was involved in diversification through natural selection when it was introduced to new environmental and ecological conditions.

Keywords *Andrographis* · Domestication · Diversification · Historical evidences · Phytogeogrphical evidences · Selection

3.1 Introduction

Medicinal plants are useful in the treatment of various kinds of health problems since time immemorial. Traditional system of medicine such as Ayurveda, Unani, Siddha and Traditional Chinese medicine are using medicinal plants extensively (Mukherjee et al. 2010). Medicinal plants are usually collected from nature and used in the traditional system of medicine. Nevertheless, many medicinal plants have been domesticated in the past, cultivated as field crops, either in sole cropping or in intercropping system (Schippmann et al. 2002). Ginseng (*Panax ginseng*), Ashwagandha (*Withania somnifera*) and Tulsi (*Ocimum sanctum*) are few examples which are domesticated thousands of years ago and are cultivated in fields. Collection of

H. N. Murthy (✉)
Department of Botany, Karnatak University, Dharwad, India

H. N. Murthy · S. Y. Park · K. Y. Paek
Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju, Republic of Korea
e-mail: soyapark7@chungbuk.ac.kr

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_3

medicinal plant resources from wild have several disadvantages, viz. wild collection of plant material will lead to variations in chemical ingredients in the collected material; there is a scope of adulteration of medicinal plant resources with other plant materials; controlled and regular supply of medicinal plant resources may not be possible; and wild collection of medicinal plants also leads to the scarcity of plant species. On the other hand, bringing the useful medicinal plants into cultivation has several advantages like selection and development of superior genotypes with desirable traits may offer opportunities for the economic development of the medicinal plant species as a crop; cultivation of medicinal plants allows controlled post-harvest handling with assured quality; and cultivated material will help in certification of material for assured quality and efficacy (Schippmann et al. 2002). In view of the above, the present review summarizes the domestication of *Andrographis paniculata* (AP), an important medicinal plant.

3.2 Botany and Distribution of *Andrographis paniculata*

Andrographis paniculata (Burm. F.) Wallich ex Nees. belongs to family Acanthaceae and it is popularly known as 'King of bitters', since all parts of the plant are bitter in taste because of its chemical constituents (diterpenoids). This plant is native to India and Sri Lanka. It grows in varied ecological and climatic conditions, mostly in dry deciduous and shrubby forest regions, hilly areas, plains, and occupied red and loam to lateritic soils. Naturally it is herbaceous perennial plant; old perennial underground stems regenerate new shoots and roots, with the onset of growing season. However, it can be cultivated as annual plant also. *Andrographis paniculata* is much branched, herbaceous, and erect plant (30–110 cm); stem is quadrangular in nature (Fig. 3.1a); leaves are simple, opposite, lanceolate, glabrous, 2–12 cm long, 1–3 cm wide, margin entire with short petiole (Fig. 3.1b); inflorescence is terminal or axillary panicles (10–30 cm long). Flowers are small, white in color with purple spots on the petals with small bracts, short pedicle (Fig. 3.1c); sepals 5, small, linear; corolla fused at the base, corolla tube narrow, about 6 mm long, limb longer than the tube, bilabiate, upper lip oblong, white with yellowish top, lower lip broadly cunate, 3 lobed, white with violet or purple spots; stamens 2, inserted in the throat, and far exerted, anthers basally bearded; Ovary superior, 2-celled, style far exerted. Fruit is a capsule, erect, linear-oblong, 1–2 cm long and 2–5 mm wide, compressed, longitudinally furrowed on broad faces, acute at the ends, covered by tiny glandular hairs (Fig. 3.1c, d); seeds are small (Fig. 3.1e, f) (Anonymous 2019).

The genus *Andrographis* comprises around 28 species which are having potential medicinal value and majority of these species are reported to found in India, mostly in South India (Karthikeyan et al. 2009). *Andrographis paniculata* (AP) is the prominent species among these, which is widely distributed in India and Sri Lanka, which is considered as center of origin and diversity of this species. AP is also distributed in China, Thailand, Brunei, Laos, Myanmar, Malaysia, Indonesia, and West Indies where it is introduced because of its medicinal properties (Hossain

Fig. 3.1 *Andrographis paniculata* **a** Habit; **b** Vegetative branch; **c** Flower along with green fruit; **d** Mature fruits; **e** Cut open mature fruit with seed; **f** Seeds

et al. 2014). AP is an important medicinal plant traditionally used in Indian system of medicine (Ayurveda) for treatment of dysentery, snake bites, cholera, diabetes, fever, throat infections, hypertension, piles, jaundice, and others (Hossain et al. 2014). It is also a popular plant in Japanese, Malaysian, Scandinavian, Traditional Bangladeshi, Traditional Chinese, Traditional Thai, and Unani system of medicine (Hossain et al. 2014), and hence it has vernacular names in many languages. It is called as Kalmegh/Kalamegha (meaning dark clouds, due to its dark deep green color from the distance), Bhunimba/Bhui-nimba/Bhui-neem (meaning neem of the ground, due to its bitterness), Maha-tikta (extremely bitter) in Sanskrit and Hindi, and much

of the earlier literature is available with these titles. AP is also popular as 'The creat' or 'Green chiretta' (USDA 2020), which is cultivated in Asia temperate: China; Asia-Tropical: India, Bangladesh, Nepal; Indo-China: Cambodia, Laos, Myanmar, Thailand, Vietnam; Melesia: Indonesia, Malaysia, Philippines; Australia; South America: Caribbean: West Indies; and Central America. AP is also naturalized in Africa: Mauritius, Nigeria; Asia temperate: China; Asia-tropical; Indo-China; Melesia: Indonesia, Malaysia; South America; Caribbean: West Indies; and Central America (USDA 2020).

3.3 Phytochemicals of *Andrographis paniculata* and Their Importance

Aerial parts of the AP especially leaves, stem, flowers, and roots are rich in phytochemicals such as monoterpenoids (noriridoids), ent-labdane diterpenoids, flavonoids, quinic acids, and xanthones. A total of 5 monoterpenoids (noriridoids), 55 ent-labdane diterpenoids, 30 flavonoids, 8 quinic acid, and 5 xanthones have isolated from different organs of AP by varied researchers (Subramanian et al. 2012; Mishra et al. 2007). Varied pharmacological activities are attributed toward andrographolide and its derivatives namely andrographolide (AG), neoandrographolide (NAG), and 14-deoxy-11,12-didehydroandrographolide (DDAG) (Fig. 3.2) (Dai et al. 2019; Kumar et al. 2020; Zhang et al. 2020). Anti-inflammatory activities against bacterial- and viral-induced inflammation have been well established by Gu et al. (2016), Li et al. (2017); and Peng et al. (2016). Widyawaruyanti et al. (2014) and Zaid et al. (2015) demonstrated significant antiplasmodial activity against *Plasmodium falciparum* and *P. berghei* in vitro and in vivo. Recent analyses of molecular docking studies have shown that neoandrographolide could be cost-effective drug-analog for treating SARS-CoV-2 infection (Murugan et al. 2020). Andrographolide

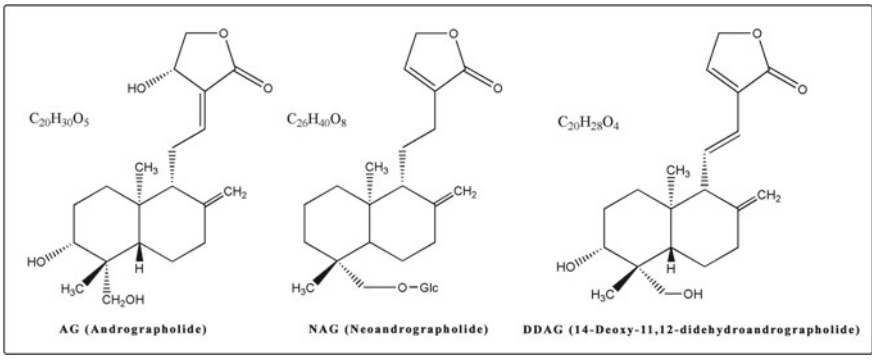


Fig. 3.2 Structure of major phytochemicals of AP: andrographolide, neoandrographolide and 14-deoxy 11, 12-didehydroandrographolie

also reported to inhibit diabetes and exhibits neuro-protection in diabetic rats (Takur et al. 2014). During diabetes progression, andrographolide decreases the activity of acetyl-cholinesterase and inhibits oxidative stress, which improves hyperglycemia and insulin deficiency (Takur and Rai 2016). Lee et al. (2014) displayed usefulness of andrographolide in hepatic fibrosis by regulating NF-kB-signaling pathway in adult rat (Khamphaya et al. 2016). Andrographolide reported to induce antileukemia activity by regulating the P13K/AKT and p38-MAPK signaling pathway in Jurkat cells (Yang et al. 2016). Varied studies illustrated that andrographolide possess antibreast cancer, anticervical cancer, antiprostate cancer, antiesophageal cancer, and anticolorectal cancer activities (Banerjee et al. 2016; Alzaharna et al. 2017; Mir et al. 2016; Wang et al. 2016).

3.4 Domestication of *Andrographis paniculata*

3.4.1 Lines of Evidences in Support of Domestication

Domestication of crop plants is an evolutionary process that occurs when wild plants are brought into cultivation by humans, leading to origin of new species and/or differentiation of populations that are critical for human survival (Purugganan 2019). This definition holds well for food/fruit/fibrous crops which are evolved into new species from their wild ancestors, whereas domestication of medicinal crops leads to differentiation of populations during course of evolution rather than origin of new species. For example, cotton is one of the most important fibrous crops that has been used for clothing and paper has evolved in different centers (center of origin) of the world from their wild ancestors, i.e., *Gossypium arboreum* and *G. herbaceum* are diploids which are mainly cultivated in Asia and Africa, whereas *G. barbadense* and *G. hirsutum*are allotetraploids which were domesticated in America and currently over 90% of world's cotton production is supplied by elite cultivars of *G. hirsutum* (Wendel and Cornn 2003; Wendel et al. 2009). In contrast, medicinal plants such as Ginseng (*Panax ginseng* C. A. Meyer) exist in wild as well as cultivated forms. Wild ginseng was once widely distributed till the end of twentieth century in Northeastern China and Korean peninsula which is very scarce now. Wild ginseng differs from cultivated ginseng in growth cycles and root morphology; wild ginseng is perennial species keep growing up to 100 years or even more, has a short and stout primary root with slender rhizome and thick branches (which resembles human body). In contrast, cultivated ginsengs are usually grows up to maximum seven years, possess an elongated primary root with numerous lateral roots and rootlets or even highly variable depending on landraces (Li et al. 2015). Further, the genetic diversity and population structure of cultivated ginseng is highly variable and these variations might be due to different conditions between natural and artificial growing environments (Ma et al. 2000). It was Charles Darwin who made thorough observations of plants and animals of various parts of the world during expedition and developed

the concept 'origin of species' and he also developed concept of domestication of species, highlighting the variation among breed, similarities of offspring and parents, and the transformative role of selection on species differentiation (Darwin 1859, 1868). Later, Vavilov defined eight 'centers of origin' for evolution and domestication of crop plants, based on high varietal diversity, co-occurrence of wild ancestors, cultivated species, and long history of crop use (Vavilov 1926, 1951, 1992). To trace the domestication process of any cultivated plant species, various lines of evidences are helpful and comparisons are made among cultivated species with their wild progenitors to deduce the mode and place of domestication (Harlan 1971, 1975). These lines of evidences are phytogeography (geographical distribution of wild and cultivated species); historical account; morphology (morphological comparison of wild/progenitor with cultivated species); cytogenetics and breeding behavior (comparison of chromosome number, ploidy, crossability, and breeding behavior of wild with that of cultivated); nomenclature; and archeology. In the recent years, comparison of genomic, biochemical, and molecular data has been used in addition to the earlier listed lines of evidences. In the following sections, domestication and diversification of AP is discussed by taking various lines of evidences available in the literature.

3.4.2 Phytogeographical Evidences

Phytogeographical (also known as biogeography) approaches focus on the present-day wild species and populations, their relationships to cultivated crops and their distribution forms, and it gives one line of evidence in support of plant domestication (Vavilov1992; Harlan 1971,1975). Various floristic evidences suggest that native populations of AP occur in South India and Sri Lanka (Karthikeyan et al. 2009). AP populations are introduced to Northern India, China, Thailand, Cambodia, Brunei, Vietnam, Malaysia, Indonesia, Java, Jamaica, Barbados, Bahamas, Christmas Island, and West Indies, where they occupied local environmental and ecological conditions and evolved as new population/s (Karthikeyan et al. 2009). The genus *Andrographis* was reported to possess 28 species in the world out of which 26 are distributed in India and 23 species are exclusively reported from peninsular region of India, which are endemic to this region (Karthikeyan et al. 2009; Index Kewensis 1977–87). Eleven species were reported from Andhra Pradesh including *A. alata*, *A. beddomei*, *A. echioides*, *A. elongata*, *A. glandulosa*, *A. lineata*, *A. longipedunculata*, *A. nallamalayana*, *A. ovata*, *A. paniculata*, and *A. serpyllifolia* (Neerja et al. 2015) and nine species are reported from Karnataka, viz. *A. alata, A. echioides A. lineata* var. *lineata*, *A. lineata* var. *lawii*, *A. macrobotrys*, *A. ovata*, *A. paniculata, A. producta*, and *A. serpyllifolia* (Dalawai et al. 2019). Other species, viz. *A. affinis, A. elongata, A. eplicata, A. glandulosa, A. lawsonii, A. lobeloides, A. neesiana var. neesiana, A. neesiana var. producta, A. neesiana var. rotundifolia, A. rothii, A. stellulata, A. stenophylla, and A. viscosulla,* along with other *Andrographis* species were reported from Kerala

and Tamil Nadu (Sabu 2002). Majority of wild relatives of AP are distributed in peninsular India/South India and the above phytogeogrpahical data clearly demonstrates that peninsular India/South India might the place of domestication of AP.

3.4.3 Historical Evidences

According to Indian Pharmacopoeia, AP has been used for centuries in India for the treatment of dysentery, carbuncles, colitis, tuberculosis, malaria, herpes, ulcer, and venomous snake bites (Hossain et al. 2014). By realizing the medicinal importance of AP, people might have introduced this plant in other parts of the world. It is very difficult to demarcate when AP has been introduced into China, Southeast Asian countries, and Persian Gulf areas, however, few historical evidences exist in the literature in this regard. Traditional Chinese medicine which is widespread in China is derived mostly from the philosophy that informs Taoist and Buddhist thought and reflects the traditional Chinese belief that the life and activity of individual human beings have a close relationship with the environment on all levels (Benn 2002). AP might have introduced into China by Buddhists during Han dynasty (206 BC–220 AD) through silk route. In Mandarin, AP is called Chuan xin lian, Yi jian xi and Lan he lian, which translate directly as 'thread-the-heart lotus', and this name might have originated in China because lotus is one of major prominent flowers in Buddhism which represents purity of body, speech and mind. Thailand, which is originally known as 'siam', has religious, linguistic, and cultural ties with India, which go back to a hoary past. Emperor Ashoka (famous Indian emperor of the Maurya Dynasty; c.268 to 232 BC) said to have sent two of his emissaries there, the monks Sona and Uttara, as part of his mission to propagate Buddhism in countries far and near. They are said to have delivered their first sermon at Nakhon Pathom (Sanskrit word meaning Nagara Prathama or the First City), so called perhaps to commemorate that event. The Puranas (Hindu religious text of ancient times) mention Indian ships laden with merchandize touching the ports in Suvaranabhumi, which is all likelihood, included Syamadesa, Siam as Thailand was called then. A Tamil inscription found in Thailand, at the site of Takua Pa, testifies to Southeast Asian commerce with the Pallava dynasty in India (Sastri 1949). Here a South Indian mercantile corporation, the 'Manikramam', had established a settlement possessing its own regiment, had constructed its own temple and tank, and lived as a self-contained colony (Shastri 1998). Therefore, it is believed that AP was brought Thailand either by Buddhists or by Tamil mercantile people. Later the Khmer Empire (officially the Angkor Empire) was ruling mainland Southeast Asia (involving Cambodia, Laos, Myanmar, Peninsula Malaysia, Thailand, and Vietnam) and parts of Southern China from 9th to fifteenth century might have spread AP in these regions. Alternatively, the British who were ruling Indian subcontinent and Southeast Asia main land brought labor from South India to Malaya to meet the needs of laborers in the sugar cane and rubber plantation in the late nineteenth century and early twentieth century. Indian workers were brought to work as porters in the rubber plantations belong to British businessmen (Jain 1970).

In the 1880s and 1890s, south Indian migrant workers in Malays faced enormous risk of health problems especially dysentery, diarrhea, and malarial fevers (Amrith 2014). To overcome these problems, Indian migrants brought few medicinal plants from India to cure such diseases and AP is one among them. Introduction AP in Persia can be perceived only through historical accounts on India and Persia relationships. India and Persia (ancient Iran) relationship goes back to sixth century, at that time Achaemenid empire was ruling Persia which was established by Cyrus the Great (Schmitt 2019). In the sixth century BC, Maghadh dynasty was ruling extensive areas of India and invasion of foreigners started on the northwest frontier of India. The Aryans who settled in India belonged to the racial stock which had first entered Persia. During this age, India exported spices, black pepper, medicinal plants and imported gold and silver coins from Iran (Jorfi 1994). The grape, introduced from Persia with the almond and walnut, was cultivated in the Western Himalayas (Jorfi 1994). On the other hand, around seventh century AD, an Arabic translation from a Persian version of Charaka Samhita (a Sanskrit text on Ayurveda which is an Indian traditional medicine system) was made which contains information on AP and its formulations (Dey et al. 2013). Traditionally, in Ayurvedic medicine, AP is used as carminative, liver stimulant, laxative, anthelmintic, blood purifier, anti-inflammatory, antileprotic, antipyretic, and antimalarial drug (Handa 1998). Knowing the medicinal value of AP, this plant might have been introduced to Persia during that period. It is insoluble to predict the exact time of domestication of AP, archeology, and other lines of evidences may be helpful in predicting the exact time of domestication.

3.4.4 Diversification

In majority of the seed crops, the evolutionary process of domestication involves four stages, viz. stage 1—predomestication cultivation of plants by humans; stage 2—spreading and adaptation of domesticated species to different agroecological and cultural environments; stage 3—adoption of domesticated species to different environments and human cultural practices including geographical radiation; stage 4—deliberate breeding to maximize the yield, ease of forming, and quality (Meyer and Purugganan 2013). In light of this, the evolutionary process and domestication of AP have surpassed stage 1 and stage 2 in the past and currently AP domestication is in stage 3, systematic breeding efforts have not been attempted yet for the improvement of AP. Darwin (1859; 68) and others (Zohary 2004; Heiser 1988) have recognized two kinds of selections, namely conscious (methodical/artificial) and unconscious (automatic/natural) selection during domestication. Certain traits, such as color and possible taste, likely were driven by conscious human selection (Heiser 1988) and other traits, however, such as seed non-shattering, seed dormancy, and synchronous germination may have occurred by unconscious selection or natural selection (Heiser 1988; Zohary 2004). Majority of wild species (relatives) of AP (including AP) are distributed in peninsular India, and it is only AP which is wide spread in other parts of India (north India, northeast India), China, Southeast Asia (Thailand, Brunei,

Vietnam, Malaysia, and Indonesia) and other countries and no systematic efforts were applied for domestication of AP, and therefore, AP domestication might be due to unconscious or natural selection. Germplasm of AP which were selected for cultivation in India and elsewhere are still depicting seed dormancy and seed shattering characteristics and these attributes certainly supports unconscious selection which was operated during domestication of AP.

Interspecific hybridization and introgressive hybridization were recognized as major mechanisms for adaptive evolution (Anderson 1949; Anderson and Stebbins 1954; Arnold 2004; Rieseberg and Carney 1998). The role of hybridization in domestication and crop diversification is widely documented (Hancock 2004). All the *Andrographis* species including AP possess hermaphrodite flowers (Hudedamani and Yadav 2013; Valdiani et al. 2012). The protandrous condition, dehiscence of anthers prior to anthesis and self-compatibility makes these species as habitual inbreeders (Valdiani et al. 2012; Lattoo et al. 2008). The above-mentioned conditions rule out intraspecific/interspecific/introgressive hybridization among *Andrographis* species and the mode of diversification of AP might be only due to natural selection. Additionally, polyploidy is yet another factor which favors evolution and speciation in many angiosperm families (Soltis and Soltis 1999) and it was estimated that 15% of speciation events in angiosperms involve polyploidization (Wood et al. 2009). Both autopolyploidy and allopolyploidy lead alteration of characteristics in such plants which could enable domesticates to adapt to disturbed agricultural environments that are not suitable for the ancestor. AP is reported to be diploid with $2n = 50$ chromosomes (Roy and Datta 1988) and chromosome counts in other wild relatives of *Andrographis* is not yet known. There are no evidences of polyploidy variation in *Andrographis* species, which suggest that diversification of AP population/s, are only due to natural selection events.

It was suggested that plant's life history may also influence the process of domestication (Setter et al. 2017) Annual plants have been very successful as domesticates because of their shortened generation time which speeds up selection process compared to perennials and annuals act as ruderal species during domestication and adapt to varied environments including disturbed environments. AP is originally a perennial species and also exists as annual species. When varied AP germplasm is examined, the annual habit might be outcome of domestication events. Setter et al. (2017) also proposed that, several crops show increased rates of self-fertilization compared to their wild ancestors, and self-fertilization also facilitates the maintenance of desired genotype combinations and lessens inbreeding depression. However, the complexity of adaptation during domestication and polygenic nature of many domestication traits suggest that at least some outcrossing likely played a pivotal role even in primarily self-fertilizing species, providing and influx of new variation and the opportunity to combine favorable alleles on different genetic background. AP is exclusively self-pollinating species and various studies, i.e., comparative experimental pollination methods, including bagging, netting, hand self-pollination, hand cross-pollination, emasculation, and indoor pollination reveled that AP is self compatible species (Valdiani et al. 2012), however, application of delayed pollination techniques showed crossability between different genotypes may be up to

13.33% (Lattoo et al. 2008). These features of AP might be product of evolution and differentiation of populations during domestication.

3.5 Opportunities and Challenges

3.5.1 Assessment of Genetic Diversity and Its Utilization

In the recent past various attempts have been made by researchers to assess the genetic diversity of AP landraces from India, China, Thailand, and Malaysia. Morphological, biochemical, and molecular evaluation of landraces revealed low to moderate diversity in China, Malaysia, and Thailand landraces, whereas moderate to significant diversity in India landraces. India, being the place of domestication of AP and have varied agroclimatic zones, certainly AP should depict significant variation due to natural selection events. As mentioned in the above section, AP is still in the stage 3 of domestication, i.e., adoption of domesticated species to different environments and human cultural practices including geographical radiation. Therefore, it is essential to collect, conserve, and utilize varied landraces of AP involve in deliberate breeding attempts to maximize the yield, ease of forming, and quality.

3.5.2 Use of Wild Relative for Breeding and Domestication

Wild relatives of crop plants represent the largest gene pool and it should be utilized for crop improvement through hybridization programs (Dulloo et al. 2013; Hajjar and Hodgkin 2007). Plant breeders have developed breeding programs and transferred disease and pest-resistant genes from wild to cultivated species through introgression (Hajjar and Hodgkin 2007). Many polygenic characteristics such as yield, quality, and adaption are yet to be transferred to cultivated species from wild germplasm. Majority of wild *Andrographis* species are distributed in South India, some of them have andrographolide content on par with AP and some other species rich in neoandrographolide and 14-deoxy-11,12-didehydroandrographolide in higher concentration than AP (Dalawai et al. 2019). These characteristics should to be transferred to AP via interspecific hybridization. Some of wild relative/s of AP, for example, *Andrographis lineata* var. *lawii* was reported to have andrographolide content (40.85 mg/g DW) on par with AP and *Andrographis macrobotrys* contain neoandrographolide in highest concentrations (98.43–102.03 mg/g DW) (Dalawai et al. 2019). These wild species could be domesticated and further cultivated and utilized.

3.6 Conclusions

In the present study, efforts have been made to trace the domestication of AP based on phytogeographical and historical evidences. One of the fundamental challenges of domestication research is filling gaps that remain in the areas such archeology, ecology, evolution, genetics, and genomics. Therefore, systematic research efforts should be undertaken in these areas to elucidate domestication syndrome of *Andrographis* species. Further, germplasm of *Andrographis paniculata* and wild relatives are threatened by progressive climate change, habitat loss, and agricultural intensification, and efforts should be focused on in situ and *ex situ* conservation of this valuable germplasm. Insights into the evolutionary origin and diversification of AP and its wild relatives can help us in developing new varieties and even possible new species for the developing world.

Acknowledgements This work was supported by UGC-BSR mid-career award grant [No. F. 19-223/2018(BSR)].

References

Alzaharna M, Alqouqa I, Cheung HY (2017) Toxifolin synergizes Andrographolide-induced cell death by attenuation of autophagy and augmentation of caspase dependent and independent cell death in HeLa cell. PLoS ONE 12:

Amrith SS (2014) The art of medicine: Migration and health in southeast Asian history. The Lancet 384:1569–1570

Anderson E (1949) Introgressive hybridization. Wiley and Sons, New York

Anderson E, Stebbins GL (1954) Hybridization as an evolutionary stimulus. Evolution 8:378–388

Anonymous (2019) *Andrographis paniculata*, Plants of the World online. Royal Botanic Garden, Kew, UK

Arnold ML (2004) Natural hybridization and the evolution of domesticated, pest and disease organisms. Mol Ecol 13:997–1007

Banerjee M, Chattopadhyay S, Choudhuri T, Bera R, Kumar S, Chakraborty B, Mukherjee SK (2016) Cytototoxicity and cell cycle arrest induced by andrographolide lead to programmed cell death of MDA-MB-231 breast cancer cell line. J Biomed Sci 16:23–40

Benn C (2002) China's golden age: everyday life in the Tang Dynasty. Oxford University Press, London

Dai Y, Chen SR, Chai L, Zhao J, Wang Y, Wang Y (2019) Overview of pharmacological activities of *Andrographis paniculata* and its major compound andrographolide. Food Sci Nutrition 59(sup1):S17–S29

Dalawai D, Aware C, Jadhav JP, Murthy HN (2019) RP-HPLC analysis of diterpene lactones in leaves and stem of different species of *Andrographis*. Nat Prod Res 9:1–4. https://doi.org/10.1080/14786419.2019.1662004

Darwin C (1859) On the origin of species by means of natural selection, or The preservation of favoured races in the struggle for life. Murray J, London

Darwin C (1868) The variation of animals and plants under domestication. Murrary J, London

Dey YN, Kumari S, Ota S, Srikanth N (2013) Phytopharmacological review of *Andrographis paniculata* (Burm. f.) Wall. ex Nees. Int J Nutr Pharmacol Neurol Dis 3:3–10

Dulloo ME, Thormann I, Fiorino E, De Felice S, Rao VR, Snook L (2013) Trends in research using plant genetic resources from germplasm collections: from 1996 to 2006. Crop Sci 53:1217–1227
Gu LL, Zhang XY, Xing WM, Xu JD, Lu H (2016) Andrographolide-induced apoptosis in human renal tubular epithelial cells: roles of endoplasmic reticulum stress and inflammatory response. Environ Toxicol Phamacol 45:257–264
Hajjar R, Hodgkin T (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156:1–13
Hancock JF (2004) Plant evolution and the origin of crop species. CABI, Wallingford, UK
Handa SS (1998) Indian herbal pharmacopoeia, vol 1. Indian Drug Manufacturers Association, Mumbai
Harlan JR (1971) Agricultural origins: centres and non-centres. Science 174:468–474
Harlan JR (1975) Crops and man. American Society of Agronomy, Wisconsin, USA
Heiser CB (1988) Aspects of unconscious selection and the evolution of domesticated plants. Euphytica 37:77–81
Hossain MS, Urbi Z, Sule A, Hafizur Rahamn KM (2014) *Andrographis paniculata* (Burm. f.) Wall. Ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. Sci World J 2014:274905
Hudedamani UB, Yadav OP (2013) Floral biology of Kalmegh (*Andrographis paniculata* Nees.). Environ Ecol 31:76–79
Index Kewensis (1977–87) Royal botanic garden, vol 1–18. Oxford University Press, Oxford
Jain RK (1970) South Indians on the plantation frontier in Malaya. Yale University Press, New York
Jorfi AM (1994) Iran and India: age old friendship. India Q A J Int Aff 50:65–92
Karthikeyan S, Sanjappa M, Moorthy S (2009) Flowering plants of India, Dicotyledons. Bot Surv India 1:1–4
Khamphaya T, Chansela P, Piyachaturawat P, Suksamaranan A, Nathanson MH, Weerachayaphorn J (2016) Effects of andrographolide on intrahepatic cholestasis induced by alpha-naphthylisothiocyante in rats. Eur J Pharmacol 789:254–264
Kumar G, Singh D, Tali JA, Dheer D, Shankar R (2020) Andrographolide: chemical modification and its effect on biological activities. Bioorganic Chem 95:
Lattoo SK, Dhar RS, Khan S, Bamotra S, Bhan MK, Dhar AK, Gupta KK (2008) Comparative analysis of genetic diversity using molecular and morphometric markers in *Andrographis paniculata* (Baum. F.) Nees. Genet Resour Crop Evol 55:33–43
Lee TY, Chang HH, Wen CK, Huang TH, Chang YS (2014) Modulation of thioacetamide-induced hepatic inflammations, angiogenesis and fibrosis by andrographolide in mice. J Ethanopharmacol 158:423–430
Li MR, Shi FX, Zhou YX, Li YL, Wang XF, Zhang C, Wang XT, Liu B, Xiao HX, Li LF (2015) Genetic and epigenetic diversities shed light on domestication of cultivated ginseng (*Panax ginseng*). Mol Plant 8:1612–1622
Li Y, He S, Tang J, Ding N, Chu X, C L, Ding X, Feng S, Rahman SU, Wang X, Wu J (2017) Andrographolide inhibits inflammatory cytokines secretion in LPS-stimulated RAW264.7 cells through suppression NF-kB/MAPK signaling pathway. Evid Based Complemetn Alternat Med 2017:8248142
Ma XJ, Wang XQ, Xiao PG, Hong DY (2000) A study on AFLP fingerprinting of landraces of *Panax ginseng* L. Zhongguo Zhong Yao Za Zhi 25:707–710
Meyer RS, Purugganan MD (2013) Evolution of crop species: genetics of domestication and diversification. Nat Rev Genet 14:840–852
Mir H, Kapur N, Singh R, Sonpavde G, Lillar JW, Singh S (2016) Andrographolide inhibits Prostate cancer by targeting cell cycle regulators, CXCR3 and CXCR7 chemokine receptors. Cell Cycle 15:819–826
Mishra SK, Sangwan NS, Sangwan RS (2007) *Andrographis paniculata* (Kalmegh): a review. Phcog Rev 1:283–298
Mukherjee PK, Pitchairajan V, Murugan V, Sivasankarna P, Khan Y (2010) Strategies for revitalization of traditional medicine. Chinese Herbal Med 2:1–15

Murugan NA, Pandian CJ, Jeyakanthan J (2020) Computational investigation on Adrographolide paniculata phytochemicals to evaluate their SARS-CoV-2 in comparision to known antivial components in drug trials. J Biomol Structre Dynamics. https://doi.org/10.1080/07391102.2020.1777901

Neeraja C, Hari Krishna P, Sudhakar Reddy C, Giri CC, Rao KV, Reddy VD (2015) Distribution of *Andrographis* species in different districts of Andhra Pradesh. Proc Natl Acad Sci India Sect B Biol Sci 85:601–606

Peng S, Hang N, Liu W, Guo W, Jiang C, Yang X, Xu Q, Sun Y (2016) Andrographolide sulfonate ameliorates lipopolysaccharide-induced acute lung injury in mice by down-regulating MAPK and NF-kB pathways. Acta Pharm Sin B 6:205–211

Purugganan MD (2019) Evolutionary insights into the nature of plant domestication. Curr Biol 29:R705–R714

Rieseberg L, Carney SE (1998) Plant hybridization. New Phytol 140:599–624

Roy SK, Datta PC (1988) Chromosomal biotypes of *Andrographis paniculata* in India and Bangladesh. Cytologia 53:369–378

Sabu KK (2002) Intraspecific variations in *Andrographis paniculata* Nees. Ph. D. thesis, Kerala University, Thiruvananthapuram, India

Sastri KAN (1949) Takuapa and its Tamil inscriptions. J Malayan Roy Asiatic Soc 22:25–30

Schippmann U, Leaman DJ, Cunningham AB (2002) Impact of cultivation and gathering of medicinal plants on biodiversity: global trends and issues. In: Biodiversity and the ecosystem approach in agriculture, forestry and fisheries. Satellite event on the occasion of the ninth regular session of the commission on genetic resources for food and agriculture, Rome, Food and Agriculture Organization, Rome, pp 1–21

Schmitt R (2019) Achaemenid dynasty. Encyclopedia Iranica, pp 414–426; an updated version available online at http://www.iranicaonlin.org/articles/achaemeind-dynasty

Setter MG, Gates DJ, Mei W, Ross-Ibarra J (2017) How to make a domesticate. Curr Biol 27:R896–R900

Shastri SV (1998) Hindu culture. In: Vohra NN, Dixit JN (eds) Religion and politics, in south and southeast Asia. Konark Publishers, New Delhi, p 51

Soltis DE, Soltis PS (1999) Polyploidy: recurrent formation and genome evolution. Trends Ecol Evol 14:348–352

Subramanian R, Asmawi MZ, Sadikun A (2012) A bitter plant with a sweet future? A comprehensive review of an oriental medicinal plant: *Andrographis paniculata.* Phytochem Rev 11:39–75

Takur A, Chterjee S, Kumar V (2014) Antidepressant-like activity of Andrographis paniculata in type-2 diabetic rats. Clinic Pharmcol Biopharmaceut S 2:2

Takur A, Rai G (2016) Beneficial effects of *Andrographis paniculata* extract and andrographolide on congnitive functions in streptozotocin-induced diabetic rats. Pharm Biol 1:11. https://doi.org/10.3109/13880209.2015.1107107

USDA (2020) Agricultural Research Service, National Plant Germplasm System. Germplasm Resources Information Netwrok (GRIN-Taxonomy). National Germplasm Resources Laboratory, Beltsville, Maryland, USA. https://npgsweb.ars-grain.gov/gringlobal/taxonomydetail.aspx?id=41428. Accessed 8 Apr 2020

Valdiani A, Kadir MA, Saad MS, Talei D, Omidvar V, Hua CS (2012) Intraspecific crossability in *Andrographis paniculata* Nees. A barrier against breeding of the species. Sci World J 2012:297545

Vavilov NI (1926) Studies on the origin of cultivated plants. Institute of Applied Botany and Plant Improvement, Leningrad, USSR

Vavilov NI (1951) The origin, variation, immunity and breeding of cultivated plants. Soil Sci 72:482

Vavilov NI (1992) Origin and geography of cultivated plants. (trans: Love D). Cambridge University Press, Cambridge, UK

Wang ZM, Kang YH, Yang X, Wang JF, Zhang Q, Yang BX, Zhao KL, Xu LP, Ma JX, Huang GH, Cai J, Sun XC (2016) Andrographolide radiosensitizes human esophageal cancer cell line ECA109 to radiation in vitro. Dis Esophagus 29:54–61

Wendel JF, Brubaker C, Alvarez I, CronnR Stewart JM (2009) Evolution and natural history of the cotton genus. In: Patterson AH (ed) Genetics and genomics of cotton: crops and models, vol 3. Springer, New York, pp 3–22

Wendel JF, Cronn RC (2003) Polyploidy and the evolutionary history of cotton. Adv Agron 78:139–186

Widyawaruyanti A, Asrory M, Ekasari W, Setiawan D, Radjaram A, Tumewu L, Hafid AF (2014) In vivo antimalarial activity of Andrographis paniculata tablets. Procedia Chem 13:101–104

Wood TE, Takebayashi N, Barker MS, Mayrose I, Greenspoon PB, Rieseberg LH (2009) The frequency of polyploid speciation in vascular plants. Proc Natl Aca Sci 106:13875–13879

Yang T, Yao S, Zhang X, Guo Y (2016) Andrographolide inhibits growth of human T-cell acute lymphoblastic leukemia Jurkat cells by downregulation of P13K/AKT and upregulation of p38 MAPK pathways. Drug Des Devel Ther 10:1389–1397

Zaid OI, Abd Majid R, Sabarih MN, Hasidah MS, Al-Zihiry K, Yam MF, Basir R (2015) Andrographolide effect on both *Plamodium falciparum* infected and non infected RBCs membranes. Asian Pacific J Trop Med 8:507–512

Zhang L, Bao M, Liu B, Zhao H, Zhang Y, Ji XY, Zhao N, Zhang C, He X, Yi J, Tan Y, Li L, Lu C (2020) Effect of andrographolide and its analogs on bacterial infection: a review. Pharmacology 105:123–134

Zohary D (2004) Unconscious selection and the evolution of domesticated plants. Econ Bot 58:5–10

Chapter 4
Successful Cultivation and Utilization of *Aronia melanocarpa* (Michx.) Elliott (Black Chokeberry), a Species of North-American Origin, in Poland and the Biosynthetic Potential of Cells from In Vitro Cultures

Halina M. Ekiert, Paweł Kubica, and Agnieszka Szopa

Abstract *Aronia melanocarpa* is a medicinal, culinary, and ornamental plant known for many years in the Central, Eastern, and Southern European countries, in Scandinavia and Russia, but is native to North America. At the end of the eighteenth century, it was introduced to Europe and Asia where it has become naturalized and successfully cultivated on an increasingly large scale. This species is a source of the raw material, i.e., fruits rich in antioxidants, most of all anthocyanins, procyanidins, phenolic acids, catechins and flavonoids, as well vitamins and bioelements. This article reviews basic information on the morphology, ecology, and distribution of *A. melanocarpa* in natural habitats. The requirements for cultivation of this species are also characterized. Much attention has been paid to the chemical composition of the fruits and their consequent therapeutic, health-promoting, culinary and cosmetic applications as confirmed by scientific studies. The current state of the art in biotechnological studies of this species is described, with a special focus on the investigations of the biosynthetic potential of cells cultured in vitro. The study aimed to establish the most beneficial culture conditions for the accumulation of phenolic acids, which are well-known strong antioxidants showing also many other important directions of biological activity. The optimization of culture conditions comprised testing the basal media, concentrations of plant growth regulators, supplementation of biosynthetic precursors, as well as examination of the impact of light conditions (monochromatic lights, white light, darkness, UV-A irradiation), and culture type (agar callus cultures and agar, agitated and bioreactor shoot cultures). In addition, the biotransformation potential of cells from agitated shoot cultures and high production of arbutin from exogenous hydroquinone were presented. Finally, the evaluation

H. M. Ekiert (✉) · P. Kubica · A. Szopa (✉)
Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, ul. Medyczna 9, 30-688 Kraków, Poland
e-mail: halina.ekiert@uj.edu.pl

A. Szopa
e-mail: a.szopa@uj.edu.pl

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_4

of efficacy and potential applications of biotechnological studies have been outlined. The obtained biotechnological results have documented that shoot cultures of *A. melanocarpa* could be a rich potential source of phenolic acids and arbutin, which are valuable products with therapeutic, health-promoting, and cosmetological values.

Keywords Black aronia · Botanical characteristics · Chemical composition · Biological activities · In Vitro cultures · Endogenous production of phenolic acids · Biotransformation potential

Abbreviations

BAP	6-benzylaminopurine
DW	Dry weight
FW	Fresh weight
HPLC-DAD	High-pressure liquid chromatography with diode array detector
LS	Linsmaier and Skoog
MS	Murashige and Skoog
NAA	1-naphthaleneacetic acid
PGRs	Plant growth regulators

4.1 Introduction

Aronia melanocarpa (Michx.) Elliott (black chokeberry, black aronia), a shrub (Fig. 4.1) of the *Spiraeoideae* (earlier *Pomoideae*) subfamily (*Rosaceae* family) is a North American autochthonous species. It is distributed along the east coast of Canada and the USA, and from the Great Lakes as far as to Florida. This species was introduced to Scandinavia and Russia at the end of the eighteenth century and was successfully naturalized in Europe and Northwestern Asia. It is a long known popular medicinal, culinary, cosmetic, and ornamental plant, cultivated mostly in Central, Eastern, and Southern European countries and in Scandinavia and Russia (Kulling and Rawel 2008; Valcheva-Kuzmanova and Belcheva 2006; Walther and Schnell 2009; Wawer 2006).

The fruits are the essential part of this plant (Fig. 4.1). They are an exceptionally rich source of a variety of subgroups of antioxidants, including polyphenols (anthocyanins, procyanidins, phenolic acids, catechins and flavonoids), carotenoids and vitamin (among others C and E) and numerous bioelements. Many professional studies of biological activity of fresh and dry fruits, fruit extracts and juice have proven their very numerous valuable properties—antioxidant, anti-inflammatory, hepatoprotective, gastroprotective, antimicrobial, and anticancer. They could be used in the prevention of ophthalmologic and circulatory diseases and in diabetes

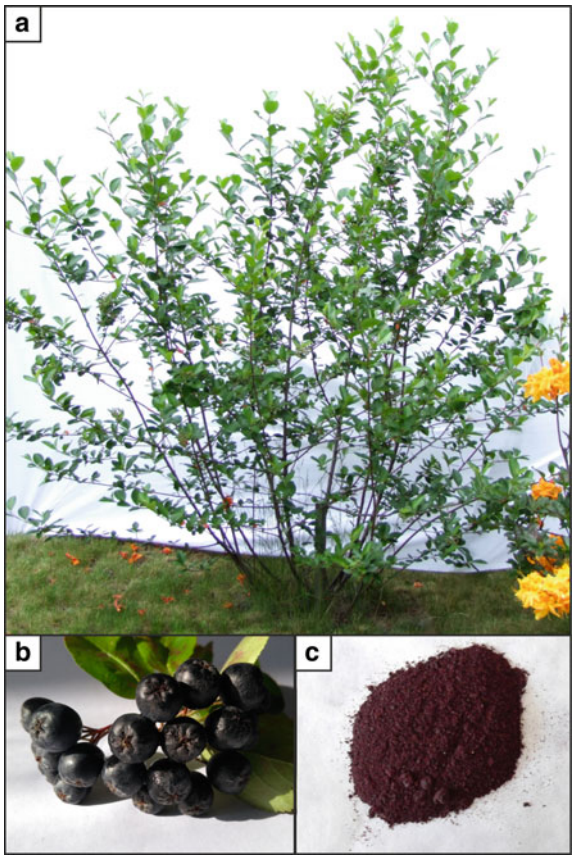


Fig. 4.1 *A. melanocarpa;* the appearance of the shrub cultivated in Poland in spring (**a**), fresh fruits of Viking cultivar (**b**), powdered dried fruits used in industry (**c**)

(Augustyniak et al. 2010; Brand 2010; Brewer 2011; Chrubasik et al. 2010; Kattappagari et al. 2015; Kokotkiewicz et al. 2010; Kulling and Rawel 2008; Oszmianski et al. 2005; Szopa et al. 2017a).

Great interest in this species, its importance and popularity in the European medicine and food industry can be corroborated by review article which were published in the last decade (e.g., Sidor et al. 2019; Szopa et al. 2017a; Denev et al. 2012; Kokotkiewicz et al. 2010) and the newest reviews of phytochemical and pharmacological studies (Borowska and Brzóska 2016; Denev et al. 2012, 2019; King and Bolling 2020; Kokotkiewicz et al. 2010; Sidor et al. 2019; Szopa et al. 2017b).

The raw material, i.e., the fruit is sourced from plants, especially of the cultivars "Nero", "Galicjanka", and *Aronia mitschurinii*, commercially cultivated with great success in Central and Eastern European countries, including Poland. Recently, these crops have become very popular and are cultivated on a mass scale, also as organic farming (Michalak 2015).

The conspicuously growing interest in *A. melanocarpa* fruits and the constantly rising demand for this raw material result principally from the rich composition of antioxidants important in the prevention and treatment of different civilization diseases. These compounds can be useful in hypertension, angina, stroke prevention, neurodegenerative disorders, and neoplastic diseases and control of lipid and cholesterol levels. Moreover, these compounds show antiaging effect. They are a focus of interest of the pharmaceutical, health food, and cosmetic industries (Cai et al. 2004; Chrubasik et al. 2010; Denev et al. 2019; Heleno et al. 2015; Kakkar and Bais 2014; Sidor et al. 2019; Szopa et al. 2018a).

A substantial need for the search for new natural sources of antioxidants, natural phenomenon of chemical variability as well as environmental pollution and current rapid climate changes have also inspired interest in the morphological and biosynthetic potential of in vitro cultures of this species. Earlier biotechnological studies of *A. melanocarpa* carried out by other research groups concentrated on the development of micropropagation protocols (Brand and Cullina 1992; Litwinczuk 2002; Petrovic and Jacimovic-Plavšic 1992; Ruzic 1993). The wide-ranging studies of our team have aimed to optimize favorable conditions for the synthesis of one of group of antioxidants, namely phenolic acids. Optimization has involved agar (callus and shoot) cultures, agitated shoot cultures, and shoot cultures carried out in commercially available bioreactors—RITA and PlantForm (temporary immersion systems) (Rugină et al. 2012; Szopa et al. 2018a, 2020; Zheng and Wang 2003). Some studies examined biotransformation potential of cells cultured in vitro. They were concentrated on biotransformation of exogenous hydroquinone into its β-D-glucoside, arbutin (Kwiecień et al. 2013).

4.2 Synonyms and Names in Other Languages

Black chokeberry and black aronia are the most popular and commonly used English names of *Aronia melanocarpa* (Michx.) Elliott. This species possesses several other Latin synonymous names: *Aronia arbutifolia* (L.) Pers. var. nigra (Willd.) Seymour, *Aronia nigra* (Willd.) Koehne, *Photinia melanocarpa* (Michx.) K.R. Robertson and Phipps., *Pyrus arbutifolia* (L.) L. f. var. nigra Willd., *Pyrus melanocarpa* (Michx.) Willd. and *Sorbus melanocarpa* (Michx.) Heynh (University of Maine 2020). Other foreign names of the species are as follows: aronia czarna, aronia czarnoowocowa (Polish), schwarze Apfelbeere (German), aronie, aronia à fruits noirs, aronie noire, (French).

4.3 Morphology

Aronia melanocarpa is a perennial shrub growing to ca. 3 m tall and up to ca. 2.5 m wide (Fig. 4.1). It develops an extensive but shallow root system within the

perimeter of the crown. The taproot penetrates to 1.5 m deep. The lateral roots are thin and spread horizontally. The plant grows vigorously and spontaneously develops numerous suckers, thus forming dense colonies. Shrubs assume a compact form, bushier during fruiting. The shrubs are densely branched and are capable of regeneration and thickening (Celka and Szkudlarz 2010; Kleparski and Domino 1990; Kokotkiewicz et al. 2010; Rumińska 1984).

Both leaf buds and flower buds closely adhere to the shoots. They are ca. 1 cm long and 3–4 mm wide. In European climate conditions, the flower buds blossom much later than in other fruit trees and shrubs. Blooming begins most often in April when the temperature exceeds 5 °C (Celka and Szkudlarz 2010; Kleparski and Domino 1990; Kokotkiewicz et al. 2010; Rumińska 1984).

Young one-year twigs are thin, flaccid, slightly hairy, dark gray in color, not branched. Older shoots are dark brown. Leaves are elliptic, leathery, glossy. Leaf blade top is glabrous, while the bottom is covered by whitish delicate tomentum. Midrib is conspicuous. Leaves have toothed margins. Leaves are borne on short stalks with two bracts. Leaves on vegetative shoots are ca. 6–8 cm long and ca. 4–6 cm wide. In European conditions, in September leaves turn yellow-orange and red and fall relatively quickly (Celka and Szkudlarz 2010; Kleparski and Domino 1990; Kokotkiewicz et al. 2010; Rumińska 1984).

Aronia flowers are bisexual, with five petals. Flowers are small, inconspicuous, ca. 1 cm across, white or pinkish-white. They are gathered in corymbs. Freshly opened flowers are distinguished by violet anthers. In Europe, flowering starts at the end of June and lasts ca. 10 days. In general, flowers are entomophilous, however, in unfavorable circumstances, they can self-pollinate (Kleparski and Domino 1990; Rumińska 1984).

Unripe fruits are green, during ripening they turn dark red, and then almost black, from matt they change into glossy. Most often they ripen at the beginning of September. Fully ripe aronia fruits are black or dark blue, and they are covered by thick wax coating. One fruit is ca. 6–15 mm across and weights ca. 1 g. Each fruit contains ca. 5 seeds. Aronia fruits do not fall and can remain on plants to the first frost (Kleparski and Domino 1990; Rumińska 1984).

For medicinal and culinary purposes, chokeberry fruits should be harvested when fully ripe, i.e., almost black. They are usually harvested in September. The fruits are first dried on sieves at the beginning at a temperature of 30 °C and then slightly higher but not exceeding 45 °C (Rumińska 1984; Senderski 2004) (Fig. 4.1).

4.4 Natural Habitats in North America and Ecology

Aronia is a species native to North America. Its natural habitats are located in the eastern part of North America from Great Lakes extending south as far as to Florida. Its natural locations can be found both in Canada and the USA (Wawer 2006).

Aronia is cold hardy, and it tolerates temperatures even below −30 °C. It does not have special soil requirements. Since it develops a shallow root system, it can be

cultivated even in areas unsuitable for other more demanding fruit crops. It is tolerant both to drought and excessive humidity (Kleparski and Domino 1990; Kokotkiewicz et al. 2010).

4.5 Successful Cultivation in European Countries

The first *A. melanocarpa* plants were introduced to Europe at the end of the eighteenth century. Cultivation of black chokeberry first developed in Scandinavia and Russia. Farm cultivation on industrial scale became popular especially in the Altai Mountains and in the area of Moscow and Petersburg (Kulling and Rawel 2008; Valcheva-Kuzmanova and Belcheva 2006; Walther and Schnell 2009).

At present, chokeberry is a commonly grown shrub in Central, Eastern, and Southern European countries and in Scandinavia. It is suitable as well for amateur cultivation in backyards as for industrial-scale crop production. Commercial plantings are based on most popular cultivars, such as the Czech cultivar "Nero" and Polish cultivar "Galicjanka". These cultivars owe their popularity to a high yield and high resistance to harmful environmental conditions. The Russian cultivar *Aronia mitschurinii* Amit is another known and popular cultivar which was obtained by grafting *Aronia melanocarpa* on a rootstock of mountain ash (*Sorbus aucuparia*). This cultivar is distinguished by especially high cold-tolerance level. Its fruits are very large and sweet (Kleparski and Domino 1990).

Due to its attractive appearance, in particular changeable leaf color, aronia is also cultivated as an ornamental plant. For its esthetic beauty, it is planted in gardens and parks. The cultivars available in Sweden—"Viking" (originating from Finland), "Aron" (from Denmark), and "Hugin" (from Sweden) are appreciated not only as medicinal but also as ornamental plants (Jeppsson 1999). There are also other cultivars of black chokeberry bred in various countries. Few of them were developed in Poland: "Albigowa", "Dąbrowice", "Egerta", "Kutno", and "Nowa Wieś". Another was developed in Hungary—"Fertödi". The hybrid between Russian and Finnish plants named "Rubina" is the next cultivated cultivar. The most popular cultivars in the USA include "Autumn Magic", "McKenzie", and "Morton" (University of Maine 2020).

4.6 Cultivation Requirements

As already mentioned, aronia is a cold-hardy species. However, it applies to its aboveground parts while the roots can be damaged by frost below −11 °C, and this is why snow cover is vital (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

Chokeberry is best grown on fertile humus, high-humidity, medium-cohesive soils, on permeable substrate, rich in nutrients. It is important to choose an area free of frost basins, unflooded in the spring, with a low level of groundwater. Light

conditions should also be taken into account because aronia is a sun-loving plant and should not be shaded by other plants (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

The best sites for chokeberry plantings are those where root crops were grown on manure-amended soils or after horticultural crops. If plantations are to be established on fallow land, the application of manure is indispensable. If manure is not available, it can be replaced by compost. Presowing tillage procedures should include deep plowing and harrowing (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

Plantations can be established both from seeds and from plantlets. The vegetative propagation is based on three methods: division of old shrubs, root suckers, and rooted softwood or hardwood stem cuttings. One parent plant several years old can yield ca. 20 rooted plantlets. Vegetative propagation accelerates plant growth and boosts yield. Vegetatively propagated plants begin yielding already after 2–3 years while shrubs grown from seed require 3 or even 5 years. In the case of propagation by seeds, they have to be stratified for 3–5 months (by storage in humid coarse sand at a temperature of 2–3 °C) (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

The plantlets used for establishing aronia crops should be 50 cm tall and should have 2–3 lateral shoots. The stem at the neck should be 1 cm across. The root system should be highly branched, reaching ca. 20 cm in length (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

Planting can be done both in autumn and early spring. Spring planting is preferred in the regions which lack the snow cover in winter. Planting should be carried out immediately after the soil thaws—not later than till mid-April. If autumn planting is planned and when planting material has to be stored, mulching or covering with snow is recommended before frost occurs (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

The raw material, namely aronia fruits, is harvested when the fruits are fully colored and almost black. Depending on atmospheric conditions in a given year and plantation age, yields can reach from several tens of kilograms to several tons per hectare. A 3-year well-growing plantation can yield ca. 3 tons of chokeberry fruits per 1 ha. A plantation can be used for 10–15 years, and then it should be liquidated (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

Aronia is a species resistant to plant diseases. Brown spot disease and brown rot are observed the most often. In addition, the following insects can feed on chokeberry: apple aphid (*Aphis pomi de Geer*), woolly aphid (*Eriosoma lanigerum* Hasm.) and leaf blister mite (*Eriophyes piri* Pgst.)—a mite species (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

4.7 Chemical Composition

Aronia fruits are the most valuable part of this species and are used as the raw material for pharmaceutical, culinary, and/or cosmetic purposes as they are a rich source of polyphenols—anthocyanins, procyanidins, phenolic acids, catechins, and

Table 4.1 Maximal contents of antioxidant compounds in fresh [mg/g FW] and dried [mg/g DW] fruits and fruit extracts of *A. melanocarpa*

Group of metabolites	Compound	Fresh fruits	Dried fruits	Dried fruit extracts
Anthocyanins	Cyanidin-3-galactoside	9.9	12.8	314.0
	Cyanidin-3-arabinoside	4.0	5.8	159.6
	Cyanidin-3-xyloside	0.5	0.5	40.0
	Cyanidin-3-glucoside	0.4	0.4	14.5
	Pelargonidin-3-arabinoside with pelargonidin-3-galactoside	nd[a]	nd	0.5
Proanthocyanidins	Procyanidin B_1	nd	nd	25.4
Phenolic acids	Hydroxycynnamic acid	0.01	nd	nd
	Chlorogenic acid	2.8	3.02	79.0
	Neochlorogenic acid	1.8	2.9	44.7
	3,4-Dihydroxyphenylacetic acid	0.1	nd	nd
	Protocatechuic acid	0.1	nd	nd
	Rosmarinic acid	0.1	nd	nd
	Caffeic acid	1.4	nd	0.7
Catechins	(+)-catechin	nd	nd	19.9
	(−)-epicatechin	nd	0.2	12.8
Flavonoids	Quercetin	0.1	nd	1.8
	Quercetin-3-rutinoside	nd	18.0	18.3
	Quercetin-3-galactoside	0.3	0.4	8.9
	Quercetin-3-glucoside	0.3	nd	21.5

[a]nd—no data

flavonoids (Table 4.1) (Bijak et al. 2013; Brzóska et al. 2015; Jakobek et al. 2012; Jodynis-Liebert et al. 2014; Jurgoński et al. 2008; Kim et al. 2013; Pérez-Jiménez et al. 2010; Rugină et al. 2012; Ryszawa et al. 2006; Wangensteen et al. 2014).

Phytochemical studies have concentrated on the chemical composition of fresh and dried fruits, dry fruit extracts, and juice (Benvenuti et al. 2006; Bijak et al. 2013; Brzóska et al. 2015; Jakobek 2007; Jodynis-Liebert et al. 2014; Jurgoński et al. 2008; Kim et al. 2013; Pérez-Jiménez et al. 2010; Rugină et al. 2012; Ryszawa et al. 2006; Taheri et al. 2013; Vlachojannis et al. 2015; Wang et al. 1996; Wangensteen et al. 2014; Wu et al. 2004; Zheng and Wang 2003) (Table 4.1).

Anthocyanins in aronia fruits have been confirmed to comprise mostly cyanidin glycosides with dominating cyanidin-3-galactoside and cyanidin-3-arabinoside while cyanidin-3-xyloside and cyanidin-3-glucoside are present at lower amounts. Cyanidin glycosides are accompanied by glycoside compounds of pelargonidin: arabinoside and galactoside. The group of procyanidins includes mostly procyanidin B_1 (Table 4.1).

Phenolic acids are represented by caffeic acid and hydroxycinnamic acid and depsides—chlorogenic acid and neochlorogenic acid (Fig. 4.2). The catechin group has been shown to include (+)catechin and (−)epicatechin. On the other hand, among flavonoids present in aronia fruits, mostly quercetin glycosides (rutinoside, galactoside, and glucoside) and an aglycone, namely quercetin, were identified (Table 4.1) (Kulling and Rawel 2008; Sidor and Gramza-Michałowska 2019; Szopa et al. 2017a). Other flavonoid compounds confirmed to be present in chokeberry fruits include: isorhamnetin 3–O–: -galactoside and –glucoside; isorhamnetin–3–: -galactoside, -glucoside, -neohesperidoside, and –rutinoside; kaempferol 3–O–galactoside; kaempferol-3-: -galactoside and –glucoside; myricetin 3–O–: -galactoside and –glucoside; and quercetin–3–: -robinobioside and –vicianoside (Gramza-Michałowska et al. 2017; Sidor et al. 2019).

Aronia fruits are also a rich source of vitamins, in particular, vitamin C, E, K, folic acid, many vitamins of B complex (B_1, B_2, B_6), niacin (vitamin B_3) and pantothenic acid (vitamin B_5). They also contain β-carotene (provitamin A) as well as other carotenoids—β-cryptoxanthine and violaxanthine. In addition, the fruits are a generous supply of bioelements, including zinc, magnesium, potassium, sodium, calcium, copper, selenium, and iron (Andrzejewska et al. 2015; Benvenuti et al. 2006; Kulling and Rawel 2008; Razungles et al. 1989; Sikora et al. 2009; Stralsjoe et al. 2003; Tanaka and Tanaka 2001) (Table 4.2).

The above-mentioned metabolites and bioelements are accompanied by organic acids (citric acid and malic acid), fiber, pectins, fatty acids, and sterols.

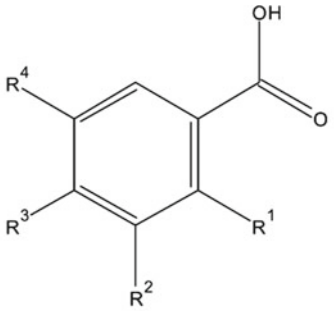
Phytochemical studies of the dried fruits and leaves of aronia specimens acquired from the Arboretum of the Warsaw University of Life Sciences (SGGW) in Rogów (central part of Poland), as carried out by our team, have proven that the leaves of this species are very rich in polyphenols while some polyphenolic compounds were demonstrated in fruits for the first time. The quantitatively dominating group in the analyzed leaves was identified as flavonoids. The presence of quercetin and its two glycosides—quercitrin and rutoside was confirmed. The group of phenolic acids was documented to include two depsides: chlorogenic and neochlorogenic acids and additionally 3,4-dihydroxyphenylacetic and protocatechuic acids. Among anthocyanins, cyanidin arabinoside, and galactoside were identified (Szopa et al. 2017a).

The studies of other authors (Tian et al. 2017) underlined also the biosynthetic potential of the leaves of various berry plants, among them also black chokeberry. The leaves extracts of some berry plants could also be a potential rich source of phenolic compounds (Tian et al. 2017).

Our analysis of fruit extracts confirmed the presence of chlorogenic acid and neochlorogenic acid, while rosmarinic acid, protocatechuic acid, and 3,4-dihydroxyphenylacetic acid were identified for the first time. The anthocyanin group was shown to be represented by three cyanidin glycosides, namely galactoside, arabinoside, and glucoside. On the other hand, only the aglycone quercetin was found among flavonoids (Szopa et al. 2017a).

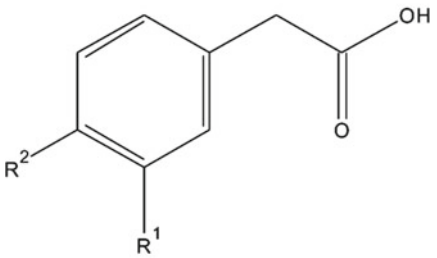
Fig. 4.2 Chemical structures of the main subgroups of phenolic acids

Benzoic acid



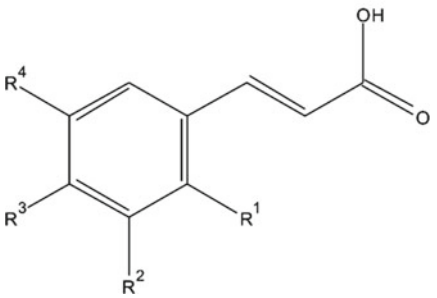
Gallic acid	R^1=H, R^2=OH, R^3=OH, R^4=OH
Protocatechuic acid	R^1=H, R^2=OH, R^3=OH, R^4=H
Salicylic acid R^1=OH,	R^2=H, R^3=H, R^4=H
Syringic acid R^1=H,	R^2=OCH_3, R^3=OH, R^4=OCH_3
Vanillic acid R^1=H,	R^2=OCH_3, R^3=OH, R^4=H

Phenylacetic acid



3-Hydroxyphenylacetic acid	R^1=OH, R^2=H
3,4-Dihydroxyphenylacetic acid	R^1=OH, R^2=OH

Cinnamic acid



Ferulic acid	R^1=H, R^2=OCH_3, R^3=OH, R^4=H
Caffeic acid	R^1=H, R^2=OH, R^3=OH, R^4=H
o-Coumaric acid	R^1=OH, R^2=H, R^3=H, R^4=H
p-Coumaric acid	R^1=H, R^2=H, R^3=OH, R^4=H
Sinapic acid	R^1=H, R^2=OCH_3, R^3=OH, R^4=OCH_3

Table 4.2 Content of vitamins and bioelements in fruits of *A. melanocarpa*

Vitamins and bioelements		Content
Vitamins	C	0.013–0.27 mg/g DW
	B_1 (thiamine)	0.0002 mg/g DW
	B_2 (riboflavine)	0.0002 mg/g DW
	B_3 (niacin)	0.003 mg/g DW
	B_5 (pantothenic acid)	0.0028 mg/g DW
	B_6 (pirydoxine)	0.0003 mg/g DW
	B_9 (folic acid)	0.0002 mg/g DW
	E	0.008–0.31 mg/g DW
	K	0.0002 mg/g DW
Carotenoids	β-carotene	0.0077–0.0168 mg/g FW
	β-cryptoxanthin	0.0046–0.0122 mg/g FW
	Violaxanthin	0.013 mg/g FW
Bioelements	Zinc	0.0015 mg/g DW
	Magnesium	0.162 mg/g DW
	Potassium	2.18 mg/g DW
	Sodium	0.026 mg/g DW
	Calcium	0.322 mg/g DW
	Iron	0.0093 mg/g DW

The extraction procedures of chokeberries are decisive for the quantities of individual subgroups of phenolics and for their percent content in total phenolics. Methanolic extracts are prepared most often (Vázquez-Espinosa et al. 2019).

According to the newer investigations of the Finland team (Tian et al. 2017), anthocyanins in fresh chokeberries collected in Finland (2013–2014) represented 50% of total phenolics with two main cyanidin glycosides: 3-*O*-galactoside (222 mg/100 g FW) and 3-*O*-arabinoside (159 mg/100 g FW). 3-*O*-Caffeoylquinic acid (23%) and 5-*O*-caffeoylquinic acid (11%) were the next dominant group among the total phenolics. Flavonoid glycosides made up ca. 10% of total phenolics. The quantitatively dominant compounds include quercetin 3-*O*-galactoside and quercetin 3-*O*-glucoside. These results were obtained by the authors after extraction of fresh plant material with acidic aqueous ethanol (Tian et al. 2017).

Other authors after extraction of the fresh fruits with 0.1% hydrochloric acid in methanol documented a lower anthocyanin content in chokeberries (481 mg/100 g FW), which included also cyanidin-3-*O*-galactoside (65% of total anthocyanins) and cyanidin-3-*O*-arabinoside (30%), as the main compounds (Slimestad et al. 2005).

Some scientific investigations documented differences in the quantity of secondary metabolites between individual aronia cultivars. Five cultivars (“Aron”, “Fertödi”, “Hugin”, “Nero”, and “Viking”) grown in an experimental orchard in Zlin

(Czech Republic) in 2008–2010 (three vegetation periods) were shown to differ in total content of phenolic compounds and total antioxidant activity. The highest values were found for cultivars "Viking" and "Nero". Those cultivars were proposed by the authors as the most promising for further use in food and pharmaceutical applications (Rop et al. 2010).

On the other hand, three different cultivars—"Nero", "Viking", and "Galicjanka" and wild chokeberry, all grown in the Slavonia region (Croatia) in 2010–2011, had the same profile of phenolic compounds, but the contents of subgroups of compounds were different. The total phenolic and total anthocyanin contents were higher in "Nero" and "Viking", while in "Galicjanka" the lowest content was confirmed. Flavonoid content was comparable in all chokeberries. Phenolic acid contents were the highest in "Viking" and wild chokeberries. The antiradical activity was the strongest (in DPPH and ABTS tests) also for "Viking" and wild-chokeberry berries (Jakobek et al. 2012).

The more recent research on this issue documented that qualitative composition of anthocyanins did not differ between the wild-grown and cultivated cultivars, but, in general, their contents were different (Veberic et al. 2015).

The quantitative content of bioactive compounds in aronia fruits is affected by weather conditions during the growth and ripening of the fruits, which is obvious. The investigations by another Croatian team (fruits from experimental orchard, Donja Zelina, 2012–2014) documented that the mean monthly temperature and bright sunshine hours (May–September) had a positive impact on the concentrations of phenolic substances (total phenolics and total flavonoids) and, therefore, on the antioxidant activity of juice from aronia fruits (FRAP test) (Tolić et al. 2017).

Phenolic acids—the object of our biotechnological approach

Our biotechnological studies presented below in this article focused specifically on phenolic acids. Therefore, we decided to present this group in more detail specifying their characteristics—chemical division, biogenesis, and biological activity. These compounds are antioxidants widespread in the plant kingdom. Structurally they are polyphenols and are divided into derivatives of cinnamic acid, benzoic acid, and phenylacetic acid (Fig. 4.2). The derivatives of cinnamic acid are the most prevalent in the plant kingdom. They include mostly *p*-coumaric acid, *o*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid. Benzoic acid derivatives include principally salicylic acid, *p*-hydroxybenzoic acid, protocatechuic acid, and vanillic, syringic, and gallic acids. On the other hand, phenylacetic acid derivatives are represented predominantly by 3-hydroxyphenylacetic and 3,4-dihydroxyphenylacetic acid.

Phenolic acids occur in the plant kingdom in the free form or often as depsides. They may also be a part of glycosidic linkages. Depsides are compounds built of two or rarely more of molecules of phenolic acids linked by an ester bond. They often contain caffeic acid or its derivatives. The most commonly known depsides include chlorogenic acid and its numerous isomers—e.g., isochlorogenic acid, neochlorogenic acid, and cryptochlorogenic acid. Rosmarinic acid, ellagic acid, and *m*-digallic acid are other well-known and popular representatives of this group. Chicory acid,

cinarine, cetraric acid, and lecanoric acid are less common depsides in the plant kingdom.

Biogenesis of phenolic acids is closely associated with the shikimic acid pathway (Fig. 4.3). Shikimic acid gives rise to chorismic acid, and in the next step, to prephenic acid. Prephenic acid is a precursor of the path leading to phenylalanine and tyrosine—aromatic amino acids. Deamination of phenylalanine yields cinnamic acid, the parent compound of one subgroup of phenolic acids. The next stage involves the formation of hydroxy and methoxy derivatives of cinnamic acid—*p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid. Phenylalanine is the main direct biogenetic precursor of phenolic acids. *p*-Coumaric acid can also be formed from tyrosine.

Benzoic acid derivatives can be formed *via* chorismic acid transformations. This path leads to salicylic acid formation. Salicylic acid and other benzoic acid derivatives can be produced also from cinnamic acid by shortening of the side chain of this compound by 1–3 carbon atoms.

In addition to strong antioxidant properties phenolic acids show a multitude of therapeutically important biological actions. They exhibit cholagogic, choleretic, hypolipemic, hypocholesterolemic, and hepatoprotective activities. They also cause spasmolytic effect. They were proven to have anxiolytic, chemoprotective, and immunostimulating properties. Their antiviral, bacteriostatic, fungistatic, cytotoxic, and anti-inflammatory activities are also known. However, these are antioxidant and anticancer effects documented for some phenolic acids that have attracted the greatest interest. It is a group of plant metabolites which receives tremendous attention of many scientific centers worldwide (Brewer 2011; Kattappagari et al. 2015; Krishnaiah et al. 2011; Szopa et al. 2018a; Willcox et al. 2004; Zhang and Tsao 2016).

A wide range of biological activities and resulting possible therapeutic applications of phenolic acids outlined above prompted us to choose this group of compounds as the object of our biotechnological studies.

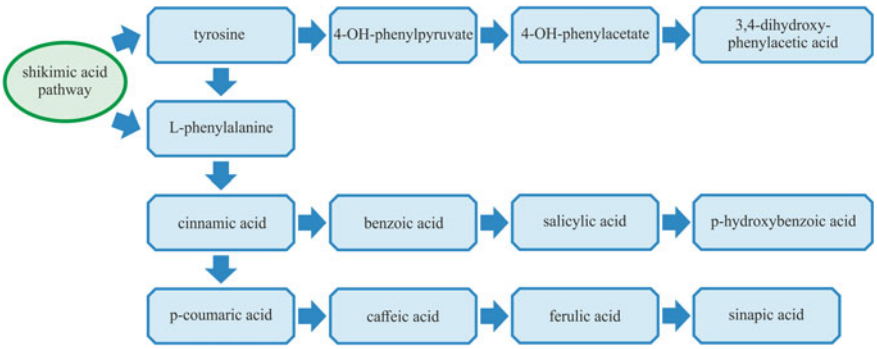


Fig. 4.3 Biosynthesis of chosen phenolic acids

4.8 Medicinal and Health-Promoting Properties

The above-presented chemical composition of chokeberry fruits underlies their multidirectional health-promoting properties and efficacy in supportive treatment of many of civilization diseases (Wawer 2006). Most of the directions of their biological activity were confirmed by scientific studies.

In the last years, three valuable review articles (Sidor et al. 2019, Sidor and Gramza-Michałowska 2019; King and Bolling 2020) presented the current knowledge about different directions of biological activity of different forms of fruits (fresh, dry, juice, extract) based on black chokeberry fruits, as evidenced by animal experiments and/or human trials. The first article paid a special attention on health-promoting activity of those products in cardiovascular diseases, hyperlipemia, hypercholesterolemia, hypertension, and diabetes (Sidor et al. 2019). The second article documented most of all the antioxidant activity of aronia products measured with use of different tests (DPPH, FRAP, ABTS, CUPRAC) (Sidor and Gramza-Michałowska 2019). Objective of the third article is to analyze aronia berry composition, including polyphenols nutrients, summarize available studies on the polyphenol bioavailability and health benefits (King and Bolling 2020). The most important biological activities of chokeberry products confirmed by scientific research are presented below.

Antioxidant action

Both fresh, dried, and/or powdered fruits and their products show a strong antioxidant activity, which is associated with a high content of polyphenols, known antioxidants, which include anthocyanins, proanthocyanidins, phenolic acids, catechins and flavonoids. Some other ingredients present in fruits, such as vitamin C and E, β-carotene, and bioelements—zinc, copper, and selenium, have also antioxidant properties.

A comparison of antioxidant actions of fresh *A. melanocarpa* fruits with other fruits—blueberries, apples, strawberries, cranberries, and even grapes demonstrated ca. four times stronger action of aronia fruits. Antioxidant activity of chokeberry fruits was documented in in vitro tests (ABTS, DPPH, FRAP, CUPRAC, ORAC), in vivo experiments on laboratory animals and in clinical trials (Denev et al. 2012, 2019; Kokotkiewicz et al. 2010; Kulling and Rawel 2008; Oszmianski et al. 2005; Tolić et al. 2015).

The studies carried out by our research team also proved (by DPPH and FRAP tests) a high antioxidant activity of both fruit and leaf extracts (Szopa et al. 2017a).

Beneficial effect on the cardiovascular system

Scientific studies confirmed the protective and stimulating effect of aronia polyphenol compounds on the cardiovascular system function, in particular their anticoagulant, vasoprotective, cardioprotective, hypotensive and blood triglyceride, and cholesterol-lowering effects. In vitro studies have proven the protective and regenerative actions of polyphenols present in aronia products on the endothelial cell function due to their

antioxidant and anti-inflammatory potential. Supplementation of the diet with chokeberry juice in men with mild hypercholesterolemia resulted in a marked reduction of the total cholesterol, LDL cholesterol, and triglyceride levels, which was accompanied by a rise in HDL cholesterol. Moreover, a decrease in both systolic and diastolic blood pressure was documented. Similar effects of the fruit extract were observed in patients with type II diabetes, after myocardial infarction and co-treated with statins (Bell and Gochenaur 2006; Broncel et al. 2010; Kokotkiewicz et al. 2010; Kulling and Rawel 2008; Naruszewicz et al. 2007).

Diabetes prevention and treatment

Numerous studies have documented a beneficial effect of aronia fruit extracts on prevention and outcomes of type II diabetes. Administration of aronia products to patients reduced blood levels of glucose, glycated hemoglobin (HbA1c), cholesterol, and lipids (Badescu et al. 2015; Jurgoński et al. 2008; Kokotkiewicz et al. 2010; Kulling and Rawel 2008; Valcheva-Kuzmanova et al. 2007). It was shown that chlorogenic acid present in chokeberry juice and fruit extracts stimulated glucose and lipid metabolism. On the other hand, cyanidin-3-arabinoside inhibited the activity of α-glucosidase, which is the enzyme participating in carbohydrate breakdown (Meng et al. 2013).

A Japanese research team has recently documented the beneficial effect of the identified cyanidin-3,5-*O*-diglucoside from aronia juice on hyperglycemia in mice-administered aronia juice. This diglucoside is a dipeptidyl peptidase IV inhibitor. The investigations in male and female adult healthy Japanese who consumed aronia juice demonstrated the reduction of postprandial blood glucose levels. In addition, the authors documented that activities of dipeptidyl peptidase IV, α-glucosidase, and angiotensin-converting enzyme were reduced by aronia juice. Probably aronia juice suppresses the elevation of postprandial blood glucose levels through the inhibition of those enzyme activities and could be useful for prevention of metabolic disease in adult healthy Japanese (Yamane et al. 2017).

Hepatoprotective activity

Studies on experimental animals (rats) have demonstrated the protective action of both juice and nectar from aronia fruits on hepatocytes. In animals, lipid peroxidation was induced by the administration of CCl_4, aminophenazone, and sodium nitrite which caused hepatocyte death while anthocyanins and other phenolic compounds from aronia fruits were proven to suppress lipid peroxidation. Moreover, efficiency of chokeberry fruit extracts was evidenced in the treatment of non-alcoholic hepatic steatosis (Park et al. 2016; Pool-Zobel et al. 1999; Valcheva-Kuzmanova et al. 2004).

Recently, another research team has evaluated the hepatoprotective activity of aronia juice and silymarin in the rat model of fibrosis (induced by CCl_4). After administration of aronia juice, the peroxidation of lipids was suppressed. The beneficial effect of aronia juice was also confirmed during histological examination. In this model, the effect of silymarin used as a positive control was very limited (Piotrowska-Kempisty et al. 2020).

Gastroprotective activity

Animal studies in the rat documented antiulcer activity and protective action on the gastrointestinal mucosa after the administration of aronia juice. Animals which did not receive the juice more often showed indomethacin-induced stomach injury (Valcheva-Kuzmanova et al. 2005).

Ophthalmological applications

Aronia anthocyanins accelerated regeneration of rhodopsin in rods of the retina of the eye and improved color vision, mesopic vision, and image registration. On the other hand, flavonoids present in chokeberry fruits improved elasticity of the capillary walls in the eyeball by interacting with collagen. In this way, they reduced fragility and permeability of the vascular wall. The complex of antioxidants in aronia preparations shows a beneficial effect in the case of progressing cataract, glaucoma, and macular degeneration (Wawer et al. 2012; Wolski et al. 2007).

Anti-inflammatory activity

Anti-inflammatory activity was evidenced for dry extract from aronia fruit. This activity was multidirectional. Flavonoids and anthocyanins present in the extract inhibited the activity of cyclooxygenase (COX-2) and inflammatory reactions in which it participates. On the other hand, research on mouse macrophage cultures documented anthocyanin-induced inhibition of mastocyte degranulation and reduction of tumor necrosis factor (TNF-α) level (Valcheva-Kuzmanova et al. 2005).

Antibacterial and antiviral activity

Bacteriostatic activity of aronia fruit juice was proven against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Antiviral activity was demonstrated for influenza A virus (Valcheva-Kuzmanova and Belcheva 2006).

The mechanisms of antiviral activity of chokeberry extract against influenza viruses have been presented recently. It was shown that this effect is connected with the presence of anthocyanins, proanthocyanidins, and other classes of flavonoids and phenolic acids in fruits. Polysaccharides could also play an important role. The components of fruits could inhibit replication of the virus directly, e.g., by blocking surface glycoproteins of influenza virus or indirectly by stimulating the immune system of the host. Similar properties were documented for other popular berry fruits, elderberry, goji berry, cranberry, and black currant (Gramza-Michałowska et al. 2017; Sidor et al. 2019).

Chelation of heavy metals

Chelating actions of anthocyanins was revealed in experimental animals (rats) intoxicated with cadmium. Aronia juice supplementation was shown to decrease the accumulation and toxicity of this element (Kokotkiewicz et al. 2010). A similar chelating action was also proven in lead poisoning in which a drop in the concentration of lead compounds in serum and internal organs was observed. This effect was also attributed to aronia anthocyanins (Jan Niedworok 2001).

Radioprotective activity

It has been evidenced that anthocyanins contained in aronia fruits have a beneficial effect on the outcomes of acute radiation syndrome experimentally induced in rats. Anthocyanins suppressed the generation of free radicals and rapid drop in leukocyte count. The enhanced regenerative potential of cell was also documented (Wawer et al. 2012).

Another study confirmed the radioprotective activity of cyanidin and its glycosides as well as the extract containing aronia anthocyanins. This effect was observed on monkey renal cell line exposed to the radioactive complex of technetium and 2,3-dimercaptosuccinic acid (Wawer et al. 2012).

In addition, gel from chokeberry fruits applied to the skin protected it from the harmful effect of UV-B radiation (Niedworok et al. 1999; Pratheeshkumar et al. 2014).

Antimutagenic activity

Studies on human lymphocyte culture exposed to mutagenic compounds (benzopyrene and 2-aminofluorene) have demonstrated antimutagenic action of aronia fruit extracts. Anthocyanins present in the studied extracts produced antigenotoxic effect which resulted from the ability of anthocyanidins to neutralize free radicals and to inhibit enzymes activating mutagenic substances (Gasiorowski et al. 2000).

Anticancer activity

Anticancer action of products from aronia fruits is closely linked with their strong antioxidant potential (Lala et al. 2006; Thani et al. 2014). The presence of various groups of polyphenols is decisive for cell protection against oxidative stress and apoptosis leading to carcinogenesis. Furthermore, these compounds suppress cell cycle of abnormal cancer cells (Sharif et al. 2012; Zhao et al. 2004).

In vitro studies proved the inhibition of human colon adenocarcinoma cells HT29 and Caco-2 by extracts and juice from chokeberry fruit (Bermúdez-Soto et al. 2007a, b; Jing et al. 2008; Lala et al. 2006; Malik et al. 2003; Saruwatari et al. 2008; Zhao et al. 2004). In addition, acetone extracts from aronia fruits and extracts from its leaves were observed to inhibit the development of leukemia cell line L1210 and HL60 (Skupień et al. 2008; Sueiro et al. 2006).

Safety

Despite a common use of the processed and unprocessed aronia fruits, there are no data on effective and safe dosages. No records are kept on undesired reactions and possible toxicity of the chokeberry fruits and products. It is only known that procyanidins (mostly procyanidin B_1) and anthocyanins (mostly cyanidin-3-arabinoside) are inhibitors of cytochrome P450 3A4 (CYP3A4) which participates in biotransformations of some drugs. Therefore, it is important not to take some drugs with aronia fruits and their products.

4.9 Significance in the Production of Dietary Supplements and in Food Industry

A. melanocarpa is an ingredient of numerous dietary supplements in Europe produced with the addition of dried fruits, powdered fruits, aqueous extracts, juice or juice concentrate. They are manufactured by European, American, Canadian, and even South Korean companies. Products of Polish, German, Swiss, Italian, Ukrainian, and Turkish enterprises dominate in European countries. Table 4.3 presents important data on some chosen chokeberry-based dietary supplements.

According to the opinion of the European Food Safety Authority (EFSA) presented at the Panel on Dietetic Products, Nutrition and Allergies (NDA), *A. melanocarpa* possesses antioxidant properties and is a source of anthocyanins and polyphenols with antioxidant activity. It states that *A. melanocarpa* "helps to protect cells from the free-radical damage and oxidative stress" (EFSA 2011).

In food industry and cuisine, fresh aronia fruits are used to prepare jams, marmalades, jellies, juices, infusions, and wines. On the other hand, chokeberry juice is used to colorating other products as a very intense natural colorant. Aronia fruit juice is a much richer source of colorants than grapes and black currant fruits (Jeppsson 1999; Wawer et al. 2012).

A new interesting proposal is to use not only aronia juice as a source of colorants for food industry, but also aronia juice pomace, the by-product in juice processing. Total anthocyanin levels in the pomace is affected mostly by enzyme treatment followed by maceration temperature (2 °C and 50 °C were tested). Cold maceration of frozen berries without enzyme addition yielded the highest concentrations of pigments in the pomace (Kitrytė et al. 2017).

Currently, also the possibility of black chokeberry pomace uses as a source of food ingredients, not only as a source of colorants, is intensively explored. With the use of commercially available cellulolytic and xylanolytic enzyme preparation, the cell walls in the pomace are broken down resulting in an increase in the yield of water

Table 4.3 Chosen diet supplements based on *A. melanocarpa,* and their description in accordance with the data provided by the manufacturers (according to web sites of producers and online stores)

Manufacturer and country of production	Trade name and form	Composition	Activity profile recommended by manufacturer
Pharmovit, Poland	Aronia Gold (capsules)	Black chokeberry extract (*A. melanocarpa*) standardized for 25% anthocyanins	Antioxidant
Herbapol Poznań, Poland	Aronia żel active 100 g (food gel)	Water extract composed of cistus, lemon verbena and lemongrass, chokeberry juice, gelling agent: xanthan gum; zinc gluconate, stevia extract	Support the body's immunity and vitality

(continued)

Table 4.3 (continued)

Manufacturer and country of production	Trade name and form	Composition	Activity profile recommended by manufacturer
Herbapol Kraków, Poland	Aronia (capsules)	Aronia fruit powder (194 mg/caps.), potassium chloride (130 mg/capsule) (equivalent to 68 mg of potassium)	The preparation contains powdered chokeberry fruit enriched with potassium chloride. Potassium is an element that helps in maintaining proper blood pressure and in the proper functioning of muscles and the proper functioning of the nervous system
Pure nature, Poland	Aronia berry—black chokeberry—Superfood (capsules)	*A. melanocarpa* fruit, microcrystalline cellulose, magnesium stearate	Support for immune system, antioxidant
URSAPHARM Arzneimittel GmbH, Germany	Aronia + Immun (drinking ampoules)	Aronia juice (from 87.3% concentrate), sucrose, aronia juice concentrate (2.7%), acid stabilizers (citric acid), preservatives (sodium benzoate, potassium sorbitate), zinc gluconate, natural flavor, niacin, pantothenic acid, vitamin B_6, vitamin B_2, sodium selenate, vitamin D_3.	Allow to maintain proper, high resistance to diseases and colds
Aronia ORIGINAL, Germany	Aronia Original: Zellschutzkapseln Bio Aronia + Acerola (capsules)	76% powder from pressed, dried aronia berries, 6.4% acerola extract, coating agent (capsule shell): hydroxypropylmethylcellulose, separating agent: talc	Protects cells from oxidative stress, source of vitamin C
Sanoctua GmbH & Co. KG, Germany	BASIS 7 GRÄSLER PLUS (drinking ampoules)	Water, extract from multifloral mountain blossom pollen, bee honey, orange juice concentrate, wheat germ extract, royal jelly, barley malt extract, acidifier citric acid, aronia juice concentrate, mountain ash fruit juice concentrate, blueberry juice concentrate, mixed juice, rose fruit concentrate, concentrated elderberry juice preservative potassium sorbate, vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, niacin NE, pantothenic acid, folic acid, biotin, vitamin C, L-carnitine	Help to restore the physicological balance

(continued)

Table 4.3 (continued)

Manufacturer and country of production	Trade name and form	Composition	Activity profile recommended by manufacturer
Aronia-Swiss, Switzerland	Aronia Kapseln (capsules)	Capsules with aronia pomace (the residue that remains after pressing or juice production)	High concentration of antioxidant ingredients
Vegetal Progress, Italy	Vedyben® (capsules)	Aronia (*A. melanocarpa* L., EU origin) concentrated berry micronized powder, elderberry (*Sambucus nigra* L., EU origin) berry concentrated juice dehydrated micronized powder, blueberry (*Vaccinium myrtillus* L., EU origin) berry concentrated juice dehydrated micronized powder, currant black (*Ribes nigrum* L., EU and/or Asian origin) berry juice concentrate dehydrated micronized powder, aronia (*A. melanocarpa* L., EU origin) berry juice concentrated dehydrated micronized powder, maize maltodextrin, calcium carbonate	High content of polyphenols, anthocyanins, and vitamin C. Valuable aid to support the physiological functionality of the microcirculation and sight
Liktravy, Ukraine	*A. melanocarpa* fructus herbal tea, Ashberry blackheaded fruits (tea)	Ashberry blackheaded fruits	In complex therapy with hypo- and avitaminosis, hemorrhagic diathesis, bleeding of different origins, initial stages of arterial hypertension, thyrotoxicosis, atherosclerosis
L'ACTONE, Turkey	Life Besleyici Set Shake Karışım Thermo Çay Shaker, Aronia thermo (powder)	Powered aronia fruits	Activates the metabolism and supports fat burning
Eclectic Institute, USA	Aronia berry freeze-dried, 450 mg, 90 VegCap, great antioxidant (capsules)	Organic freeze-dried aronia berries	Antioxidant
Eclectic, USA	Eclectic Aronia Cog O, red, 1 fluid ounce (drops)	Organic freeze-dried aronia berry (*A. melanocarpa*), organic grain-free alcohol, filtered water. Dry herb strength 1:4 (250 mg/ml)	Antioxidant

(continued)

Table 4.3 (continued)

Manufacturer and country of production	Trade name and form	Composition	Activity profile recommended by manufacturer
Brownwood Acres Foods, USA	Pure aronia berry juice concentrate (juice)	*A. melanocarpa* fruit juice	Antioxidant
Swanson, USA	Full spectrum aronia (chokeberry)	Black chokeberry fruit (*A. melanocarpa*) 400 mg	Antioxidant
Nutridom, Canada	Nutridom Aronia 2000 300 Vcaps—powdered fruit of *A. melanocarpa* (capsules)	*A. melanocarpa* (Black chokeberry) 500 mg (4:1 concentrated, equivalent to 2000 mg)	Provides antioxidants
Natural One, Canada	Aronia extract 4:1 (capsules)	*A. melanocarpa* 500 mg Extract 4:1 DHE: 2000 mg dry	Provides antioxidants
GNM dignity of Nature, South Korea	GNM pure aronia/aronia juice/aronia extract/fruit juice (powder)	Aronia concentrate mix 100% (aronia, apple, grape, cranberries)	Good at antioxidative effect

soluble fractions (max. 113%), monosaccharide content (max. 140%), total phenolic content (max. 41%), and radical scavenging capacity (max. 39%). Solid residues from the enzyme-treated berry pomace possess also high antioxidant potential. These investigations indicated that all fractions after juice pressing could be utilized as a low-cost source of highly valuable functional food ingredients (Vagiri and Jensen 2017).

4.10 Cosmetic Applications

Constituents of *A. melanocarpa* fruit are decisive for their growing popularity in the manufacture of cosmetics.

In the European Cosmetic Ingredients (CosIng) database developed by the European Commission, as many as five forms of aronia have been authorized for use in cosmetic production, namely fruits extract, juice, fruit and leaf extracts, filtrates of products obtained by fermentation with *Acetobacter* and *Saccharomyces*. It is noteworthy that callus cultures can be used for the production of cosmetics (Table 4.4) (CosIng 2020).

Some cosmetic preparations are based on oil derived from the seeds; they have not yet been listed in the CosIng database. It is a rich source of essential unsaturated fatty acids. This oil is well absorbed through the skin and prevents comedone formation by suppressing the blockade of the sebaceous gland ducts. The oil is used in cosmetics as an emollient, i.e., a product moisturizing and strengthening the lipid barrier of the skin and inhibiting the transepidermal water loss (TEWL). It is also used in

Table 4.4 *A. melanocarpa* in cosmetic products according to CosIng

Form	Activity
Aronia melanocarpa fruit extract	Skin conditioning
Aronia melanocarpa fruit juice	Skin conditioning
Aronia melanocarpa fruit/leaf extract	Skin conditioning
Aronia melanocarpa callus extract	Antioxidant Hair conditioning Skin protecting
Acetobacter/Saccharomyces/(Aronia melanocarpa/Pyrus serotina) fruit juice extract ferment filtrate *(Acetobacter/Saccharomyces/(Aronia melanocarpa/Pyrus serotina)* fruit juice extract ferment filtrate is a filtrate of the product obtained by the fermentation of the extract of the juice obtained from the fruit of *Aronia melanocarpa* and *Pyrus serotina* by the microorganisms *Acetobacter* and *Saccharomyces)*	Antioxidant

medicine because it accelerates wound and burn healing and inhibits degenerative changes (BIOnly 2020).

The characteristics of chokeberry-derived raw materials particularly useful in cosmetics include their antioxidant actions and the ability to strengthen the vascular wall and to reduce erythematous changes. Anthocyanins are decisive for their UV protection property and anti-photoaging activity. Antibacterial actions are used in the treatment of acne lesions. Vitamin C and zinc contribute to strengthening the skin. Aronia preparations also reduce the sebaceous gland activity (BIOnly 2020).

Table 4.5 presents examples of the cosmetic products manufactured in different countries. In Europe, the main manufacturers are based in Poland, Austria, Germany, Switzerland, Italy, and Turkey. In the global market, the products of manufacturers from the USA and Korea also appear and can be bought via the internet.

4.11 Biotechnological Studies

4.11.1 Micropropagation

Micropropagation studies have been carried out by agricultural and horticultural research institutions. *A. melanocarpa* micropropagation protocols were developed, indicating that the standard Murashige and Skoog (MS) medium supplemented with indolebutyric acid (IBA), 6-benzylaminopurine (BAP), and gibberellic acid (GA_3) can create the conditions stimulating the development of microseedling (Brand and Cullina 1992; Murashige and Skoog 1962; Petrovic and Jacimovic-Plavšic 1992; Ruzic 1993).

Table 4.5 Chosen cosmetics based on *A. melanocarpa,* and their description in accordance with the data provided by the manufacturers (according to web sites of producers and online stores)

Manufacturer and country of production	Trade name and form	Used form of *A. melanocarpa*	Activity profile recommended by manufacturer
Produkty Naturalne Berezińscy Sp. Jawna, Poland	Naturalis Beauty Aronia—Łagodzące mleczko do twarzy (face milk)	*A. melanocarpa* anthocyanins	Aronia anthocyanins—natural strong antioxidants, strengthen capillary walls and reduce swelling
AJEDEN Sp. z o.o., Poland	Olej z pestek aronii (*A. melanocarpa* seed oil)	Cold-pressed chokeberry seed oil	For sensitive skin with a tendency to rosacea, vascular, mature, mixed skin. Soothing, healing, anti-inflammatory, and regenerative properties protect the skin from exposure to free radicals and antioxidant properties. Restores the hydrolipidic balance of the skin, protects it from dehydration
Aronialand, Austria	Chokeberry eye balm	*A. melanocarpa* fruit powder	Daily care for the eyes, contains numerous vitamins and minerals
Gerlinde Hofer—Florex GmbH, Austria	Flüssige Schafmilchseife Aronia (liquid soap)	*A. melanocarpa* fruit extract	–
Original Florex®, Austria	Aronia Badesalz (bath salt)	–	–
RAUSCH Ges.mbH, Austria	Aronia anti-grau intensiv-fluid (hair fluid)	–	Promotes melanin production, thus preserving and reactivating the natural hair color
Dr. Eckstein, Germany	Ultimate supreme aronia concentrate	*A. melanocarpa* fruit extract	Reduces and protects the visible signs of age, protects against the visible consequences of stress and negative environmental influences, supports the natural functions of the skin, supports the skin's own regeneration, especially after the sun

(continued)

Table 4.5 (continued)

Manufacturer and country of production	Trade name and form	Used form of *A. melanocarpa*	Activity profile recommended by manufacturer
Bioksama, Switzerland	Extra-rich body cream without palmoil, organic edelweiss, organic chokeberry (body cream)	*A. melanocarpa* fruit extract	–
Alma Briosa, Italy	Filler Riempirughe contorno occhi e labbra (eye and lip contour filler)	Aronia fruit extract	Antioxidant Moisturizer
MOR—Miracle Optimum Result, Turkey	MOR Aronia Özlü El Kremi (hand cream)	*A. melanocarpa* extract	Antioxidant
Farmhouse Fresh®, USA	Vitamin berry facial tonic—Instant pore-refining & replenishing facial toner	*A. melanocarpa* fruit juice	Antioxidant
Bianca Rosa, USA	Black chokeberry cream	*A. melanocarpa* fruit	–
ReinPlatz, South Korea	Hydro aid moisturizing aronia berry essence facial sheet mask	*A. melanocarpa* fruit extract rich in anthocyanins	Soothing and moisturizes antioxidant

4.11.2 Endogenous Production of Phenolic Acids in Various Types of in vitro Cultures

Studies on the biosynthetic potential of *A. melanocarpa* cells in in vitro cultures have been conducted very intensively since 2011 by our team representing the Chair and Department of Pharmaceutical Botany, Jagiellonian University, Medical College, Kraków (Poland). These studies were focused on the optimization of in vitro culture conditions favoring the accumulation of one group of antioxidants, namely phenolic acids. The optimization involved testing the basal media—Linsmaier and Skoog—LS (Linsmaier and Skoog 1965), and Murashige and Skoog—MS (Murashige and Skoog 1962), concentrations of PGRs (NAA and BAP), supplementation of biosynthetic precursors, light conditions (monochromatic lights, white light, darkness and UV-A irradiation), type of culture—agar callus culture, agar, agitated, and bioreactor shoot culture of aronia (Fig. 4.4) (Szopa et al. 2013, 2018a, 2020; Szopa and Ekiert 2014). A separate research direction regarding biotransformation potential of cells cultured in vitro concentrated on β-D-glucosylation of hydroquinone into arbutin (Kwiecień

et al. 2013). The phenolic acids and arbutin contents were estimated by DAD-HPLC methods.

4.11.2.1 The Biosynthetic Potential of Agar Callus Cultures

Testing of LS media variants

Callus cultures (Fig. 4.4) maintained on five different variants of agar LS medium containing NAA and BAP as growth regulators (in the concentration range 0.1–3 mg/l; NAA/BAP [mg/l]: 0.1/0.1, 0.5/1.0, 1.0/1.0, 2.0/2.0, 1.0/3.0) was shown to be capable of producing 5 (of 19 tested) phenolic acids—caffeic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, syringic acid, and vanillic acid. Their contents varied depending on PGR concentrations. On the LS medium variants with 1:1 auxin/cytokinin ratio, the patterns of phenolic acids were very similar, just as on the LS media with 1:2 and 1:3 PGRs ratio, where the pattern of the estimated compounds was also similar. Syringic acid was the dominant compound with maximum amounts of 46.26 and 41.20 mg/100 g DW. p-Hydroxybenzoic acid contents were substantial and ranged from 17.41 to 25.60 mg/100 g DW. The maximum contents of the remaining compounds were as follows: 7.31 mg/100 g DW (caffeic acid), 6.65 mg/100 g DW (vanillic acid), and ca. 12 mg/100 g DW (*p*-coumaric acid). The

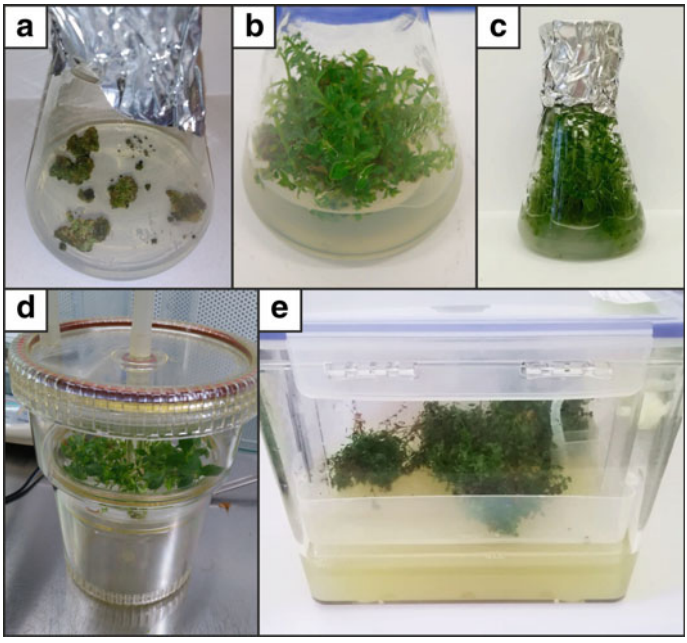


Fig. 4.4 *A. melanocarpa;* agar callus cultures (**a**), agar shoot cultures (**b**), agitated shoot cultures (**c**), and shoot cultures in RITA (**d**) and PlantForm (**e**) bioreactors

Table 4.6 Best media variants stimulating the biomass growth and phenolic acids production in *A. melanocarpa* in vitro cultures

Culture type	Medium	BAP/NAA [mg/l]	Total content of phenolic acids (mg/100 g DW)	Biomass increment
Agar callus culture	LS	1/0.5 and 3/1	78.82 and 81.56	3.5
Agar shoot culture	MS	0.5/2	83.84	5.0
	LS	1/0.5 and 1/1	150.95 and 145.49	15.3 and 10.0
Agitated shoot culture	MS	2/2 and 2/0.5	206.19 and 217.50	17.2 and 15.0
	LS	3/1	130.44	2.6
	MS	2/2	324.78	3.5

total content of the tested compounds was consistently dependent on LS medium variant and ranged from 50.23 to 81.56 mg/100 g DW. Biomass increments in 4-week growth cycles were relatively low in this type of culture (from 2.0- to 4.8-fold) (Szopa et al. 2013).

Two LS medium variants, containing 0.5 mg/l NAA + 1 mg/l BAP and 1 mg/l NAA + 3 mg/l BAP were selected as universal both "productive" and "growth-promoting" media (total phenolic acids was of about 80 mg/100 g DW and biomass increments were over 3.5-fold) (Table 4.6) (Szopa et al. 2013).

Testing of MS media variants

Callus cultures carried out on seven different variants of agar MS medium containing NAA and BAP in the concentration range 0.1–3 mg/l, BAP/NAA [mg/l]: 0.1/2.0, 0.5/2.0, 1.0/0.5, 2.0/0.5, 2.0/1.0, 2.0/2.0, 3.0/1.0 were demonstrated to be capable to producing the same five phenolic acids as in the case of LS media. Their contents were very diverse, differing by 1.8–4.0 times depending on PGRs concentration. On MS media with 2:1, 3:1 and 4:1 cytokinin/auxin ratio, the pattern of phenolic acids was similar. On MS media with 1:4 and 1:20 cytokinin/auxin ratio, analogous metabolite patterns were also obtained.

Syringic acid (max. 40.16 mg/100 g DW) and *p*-hydroxybenzoic acid (max. 23.59 mg/100 g DW) were the dominant metabolites. The contents of the remaining three phenolic acids did not exceed 11 mg/100 g DW. The total content of phenolic acids varied 1.78-fold from about 47 to 84 mg/100 g DW depending on the PGR concentration. Biomass increments during a 4-week growth cycle were relatively low and diverse (3.3–5.0-fold) (Szopa and Ekiert 2014).

The MS medium variant containing 0.5 mg/l BAP and 2 mg/l NAA was proposed as a "universal" medium (total content of phenolic acids was over 80 mg/100 g DW and the increase in biomass was one of the highest) (Table 4.6) (Szopa and Ekiert 2014).

In the callus cultures of aronia, grown on LS and MS tested media variants, predominant biosynthetic pathways were those of the benzoic acid derivatives. Syringic acid (dimethoxy-derivative of benzoic acid) and *p*-hydroxybenzoic acid were the metabolites accumulated in the greatest quantities (Szopa and Ekiert 2014).

The main compounds in the fruit extracts included salicylic acid (15.6 mg/100 g DW) and *p*-hydroxybenzoic acid—15.29 mg/100 g DW. Other four—caffeic, *p*-coumaric, syringic, and vanillic acids were present in quantities below 4.2 mg/100 g DW. The maximum total amounts of phenolic acids in callus cultures on the tested LS and MS medium variants were 2.51-times and 2.59-times higher than in the fruit extracts (32.43 mg/100 g DW), respectively (Szopa and Ekiert 2014).

The total amounts of phenolic acids in callus cultures cultivated on two LS and MS media variants with the same PGRs were almost the same. Only on one variant, namely that containing 3 mg/l BAP and 1 mg/l NAA, the total content was 1.22-times higher on MS basal medium than on LS basal medium (Szopa and Ekiert 2014).

4.11.2.2 The Biosynthetic Potential of Agar Shoot Cultures

Testing of LS medium variants

The production of phenolic acids was investigated in *A. melanocarpa* shoot cultures (Fig. 4.4) maintained on five agar variants of LS medium enriched in PGRs—NAA and BAP (in the concentration range 0.1–3.0 mg/l). The tested LS medium variants were identical as in the case of agar callus cultures. HPLC analysis was used to analyze 19 phenolic acids and cinnamic acid.

Methanolic extracts of biomass harvested after 4-week growth cycles were shown to contain five compounds: caffeic acid, *p*-hydroxybenzoic acid, syringic acid, vanillic acid, and salicylic acid. Salicylic acid (max. 78.25 mg/100 g DW) and *p*-hydroxybenzoic acid (max. 55.14 mg/100 g DW) were the main metabolites accumulated in the shoots (Szopa et al. 2013, 2018a).

The total content of the tested compounds ranged from 105.05 to 150.96 mg/100 g DW and was evidently dependent on the concentrations of PGRs in the LS media (Szopa et al. 2013, 2018a).

Biomass increments on the tested LS medium variants during 4-week growth cycles were very good (from 8.2- to 15.3-fold) (Szopa et al. 2013, 2018a).

Two LS medium variants were proposed as "universal" media, namely that containing 0.5 mg/l NAA and 1 mg/l BAP, and the second one supplemented with 1 mg/l NAA and 1 mg/l BAP (Table 4.6) (Szopa et al. 2013, 2018a).

Testing of MS medium variants

Shoot cultures of *A. melanocarpa* carried out on seven variants of MS medium containing NAA and BAP (0.1–3.0 mg/l), identical as in the case of agar callus cultures were demonstrated to produce 6 of 20 tested compounds. They included caffeic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, syringic acid, vanillic acid, and salicylic acid. Salicylic acid (max. 91.86 mg/100 g DW), *p*-coumaric acid (max.

62.39 mg/100 g DW), and *p*-hydroxybenzoic acid (max. 50.66 mg/100 g DW) were the quantitatively dominant metabolites. The amounts of individual compounds and consequently the total amounts of phenolic acids depended on the PGR concentrations in MS medium variants. The total amounts reached from 93.52 to 217.50 mg/100 g DW (Szopa et al. 2018a; Szopa and Ekiert 2014).

Increments of shoot biomass grown on the tested MS medium variants during 4-week growth cycles were very high (from 11.4- to 17.2-fold). Two variants were proposed as "universal" media—these supplemented with 2 mg/l NAA + 2 mg/l BAP and 2 mg/l NAA + 0.5 mg/l BAP (Table 4.6) (Szopa et al. 2018a; Szopa and Ekiert 2014).

The maximum total amounts of phenolic acids accumulated on the tested LS and MS medium variants were ca. 4.65-times and 6.69-times higher than in the fruit extracts (32.43 mg/100 g DW), respectively (Szopa et al. 2018a; Szopa and Ekiert 2014).

Three variants of LS and MS medium contain identical concentrations of PGRs. The obtained comparative results show that 1.6–2.0-times higher total amounts of phenolic acids can be obtained on MS medium variants. MS medium variants richer in various vitamins—co-enzymes of different enzymes can stimulate different reactions in the shoots grown in vitro (Szopa et al. 2018a; Szopa and Ekiert 2014).

The biosynthetic potential under different light conditions

Stationary shoot cultures were maintained on the MS medium variant with 1 mg/l NAA and 1 mg/l BAP in the presence of monochromatic light (far-red, red, blue lights, UV-A irradiation) in darkness and under multispectral white light during 4-week cycles (Szopa et al. 2018a, b).

In biomass extracts, the presence of four compounds (out of 20 analyzed) was confirmed, namely three depsides—chlorogenic, neochlorogenic and rosmarinic acids, and also protocatechuic acid. The total amounts of phenolic acids changed 3.2-fold, from 167.14 mg/100 g DW (UV-A irradiation) to 527.40 mg/100 g DW (blue light). Under control conditions (white light), the total content reached 339.45 mg/100 g DW while in the darkness it amounted to 289.64 mg/100 g DW. The total contents under red and far-red lights were similar—293.27 and 256.00 mg/100 g DW, respectively (Szopa et al. 2018a, b).

During 4-week growth cycles, biomass increased from 3.78-fold (darkness) to 8.06-fold (blue light), and in the control culture (white light)—4.77-fold (Szopa et al. 2018a, b).

The experiment evidenced the stimulating effect of blue light both on biomass growth and accumulation of phenolic acids. Under blue light, the total content of phenolic acids was 1.58-fold higher than under white light. The maximum content was ca. 16.3-fold higher than in the fruit extract (32.43 mg/100 g DW) (Szopa et al. 2018a, b).

4.11.2.3 The Biosynthetic Potential of Agitated Shoot Cultures

Testing of LS medium variants

Agitated cultures (Fig. 4.4) were maintained on three variants of LS medium (NAA/BAP [mg/l]: 2/0.5, 2/2, 1/3) during 4-week growth cycles (Szopa et al. 2015, 2018a).

Shoot extracts were shown to contain four phenolic acids—three depsides (rosmarinic, neochlorogenic, and chlorogenic acids) and also protocatechuic acid. Rosmarinic acid (max. 53.40 mg/100 g DW) was the main metabolite. The maximum amounts of two other depsides were also high (35.18 and 41.58 mg/100 g DW, respectively). The total amounts on three tested LS variants varied from 67.22 to 130.44 mg/100 g DW. The maximum amount was 4.02-times higher than the content in the fruit extract (32.43 mg/100 g DW) (Szopa et al. 2015, 2018a). In analyzed plant media, trace amounts of phenolic acids were confirmed.

The biomass increments were low and ranged from 2.33 to 2.55-fold. The LS variant containing 1 mg/l NAA and 3 mg/l BAP was proposed as "universal" medium, stimulating the growth and production of phenolic acids (Table 4.6) (Szopa et al. 2015, 2018a).

Testing of MS medium variants

Agitated cultures were maintained on three variants of MS medium supplemented with the identical concentrations of PGRs as those added of LS medium for agitated shoot cultures. The tested shoot extracts were evidenced to contain four compounds, the same as those accumulated on LS medium variants, namely three depsides and protocatechuic acid. Depsides including rosmarinic acid, chlorogenic acid, and neochlorogenic acid were accumulated in high amounts (max. 134.24, 105.45, and 82.00 mg/100 g DW, respectively) (Szopa et al. 2015, 2018a).

The total contents of phenolic acids synthesized on the tested MS medium variants ranged from 189.02 to 324.78 mg/100 g DW depending on PGR concentrations and were decidedly higher than those obtained on LS medium variants. The maximum amount was 10.1-times higher than in the fruit extract (Szopa et al. 2015, 2018a). In analyzed plant media, only trace amounts of phenolic acids were confirmed.

The biomass increments were relatively low but slightly higher than on identical LS medium variants and varied between 2.61- and 3.52-fold. The MS medium variant containing 2 mg/l NAA and 2 mg/l BAP was proposed as a "universal" medium (Table 4.6) (Szopa et al. 2015, 2018a).

Agitated cultures maintained with the addition of biosynthetic precursors

Agitated cultures of *A. melanocarpa* were maintained on MS medium with the addition of 1 mg/l NAA and 1 mg/l BAP for 20 days. Biosynthetic precursors of phenolic acids—phenylalanine, cinnamic acid, and benzoic acid at five concentrations 0.1, 0.5, 1.0, 5.0, and 10.0 mmol/l were added to culture flasks at the time "0" (at culture initiation) and on day 10th of culture. Moreover, the caffeic acid at the above-mentioned

five concentrations was used as a precursor in order to stimulate the production of depsides (Szopa et al. 2018a, 2020).

Out of the 26 tested phenolic acids (the analysis was extended by seven compounds, for which standards were available for purchase) assayed in the extracts from biomass harvested after 20-day growth cycles, seven compounds were identified—five depsides: neochlorogenic, chlorogenic, cryptochlorogenic, isochlorogenic, and rosmarinic acids, as well as caffeic and syringic acids (Szopa et al. 2018a, 2020).

In control cultures maintained without precursors, the total content of phenolic acids amounted to 290.10 mg/100 g DW. Precursor supplementation distinctly stimulated biosynthesis and accumulation of the tested compounds. The maximum total contents of phenolic acids after the addition of each of the precursors estimated at time "0" were as follows: 481.06 mg/100 g DW (phenylalanine—5 mmol/l), 543.35 mg/100 g DW (cinnamic acid—0.5 mmol/l), 439.43 mg/100 g DW (benzoic acid—1 mmol/l), and 660.63 mg/100 g DW (caffeic acid—1 mmol/l). The maximum total contents of phenolic acids after precursor administration assessed on the 10th day of culture were higher and reached: 592.27 mg/100 g DW (phenylalanine—0.1 mmol/l), 989.79 mg/100 g DW (cinnamic acid—5 mmol/l), 503.02 mg/100 g DW (benzoic acid—1 mmol/l), and 854.99 mg/100 g DW (caffeic acid—5 mmol/l), respectively (Table 4.7) (Szopa et al. 2018a, 2020). In all extracts from biomass growing with precursors, three depsides were the main metabolites, namely neochlorogenic acid (max. 127.00–163.97 mg/100 g DW), chlorogenic acid (max. 119.08–450.35 mg/100 g DW), and isochlorogenic acid (max. 168.23–249.88 mg/100 g DW) (Szopa et al. 2018a, 2020).

Cinnamic acid and caffeic acid supplementation at a concentration of 5 mmol/l on the 10th day of culture was the most efficient in stimulating the biosynthesis and accumulation of phenolic acids. Precursor supplementation raised phenolic acid contents by 3.41- and 2.95-fold, respectively, compared with precursor-free cultures. The obtained results have a potentially applicable nature (Szopa et al. 2018a, 2020).

In general, the addition of precursors did not suppress biomass growth. Only higher concentrations of cinnamic acid and benzoic acid (5 and 10 mmol/l) and

Table 4.7 Maximal total contents [mg/100 g DW] (MTC), precursor concentration [mmol/l] (PC) and increase versus control[a] (IC) of phenolic acids produced in the agitated shoot cultures of *A. melanocarpa* after feeding with the tested precursors

Precursor	Point "0"			10th day		
	MTC	PC	IC	MTC	PC	IC
Phenylalanine	481.06	5	1.66	592.27	0.1	2.04
Cinnamic acid	543.35	0.5	1.87	989.79	5	3.41
Benzoic acid	439.43	1	1.51	503.02	1	1.73
Caffeic acid	660.63	1	2.28	854.99	5	2.95

[a]Control culture—290.10 mg/100 g DW

the highest concentration of phenylalanine (10 mmol/l) added at time "0" reduced biomass increments (Szopa et al. 2018a, 2020).

Dynamics of accumulation of phenolic acids in agitated cultures—preliminary results

Agitated *A. melanocarpa* shoot cultures were maintained on MS medium enriched in 1 mg/l NAA and 1 mg/l BAP for 8 weeks (3 series). Biomass was harvested at 7-day intervals for determination of phenolic acid contents. Out of 26 assayed compounds, methanolic extracts were confirmed to contain 11 metabolites: 3-phenylacetic acid, 3,4-dihydroxyphenylacetic acid, syringic acid, caftaric acid, protocatechuic acid, caffeic acid and depsides—chlorogenic, cryptochlorogenic, neochlorogenic, isochlorogenic acids and rosmarinic acid (Kubica et al. 2019b).

The total content of phenolic acids, after an initial decrease (2nd week) gradually rose through 1128.25 mg/100 g DW (third week of the growth cycle) to reach the maximum value of 1237.62 mg/100 g DW in the 5th week. Beginning from the sixth week, the contents of the compounds under study drastically dropped to 710.84 mg/100 g DW in the sixth week, 383.02 mg/100 g DW in the 7th week and 75.15 mg/100 g DW in the eighth week of culture (Fig. 4.5) (Kubica et al. 2019b).

The dominant compounds quantified in the biomass included: 3-phenylacetic acid (max. 424.52 mg/100 g DW—third week), 3,4-dihydroxyphenylacetic acid (317.76 mg/100 g DW—fifth week), isochlorogenic acid (380.01 mg/100 g DW—fourth week), and cryptochlorogenic acid (228.73 mg/100 g DW—fourth week) (Kubica

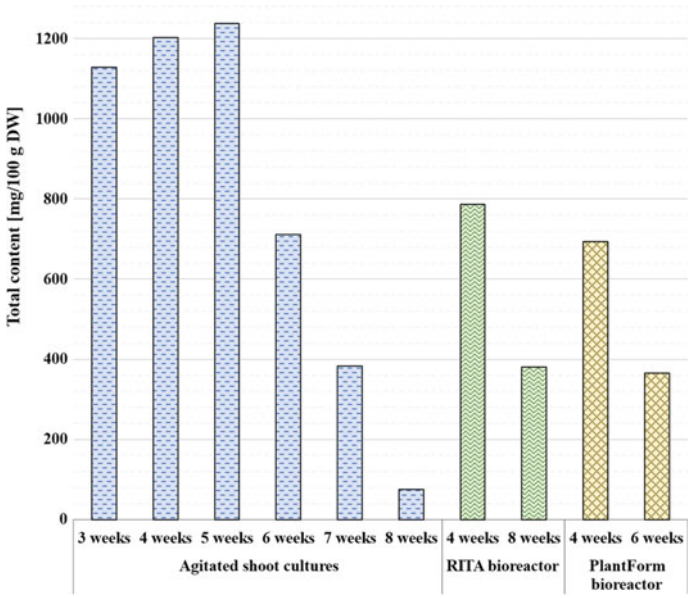


Fig. 4.5 Dynamic of phenolic acid accumulation in *A. melanocarpa* agitated and bioreactor in vitro cultures during the tested growth cycles

et al. 2019b). In analyzed plant media only trace amounts of phenolic acids were confirmed.

Dry shoot biomass increments (determined also at 7-day intervals) gradually increased by 4.27-fold (second week) and 6.59-fold (third week) to reach the maximum value of 9.08-fold (4th week), 8.31 (fifth week) and then steadily decreased to 6.84 (sixth week) and 5.44 (eighth week) (Kubica et al. 2019b).

The highest contents of phenolic acids were obtained during the stationary phase of the culture growth.

The obtained results are potentially applicable. They indicate that the maximum contents of the studied compounds are accumulated after 5-week culture growth cycles. However, reproducibility of these data is needed to be checked (Kubica et al. 2019b).

4.11.2.4 Shoot Cultures in Bioreactors—Preliminary Results

A. melanocarpa shoot cultures were carried out in two types of commercially available bioreactors—RITA (VITROPIC S.A., France) and PlantForm (Plant Form AB, Sweden) (Fig. 4.4), operating as temporary immersion systems, on MS medium containing 1 mg/l NAA and 1 mg/l BAP for 8 weeks (RITA) or 6 weeks (PlantForm). Biomass was harvested at 2 time points: after 4 weeks from both bioreactors, and after 8 weeks and 6 weeks, respectively (Kubica et al. 2019a, 2020).

HPLC analysis of methanolic extracts from the biomass growing in both types of bioreactors confirmed the presence of 11 metabolites of 26 tested compounds. They were the same compounds as those identified in the agitated cultures maintained with the aim to investigate the dynamics of phenolic acid accumulation (Kubica et al. 2019a, 2020).

In the RITA bioreactors, the total content of phenolic acids in extracts from the biomass growing for 4 weeks (786.88 mg/100 g DW) was over twice as high as their amount after 8-week growth cycles (380.66 mg/100 g DW) (Fig. 4.5) (Kubica et al. 2019a, 2020).

Three compounds were identified as the dominant metabolites, namely: isochlorogenic acid (max. 236.16 mg/100 g DW), cryptochlorogenic acid (max. 153.96 mg/100 g DW), and 3,4-dihydroxyphenylacetic acid (max. 151.80 mg/100 g DW). The maximum contents of the mentioned compounds were obtained after 4-week growth cycles (Kubica et al. 2019a, 2020). In analyzed plant media, no significant amounts of phenolic acids were confirmed. Dry biomass increments obtained during 4-week culture cycles were high (7.6-fold). The increments after 8-week cycle were lower but satisfactory (4.5-fold) (Kubica et al. 2019a, 2020).

These preliminary results indicate that it will be necessary to conduct quantitative analyses at other time points, in particular after 5-, 6-, and 7-week culture cycles (Kubica et al. 2019a, 2020). It would be also recommended to examine reproducibility of the data obtained so far. These studies are planned to be performed in the near term.

In the PlantForm bioreactor, the total content of phenolic acids in the extracts from biomass after 4-week culture cycles was almost double (693.29 mg/100 g DW) the content obtained after 6 weeks of culture (365.11 mg/100 g DW) (Fig. 4.5) (Kubica et al. 2019a, 2020).

The main compounds accumulated in shoots included: 3-phenylacetic acid (max. 374.87 mg/100 g DW), isochlorogenic acid (max. 77.84 mg/100 g DW), and dihydroxyphenylacetic acid (max. 58.64 mg/100 g DW). These maximum contents of the above-mentioned phenolic acids were documented after 4-week culture cycles. With regard to dry shoot biomass increments, the biomass increased over 5-fold (5.2-fold) after 4-week culture cycles; hence, the result was satisfactory. Extension of the culture cycle till 6 weeks resulted in a distinct reduction of biomass increments (3.3-fold biomass growth) (Kubica et al. 2019a, 2020).

To sum up, it appears that also in this type of bioreactor cultures, quantitative analyses will need to be performed after 5 weeks and reproducibility of study results should be checked, as well (Kubica et al. 2019a, 2020).

4.11.3 Biotransformation Potential of Cells in Agitated Shoot Cultures

A. melanocarpa agitated shoot cultures were investigated for the ability to transform exogenous hydroquinone into its β-D-glucoside, arbutin (Fig. 4.6). Arbutin has an important position both in medicine (as a urinary tract disinfectant) and in cosmetology (as a safe skin-lightening agent). In vitro cultures of numerous plant species representing very different taxa are able to produce arbutin via biotransformation by β-D-glucosylation of exogenous hydroquinone (Fig. 4.6). It is due to a common occurrence of enzymes belonging to β-glucosidases in the plant kingdom and the lack of substrate specificity of these enzymes.

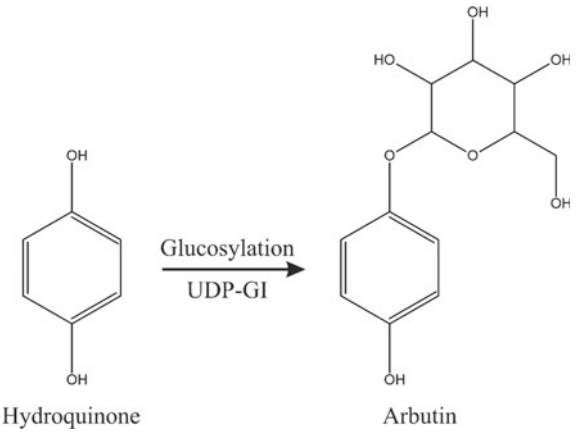


Fig. 4.6 Biotransformation of hydroquinone into arbutin

Cultures were maintained on MS medium supplemented with 2 mg/l BAP and 2 mg/l NAA. After a 2-week culture growth period, hydroquinone was added to culture flasks at different doses (100–400 mg/l) either as a single dose or divided into 2 or 3 doses given at 24-hour intervals. The contents of reaction products were determined by HPLC in methanolic extracts from biomass and in lyophilized media harvested at 24 h after the last precursor dose (Kwiecień et al. 2013).

The cells from in vitro cultures exhibited the ability to transform hydroquinone into arbutin. The product was accumulated mainly in the cultured biomass (72.09–93.42%) and was released to the medium at lower quantities. The total contents of the product were very diverse varying from 2.71 to 8.27 g%. Arbutin production gradually rose with increasing concentration of hydroquinone. The maximum contents of arbutin were obtained at the maximum concentration of hydroquinone (400 mg/l) and amounted to 7.94 g% (single dose), 8.27 g% (2 doses), and 7.76 g% (3 doses). The analyzed media contained arbutin in amounts ranging from 6.58% (150 mg, 3 doses) to 30.04% (400 mg, 2 doses) of the total content of this compound in biomass and medium (17.24 and 150.32 mg/l, respectively). The yield of the biotransformation process was very divergent ranging from 37.04% (400 mg/l, single dose) to 73.80% (100 mg/l, 3 doses). The identity of the product, i.e., arbutin was confirmed by spectral analysis (^{1}H-NMR spectrum) (Kwiecień et al. 2013).

The obtained maximum content of arbutin (8.27 g%) was higher than its minimum content in classical arbutin-containing raw material *Uvae ursi folium* (7.0 g%) required by the newest edition of the European Pharmacopoeia and higher than its minimal content in *Vitis idaeae folium* (4.0 g%), acc. to national monograph in the newest Polish Pharmacopoeia. This result is potentially applicable in practice (Kwiecień et al. 2013).

4.11.4 Evaluation of Our Biotechnological Investigations

- Stationary agar callus cultures of *A. melanocarpa* accumulate comparable amounts of phenolic acids on the tested variants of LS and MS medium. Culture on the MS media yielded slightly greater callus biomass increments (Table 4.8).
- Stationary agar and agitated shoot cultures accumulate larger amounts of phenolic acids growing on the MS medium variants compared with the LS medium variants. Shoot biomass increments are also higher on the MS medium variants (Table 4.8).
- The contents of PGRs—NAA (auxin) and BAP (cytokinin) and auxin/cytokinin ratio in the tested variants of LS and MS medium conspicuously influenced phenolic acid accumulation and biomass increments both in agar callus cultures and agar shoot and agitated shoot cultures.
- Both in agar callus and shoot cultures, cell metabolism was oriented toward the production of benzoic acid and/or cinnamic acid derivatives while the production of depsides prevailed in agitated shoot cultures (Table 4.9).
- Supplementation with biosynthetic precursors of phenolic acids (phenylalanine, cinnamic acid, and benzoic acid) and depside precursor (caffeic acid) of the

agitated shoot cultures evidently stimulated phenolic acid accumulation. The addition of cinnamic acid and/or caffeic acid was the most beneficial (Table 4.10).

- Among light conditions tested in stationary agar shoot cultures (monochromatic lights, UV-A irradiation, darkness, and multispectral white light), it was the blue light that clearly stimulated the accumulation of phenolic acids and biomass growth (Table 4.10).
- Callus biomass increments were relatively low. In contrast, shoot biomass increments in stationary agar culture were exceptionally high but in agitated cultures, they were low. The tested types of shoot culture, namely agar and agitated cultures, accumulated high amounts of phenolic acids. Particularly high metabolite contents were documented in agitated shoot cultures on the MS media. Callus cultures have proven to be a much less productive source of phenolic acids compared with shoot cultures (Table 4.10).
- The studies of the dynamics of phenolic acid accumulation in agitated shoot cultures over a period of 8 weeks demonstrated the maximum accumulation of phenolic acids after 5-week culture growth cycles.

Table 4.8 Minimal and maximal total contents [mg/100 g DW] of phenolic acids estimated after testing of basal media and PGRs combinations in agar callus, agar shoot, and agitated shoot cultures of *A. melanocarpa*

Type of culture	Medium	Total content of phenolic acids	
		Minimal	Maximal
Agar callus culture	LS	50.23	81.56
	MS	47.00	84.00
Agar shoot culture	LS	105.05	150.95
	MS	93.52	217.00
Agitated shoot culture	LS	67.22	130.44
	MS	189.02	324.78

Table 4.9 Maximal contents [mg/100 g DW] of individual phenolic acids in different types of studied *A. melanocarpa* in vitro cultures

Phenolic acid	Agar callus culture		Agar shoot culture		Agitated shoot culture	
	LS	MS	LS	MS	LS	MS
p-Coumaric acid	12.15	10.93	-[a]	62.39	–	–
p-Hydroxybenzoic acid	25.60	23.59	55.14	50.66	–	–
Syringic acid	46.26	40.16	15.82	28.72	–	–
Vanillic acid	6.65	7.47	16.37	14.36	–	–
Salicylic acid	–	–	78.25	91.86	–	–
Rosmarinic acid	–	–	–	–	53.40	134.24
Neochlorogenic acid	–	–	–	–	35.18	82.00
Chlorogenic acid	–	–	–	–	41.58	105.45

[a]"-"—not confirmed

Table 4.10 Maximal total contents of phenolic acids [mg/100 g DW] in the tested types of *A. melanocarpa* in vitro cultures

Type of culture	Medium/conditions	Total content
Agar callus culture	LS	81.56
	MS	84.00
Agar shoot culture	LS	150.95
	MS	217.00
Agar shoot culture	Blue light	527.40
Agitated shoot culture	LS	130.44
	MS	324.78
Agitated shoot culture with precursor addition	MS medium supplemented with 5 mmol/l of cinnamic acid on 10th day	989.79
Bioreactors (preliminary results)	RITA bioreactor	787.88
	PlantForm bioreactor	693.29

A. melanocarpa fruits—32.43 mg/100 g DW

- *A. melanocarpa* bioreactor shoot cultures produced satisfactory amounts of phenolic acids and biomass increments. Decidedly greater amounts of phenolic acids were obtained after 4-week growth cycles compared with 8-week or 6-week cycles (in RITA and PlantForm bioreactors, respectively). The main metabolites in this culture type, besides depsides, were identified as phenylacetic acid derivatives. Slightly greater amounts of phenolic acids were obtained in the RITA bioreactors (Table 4.10).
- In all tested types of cultures, the total content of phenolic acids was greater than in fruit extracts—the pharmaceutical raw material (Table 4.10).
- At the present stage of research, agitated or agar shoot cultures of *A. melanocarpa* on the MS media can be proposed as a potential rich source of phenolic acids, including depsides (Table 4.10).
- Apart from the ability of cells from agitated shoot cultures to endogenously accumulate phenolic acids, they are also capable of exogenous hydroquinone biotransformation into its β-D-glucoside, arbutin. The obtained amounts of the product are interesting from a practical point of view.
- The types of *A. melanocarpa* in vitro cultures maintained in the course of these studies are very good model cultures for further biotechnological studies.

4.12 Summary and Prospects

A. melanocarpa, a species native to North America, was introduced to Northwestern Asia and Europe at the end of the eighteenth century and became naturalized in some European (Central and South Europe and Scandinavia) and Asian (Russia) countries. The fruits of this species are exceptionally rich in a wide variety of chemical

subgroups of antioxidants, vitamins and bioelements, hence in recent years they have been increasingly used for the production of dietary supplements and has important position in the food and cosmetics industries. This raw material has been documented by scientific research to exhibit invaluable directions of biological activity, including the prevention of civilization diseases. Tremendous interest in this raw material in Central, South European countries, including Poland and in Scandinavia, has contributed to its cultivation as a commercial crop on an increasingly wide scale in Europe. Recently, this species has been grown in ecological farming systems with great success.

Considering a very wide range of possible therapeutic applications, the raw material, *Aroniae fructus,* should be included into the list of pharmacopoeial plant raw materials in the European Pharmacopoeia so that is can be used in official medicine in European Union countries.

Phytochemical and pharmacological studies are paralleled by professional biotechnological investigations aimed at developing micropropagation protocols and examining the biosynthetic potential of in vitro cultures of this species. The results of the studies on the biosynthetic potential, so far focused on the endogenous accumulation of phenolic acids—one of the subgroups of antioxidants—clearly indicate that shoot cultures of this species can be a potential rich source of these metabolites and an alternative to plants growing in the open air. The biosynthetic potential of cells from in vitro cultures can be utilized for biotransformation of exogenous hydroquinone into its β-D-glucoside, arbutin, which is an important compound of high medicinal and cosmetic value. However, only the full phytochemical analysis of the biomass to estimate other subgroups (flavonoids, anthocyanins) of antioxidants will provide a full picture of the biosynthetic capabilities of the cells cultured in vitro.

Among a wide range of biotechnological approaches, the development of micropropagation protocols is of crucial significance. They can ensure the supply of high-producing genotypically identical plants. The plants from in vitro cultures could be used for establishing professional open-field plantations.

Considering the well-known natural phenomenon of chemical variability, still high environmental pollution and the rapid rate of climate change, the proposals of biotechnological solutions, most of all to use the biomass grown in large-scale bioreactor installations as a source of antioxidants, seems to be a very significant and future-oriented approach.

Currently the cosmetics industry makes use of the biosynthetic potential of the callus tissue cells of *A. melanocarpa* for the manufacture of cosmetics. Therefore, it can be expected that in the near future in vitro cultures of this species will also be used for the production of pharmaceuticals and food ingredients, as an alternative to field-grown crops.

Acknowledgements Funding This study was supported by the financial support of the funds of the Ministry of Science and Higher Education Programs: K/DSC/003506, N42/DBS/000010, N42/DBS/000011.

Author Contribution Statement All the authors read and approved the manuscript in its final form. All authors declare that they have no conflict of interest.

References

Andrzejewska J, Sadowska K, Klóska Ł, Rogowski L (2015) The effect of plant age and harvest time on the content of chosen components and antioxidative potential of black chokeberry fruit. Acta Sci Pol Hortorum Cultus 14(4):105–114

Augustyniak A, Bartosz G, Čipak A, Duburs G, Horáková U, Łuczaj W et al (2010) Natural and synthetic antioxidants: an updated overview. Free Radical Res 44(10):1216–62 https://www.bib.irb.hr/487622

Badescu M, Badulescu O, Badescu L, Ciocoiu M (2015) Effects of *Sambucus nigra* and *Aronia melanocarpa* extracts on immune system disorders within diabetes mellitus. Pharm Biol Informa Healthc 53(4):533–539

Bell DR, Gochenaur K (2006) Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. J Appl Physiol 100(4):1164–1170

Benvenuti S, Pellati F, Melegari M, Bertelli D (2006) Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of rubus, ribes, and aronia. J Food Sci 69(3):164–169

Bermúdez-Soto MJ, Larrosa M, García-Cantalejo JM et al (2007a) Transcriptional changes in human Caco-2 colon cancer cells following exposure to a recurrent non-toxic dose of polyphenol-rich chokeberry juice. In: Genes & nutrition. Springer, pp 111–113

Bermúdez-Soto MJ, Larrosa M, Garcia-Cantalejo JM et al (2007b) Up-regulation of tumor suppressor carcinoembryonic antigen-related cell adhesion molecule 1 in human colon cancer Caco-2 cells following repetitive exposure to dietary levels of a polyphenol-rich chokeberry juice. J Nutr Biochem 18(4):259–271

Bijak M, Saluk J, Antosik A, Ponczek MB, Zbikowska HM, Borowiecka M et al (2013) *Aronia melanocarpa* as a protector against nitration of fibrinogen. Int J Biol Macromol 55:264–268

BIOnly (2020) Aronia czarnoowocowa (*Aronia melanocarpa*) [Internet]. Kosmet. Nat. Org. bio. [cited 2020 Dec 27]. Available from: https://www.bionly.pl/pl/articles/details/72/porady

Borowska S, Brzóska MM (2016) Chokeberries (*Aronia melanocarpa*) and their products as a possible means for the prevention and treatment of noncommunicable diseases and unfavorable health effects due to exposure to xenobiotics. Compr Rev Food Sci Food Saf 15(6):982–1017

Brand MH (2010) Aronia: native shrubs with untapped potential. Aroldia 67:14–25

Brand MH, Cullina WG (1992) Micropropagation of red and black chokeberry (*Aronia spp.*). Hort Sci 27(1):81–81

Brewer MS (2011) Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Compr Rev Food Sci Food Saf 10(4):221–247

Broncel M, Kozirog M, Duchnowicz P, Koter-Michalak M, Sikora J, Chojnowska-Jezierska J (2010) *Aronia melanocarpa* extract reduces blood pressure, serum endothelin, lipid, and oxidative stress marker levels in patients with metabolic syndrome. Med Sci Monit Inter Sci Info Inc 16(1):CR28–CR34

Brzóska MM, Rogalska J, Galazyn-Sidorczuk M, Jurczuk M, Roszczenko A, Tomczyk M (2015) Protective effect of *Aronia melanocarpa* polyphenols against cadmium-induced disorders in bone metabolism: A study in a rat model of lifetime human exposure to this heavy metal. Chem Biol Interact 229:132–146

Cai Y, Luo Q, Sun M, Corke H (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 74(17):2157–2184

Celka Z, Szkudlarz P (2010) Spontaneous occurrence and dispersion of *Aronia x prunifolia* (Marshall) Rehder (Rosaceae) in Poland on the example of the "Bagna" bog complex near Chlebowo (Western Poland). Acta Soc Bot Pol 79(1):37–42

Chrubasik C, Li G, Chrubasik S (2010) The clinical effectiveness of chokeberry: a systematic review. Phytother Res 24(8):1107–11014

CosIng (2020) CosIng—cosmetic ingredients database [Internet]. European Communications [cited 2 Dec 2020]. Available from: http://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.results

Denev PN, Kratchanov CG, Ciz M, Lojek A, Kratchanova MG (2012) Bioavailability and antioxidant activity of black chokeberry (*Aronia melanocarpa*) polyphenols: in vitro and in vivo evidences and possible mechanisms of action: a review. Compr Rev Food Sci Food Saf 11(5):471–489

Denev P, Číž M, Kratchanova M, Blazheva D (2019) Black chokeberry (*Aronia melanocarpa*) polyphenols reveal different antioxidant, antimicrobial and neutrophil-modulating activities. Food Chem 30(284):108–117

EFSA (2011) Scientific opinion part I on the substantiation of health claims related to various food(s)/food constituent(s) not supported by pertinent human data (ID 411, 559, 1174, 1184, 1197, 1380, 1409, 1656, 1667, 1670, 1763, 1767, 1806, 1884, 1908, 1997, 2141 2). EFSA J 9(6)

Gasiorowski KB, Brokos B, Kozubek A, Oszmianski J (2000) The antimutagenic activity of two plant—derived compounds. A comparative cytogenetic study. Cell Mol Biol Lett 2(05)

Gramza-Michałowska A, Sidor A, Kulczyński B (2017) Berries as a potential anti-influenza factor—a review. J Funct Foods 37:116–137

Gruszczyk M, Kołodziej B, Król B, Sugier D, Wiśniewski J, Nurzyńska-Widerek R et al (2018) Uprawa ziół. Poradnik dla plantatorów. wydanie II. Warszawa: Powszechne Wydawnictwo Rolnicze i Leśne

Heleno SA, Martins A, Queiroz MJRP, Ferreira ICFR (2015) Bioactivity of phenolic acids: metabolites versus parent compounds: a review. Food Chem 173:501–513

Jakobek L (2007) Antioxidant activity and polyphenols of aronia in comparison to other berry species. Agric Conspec Sci 72(4):301–306

Jakobek L, Drenjančević M, Jukić V, Šeruga M (2012) Phenolic acids, flavonols, anthocyanins and antiradical activity of "Nero", "Viking", "Galicianka" and wild chokeberries. Sci Hortic 147:56–63

Jan Niedworok FB (2001) The investigation of a biological and phytotherapeutical properties of the *Aronia melanocarpa* E. anthocyanins. Postępy Fitoter 1:20–24

Jeppsson NJ (1999) Evaluation of black chokeberry, *Aronia melanocarpa*, germplasm for production of natural food colourants. Acta Hortic Int Soc Hort Sci 484:193–198

Jing P, Bomser JA, Schwartz SJ, He J, Magnuson BA, Giusti MM (2008) Structure-function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth. J Agric Food Chem 56(20):9391–9398

Jodynis-Liebert J, Adamska T, Ewertowska M et al (2014) Effects of long-term administration of freeze-dried chokeberry juice to rats. J Pharm Nutr Sci 4(2):154–161

Jurgoński A, Juśkiewicz J, Zduńczyk Z (2008) Ingestion of black chokeberry fruit extract leads to intestinal and systemic changes in a rat model of prediabetes and hyperlipidemia. Plant Foods Hum Nutr 63(4):176–182

Kakkar S, Bais S (2014) A review on protocatechuic acid and its pharmacological potential. 2014, Article ID 952943, 9 pages http://dx.doi.org/10.1155/2014/952943

Kattappagari K, Ravi Teja C, Kommalapati R et al (2015) Role of antioxidants in facilitating the body functions: a review. J Orofac Sci Medknow Publications 7(2):75

Kim B, Ku CS, Pham TX, Park Y, Martin DA, Xie L et al (2013) *Aronia melanocarpa* (chokeberry) polyphenol-rich extract improves antioxidant function and reduces total plasma cholesterol in apolipoprotein E knockout mice. Nutr Res 33(5):406–413

King ES, Bolling BW (2020) View of composition, polyphenol bioavailability, and health benefits of aronia berry: a review. J Food Bioact 11:13–30

Kitrytė V, Kraujalienė V, Šulniūtė V, Pukalskas A, Venskutonis PR (2017) Chokeberry pomace valorization into food ingredients by enzyme-assisted extraction: process optimization and product characterization. Food Bioprod Process 105:36–50

Kleparski J, Domino Z (1990) Aronia. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa

Kokotkiewicz A, Jaremicz Z, Luczkiewicz M (2010) Aronia plants: a review of traditional use, biological activities, and perspectives for modern medicine. J Med Food 13(2):255–269

Krishnaiah D, Sarbatly R, Nithyanandam R (2011) A review of the antioxidant potential of medicinal plant species. Food Bioprod Process 89(3):217–233

Kubica P, Pałka A, Szopa A, Ekiert H (2019a) Production of phenolic acids in shoot cultures of black aronia (*Aronia melanocarpa*) cultivated in RITA bioreactors. In: 67th International Congress Annual Meeting of the Society for Medicinal Plant and Natural Product Research, Innsbruck

Kubica P, Szopa A, Żywko J, Pałka A, Ekiert H (2019b) Agitated and bioreactor's cultures of aronia species—investigations on accumulation dynamics of bioactive phenolic acids during the growth cycles. In: XI Conference "In vitro cultures in biotechnology plant physiology". 4–6 December 2019, Kraków (Poland)

Kubica P, Pałka A, Szopa A, Ekiert H (2020) Accumulation of phenolic acids in in vitro cultures of black aronia (*Aronia melanocarpa*) cultivated in PlantForm bioreactors. 3rd ICPMS Martin, Kraków, Szeged

Kulling SE, Rawel HM (2008) Chokeberry (*Aronia melanocarpa*)—a review on the characteristic components and potential health effects. Planta Med 74(13):1625–1634

Kwiecień I, Szopa A, Madej K, Ekiert H (2013) Arbutin production via biotransformation of hydroquinone in in vitro cultures of Aronia melanocarpa (Michx.) Elliott. Acta Biochim Pol 60(4):865–870

Lala G, Malik M, Zhao CW, He J, Kwon Y, Giusti MM et al (2006) Anthocyanin-rich extracts inhibit multiple biomarkers of colon cancer in rats. Nutr Cancer Int J 54(1):84–93

Linsmaier EM, Skoog F (1965) Organic growth factor requirements of tobacco tissue cultures. Physiol Plant 18(1):100–127

Litwinczuk W (2002) Propagation of black chokeberry (*Aronia melanocarpa* Elliot) through in vitro culture. Electron J Polish Agric Univ Ser Hortic 5(2)

Malik M, Zhao C, Schoene N, Guisti MM, Moyer MP, Magnuson BA (2003) Anthocyanin-rich extract from *Aronia meloncarpa* E. induces a cell cycle block in colon cancer but not normal colonic cells. Nutr Cancer 46(2):186–196

Meng S, Cao J, Feng Q, Peng J, Hu Y (2013) Roles of chlorogenic acid on regulating glucose and lipids metabolism: a review. Evidence-based Complement Altern Med Hindawi Pub Corp 801457

Michalak P (2015) Aronia planter's guide, 1st edn. Dąbie

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15(3):473–479

Naruszewicz M, Łaniewska I, Millo B, Dłuzniewski M (2007) Combination therapy of statin with flavonoids rich extract from chokeberry fruits enhanced reduction in cardiovascular risk markers in patients after myocardial infraction (MI). Atherosclerosis 194(2):179–184

Niedworok J, Gwardys A, Jankowski A, Kowalczyk E, Oszmański J, Skośkiewicz J (1999) Badania nad protekcyjnym wpływem żelu antocyjaninowego na fototoksyczne działanie promieni UV. Ochr Środ Zas Nat 18:83–87

Oszmianski J, Wojdylo A, Oszmiański J, Wojdylo A (2005) Aronia melanocarpa phenolics and their antioxidant activity. Eur Food Res Technol 221(6):809–813

Park H, Liu Y, Kim HS, Shin JH (2016) Chokeberry attenuates the expression of genes related to *de novo* lipogenesis in the hepatocytes of mice with nonalcoholic fatty liver disease. Nutr Res 36(1):57–64

Pérez-Jiménez J, Neveu V, Vos F, Scalbert A (2010) Identification of the 100 richest dietary sources of polyphenols: an application of the phenol-explorer database. Eur J Clin Nutr 64(3):112–120

Petrovic DM, Jacimovic-Plavšic MM (1992) *Aronia melancarpa* and propagation in vitro. Acta Hortic 300:133–136

Piotrowska-Kempisty H, Nowicki M, Jodynis-Liebert J et al (2020) Assessment of hepatoprotective effect of chokeberry juice in rats treated chronically with carbon tetrachloride. Mol MDPI AG 25(6):1268

Pool-Zobel BL, Bub A, Schröder N, Rechkemmer G (1999) Anthocyanins are potent antioxidants in model systems but do not reduce endogenous oxidative DNA damage in human colon cells. Eur J Nutr 38(5):227–234

Pratheeshkumar P, Son YO, Wang X, Divya SP et al (2014) Cyanidin-3-glucoside inhibits UVB-induced oxidative damage and inflammation by regulating MAP kinase and NF-κB signaling pathways in SKH-1 hairless mice skin. Toxicol Appl Pharmacol 280(1):127–137

Razungles A, Oszmański J, Sapis J-C (1989) Determination of Carotenoids in Fruits of Rosa sp. (*Rosa canina* and *Rosa rugosa*) and of Chokeberry (*Aronia melanocarpa*). J Food Sci 54(3):774–775

Rop O, Mlcek J, Jurikova T, Valsikova M et al (2010) Phenolic content, antioxidant capacity, radical oxygen species scavenging and lipid peroxidation inhibiting activities of extracts of five black chokeberry (*Aronia melanocarpa* (Michx.) Elliot) cultivars. J Med Plants Res 4(22):2431–2437

Rugină D, Sconţa Z, Leopold L, Pintea A, Bunea A, Socaciu C (2012) Antioxidant activities of chokeberry extracts and the cytotoxic action of their anthocyanin fraction on HeLa human cervical tumor cells. J Med Food 15(8):700–706

Rumińska A (1984) Poradnik plantatora ziół. Państwowe Wydawnictwo Rolnicze i Leśne—oddział w Poznaniu, Poznań

Ruzic D (1993) In vitro rooting and subsequent growth of black chokeberry (*Aronia melanocarpa*) plants *ex vitro*. J Fruit Ornam Plant Res. Research Institute of Pomology and Floriculture 1:1–8

Ryszawa N, Kawczyńska-Drózdz A, Pryjma J et al (2006) Effects of novel plant antioxidants on platelet superoxide production and aggregation in atherosclerosis. J Physiol Pharmacol 57(4):611–626

Saruwatari A, Isshiki M, Tamura H (2008) Inhibitory effects of various beverages on the sulfoconjugation of 17β-estradiol in human colon carcinoma caco-2 cells. Biol Pharm Bull Pharmaceu Soc Japan 31(11):2131–2136

Senderski ME (2004) Prawie wszystko o ziołach. Wydanie pi. Mateusz E. Senderski, Podkowa Leśna

Sharif T, Alhosin M, Auger C, Minker C et al (2012) *Aronia melanocarpa* juice induces a redox-sensitive p73-related caspase 3-dependent apoptosis in human leukemia cells. PLoS One 7(3)

Sidor A, Gramza-Michałowska A (2019) Qualitative composition, phenolic profile and antioxidant potential. Molecules 24(20):3710. https://doi.org/10.3390/molecules24203710

Sidor A, Drożdżyńska A, Gramza-Michałowska A (2019) Black chokeberry (*Aronia melanocarpa*) and its products as potential health-promoting factors—an overview. Trends Food Sci Technol 89:45–60

Sikora J, Markowicz M, Mikiciuk-Olasik E (2009) Rola i właściwości lecznicze aronii czarnoowocowej w profilaktyce chorób cywilizacyjnych. Bromat Chem Toksykol XLII(1):10–17

Skupień K, Kostrzewa-Nowak D, Oszmiański J, Tarasiuk J (2008) In vitro antileukaemic activity of extracts from chokeberry (*Aronia melanocarpa* (Michx) Elliott) and mulberry (*Morus alba* L.) leaves against sensitive and multidrug resistant HL60 cells. Phyther Res 22(5):689–694

Slimestad R, Torskangerpoll K, Nateland HS, Johannessen T, Giske NH (2005) Flavonoids from black chokeberries, *Aronia melanocarpa*. J Food Compos Anal 18(1):61–68

Stralsjoe L, Ahlin H, Witthoeft CM, Jastrebova J (2003) Folate determination in Swedish berries by radioprotein-binding assay (RPBA) and high performance liquid chromatography (HPLC). Eur Food Res Technol 216(3):264–269

Sueiro L, Yousef GG, Seigler D, De Mejia EG, Grace MH, Lila MA (2006) Chemopreventive potential of flavonoid extracts from plantation-bred and wild *Aronia melanocarpa* (black chokeberry) fruits. J Food Sci 71(8):C480–C488

Szopa A, Ekiert H (2014) Production of biologically active phenolic acids in *Aronia melanocarpa* (Michx.) Elliott in vitro cultures cultivated on different variants of the Murashige and Skoog medium. Plant Growth Regul 72(1):51–58

Szopa A, Ekiert H, Muszyńska B (2013) Accumulation of hydroxybenzoic acids and other biologically active phenolic acids in shoot and callus cultures of *Aronia melanocarpa* (Michx.) Elliott (black chokeberry). Plant Cell Tiss Org Cult 113(2):323–329

Szopa A, Setkiewicz A, Ekiert H (2015) *Aronia melanocarpa* (black chokeberry) in vitro cultures—potential source of bioactive phenolic acids for phytotherapy. In: Conference. Bonn

Szopa A, Kokotkiewicz A, Kubica P, Banaszczak P et al (2017a) Comparative analysis of different groups of phenolic compounds in fruit and leaf extracts of Aronia sp.: *A. melanocarpa, A. arbutifolia and A. x prunifolia*, and their antioxidant activities. Eur Food Res Technol 243(9):1645–1657. https://doi.org/10.1007/s00217-017-2872-8

Szopa A, Kubica P, Ekiert H (2017b) Ecology, chemical composition, health-promoting effects and biotechnological studies on black chokeberry (*Aronia melanocarpa*), red chokeberry (*Aronia arbutifolia*) and purple chokeberry (*Aronia* × *prunifolia*). Postępy Fitoter 18(2):145–157

Szopa A, Kubica P, Ekiert H (2018a) High production of depsides and other phenolic acids in different types of shoot cultures of three aronias: *Aronia melanocarpa, A. arbutifolia and A.* × *prunifolia.* In: Ramawat KG, Ekiert MH, Goyal S (eds) Plant cell tissue differentiation and secondary metabolites. Springer, Berlin Heidelberg, pp 309–336

Szopa A, Starzec A, Ekiert H (2018b) The importance of monochromatic lights in the production of phenolic acids and flavonoids in shoot cultures of *Aronia melanocarpa, Aronia arbutifolia* and *Aronia* × *prunifolia.* J Photochem Photobiol B Biol 179:91–97

Szopa A, Kubica P, Komsta Ł, Walkowicz-Bożek A, Ekiert H (2020) The effect of feeding culture media with biogenetic precursors on high production of depsides in agitated shoot cultures of black and red aronias. Plant Cell Tiss Org Cult 142:379–399

Taheri R, Connolly BA, Brand MH, Bolling BW (2013) Underutilized chokeberry (*Aronia melanocarpa, Aronia arbutifolia, Aronia prunifolia*) accessions are rich sources of anthocyanins, flavonoids, hydroxycinnamic acids, and proanthocyanidins. J Agric Food Chem 61(36):8581–8588

Tanaka T, Tanaka A (2001) Chemical components and characteristics of black chokeberry. J Jpn Soc Food Sci Technol 48:606–610

Thani NAA, Keshavarz S, Lwaleed BA, Cooper AJ, Rooprai HK (2014) Cytotoxicity of gemcitabine enhanced by polyphenolics from *Aronia melanocarpa* in pancreatic cancer cell line AsPC-1. J Clin Pathol 67(11):949–954

Tian Y, Liimatainen J, Alanne AL, Lindstedt A, Liu P, Sinkkonen J et al (2017) Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. Food Chem 220:266–281: http://dx.doi.org/10.1016/j.foodchem.2016.09.145

Tolić MT, Jurčević IL, Krbavčić IP, Marković K, Vahčić N (2015) Phenolic content, antioxidant capacity and quality of chokeberry (*Aronia melanocarpa*) products. Food Technol Biotechnol 53(2):171–179

Tolić MT, Krbavčić IP, Vujević P, Milinović B, Jurčević IL, Vahčić N(2017) Effects of weather conditions on phenolic content and antioxidant capacity in juice of chokeberries (*Aronia melanocarpa* L.). Polish J Food Nutr Sci 67(1):67–74

University of Maine (2020) Plant description and habitat of aronia (black chokeberry) Orono, Maine. https://extension.umaine.edu/agriculture/aronia/plant-description-and-habitat/

Vagiri M, Jensen M (2017) Influence of juice processing factors on quality of black chokeberry pomace as a future resource for colour extraction. Food Chem 217:409–417

Valcheva-Kuzmanova S, Belcheva A (2006) Current knowledge of *Aronia melanocarpa* as a medicinal plant. Folia Med (Plovdiv) 48(2):11–17

Valcheva-Kuzmanova S, Borisova P, Galunska B, Krasnaliev I, Belcheva A (2004) Hepatoprotective effect of the natural fruit juice from *Aronia melanocarpa* on carbon tetrachloride-induced acute liver damage in rats. Exp Toxicol Pathol 56(3):195–201

Valcheva-Kuzmanova S, Marazova K, Krasnaliev I, Galunska B, Borisova P, Belcheva A (2005) Effect of *Aronia melanocarpa* fruit juice on indomethacin-induced gastric mucosal damage and oxidative stress in rats. Exp Toxicol Pathol 56(6):385–392

Valcheva-Kuzmanova S, Kuzmanov K, Tancheva S, Belcheva A (2007) Hypoglycemic and hypolipidemic effects of *Aronia melanocarpa* fruit juice in streptozotocin-induced diabetic rats. Methods Find Exp Clin Pharmacol 29(2):101–105

Vázquez-Espinosa M, V. González-de-Peredo A, Espada-Bellido E et al (2019) Ultrasound-assisted extraction of two types of antioxidant compounds (TPC and TA) from black chokeberry (*Aronia*

melanocarpa L.): optimization of the individual and simultaneous extraction methods. Agronomy. MDPI AG 9(8):456
Veberic R, Slatnar A, Bizjak J, Stampar F, Mikulic-Petkovsek M (2015) Anthocyanin composition of different wild and cultivated berry species. LWT 60(1):509–517
Vlachojannis C, Zimmermann BF, Chrubasik-Hausmann S (2015) Quantification of anthocyanins in elderberry and chokeberry dietary supplements. Phyther Res 29(4):561–565
Walther E, Schnell S (2009) Black chokeberry (*Aronia melanocarpa*)-a special fruit crop. Zeitschrift für Arznei- & Gewürzpflanzen 14(4):179–182
Wang H, Cao G, Prior RL (1996) Total antioxidant capacity of fruits. J Agric Food Chem 44:701–705
Wangensteen H, Bräunlich M, Nikolic V et al (2014) Anthocyanins, proanthocyanidins and total phenolics in four cultivars of aronia: antioxidant and enzyme inhibitory effects. J Funct Foods 7(1):746–752
Wawer I (2006) Aronia Polski Paradoks. Agropharm, Warszawa
Wawer I, Eggert P, Hołub B (2012) Aronia super owoc. Wektor, Warszawa
Willcox JK, Ash SL, Catignani GL (2004) Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr 44(4):275–295
Wolski T, Kalisz O, Prasał M (2007) Black chokeberry—*Aronia melanocarpa* (Michx.) Elliott—the rich source of antioxidants. Postępy Fitoter 3:145–154
Wu X, Gu L, Prior RL, McKay S (2004) Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. J Agric Food Chem 52(26):7846–7856
Yamane T, Kozuka M, Wada-Yoneta M, Sakamoto T et al (2017) Aronia juice suppresses the elevation of postprandial blood glucose levels in adult healthy Japanese. Clin Nutr Exp 12:20–26
Zhang H, Tsao R (2016) Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Curr Opin Food Sci 8:33–42
Zhao C, Giusti MM, Malik M, Moyer MP, Magnuson BA (2004) Effects of commercial anthocyanin-rich extracts on colonic cancer and nontumorigenic colonic cell growth. J Agric Food Chem 52(20):6122–6128
Zheng W, Wang SY (2003) Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. J Agric Food Chem 51(2):502–509

Chapter 5
Cultivation and Utilization of *Valeriana jatamansi* Jones for Conservation Planning and Management

Arun Kumar Jugran, Indra D. Bhatt, and Ranbeer S. Rawal

Abstract *Valeriana jatamansi* is a medicinal herb generally known as Indian Valerian belongs to family Caprifoliaceae. This is an herbal plant blended with many medicinal properties such as stimulant, carminative, antispasmodic, cytotoxic and aromatic property. The herb is used in custom and advanced medicine system for curing various ailments and in flavor and perfume industries. In this study, brief phytochemical, traditional and pharmacological studies of species and prospects of the species in future are envisaged. This study also evaluated domestication and cultivation practices available on *V. jatamansi*. Six major groups of active constituents, namely valepotriates (145), lignans compound (18), flavones or its glycosides (18), sesquiterpenoids or its glycoside (12), bakkenolide-type sesquiterpenoids (6), phenolic constituents (6), miscellaneous compounds (12) and major oil constituents (294) have been listed from *V. jatamansi*. Several therapeutic properties, e.g., neurotoxic, cytotoxic, sedative, anti-inflammation, antidepressant, antidiarrheal, anti-HCV, adaptogenic, analgesic, antioxidant and antimicrobial effects and key compounds responsible for these properties have been described. The present study clearly indicated that this plant comprises huge potential for future research and drug development. Numerous active constituents discussed in the present study can be vital for further in vivo and in vitro experimentation. The study suggested the need to promote research on identification of active component from the species and their mechanism of action. Methods available for cultivation and domestication of *V. jatamansi* will be applicable for multiplication of this valuable species to meet out domestic and industrial demand. Findings from the study will be useful for conservation planning and sustainable uses of this valuable species.

Keywords *Valeriana jatamansi* · Ethnopharmacology · Phytochemistry · Active constituents · Domestication · Cultivation

A. K. Jugran (✉)
G.B. Pant, National Institute of Himalayan Environment, Garhwal Regional Centre, Srinagar, Uttarakhand 246174, India
e-mail: arunjugran@gbpihed.nic.in

I. D. Bhatt · R. S. Rawal
Center for Biodiversity Conservation and Management, G.B. Pant National Institute of Himalayan Environment, Kosi-Katarmal, Almora, Uttarakhand 263643, India

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_5

Abbreviations

2,4-D	2, 4-dichlorophenoxyacetic acid
Azoto	Azotobacter
BA	Benzyl adenine
DW	Dry weight
IHR	Indian Himalayan Region
FYM	Farm yard manure
GABA	Gamma Aminobutyric Acid
IAA	Indole-acetic acid
IBA	Indole-3-butyric acid
MAPs	Medicinal and Aromatic Plant
NAA	α-naphthalene acetic acid
PSB	Phosphate Solubilizing Bacteria
VAM	Vesicular-Arbuscular Mycorrhiza

5.1 Introduction

Valeriana jatamansi (Indian Valerian or Tagar) is an herbaceous plant of family Caprifoliaceae used for treatment of several ailments. Various active constituents like valepotriates (iridoids) are the major active component of this herb which comprises several properties like anticancer, anti-inflammatory, hepatoprotective, anticoagulant, antioxidative, antibacterial, antifungal, antiprotozoal and neuroprotective (Dinda et al. 2009; Jugran et al. 2019). The uses of *V. jatamansi* are mentioned in Charaka Samhita, Rigveda, and modern medicine system. The most of the demand for the species is generally fulfilled from wild by harvesting its natural populations which has resulted into a huge pressure on the natural habitats/populations. Therefore, there is the need of use species sustainably for its conservation in present as well as in future. Cultivation of medicinal plants is a vital procedure applied to conserve the medicinal plants under endangered status. The cultivation of MAPs is helpful to meet out ever-increasing demand for material of a particular species without disturbing natural environment (IUCN 1993). Medicinal plants including *V. jatamansi* are harvested from wild, and only a few reports are available on the cultivation of this species. The studies have been attempted to cultivate *V. jatamansi* plants growing in wild conditions to semi-natural agroforestry system. However, proper agrotechniques required knowledge on ecology, adaptation and pollination, conditions on *V. jatamansi* before the cultivation and raising nursery. The species is mostly collected from its natural sources in wild which has imposed a huge pressure on their natural habitats. Therefore, domestication of medicinal plant for conservation is a viable option, but quality of a species depends mainly on the amount of effective constituents present in a species. Hence, determination of phytochemicals for selecting elite genotypes/individuals/populations is immediately needed.

Studies demonstrated that quality and consistency of the secondary metabolites in wild and field grown plants of a species are varied (Bhatt et al. 2012). Similarly, habitat and altitude also impact the quality of the active principals and genetic diversity of this valuable species (Jugran et al. 2013a, 2015a, 2016a, 2018). This finally impacts the market and economic returns of a valuable species. Hence, identification of elites and development of appropriate, cultivation packages will be highly beneficial for prioritization and planning conservation strategies of such species especially for successful reestablishment in wild. Once, elite plants/populations are identified conventional and modern tools can be attempted for large-scale multiplication of high yielding individual/population to obtain quality material for planting. However, in vitro methods of propagation using shoot tip and axillary bud explants of *V. jatamansi* have been employed to develop and raised plantlet which then effectively shifted to the field plots for adaptation (Mathur et al. 1988; Mathur and Ahuja 1991). Likewise, Purohit et al. (2015) developed methods for proliferation of *V. jatamansi* by explant collected from leaf. Moreover, further improvement in these protocols is required. Keeping this background in mind, the present study is attempted.

5.2 Geographic Distribution

V. jatamansi is a high-value perennial medicinal plant widely distributed in India, Burma, Bhutan, China, Nepal and Afghanistan (Polunin and Stainton 1987; Jugran et al. 2013a). The species grow in varied habitat condition and demonstrated high adaptability toward diverse environmental conditions (Rather et al. 2011). Studies from Uttarakhand, a Western Himalayan state, reported that the species grows between 1000 and 3000 m asl (Jugran et al. 2013a, b, 2015a, b, c, 2016a). Another study revealed that this plant is distributed naturally Western Himalaya particularly under canopy of *Pinus roxburghii, Cedrus deodara, Quercus leucotrichophora* and grassy habitat and mixed forests habitat (Pande and Shukla 1993; Jugran et al. 2013a). *V. jatamansi* preferentially grow under sloppy, wet and humid places, ditches, soggy woods and alongside the streams. However, in the Eastern Himalaya *V. jatamansi* is observed to associate with *Castonopsis indica, Ficus nemoralis, Rhododendron arboreum* and *Ficus cordata,* ranging from 1290 to 2000 m (Mukherjee and Chakraborty 2014). Flowering and fruiting period for the species is March–June. Sexual (through seeds) and asexual (through rhizome) means of reproduction approaches are reported for this species. Pollination in *V. jatamansi* is affected by insects belonging to order Diptera and Hymenoptera (Khajuria et al. 2011; Jugran et al. 2018). *V. jatamansi* seeds are observed to be very small in size with feathery follicle and produced in large number. Further, stylar movement is a vital phenomenon in *V. jatamansi* which is helpful to maintain the survival and growth of the plant in harsh climatic condition (Khajuria et al. 2011). As no or little cultivation practices for the species are available, it is collected from wild at large scale which has resulted into overutilization and loss of habitats (Samant et al. 1998). However, cultivation practices for *V. jatamansi* species are started in Uttarakhand at the farmer

level in few village clusters (Phondani et al. 2016) but it was not attempted at larger scale till date. Moreover, above the altitude of 1800 m this species demonstrated low adaptability (Mukherjee 2015). Comparative analysis of these finding revealed an additional adaptability of the plants growing in Western Himalayan region then the eastern region. The reason of differences in adaptation can be associated with the variations in climatic situations.

5.3 Brief Phytochemistry of *V. jatamansi*

Numerous compounds obtained from *V. jatamansi* are belonging to family of phenolics, flavonoids, iridoids, bakenolloids, etc. as the major compounds. The dominant compound categories were valepotriates, linarines, bakkenollide and essential oils. Recently, Jugran et al. (2019) reported that 511 compounds were present in this species, of which 298 were oil components. The dominant constituents of *V. jatamansi* are linarin-isovalerianate (Thies 1968), valepotriates (Becker and Chavadeoi 1985), sesquiterpenoids (Ron et al. 2000), dihydrovaltrate (Bounthanh et al. 1981), hesperidin and 6-methylapigenin, etc. (Marder et al. 2003). Valepotriates are the major chemical constituents among them employed for several medicines. These are a group of monoterpenoids containing iridoid-type compound containing an epoxy group and β-acetoxy isovaleric acids. Iridoid compound extracted from Caprifoliaceae members is also known as valeriana-epoxy triesters, generally abbreviated as valepotriates for convenience (Thies and Funke 1966). Thies (1968) isolated the valeopotriates from *V. jatamansi* and named them as valtrate, acevaltrate and didrovaltrate. New acylated iridoids, jatamanvaltrates A-M were extracted from *V. jatamansi* recently (Lin et al. 2009). Xu et al. (2011a) isolated two bakkenollide-type sesquiterpenoids from this species. Likewise, valeriandoids A–C along with three known analogs were extracted from the *V jatamansi* roots (Xu et al. 2011b). Active components like valerianine, valerenic acid, valeranone, isovalerenic acid, 1-camphene, ar-cucumene, xanthorrizol, alpha-santalene, 1-pinene, terpineol, bornylisovalerinate, alkaloids, chatinine formate glucoside, etc. are also detected in rhizomes and roots oil of the species (Arora and Arora 1963; Nadkarni 1976; Bos et al. 1996; Rawat et al. 2017; Bhatt et al. 2012). Maaliol, citric acid, succinic acid, tartaric acid, and malic acid are also occurred in the rhizome of the species (Kapoor 1990). Valtrate, didrovaltrate, maaliol, 8-acetoxy patchouli alcohol and patchouli alcohol have also been recorded from *V. jatamansi* (Keochanthala-Bounthanh et al. 1993; Mathela et al. 2005). Besides, sesquiterpene hydrocarbons (ar-curcumene, α- and β-patchouolenes, β-fornesene and sesquifenchene) valerenone, cryptomeridiol, patchouli alcohol, etc. are determined (Houghton 1999).Valerenic acid was also estimated in the aerial and root parts of *V. jatamansi* (Singh et al. 2006; Jugran et al. 2016a) and considered as a marker compound which possess sedative and spasmolytic activity. An alkaloid valeranine is also derived from this species. The structures of few principle chemical

components obtained from the species are presented (Fig. 5.1). Likewise, details of major chemical methods used to detect dominant constituents from *V. jatamansi* are provided (Table 5.1).

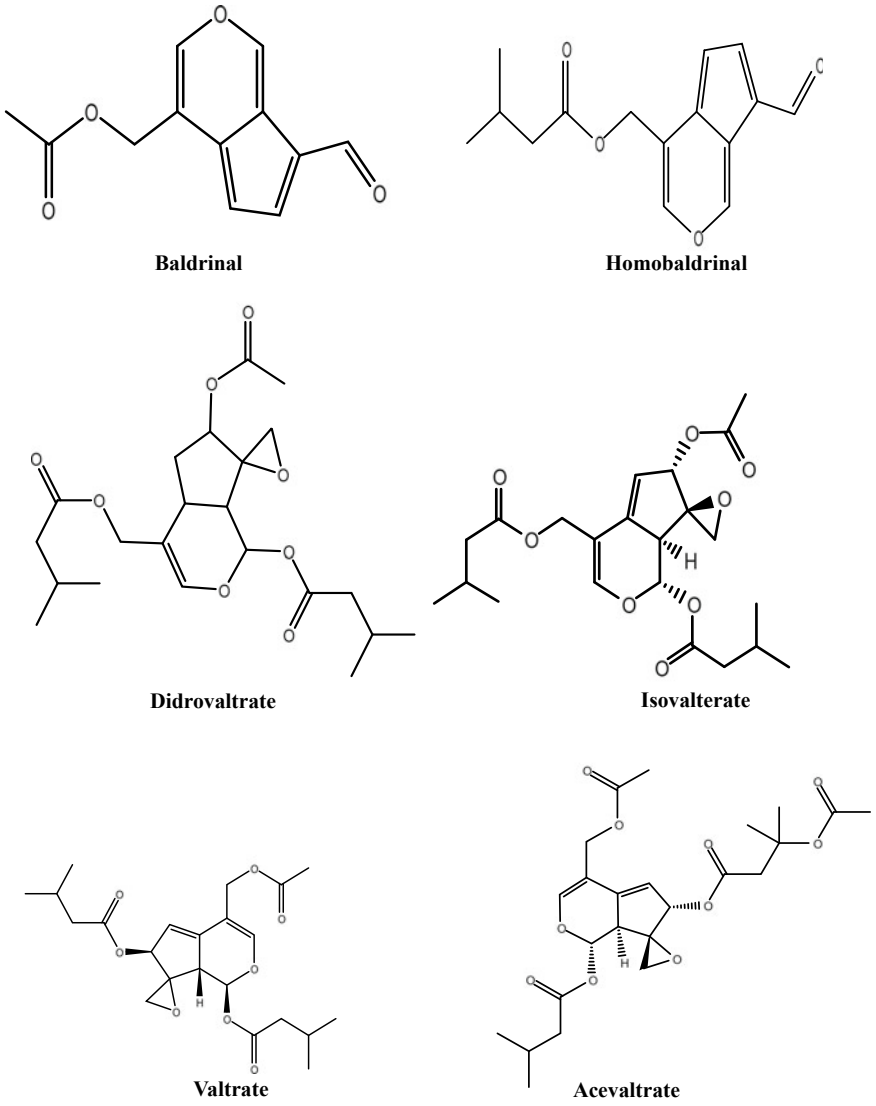
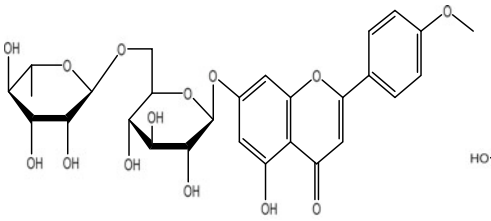
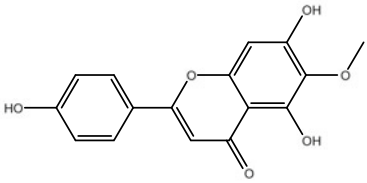


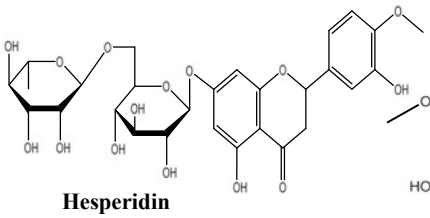
Fig. 5.1 Major active constituents reported from *V. jatamansi*



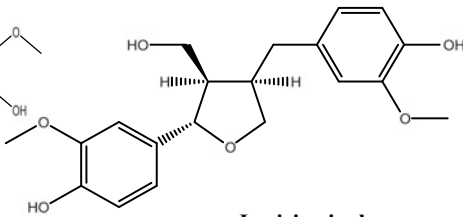
Linarin



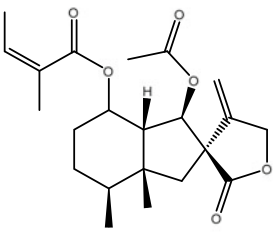
6- methylapigenin/Hispidulin



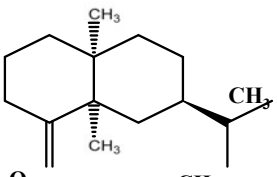
Hesperidin



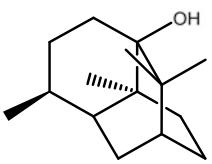
Lariciresinol



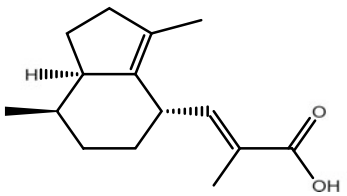
Bakkenolide B



Valeranone



Patchouli alcohol



Valerenic acid

Fig. 5.1 (continued)

Table 5.1 Chemical analysis of *Valeriana jatamansi*

S. No.	Method	Type	Part	Marker	Solvent	Mobile phase	Sample preparation	References
1	HPLC-UV	a	Roots	Hesperidin	Dimethyl sulfoxide	Solution A; Water: O-phosphoric acid (99.7: 0.3) and Solution B; acetonitrile: methanol (75: 25)	Powdered root sample (100 g) was saturated in water (500 mL). Supernatants were pooled, filtered and consequently centrifuged. The collected supernatant was lyophilized, dried and stored at 5 °C for the further evaluation	Katoch et al. (2012)
		b	Aerial and root portions	Gallic acid, catechin, hydroxyl benzoic acid, caffeic acid, chlorogenic acid and coumaric acid	Methanol	Water: methanol: acetic acid	Dried powder (1.0 g) was mixed with 25 mL methanol, sonicated and centrifuged. Supernatants were collected, filtered and stored at 4 °C for analyses within 24 h	Bhatt et al. (2012), Jugran et al. (2015d)

(continued)

Table 5.1 (continued)

S. No.	Method	Type	Part	Marker	Solvent	Mobile phase	Sample preparation	References
		c	Root and rhizome	Homoisovaltrate, 1-acevaltrate, isovaleroxyhydroxy didrovaltrate, didrovaltrate	Dichloromethane	Acetonitrile: water (80:20)	The dried plant material was subjected to dichloromethane extraction. The extract was concentrated on rotary evaporator to yield a brown dry mass	Sah et al. (2011)

(continued)

Table 5.1 (continued)

S. No.	Method	Type	Part	Marker	Solvent	Mobile phase	Sample preparation	References
2	HPLC-PDA	a	Roots and rhizome	Chlorogenic acid, *massoniresinol*-4′-*O*-β-D-glucoside, berchemol-4′-*O*-β-D-glucoside, pinoresinol-4,4′-di-*O*-β-D-glucoside, 8-*hydroxypinoresinol*-4′-*O*-β-D-glucoside, pinoresinol- 4-*O*-βD-glucoside, hesperidin, linarin, hydroxyvalerenic acid, acetoxyvalerenic acid and valerenic acid	Methanol	Water and acetonitrile-methanol (1 + 1), both with 0.05% phosphoric acid	Dried powdered material (0.2 g) was sonicated in 2.5 mL methanol for 15 min. Centrifuged and supernatant was collected. The supernatants were pooled, and final volume was adjusted to 10 mL with methanol. All samples were diluted and filtered through a 0.45-μm nylon membrane filter prior to use	Navarrete et al. (2006)

(continued)

Table 5.1 (continued)

S. No.	Method	Type	Part	Marker	Solvent	Mobile phase	Sample preparation	References
3	HPTLC	a	Rhizomes; aerial and root portion	Valerenic acid	Methanol	Hexane: ethyl acetate: acetic acid (75:25:0.5v/v)	The air-dried powdered material (0.2 gm) was extracted in 20 mL of methanol. The extracts were filtered, concentrated and dissolved in 2 mL of methanol	Singh et al. (2006), Jugran et al. (2016a)
		b	Rhizome	Hesperidin	Methanol	Ethyl acetate: methanol: water (10:1.7:1.3, v/v)	100 mg of the extract was dissolved in 2 mL of methanol. The mixture was sonicated, diluted and centrifuged. The supernatant was analyzed for hesperidin content	Sharma et al. (2012a)

(continued)

Table 5.1 (continued)

S. No.	Method	Type	Part	Marker	Solvent	Mobile phase	Sample preparation	References
4	GC and GC-MS	a	Roots and rhizomes	Sesquiterpene hydrocarbon, α-santalene, ar-curcumene, xanthorrhizol, patchouli alcohol	Water	Nitrogen as a carrier gas	20 g of air-dried, freshly ground material or 10.0 g of dry and ground root material hydrodistilled for 4 h in 300 mL water. The oil samples were stored at − 20 °C until analyzed	Bos et al. (1997)
		b	Leaf and root	3-Methylvaleric acid, maaliol (leaf oil), maaliol, β-gurjunene (root oil)	Water	Helium as a carrier gas	Fresh leaves and roots were collected and subjected separately for steam distillation to obtain oil	Sati et al. (2005)

(continued)

Table 5.1 (continued)

S. No.	Method	Type	Part	Marker	Solvent	Mobile phase	Sample preparation	References
		c	Roots and rhizomes	Patchouli alcohol, maaliol, isovaleric acid, viridiflorol (in rhizome oil), α-bulnesene, α-guaiene, bornyl acetate, 7-*epi*-α-selinene, γ-patchoulene and β-elemene (in root oil)	Water	Hydrogen as a carrier gas	Freshly harvested, cleaned and crushed rhizomes and roots separately hydrodistilled. The oil samples obtained were kept in a cool and dark place before analyses	Verma et al. (2013)
		d	Roots	Patchouli alcohol, maaliol, seychellene, calarene/s-gurjunene, α-santalene	Water	Helium as a carrier gas	Freshly harvested roots were air-dried in the shade at room temperature. The essential oil was extracted by hydrodistillation and stored at 4° till further analysis	Raina and Negi (2015)

(continued)

Table 5.1 (continued)

S. No.	Method	Type	Part	Marker	Solvent	Mobile phase	Sample preparation	References
		e	Whole plants	3-Methylvaleric acid, maaliol and β-gurjunene	Water	Helium as a carrier gas	Fresh plant material was collected and hydrodistilled in a Clevenger to obtain essential oil	Irshad et al. (2012)
		f	Rhizome	Isovaleric acid, methylvaleric acid and seychellene	Chloroform	Helium as a carrier	Hydrodistillation of the rhizome using the clevenger-type apparatus. The essential oil was collected and stored at 4 °C until analysis	Pandian and Nagarajan (2015)

(continued)

Table 5.1 (continued)

S. No.	Method	Type	Part	Marker	Solvent	Mobile phase	Sample preparation	References
		g	Roots	β-vatirenene, dehydro aromadendrene, alcohol, α-muurolene	Water	Helium as a carrier gas	Hydrodistillation of the roots using the clevenger-type apparatus. The oil was stored at 4 °C until evaluation	Thusoo et al. (2014)
		h	Roots and rhizome	Patchouli alcohol, seychellene, α-guaiene, α-humulene, δ-guaiene	Water	Helium as a carrier gas	Fresh roots (40–60 g) were hydrodistilled, and essential oil was dried through passing over anhydrous sodium sulfate. After filtration oil was stored at 4 °C till analysis	Bhatt et al. (2012)

(continued)

Table 5.1 (continued)

S. No.	Method	Type	Part	Marker	Solvent	Mobile phase	Sample preparation	References
		i	Roots	Patchoulol, α-bulnesene, isovaleric acid, α -guaiene and 3-methylvaleric acid	Water	Helium as a carrier gas	Powdered sample was hydrodistilled and extracted with *n*-hexane. Anhydrous sodium sulfate was used to remove water. Essential oils were stored in a refrigerator at 4 °C for subsequent experiments	Liu et al. (2013)
		j	Rhizomes	Isovaleric acid, methylvaleric acid and seychellene	hydrodistillation and supercritical fluid CO_2 extraction	Helium was the carrier gas	Samples were hydrodistilled, and obtained essential oil was collected and stored at 4 °C until analysis	Pandian and Nagarajan (2015)

(continued)

Table 5.1 (continued)

S. No.	Method	Type	Part	Marker	Solvent	Mobile phase	Sample preparation	References
5	GC-FID and GC-MS	a	Whole plant	Carotol, germacrene B, cis-β-farnesene, α-humulene and humulene oxide	Water	Helium as a carrier	Plant material (500 g) was shade dried, coarsely powdered and hydrodistilled. The oil was dried over anhydrous sodium sulfate and stored at 4 °C in the dark	Agnihotri et al. (2011)

5.4 Medicinal Properties and Usage

5.4.1 Traditional Uses

V. jatamansi is a vital species used in traditional and modern medicines for its aromatic, stimulant, carminative and antispasmodic property. Various activities like anticoagulant, anticancer, hepatoprotective neuroprotective, antibacterial, antifungal, inflammatory, antioxidative and antiprotozoal are reported in naturally occurring valeopotriates/iridoid (Dinda et al. 2009). This herb as a single species or in polyherbal combination is used to prepare 39 Ayurvedic formulations (Jugran et al. 2019). *V. jatamansi* is utilized for the administration of hysteria, epilepsy and urinary disorders (Singh and Ali 1998; Sharma 2003) and for removing bad smell of mouth due to toothache (Jugran et al. 2019). In the situation of extreme headache, *Valeriana* leaves are scrubbed on forehead after crushing them (Bhattacharjee 2008; Chevallier 1999). Dried rhizomes of *V. jatamansi* are utilized for scents, blackening of hair and as an aroma (Bhattacharjee 2008). The species is beneficial for the administration of head, eye and diseases releted with blood, liver, spleen-associated diseases, kidney ulcers, cardiac weakness, wounds, dry cough, asthma, prolonged and irregular body temperature (Awan 1990; Prakash 1999). Additionally, *V. jatamansi* extract is also used for curing diseases of skin, obesity, nervousness, hysteria, failing impulses, insanity, neurosis, sciatica, tranquilizer, snake poisoning and emmenagogue (Nadkarni 1976; Baquar 1989). Diuretic (Said 1970) and hepatoprotective activities are also reported (Awan 1990). Clinical and animal trails exhibited the CNS depressant property of *V. jatamansi* (Marder et al. 2003). Flavor, medicinal and perfume productions utilized extract and essential oil derived from this plant particularly for flavoring honey, tobacco and root beer (Sah et al. 2010a). An iridoid ester called as jatamanvalterate P extracted from this plant conventionally used to cure nervous ailment (Yang et al. 2017). Traditionally, several herbal formulations of the *V. jatamansi* plant are orally supplemented for curing diarrhea (Awan 1990), hypertension (Chevallier 1996) and gastrospasms (Kapoor 1990). Nadkarni (1976) reported that *V. jatamansi* is used in inflammation like jaundice and scorpion stings.

5.4.2 Pharmacological Activities

Several studies have been conducted to investigate the pharmacological attributes of *V. jatamansi*. Numerous active components extracted from this herb exhibited different level of activities to decrease stress and nervous disorders. Bhattacharya et al. (2007) reported that *V. jatamansi* extract also attenuated anxiety, stress and depression. The extract of species is useful in cerebro-spinal coordination, and migraines, nervous unrest, wakefulness, health obsessiveness, neuralgia and neuroasthemia were also observed (Cionga 1961). Depressed CNS activity is observed using the species extract in mice when supplemented orally (Veith et al. 1986). Neurotropic

activity of valiracyl derived from the species increases the amount of mediator in GABA inhibition and reduced intensity of brain bioenergetic activities (Dunaev et al. 1987). In a study, chlorophyll as well as the water extract solution of *V. jatamansi* remarkably reduced ischemia and reperfusion-stimulated cerebral injury by decreasing infarct size, enhanced memory for small period, coordination with motor, lateral push response, etc. (Rehni et al. 2007).

Roots derived valepotriates and jatamanvaltrate N of *V. jatamansi* demonstrated weaker neuroprotective property (Xu et al. 2012a). Likewise, moderate neuroprotective activities of jatairidoids A, B and C were demonstrated against MPP^+-stimulated neuronal cell death in SH-SY5Y cells of human dopaminergic neuroblastoma (Xu et al. 2012b). Jatamandoid A, valeriotriate B and jatamanvaltrate G, extracted from plant demonstrated moderate nervous system protective property against MPP^+-induced neuronal SH-SY5Y cell death (Xu et al. 2012c). Study showed that valeriotriate B and jatamanvaltrate G exerted reasonable neuroprotective activity, whereas jatamandoid A displayed highest property (Xu et al. 2012c). Likewise, jatadoids A, jatamanvaltrate H, valerilactones A, valerilactones B, bakkenollides and bakkenollide-H isolated from this plant also demonstrated robust nervous system protective activities against MPP^+-stimulated neuroblastoma SH-SY5Y cells by MTT assay (Xu et al. 2011a, 2012d).

V. jatamansi rhizome extract was analyzed for antioxidants and anti-inflammation property in MPTP-sensitized rats with Parkinson's disease. Results displayed that administration of extract remarkably recovered the changed behavior test scores, TH + cell count in mid-brain, striatal dopamine levels and TH protein amount, enhanced expression of GFAP and the alterations detected in Parkinson's disease-stimulated rats using histopathology. Likewise, reduced antioxidants level and considerably enhanced ROS, LPO and inflammatory cytokine level following the supplementation is found. Valeric acid obtained from this herb comprises similar structure to the GABA of the extract, a well-known neurotransmitter and performed as an antagonist to NMDA receptor. Neuroprotective activity of valeric acid derived from Indian Valerian is determined by improvement of tracerebroventricular streptozotocin-stimulated neurodegeneration which is demonstrated in Wistar rats (Vishwakarma et al. 2016). *V. jatamansi* 100 and 200 mg/kg extract and 20 and 40 mg/kg of valeric acid considerably reduced the retention transfer latency and escape latency than the intracerebroventricular-STZ group. The extract and valeric acid of the species reduced lipid peroxidation amount and restored the amount of glutathione in mice brains. Picrotoxin supplementation considerably inverted the properties of species extract in addition to valeric acid in intracerebroventricular-STZ-administered mice. These findings indicated the considerable GABAergic activity of valeric acid in attenuation of dementia induced by experiments. Pharmacological properties of few dominant *V. jatamansi* constituents are presented in Table 5.2.

Table 5.2 Pharmacological activities of some major compounds extracted from *Valeriana jatamansi*

S. No.	Compound	Biological activity	Description	References
1	Valtrate	Cytotoxic activity	Valtrate (40 μM) in the cultures of HTC hepatoma cells cause the disappearance of membrane microvilli, a large distension of the endoplasmic reticulum and a marked condensation of the mitochondria	Keochanthala-Bounthanh et al. (1993)
		Cytotoxic effects	Exhibited property opposite to lung adenocarcinoma (A549), metastatic prostate cancer (PC-3 M), colon cancer (HCT-8) and hepatoma (Bel7402) cell lines, with IC_{50} values ranging from 1.0 to 7.4 μM	Lin et al. (2009)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
2	Didrovaltrate	Cytotoxic activity	Didrovaltrate (80 μM) in the cultures of HTC hepatoma cells caused disappearance of membrane microvilli, a large distension of the endoplasmic reticulum and a marked condensation of the mitochondria	Keochanthala-Bounthanh et al. (1993)
3	Valerenic acid	Sedative and anxiolytic property	Reduced the breakdown of GABA in the brain and acts as $GABA_A$ receptor substrate resulting in its sedative and anxiolytic effects	Houghton (1999)
4	6-methylapigenin	Anxiolytic effects and tranquilizing properties	Supplementation of mice at intraperitoneal dose of 30 g per mouse showed anxiolytic property in rats. The bioavailability of this compound in crude infusion partially elucidates its tranquilizing effects	Wasowski et al. (2002)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
5	2S(-)-hesperidin	Sedative and sleep-enhancing properties	Exhibited reduction in ambulatory locomotor property, reduced the exploration of holes and the number of rearing in the hole-board test and enhanced the sodium thiopental-stimulated sleeping time. The depressant activity was concentration-dependent. Noteworthy moderate activity on the time spent head-dipping and on thiopental-stimulated sleeping time was observed on 2 mg/kg of hesperidin injection i.p.	Marder et al. (2003)
6	Didrovaltrate acetoxyhydrin	Cytotoxic effects	Displayed property against lung adenocarcinoma (A549), metastatic prostate cancer (PC-3 M), colon cancer (HCT-8) and hepatoma (Bel7402) cell lines, with IC_{50} values ranging from 1.0 to 7.4 μM	Lin et al. (2009)
7	IVHD-valtrate	Cytotoxic effects	Exhibited property opposite to lung adenocarcinoma (A549), metastatic prostate cancer (PC-3 M), colon cancer (HCT-8) and hepatoma (Bel7402) cell lines, with IC_{50} values ranging from 1.0 to 7.4 μM	Lin et al. (2009)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
8	5-hydroxydidrovaltrate	Cytotoxic effects	Demonstrated effects against lung adenocarcinoma (A549), metastatic prostate cancer (PC-3 M), colon cancer (HCT-8) and hepatoma (Bel7402) cell lines, with IC_{50} values ranging from 1.0 to 7.4 μM	Lin et al. (2009)
9	Acevaltrate	Cytotoxic effects	Showed activity against lung adenocarcinoma (A549), metastatic prostate cancer (PC-3 M), colon cancer (HCT-8) and hepatoma (Bel7402) cell lines, with IC_{50} values ranging from 1.0 to 7.4 μM	Lin et al. (2009)
10	Jatamanvaltrates A-M	Cytotoxic effects	Demonstrated cytotoxic effects against the PC-3 M cell line, in the IC_{50} value range of 1.4–6.3 μM except for compounds jatamanvaltrates C and jatamanvaltrates E	Lin et al. (2009)
11	Valeriotetrate A	Cytotoxic effects	Demonstrated cytotoxic activity against the PC-3 M cell line, in the IC_{50} value range of 1.4-6.3 μM	Lin et al. (2009)
12	Valeriotriate B	Cytotoxic effects	Demonstrated cytotoxicity against the PC-3 M cell line (IC_{50} value ranged from 1.4 to 6.3 μM)	Lin et al. (2009)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
13	Didrovaltrate	Cytotoxic effects	Demonstrated cytotoxicity against the PC-3 M cell line with IC_{50} value ranged from 1.4–6.3 μM	Lin et al. (2009)
14	Valeriandoids A & C	Neuroprotective effects	Displayed moderate neuroprotective effects opposite to 1-methyl-4-phenylpyridinium (MPP^+)-stimulated neuronal cell death in dopaminergic neuroblastoma SH-SY5Y cells. These compounds (3–30 μM) neither affected the cell viability nor exhibited any cytotoxic property in the absence of MPP^+	Xu et al. (2011b)
15	Chlorovaltrate	Neuroprotective effects	Displayed moderate neuroprotective effects against 1-methyl-4-phenylpyridinium (MPP^+)-stimulated neuronal cell death in dopaminergic neuroblastoma SH-SY5Y cells. These compounds (3–30 μM) neither affected the cell viability nor demonstrated any cytotoxicity with the absence of MPP^+	Xu et al. (2011b)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
	Chlorovaltrate	Cytotoxic effects	Exhibited modest cytotoxicity against lung adenocarcinoma (A 549), metastatic prostate cancer(PC-3 M), colon cancer (HCT-8) and hepatoma (Bel 7402) cell lines, with IC_{50} values of 0.89–9.76 μM	Lin et al. (2013)
16	1,5-dihydroxy-3,8-epoxyvalechlorine	Neuroprotective effects	Presented moderate neuroprotective properties against 1-methyl-4-phenylpyridinium (MPP^+)-stimulated neuronal cell death in dopaminergic neuroblastoma SH-SY5Y cells.	Xu et al. (2011b)
17	Jatamandoid A	Neuroprotective effects	Exhibited reasonable neuroprotective effects in human dopaminergic SH-SY5Y cells (CRL-2266) pretreated with several doses (3, 10 and 30 mM) of constituents before incubation in medium containing MPP^+ (0.8 mM) for stimulating neuronal cell death in dopaminergic neuroblastoma SH-SY5Y cells	Xu et al., 2012c

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
18	Valeriotriate B	Neuroprotective effects	Demonstrated moderate neuroprotective activities in human dopaminergic SH-SY5Y cells (CRL-2266) pretreated with different doses (3, 10 and v30 mM) of compounds before incubation in medium comprising MPP^+ (0.8 mM) for inducing neuronal cell death in dopaminergic neuroblastoma SH-SY5Y cells	Xu et al. (2012c)
19	Jatamanvaltrate G	Neuroprotective effects	Exhibited moderate neuroprotective effects in human dopaminergic SH-SY5Y cells (CRL-2266) pretreated with various concentrations (3, 10 and 30 mM) of compounds before incubation in medium containing MPP^+ (0.8 mM) for stimulating neuronal cell death in dopaminergic neuroblastoma SH-SY5Y cells	Xu et al. (2012c)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
20	Volvaltrate B	Cytotoxic activity	Showed cytotoxic activity against the lung adenocarcinoma (A549), metastatic prostate cancer (PC-3 M), colon cancer (HCT-8) and hepatoma (Bel7402) cell lines, with IC_{50} values of 8.5, 2.0, 3.2 and 6.1 μM, respectively	Lin et al. (2010)
21	Valerilactones A	Neuroprotective effects	Demonstrated neuroprotective properties opposite to MPP^+-stimulated neuronal cell death in human dopaminergic neuroblastoma SH-SY5Y cells, administered with several concentrations (1.5, 5 and 15 μM) of compounds before incubation in a medium containing 0.8 mM MPP^+	Xu et al. (2011a, 2012d)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
22	Valerilactones B	Neuroprotective effects	Demonstrated neuroprotective effects against MPP^+-induced neuronal cell death in human dopaminergic neuroblastoma SH-SY5Y cells treated with various concentrations (1.5, 5 and 15 μM) of compounds before incubation in a medium containing 0.8 mM MPP^+	Xu et al. (2011a, 2012d)
23	Bakkenolide-H	Neuroprotective effects	Demonstrated neuroprotective effects against MPP^+ -induced neuronal cell death in human dopaminergic neuroblastoma SH-SY5Y cells treated with various concentrations (1.5, 5 and 15 μM) of compounds before incubation in a medium containing 0.8 mM MPP^+	Xu et al. (2011a)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
24	Jatadoids A	Neuroprotective effects	Displayed moderate neuroprotective effects against MPP^+-induced neuronal cell death in human dopaminergic neuroblastoma SH-SY5Y cells. These active compounds (3–30 μM) neither affected the cell viability nor showed any cytotoxicity in the absence of MPP^+	Xu et al. (2011a, 2012d)
25	Jatamanvaltrate H	Neuroprotective effects	Displayed moderate neuroprotective effects against MPP^+-induced neuronal cell death in human dopaminergic neuroblastoma SH-SY5Y cells. These active compounds (3–30 μM) neither affected the cell viability nor showed any cytotoxicity in the absence of MPP^+	Xu et al. (2011a, 2012d)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
26	Jatairidoids A–C	Neuroprotective effects	Displayed moderate neuroprotective effects in MPP^+-induced neuronal cell death in human dopaminergic neuroblastoma SH-SY5Y cells. The above active compounds (3–30 μm) neither affected the cell viability nor showed any cytotoxicity on SH-SY5Y cell in absence of MPP^+	Xu et al. (2012b)
27	Chlorovaltrates K-N	Cytotoxic effects	Exhibited moderate cytotoxicity against lung adenocarcinoma (A 549), metastatic prostate cancer (PC-3 M), colon cancer (HCT-8) and hepatoma (Bel 7402) cell lines with IC_{50} values of 0.89–9.76 μM	Lin et al. (2013)
28	Rupesin B	Cytotoxic effects	Exhibited moderate cytotoxicity against lung adenocarcinoma (A 549), metastatic prostate cancer (PC-3 M), colon cancer (HCT-8) and hepatoma (Bel 7402) cell lines, with IC_{50} values of 0.89–9.76 μM	Lin et al. (2013)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
29	Valtrals A–C and Jatamanvaltrates P–Y	Cytotoxic activity	Demonstrated selective cytotoxic effects against metastatic prostate cancer (PC-3 M) and colon cancer (HCT-8) cell lines	Lin et al. (2015a, b)
30	Jatamanvaltrates R-S	Acetylcholinesterase (AChE) inhibitory activity	All these compounds exhibited acetylcholinesterase activity inhibition ratios of less than 10% at the concentration of 50 μM. The positive control, tacrine, showed an inhibition rate of 47.6% at 0.33 μM	Dong et al. (2015)
31	Jatamanin Q	Acetylcholinesterase (AChE) inhibitory activity	All of the constituents showed acetylcholinesterase property prevention ratios of less than 10% at the dose of 50 μM. The positive control, tacrine, exerted an inhibition rate of 47.6% at 0.33 μM	Dong et al. (2015)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
32	Valerianananoids D–E	Acetylcholinesterase (AChE) inhibitory activity	Studied compounds displayed acetylcholinesterase property inhibition ratios of less than 10% at the dose of 50 μM. The positive control, tacrine, showed an inhibition rate of 47.6% at 0.33 μM	Dong et al. (2015)
33	Clovane-2β-isovaleroxy-9α-olvalerianananoids A-C	Acetylcholinesterase (AChE) inhibitory activity	This compound exerted acetylcholinesterase activity inhibition ratios of less than 10% at the amount of 50 μM. The positive control, tacrine, exhibited an inhibition rate of 47.6% at 0.33 μM	Dong et al. (2015)
34	Volvaltrate B	Acetylcholinesterase (AChE) inhibitory activity	The compound possesses acetylcholinesterase activity inhibition proportion of less than 10% at the quantity of 50 μM. The positive control, tacrine, showed an inhibition rate of 47.6% at 0.33 μM	Dong et al. (2015)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
35	Valeriotetrate A	Acetylcholinesterase (AChE) inhibitory activity	Exhibited acetylcholinesterase activity inhibition ratios of less than 10% at the concentration of 50 μM. The positive control, tacrine, showed an inhibition rate of 47.6% at 0.33 μM	Dong et al. (2015)
36	Valeriotetrate B	Acetylcholinesterase (AChE) inhibitory activity	Displayed acetylcholinesterase activity inhibition ratios of less than 10% at the concentration of 50 μM. The positive control, tacrine, showed an inhibition rate of 47.6% at 0.33 μM	Dong et al. (2015)
37	8, 11-desoidodidrovaltrate	Acetylcholinesterase (AChE) inhibitory activity	Showed acetylcholinesterase activity inhibition ratios of less than 10% at the concentration of 50 μM. The positive control, tacrine, showed an inhibition rate of 47.6% at 0.33 μM	Dong et al. (2015)
38	Rupesin E	Acetylcholinesterase (AChE) inhibitory activity	Demonstrated acetylcholinesterase activity inhibition ratios of less than 10% at the concentration of 50 μM. The positive control, tacrine, showed an inhibition rate of 47.6% at 0.33 μM	Dong et al. (2015)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
39	(3S, 4R, 5S, 7S, 8S, 9S)-3, 8-ethoxy-7-hydroxy-4, 8-dimethylperhydrocyclopenta [c] pyran	Acetylcholinesterase (AChE) inhibitory activity	Exhibited acetylcholinesterase activity inhibition ratios of less than 10% at the concentration of 50 μM. The positive control, tacrine, showed an inhibition rate of 47.6% at 0.33 μM	Dong et al. (2015)
40	Isopatriniosine	Neuroprotective effects	Exhibited moderate neuroprotective effects against $CoCl_2$-induced neuronal cell death in PC12 cells	Tan et al. (2016)
41	Valeric acid	Neurodegeneration	Administration of Wistar Albino rats with valeric acid 20 and 40 mg/kg, i.p. (suspended in 1% Tween 80 solution) significantly decrease escape latency and retention transfer latency, then the intracerebroventricular streptozotocin group	Vishwakarma et al. (2016)

(continued)

Table 5.2 (continued)

S. No.	Compound	Biological activity	Description	References
42	Jatamanvaltrate P	Anticancer effects	Jatamanvaltrate P prevented the development and proliferation of MCF-7 and triple-negative breast cancer (TNBC) cell lines (MDA-MB-231, MDA-MB-453 and MDA-MB-468) in a concentration-based fashion. Also whereas displayed moderately low cytotoxic effects to human breast epithelial cells (MCF-10A). Administration with jatamanvaltrate P stimulated G2/M phase arrest in TNBC and G0/G1-phase arrest in MCF-7 cells	Yang et al. (2017)
43	Jatamanvaltrates N	Neuroprotective activity	Exhibited weak neuroprotective property	Xu et al. (2012a)
44	(+)-9′-Isovaleroxylariciresinol	Cytotoxicity	Significant in vitro cytotoxicity was revealed against PC-3 M and HCT-8 cell lines with IC_{50} values of 8.1 and 5.3 μM respectively	Lin et al. (2010)

5.4.2.1 Sedative and Tranquillizing Effect

Various sleeping disorders in human are effectively treated with *V. jatamansi*. Tranquilizing property is exhibited by valerenic acids (like monoterpenes and sesquiterpenes) and glycosides of iridoid from the species. Clinical studies on Tagar have established that the species root extract reduced sleep latency, improve the quality of sleep and, therefore, observed to be beneficial in administration of nervousness and sleeplessness (Leathwood and Chauffard 1983). Valerenal and few other constituents extracted from *V. jatamansi* exerted sedative property to the valepotriates fractions and essential oil (Wagner et al. 1980; Hendricks et al. 1981). Studies demonstrated that decomposition by products of valepotriates like baldrinal, homobaldrinal, decylbaldrinal and valtroxal reduced sedative activity to some extent and property also caused considerable mortality in mice (Schneider and Willems 1982). It is reported that valerenic acid prevents the enzyme system association with central GABA catabolism (Riedel et al. 1982) and is released by [3H] GABA valerian extract through reverse of GABA transporter, which depends on Na^+ and independent on Ca^{++} (Santos et al. 1994). The enhancement in [3H] GABA discharge was not found dependent on Na^+-K^+-ATPase property and the membrane potential. Root extract of *V. jatamansi* used for commercial purpose displayed pronounced sedative activities in the rats in relation to a reduced motility and an enhanced thiopental sleeping time (Leuschner et al. 1993). Comparative analysis of *V. jatamansi* extract with chlorpromazine and diazepam exhibited moderate sedative property (Leuschner et al. 1993). Sedative property was also potentiated by flavanone glycoside 2S (-) hesperidin extracted from this herb (Marder et al. 2003). Sedative property of hesperidin and 6- methylapigenin is also validated by in vivo experiments using mice as an experimental model (Marder et al. 2003). Anticonvulsant and soothing properties are exerted by glycosides of flavonoid, namely linarin and hesperidin which probably interacted with GABAA receptors (Fernandez et al. 2004). Sleep–wake profile and EEG delta property in male Sprague-Dawley rats administered with different quantities ranged from 100 to 300 mg/kg of this plant were determined. The results exhibited that non-rapid eye movement sleep delta activity (sustained for 8 h) and sleep latency were considerable after extract supplementation at 300 mg/kg dose. The duration of wake state at 200 and 300 mg dose was significantly increased. Study exhibited that extract of the roots remarkably reduced sleep latency, NREM sleep, increased duration of total sleep and reduced wakefulness period in administered animals. Hence, root extract of *V. jatamansi* attenuates the quality of sleep and control monoamine amount in mice brain (Sahu et al. 2012).

5.4.2.2 Anxiolytic Property

4′, 5, 7-dihydroxy-6-methylflavone or 6-methylapigenin (MA) extracted from *V. jatamansi* exerted anxiolytic activity (Wasowski et al. 2002). Anxiolytic effect of valtrate extracted from this herb is investigated in mice by supplementing with varying doses of valtrate for 10 days after successive disclosure to open field test (OFT) and elevated

plus-maze (EPM) test (Shi et al. 2014). Valtrate displayed the anxiolytic property in mice by enhancing open arm entry percentage and time in the EPM assay and central entries number in the OFT. Further, remarkable decrease in amount of corticosterone in rat serum was recorded. The findings from the study indicated that valtrate exerts anxiolytic property of behavioral models which may be mediated by hypothalamus–pituitary–adrenal axis function (Shi et al. 2014).

Gene expression of apoptosis-related genes was measured in the control and administered groups of mice anxiety model using Gene chip technology. Differences in expression of gene related to apoptosis in standard mice, anxiety model mice and mice administered with *V. jatamansi* extract were recorded. Ets-1, Elk-1, Bax, Apaf-1 and Bcl-2 gene expression in the model group were up-regulated than the normal group, but in other groups the gene was expressed. Finding showed this plant plays important part to control the irregular expression of genes associated with apoptosis in rat model animal (Yan et al. 2011).

5.4.2.3 Antidepressant Activity

Tager extract is used to reduce anxiety, stress and ameliorate depression symptoms (Bhattacharya et al. 2007). The species extract considerably decreased locomotor action at 200 mg/Kg dose using tail suspension method and comprises an adverse interplay with antidepressant-like activity. Methanolic and ethanol aqueous extracts of the plant exhibited that such activity of the species is not dependent on the level of terpenoids present (Subhan et al. 2010). However, a remarkable amount of antilocomotor property was detected at the high terpenoids amount using tail swim assay or forced swim assessment (Subhan et al. 2010). Extract of patchouli alcohol chemotype of *V. jatamansi* is also displayed as antidepressant property in the extract in dichloromethane of patchouli alcohol chemotype of *V. jatamansi* (Sah et al. 2011). Rhizomes and roots of the herb were gathered, dried, extracted using dichloromethane and utilized for evaluation of antidepressant property in albino LACA rats by forced swim method. Results suggested that single dose (40 mg/kg extract) supplementation considerably prevented the immobility time in rats. In chronic study, remarkable decline in the immobility period and enhanced amount of norepinephrine and dopamine in mice forebrain was found to indicate the antidepressant property of this species.

5.4.2.4 Antispasmolytic and Blood Pressure Decreasing Activity

Crude rhizome extract derived from *V. jatamansi* and its fractions showed antspasmolytic and blood pressure decreasing property (Gilani et al. 2005). Crude extract (0.1–3.0 mg/mL) when tested against high K^+ (80 mM)-stimulated contractions in rabbit jejunum preparations, generated low preventive action but totally relax the shrinkages stimulated by small K^+ (20 mM). Plant extract produced same results in ileum of guinea pig as in jejunum of rabbit. The study also demonstrated the blood

pressure reducing property of the *V. jatamansi* in mice by intravenous supplementation of extract (10–100 mg/kg). A concentration-based decrease in average arterial blood pressure was observed in normotensive mice treated with anesthesia. Findings from the report exhibited hypertensive property of valeranone which is responsible for blood pressure reducing activity of the species (Arora and Arora 1963). Likewise, Wagner et al. (1980) reported the spasmolytic activity of iridoids valtrate and didrovaltrate of *V. jatamansi*. The marketable combination of iridoids was detected to be active than the papaverine in similar amount (Gilani et al. 2005). Blood pressure reducing and antispasmodic property of *V. jatamansi* roots displayed the facilitation of these properties by K^+ (ATP) channel activation hence warranted the utility of this herb in gastrointestinal and cardiovascular complaints (Gilani et al. 2005).

5.4.2.5 Gastrointestinal and Cardiovascular Ailment

Diverse gastrointestinal complaints like diarrhea, stomach cramp, diverticulitis, irritable bowl, dyspepsia related to nervous system, stomach cramp and stimulates digestion are treated using *V. jatamansi* (Houghton 1999). Species extract is observed to decrease blood pressure and strengthening and heart palpitations (Morazzoni and Bombardelli 1995). Antispasmodic and hypotensive property of this herbal plant was demonstrated to be probably intervened through KATP channel initiation and provide evidences on utilization of species in these diseases (Gilani et al. 2005). Irritable bowel syndrome treatment using iridoids from *V. jatamansi* was also analyzed in male Sprague-Dawley mice. The model was established by chronic stress and independent feeding. The amount of colon 5-HT content is enhanced considerably in model group, but it reduced remarkably in hypothalamic region. The three groups administered with iridoid exhibits reduced 5-HT amount in serum and colon; nevertheless, the amount of 5-HT in hypothalamic region enhanced while no remarkable alterations are screened in 5-HIAA. However, colon and serum 5-HT/5-HIAA amount are decreased. The action mechanism of iridoids in irritable bowel syndrome can be associated with controlling the influence level by gastrointestinal 5-HT to CNS (Yan et al. 2011).

5.4.2.6 Antidiarrheal and Bronchodilatory Potential

V. jatamansi is found to possess antidiarrheal and bronchodilatory effects using in vivo method (Khan and Gilani 2011). Defensive property of *V. jatamansi* crude extract was screened against castor oil-stimulated diarrhea in rats. Pre-administration of crude extract to mice developed 20% and 60% defense against diarrhea at 300 mg/kg and 600 mg/kg doses, respectively, then the control. Hence, findings exhibited that *V. jatamansi* extract prevented the diarrhea stimulated by castor oil.

5.4.2.7 Anti-inflammatory Activity

Crude *V. jatamansi* leaves extract demonstrated anti-inflammation by in vitro and in vivo assays (Khuda et al. 2013). Dried leaves were powdered, and methanol extracted material of the species was filtered and concentrated to obtain crude extract. The obtained material was dissolved in dH_2O and partitioned to obtain chloroform, n-butanol, ethyl acetate, n-hexane and aqueous fractions. The methanolic extract topical formulation (cream) was tested in male Wistar rats using carrageen stimulated hind paw edema assay and its impact on inflammation models in acute and chronic stage. All the fractions with methanolic extract screened for anti-inflammation property by in vitro lipoxygenase prevention method. The species extract displayed considerable anti-inflammatory property then the standard (10%) followed by 5 h of carrageen injection. This anti-inflammatory property was also detected in ethyl acetate fraction during in vitro testing ($IC_{50} = 76 \pm 0.14$) then the standard ($IC_{50} = 6.11 \pm 0.02$). Study exhibited that the fraction of ethyl acetate may be utilized for the extraction of novel principal compound with anti-inflammatory property. Methanol and ethanol prepared extract of *V. jatamansi* also comprises anti-inflammatory effects (Subhan et al. 2007) and found to prevent mediators generated during inflammation like prostaglandins, serotonin, histamine and bradykinins (Vinegar et al. 1969). Other studies also displayed the anti-inflammatory effects of crude extract and volatile oils of *V. jatamansi* (Subhan et al. 2007; Agnihotri et al. 2011).

5.4.2.8 Analgesic Properties

Dried material (rhizomes and roots) was extracted in dichloromethane and essential oil. LACA mice (20–40 g) were supplemented with acetic acid (1%) by intraperitoneal injection. The writhing reaction was characterized by abdominal contraction and hind limb stretching counted for 10 min (Sah et al. 2010b). Extract doses, oil and aspirin were used to treat rats for 1 h prior to assessment through oral mode. It is observed that dichloromethane extract and oil considerably prevented the number of writhing than the control group. However, extract and essential oil did not exhibit any property in tail flick model which showed only peripheral analgesic property. Further, the action mechanism of acetic acid stimulated writhing exhibited that subeffective volatile oil dosage remarkably enhances activity of aspirin, whereas such effects were not recorded in the case of extract. It was found that essential oil displayed peripheral analgesic property via inhibition synthesis of prostaglandins.

5.4.2.9 Cytotoxic Activity

Cytotoxicity of several constituents isolated from this species demonstrated different level of counteracting activities on the cancer cells growth and proliferation.

Numerous valepotriates displayed cytotoxic and antitumor properties. Jatamanvalterate, an iridoids ester extracted from the species, exerted notable antitumor activities (Yang et al. 2017). Studies (in vivo and in vitro) exhibited growth inhibition and proliferation of breast cancer (TNBC) cell lines on concentration-based fashion but displayed lower cytotoxic property to human breast epithelial cells (MCF-10A). Jatamanvaltrate P showed a potent antitumor activity in MDA-MB-231 xenografts (Yang et al. 2017). Valtrals A, B and C three decomposition products of valepotriates possessed selective cytotoxic activity opposite to PC-3M and HCT-8 (metastatic prostate cancer cell lines and colon cancer) respectively (Lin et al. 2015b). Lin et al. (2009) studied that valtrate, acevaltrate, IVHD-valtrate, didrovaltrate acetoxy hydrin and 5-hydroxydidrovaltrate derived from the species demonstrated effects against diverse cancerous cell lines. However, acevaltrate was found the most effective compound. And all compounds except jatamanvalterate C, jatamanvaltrate E and 10-acetoxyvaltrathydrin, revealed cytotoxic property against the PC-3M cell line. In vitro and in vivo method were performed to determine anticancer property of IVHD-valtrate one among highly active constituents of *V. jatamansi*, against human ovarian cancer cells (Lin et al. 2013). IVHD-valtrate prevents the development and propagation of the A2780 and OVCAR-3 cancerous cell lines in a dose-based fashion. However, comparatively lower cytotoxicity was detected into immortalize IOSE-144 (non-tumorigenic human ovarian surface epithelial cells). IVHD-valtrate administration stimulates and arrests the OVCAR-3 cells in the G2/M phase. Preclinical results on IVHD-valtrate specified its potent as a therapeutic molecule to treat ovarian cancer along with providing strong proofs for creating novel chemotherapeutic molecule (Li et al. 2013). Likewise, cytotoxicity of 10 new compounds [jatamanvaltrates P-Y (jatamanvaltrates P R, S, T-Y along with one known valepotriate (nardostachin) extracted from *V. jatamansi* demonstrated that merely nardostachin, jatamanvaltrate P and jatamanvaltrate X exhibited stronger cytotoxic property in PC-3 cells. Lignan compound (+)-9′-isovaleroxylariciresinol was screened for cytotoxic property beside four cancerous cell lines of human namely, HCT-8, PC-3M, A54 and Bel7402 demonstrated cytotoxicity against PC-3 M and HCT-8 cell lines (Lin et al. 2010). Three newly identified minor valepotriate isomers, namely jatamanvaltrates Z1, Z2 and Z3 isolated from this plant exhibited modest cytotoxic property in cancerous cell lines A549, PC-3 M, HCT-8 and Bel7402 (lung adenocarcinoma, metastatic prostate cancer, colon cancer and hepatoma cell lines), respectively (Lin et al. 2017). Hydroethanolic extract of rhizomes of Tagar was measured in Swiss albino rats (Joseph et al. 2016). Acute toxicity analysis did not exhibit any sign of irregularity, illness or death throughout the time of analysis while administered and untreated animals displayed considerable variations in auditory startle loss, fierceness (untreated > administered), nasal discharge and dyspnoea. Additionally, photoactometer test demonstrated dose-based enhancement in sedative quality. The findings revealed that hydroethanolic extract didn't exhibit any morbidity, mortality, or any other negative impact on healthy Swiss albino rat neither in single oral dose nor in chronic doses on administration.

5.4.2.10 Constipation and Antinociceptive Effect

Shade dried fresh leaves of *V. jatamansi* was coarsely powdered and soaked in 70% ethanol. Extract was obtained by filtering the solution and evaporating the filtrate followed by solubilizing in distilled water. The extract of the species caused dose-based (3–10 mg/mL) contractile activity in separated ileum of guinea pig. It was found that pre-administration of tissues with atropine (1 μM) remove the stimulatory activity of crude extract. These finding exhibited that the spasmogenic property of *V. jatamansi* is mediated possibly by the activation of muscarinic receptors, which provides strong evidences for the use of *V. jatamansi* in constipation (Khan and Gilani 2011).

V. jatamansi extract was determined for antinociceptive effect by using stimulated writhing and tail flick model (Sah et al. 2010b). Intraperitoneal injection of acetic acid to Lacamice develops writhing response depicted through constriction in abdomen and stretches in hind limb. Varying extract and volatile oil doses p.o. were supplemented 1 h prior to injecting acetic acid. Acetic acid stimulated writhing was inhibited significantly by both the extract and essential oil and enhanced the latency time after 2 h of treatment in tail flick model. Essential oil in subeffective doses considerably potentiated the effects of aspirin while no such property was detected in extract. Likewise, in tail flick test the analgesic effects of essential oil were completely antagonized by naloxone while no such effects were observed using extracts. These findings showed that both poor central and a strong peripheral antinociceptive property of the maaliol-type *V. jatamansi* chemotype.

5.4.2.11 Cure Liver Cirrhosis and Tissue Hyperproliferative Response

Rhizome extract of *V. jatamansi* was tested on the liver cirrhosis animal model rats and on cell proliferation (Prasad et al. 2010). Liver cirrhosis was stimulated in rats treated with thioacetamide (0.03%). Rats were then administrated with the extract orally for 9 weeks. Results elucidated that the extract of *V. jatamansi* partially revert the enhanced amount of alkaline phosphatase, γ-glutamyl transferase and choose biochemical markers related to hepatic injury along with the drug-metabolizing enzymes. Histopathological analysis of hepatic tissue validated the therapeutic potential of species authenticated through alterations in biochemicals.

5.4.2.12 Anti-HCV Property

The water, chloroform and methanol pulverized root samples of *V. jatamansi* were extracted and screened for anti-Hepatitis C virus (HCV) property (Ganta et al. 2017). Based on primary bioassay testing, the methanolic extract was identified and applied for fractionation through preparative TLC. Fractions (4 nos.) F1–F4 were gathered from the TLC plate, isolated separately with ethyl acetate and freeze-dried. Overnight grown cells of human hepatoma cell line (Huh-7.5 cells) were infested with viral

supernatant (J6/JFH chimeric HCV strain). PBS is used to wash cells and varying extracts doses or corresponding volume of DMSO was added as control. Antiviral activity was demonstrated by RT-PCR and western blotting. Result displayed that metabolic extract showed decline in HCV replication and F4 fraction exhibited remarkable viral prevention. Additionally, sharp quenching of inherent fluorescence with enhanced extract concentration was observed in the presence of fraction F4 using intrinsic fluorescence assay of purified HCV RNA-dependent RNA polymerase NS5B. These findings revealed that methanol extract of *V. jatamansi* and F4 fraction prevented HCV through interaction with HCV NS5B protein (Ganta et al. 2017).

5.4.2.13 Regulation of Lipid Metabolism

IRFV (Iridoids rich fraction of *V. jatamansi*) was investigated to measure the control of lipid metabolism and mechanism linked with it (Zhu et al. 2016). Hyperlipidemic mice were fed with different amount of IRFV. The findings displayed that three varying dosages of iridoid-rich fraction decreases the body weight, durenes (assurance) triglyceride amount and enhance serum high density lipoprotein cholesterol content in the supplemented animals with fraction. Low iridoid-rich dosage remarkably reduces the aspartate aminotransferase and alanine aminotransferase in serum, liver index and liver triglyceride level but increased the property of lipoprotein lipase. Medium IRFV dosage can considerably decline the LDL-C and TG level in liver. However, high iridoid dosage remarkably declines the serum LDL-C, AST, TBA and ALT level and enhanced HL activity. Considerable incline in expression of PPAR-d and ApoA5 and decline in the protein SREBP-1c expression was detected by three varying iridoid dosages. Pathological investigation of liver tissue showed that iridoid can ameliorate cell deterioration to a certain extent. The findings from this study showed that iridoid-rich fraction plays essential roles in metabolism of lipid and its mechanism might be associated with enhanced expression of ApoA5 protein.

5.4.2.14 Adaptogenic Activity

V. jatamansi extract was evaluated for adaptogenic property in inbred male Sprague–Dawley rats (Sharma et al. 2012a). Rats were kept inside the cages made up of polypropylene under a regulated environment. The results exhibited that *V. jatamansi* extract at single oral dose (200 mg/kg) displayed highest adaptiveness in cold-hypoxia-restraint rats. Moreover, supplementation of the highest effective quantity of 200 mg/kg (single dose/day, for 5 days) was unable to provide additional adaptogenic property. These finding revealed that species extract do not exhibit cumulative adaptogenic property.

5.4.2.15 Enzyme Inhibition Activity

Acetylcholinesterase, butyrylcholinesterase and α-glucosidase enzyme-preventing properties of unrefined *V. jatamansi* infusion and its subsequent fractions were performed (Khuda et al. 2014). It was observed that crude extract comprises considerable property against cholinesterases. Likewise, chloroform fractions of *V. jatamansi* showed remarkable property against enzyme acetylcholinesterase (IC_{50}: 61 μg/ml) while ethyl acetate fractions exhibits considerable property against enzymes butyrylcholinesterase (IC_{50}: 58 μg/ml). Findings from the study showed enormous therapeutic potential of *V. jatamansi* for discovery of novel active constituents for treating mental abnormality for example Alzheimer's disease.

5.4.2.16 Antioxidant and Antimicrobial Properties

Various reports are available on antioxidant properties of *V. jatamansi*. For example, Kalim et al. (2010) studied the species root extracts for antioxidant activity. Hydroxyl radical, peroxynitrite scavenging assay, non-enzymatic superoxide radical scavenging assay and nitric oxide scavenging property method exhibited high antioxidant activity. Antioxidant property of methanol extracts and essential oil derived from species roots was analyzed (Thusoo et al. 2014). *V. jatamansi* essential oil and supercritical CO_2 fluid extracts was measured for antioxidant activity using DPPH radical, superoxide radical and hydroxyl scavenging assays (Pandian and Nagarajan 2015). Bhatt et al. (2012) measured the antioxidant property of root sample obtained from planted and wild source using DPPH and FRAP assay. Likewise, antioxidant property of essential oil was measured by DPPH assay. Comparative assessment of planted root samples and wild genotypes demonstrated considerably maximum antioxidant activity (ABTS–4.87 mg/g: FRAP–10.18 mg/g AAE d.w.). While antioxidant property measured by DPPH activity was more in wild source. Essential oil displayed strong antioxidant activity than the methanolic extract. Moreover, several biochemical and ISSR (inter simple sequence repeats) markers were detected to linked with the antioxidant property and valerenic acid content of *V. jatamansi* determined by ABTS, DPPH and FRAP assay and their possible uses in selection of material with quality traits for breeding was visualized (Jugran et al. 2013b, 2015b). ABTS, DPPH and FRAP assays were conducted to measure antioxidants in aerial and root parts extract of 25 distant populations of *V. jatamansi* collected from Uttarakhand (Jugran et al. 2016a). Considerable variations in antioxidant property across the population were recorded. Results indicated that *V. jatamansi* can be considered as a natural source of antioxidant. However, no clear trend was detected in antioxidants activity across the altitudinal range in this study, but among diverse habitats (oak, pine, mixed forest type and grassy land) differences in antioxidant property were recorded. The effect of arbuscular mycorrhizal fungi (AMF) on antioxidant property in aerial and root parts investigated using DPPH, FRAP and ABTS assay demonstrated considerable variations among plants of 1st and 2nd years of plantation. The

results demonstrated that inoculation with AMF increased antioxidant activity which suggests a positive effect of AMF inoculation on species (Jugran et al. 2015d).

V. jatamansi reported to possess antibacterial and antifungal property against enormous number of bacterial and fungal pathogens (Suri and Thind 1978; Thind and Suri 1979; Girgune et al. 1980). *V. jatamansi* extract in various solvent system (methanol, chloroform, hexane and water) showed higher antimicrobial property than the standard—Ampicillin and Erythromycin (Sati et al. 2011). Extract of *V. jatamansi* aerial part was investigated for antimicrobial property. The chloroform fraction of the species exhibited considerable property over *Staphylococcus aureus* while hexane fraction displayed maximum property opposite to *Bacillus subtilus* (Khuda et al. 2012). However, hexane fraction of the herb demonstrated potent preventive activity against *Microsporum canis* while chloroform and water fraction counter the activity of *M. canis* and *Aspergillus flavus.* Essential oil derived from extract of *V. jatamansi* whole plant was studied for the potential antimicrobial property against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus pumilus* and *Candida albicans* (Agnihotri et al. 2011). In another study, hydro-alcohol (50% v/v) and hexane extract of *V. jatamansi* were tested for antimicrobial property against pathogenic and drug-resistant strains. Potent antimicrobial property was demonstrated by hydro-alcoholic extract counter *Micrococcus luteus*, *Escherichia coli*, *Escherichia coli mutans*, *Salmonella abony*, *Lactobacillus plantarum* and *Staphylococcus epidermidis.* Both the extracts displayed potent sensitivity against multi-drug-resistant *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Moreover, only hydroalcoholic extract of this plant showed good antifungal property against *Aspergillus niger* but no such property was detected against *Candida albicans* (Babu et al. 2015). Five solvent system like, water, methanol, ethanol, acetone and hexane was used for root material extraction of *V. jatamansi* to analyze the antimicrobial property. Species extract in ethanol exhibited highest property opposite to all studied bacterial strains excluding *Bacillus subtilis* that displayed sensitivity to acetone extract. Further, extracts in all type of solvents prevent the development of *Escherichia coli.* However, hexane extract of species demonstrated antifungal activity against *Aspergillus flavus*, *Aspergillus fumigatus* and *Candida albicans* whereas *Aspergillus fumigatus* exhibited sensitivity at high dose against hexane extract. Moreover, bacterial strain showed much sensitivity to the *V. jatamansi* methanol extract (Rawat et al. 2017).

5.4.2.17 Other Uses

V. jatamansi is used either alone or in combination for the preparation of several herbal formulations. The plant is used for preparation of antiwrinkle cream (Ravichandran et al. 2005), and Sumenta as an antidepressant formulation (Prakash 1999). Didrovaltrate an iridoid found in *V. jatamansi* is observed to prevent alternative synthesis in the serum complement system and its likely usage in few autoimmune illnesses (Houghton 1999; Baibado and Cheung 2011). Hesperidin is a flavonoid constituents extracted from *V. jatamansi* roots was analyzed for radioprotective activity against

γ irradiation induced severe DNA injury. Hesperidin (16.38 μM dose) was found highly active in decreasing radioactivity (Katoch et al. 2012). The impact of total flavonoids isolated from these species was analyzed on TGF-beta signaling pathway in hepatocarcinoma 22-bearing rats. Four groups of hepatocarcinoma 22-bearing rats like model group, tegafur group, low and high-dose *V. jatamansi* group were divided randomly. These groups were analyzed for differences in gene expression chart of signaling pathway of TGF-beta by gene chip technology. Findings from the study exhibit expression of 7 genes were considerably controlled in other three groups than the control group in TGF-beta signaling pathway. Of which, E2f5, Cul1, Smad7 and Myc genes expression was up-regulated, while there is down-regulation in the expression of Smad1, Comp and Thbs4 genes. Total flavonoids from the herb control the unusual expression of genes involved in hepatocarcinoma 22-bearing rats with TGF-beta signaling pathway (Zhang et al. 2012).

5.5 Agrotechnology for Cultivation

IHR is a global biodiversity hotspot encompasses enormous diversity of native, endemic, rare and endangered medicinal plants. These medicinal plants appreciated across worldwide because of the presence of unique secondary metabolites for therapeutic purposes. As *V. jatamansi* is generally gathered from its natural sites to meet out the industrial demand which has severally over exploited the species and put this plant under endangered category (NMPB 2008). Therefore, there is an urgent need to attempt for its cultivation and conservation. However, only a few studies have been attempted to adopt the cultivation of these plants. Cultivation of MAPs is beneficial to develop standard agrotechniques (Fig. 5.2). It provides chances for crop variation along with income generation to the farmers. However, development of suitable cultivation packages for any medicinal plants which is collected from natural site is challenging to promote cultivation (NMPB 2008; Phondani et al. 2016; Dhiman et al. 2020). The cultivation of the *V. jatamansi* can be understood by studying the ecology and adaption situation of this species as mentioned below.

5.5.1 Climatic Conditions

V. jatamansi is a temperate herbaceous species grows well in cold winters and mild summers. This is a shade loving species observed to grow in the temperature ranging from 15 to 25 °C and with the requirement of 80–90% relative humidity (Mukherjee 2015). Seed germination of this plant is supported by high temperature at the time of sowing (Mukherjee and Chakraborty 2014). Flowerings in *V. jatamansi* started in February month and ended till April and ripening of seeds takes place in May onward (Mukherjee and Chakraborty 2014; Jugran et al. 2019). The composition of volatile constituents was investigated under the influence of genotype and environmental

Fig. 5.2 Cultivation practices of *V. jatamansi:* A&B, wild and cultivated *V. jatamansi* plants; C & D, *V. jatamansi* plants in flowering; E&F, cultivation of *V. jatamansi* at Shri Narayan Ashram, Pithoragarh, Uttarakhand, India, by GBPNIHE

variables. Various Chinese genotypes and chemotypes of Tagar collected from seven wild areas in China and common-garden specimens were recorded based on SNP and volatile constituents. Two diverse populations were differentiated from five others based on genotypes and essential oil constituents. The uniformity of samples showed that genotype could considerably affect chemotype. Volatile profiles of wild populations were different from common-garden samples which showed that chemotypes are strongly affected by environmental variables (He et al. 2018).

5.5.2 Soil Condition

Fertile, loamy soil rich in humus along with relatively slight acidic to neutral pH (6–7) condition is preferred by *V. jatamansi*. The plant can grow over an extensive range of soils, with slopes up to 20%, if adequate water and nitrogen nutrient is there (NMPB 2008). However, shallow roots of the herb are responsible for preferential moist situation and needed proper drainage as the crop plant is unable to withstand and survive with water logging condition. Harvesting of roots can be done efficiently and easily in a relatively loose soil with low clay content. Maximum development of *V. jatamansi* plants can occur in a slope of 5–6%. In humus-rich soil under shade, strong development of species is recorded than the barren rock soil on sunny spots. The shallow roots present in herb may be responsible for this due to controlling the uptake of deep soil groundwater. This can be resulted into the adaptation of *V. jatamansi* to grow under the shelter of a tree in a forest in which moisture level is high, that decreases water requirement and allow the establishment of humus from the trees dead leaves.

5.5.3 Planting Material

V. jatamansi can be cultivated through seeds and rootstocks during the post-monsoon season. However, multiplication by rhizomatous suckers is regarded as finest due to early maturation of this plant than the plants propagated through seeds. Moreover, modern biotechnological approaches like tissue culture can be an efficient method for propagation of *V. jatamansi*.

5.5.4 Methods of Propagation

Several reports are available on propagation of *V. jatamansi* using modern biotechnological techniques. Propagation of the species was carried out through seed, rhizomes and tissue culture. The propagules are multiplied at varying periods for example, April–May month are appropriate for seed sowing while plantation of rhizomes takes place in June month. The in vitro raised plantlets are also generated using explants and PGRs in several studies (Mathur et al. 1988; Purohit et al. 2015; Dhiman et al. 2020). The plantlets were than hardened out followed by field transfer.

5.5.5 Seed Germination

V. jatamansi seeds are very small and light weighted. Approximately 0.5–1.0 kg seeds are required for sowing of the species seeds per hectare area (NMPB 2008; Dhiman et al. 2020).

The species is propagated by seeds which are sown during March–April. Sowing is done in raised beds under partial shade conditions (75% shade). Due to very small size of the seeds, sowing of seeds on surface of the nursery beds followed by covering with thin layer of soil mixture is recommended. There is the requirement of light irrigation during the seed germination to keep the soil beds moist. Once seedlings reached to 2–3 leaf stage, they can be transferred in polysleeves to remove overcrowding. The transplantation of seedlings is generally done after 3–4 months (Pal et al. 2020). Mature shade dried seeds were gathered and surface sterilized with water and than sown in bed on 25th April each year under careful investigation with sterilized clay soil. The seeds were germinated at 1290, 1550, 1800 and 2000 m asl altitude in Darjeeling, India. Maximum survival percent of *V. jatamansi* plants across 1290 to 2000 m asl displayed its adaptation at a broader altitudinal range. However, findings from this study revealed low seed germination (%) above 1800 m which prohibited its cultivation potential at lower altitudinal regions only. Study demonstrated highest seed germination when seed dipped with cow urine and also exhibited parity with the pre chilled seed treatment. It was observed that seed soaked through cow urine increases 50.3% further sprouting percentage over the normal seed sown. These findings demonstrated the baseline dataset on detection of favorable cultivation sites for developing agrotechniques for conservation of genomic resources and management approaches for this plant (Mukherjee and Chakraborty 2014). The effects of varying doses of growth hormones were evaluated on seed development parameters and seedlings vitality of *V. jatamansi*. The seeds were osmo-primed with different hormone doses of IBA, GA_3 kinetin and hydroprimed with distilled water. Maximum seed sprouting percentage was recorded with 200 ppm of kinetin-treated seeds of *V. jatamansi*.

Additionally, shortest time period in days for germination of seeds was found with 250 ppm kinetin to complete 23.33 days and displayed consistency in with 200 ppm kinetin-pretreated seeds (24.33 days). Mean germination time (33.03 days) of untreated (control) seeds of the species was considerably decreased (22.95 days) when seeds soaked with GA3 250 ppm. This time was 31.88% lesser as compared to control and was at par with 23.33 days in 200 ppm kinetin-pretreated seeds. Kinetin 200 ppm pretreated seeds exhibited highest fresh weight. Similarly, considerable variation in dry weight of 2-month-old seedling phase and significantly high dry biomass was recorded with seeds pretreated with kinetin 200 ppm. The data related to the consequence of seed vigor treatments on seedling vigor index-I in species demonstrated that control seeds exhibited minimum SV-I (51.87), that was significantly low than the rest of the treatments. Seeds pretreated with 100 ppm kinetin displayed highest SV-I (303.33). Moreover, analysis demonstrated that 50 ppm kinetin exerted lowest SV-II which was same with 250 ppm IBA and control seeds recorded. The

highest SV-II (190.06) was detected in recorded 200 ppm kinetin-pretreated seeds, which was 362.32% higher as compared to control and was significantly improved to other treatments. Maximum seedling length (3.11 cm) was recorded with 200 ppm of kinetin. Highest EI was found with 200 ppm of kinetin and displayed parity with 250 ppm kinetin-pretreated seeds (Mukherjee 2018).

5.5.6 Macropropagation

Root suckers of *V. jatamansi* which can be used as a planting material is needed to maintain in a separate mother nursery. Plantation of the sucker can be done by taking out the fresh root suckers from the mother nursery for field plantation. However, the month of June or onset of monsoon is appropriate for plantation of new sucker in the nursery. The crop of *V. jatamansi* can be raised through seeds by preparing a separate nursery in April–May. The seeds are germinated within 15–20 days and placed into polybags for further development and will be ready for planting in next three month. Planting of rhizomes in June month is most favorable. However, successful multiplication can be obtained using old rhizomes/rootstock (NMPB 2008). A simple cost-effective method has been established for vegetative production of *V. jatamansi* through macroproliferation in large amounts. This method ensures that each propagule possesses some part of shoot along with rhizome part and some roots at the time of separation, ensuring rapid establishment and practically 100 per cent survival of the propagated material. The most vital component identified for the success of this method is time of separation, portion of shoot/root/rhizome to be retained in each propagule and providing suitable growing conditions for planting the propagules. The technique has been successfully applied for the production of 2.8 lakhs nursery stock of *V. jatamansi* during 2004–2008 under National Medicinal Plants Board (NMPB) funded project completed by Himalayan Forest Research Institute. A desirable number of nursery stocks were maintained permanently in the nursery for propagation in future using this technique without depleting natural population (Sharma et al. 2012b). Propagation of *V. jatamansi* plant raised by cutting and separating the rhizomes is reported to generate various plants. These plants were sown in the month of rainy season in the well-prepared field in the month of June–July (Mukherjee 2015). It is observed that spacing between plants of this herb plays considerable role in growth of fresh aerial biomass. Findings from study revealed that spacing of 30 × 45 cm at 6-, 9-, 12-, 15- and 18-month-old plants of *V. jatamansi* possess higher biomass. Transplanting time and space was well at 9-month-old stage for rhizome weight per plant than other stages to obtain underground biomass. Likewise, higher biomass of underground part was recorded in June transplanting. Space of 30 cm × 45 cm provides considerable positive response at 9- and 12-month stages of *V. jatamansi* (Mukherjee and Chakraborty 2014; Mukherjee 2015). In a study, high-altitude germplasm of *V. jatamansi* demonstrated the presence of three sex forms, namely gynoecious, gynomonoecious and bisexual. All the sex forms displayed considerable differences among all traits studied. Bisexual form exhibited

higher (16.36 g, 13.43 g) dry aerial biomass and dry rootstock biomass under open condition followed by gynomonoecious and gynoecious forms, respectively. Hence, plantation of the bisexual form of the species in open environment is suggested for high biomass yield and for commercial cultivation (Karnwal et al. 2012).

5.5.7 Biotechnological Intervention

5.5.7.1 Micropropagation

Several studies have been attempted for faster clonal and large quantity generation of MAPs with threatened endangered and rare status using modern biotechnological tools (Abraham et al. 2010). Various protocols have been established by using different types of explants of this species (Table 5.3). Adventitious shoots and somatic embryos from embryogenic callus were induced from in vitro raised *V. jatamansi* leaves. Both morphogenic developments could be stimulated on MS basal medium supplemented with varying concentrations and combinations of PGRs. Embryogenic callus, stimulated solely in the presence of 1.0–7.5 μM 2,4-D and 5.0 μM α- NAA, differentiated into adventitious shoots and somatic embryos, respectively when left on the same medium. Callus on MS medium having IBA (5.0 μM), NAA (5.0 μM) or 2,4-D (1–5 μM) stimulated the development of adventitious roots. Cytokinins that were added singly to callus initiation medium, containing 6-BA, kinetin and thidiazuron, were unable to stimulate callus at 5.0 μM except when pooled with 0.25–1.0 μM NAA (Chen et al. 2014). Purohit et al. (2015) reported the effective in vitro regeneration protocol for this herb using Nodal explants in MS basal medium containing with PGRs. Different combinations of 1.5 μM BAP, 0.5 μM NAA and 0.1 μM GA_3 in the medium exhibited maximum mean shoot length, shoot number and leaf number. A hundred percent rooting with significantly high average root number and root length was achieved in full strength MS medium accompanied with similar quantities for example BAP (1.5 μM), NAA (0.5 μM) and GA_3 (0.1 μM) combination. A separate medium for root initiation was not required. After 1 year of adaptation, a total of 91% survival of plantlets was recorded. Similarly, a rapid multiplication protocol of *V. wallichii* was established using shoot tip and axillary bud as explants (Mathur et al. 1988). Various other studies using different explants of *V. jatamansi* like shoot buds (Kaur et al. 1999), rhizomes and leaf (Das et al. 2013) were also established.

5.5.7.2 Preparation of the Field

Fields are well drained for cultivation of *V. jatamansi*. The soil of the field should be pulverized properly by plowing the field thrice before planting the species to obtain best rhizomes and roots production. The optimum period for first plowing is June month, and then during second plowing in the same month FYM at 20 t/ha should

Table 5.3 In vitro propagation method available for *V. jatamansi*

S. No.	Plant part	Medium	Hormone combination	Results	References
1	In vitro raised leaves	MS basal medium	(i) 1.0–7.5 μM 2,4-D and 5.0 μM NAA (ii) 5.0 μM IBA, 5.0 μM NAA or 1.0–5.0 μM 2,4-D	Embryogenic callus stimulated solely in the presence of auxins, namely 1.0–7.5 μM 2,4-D and 5.0 μM NAA and differentiated into adventitious shoots and somatic embryos, respectively, when placed in the same medium	Chen et al. (2014)

(continued)

Table 5.3 (continued)

S. No.	Plant part	Medium	Hormone combination	Results	References
2	Nodal explants	MS	Medium supplemented with 1.5 μM BAP, 0.5 μM NAA and 0.1 μM GA_3 Rooting Medium: 1.5 μM BAP, 0.5 μM NAA and 0.1 μM GA_3	Medium supplemented with 1.5 μM BAP, 0.5 μM NAA and 0.1 μM GA_3 exhibited maximum mean shoot length, shoot number and leaf number. A hundred percent rooting with considerably maximum average root number (27.5 ± 1.98) and root length (50 ± 1.35 cm) was obtained in full-strength MS medium accompanied with same concentration of BAP, NAA and GA_3. A total of 91% plantlets survived after 1 year of acclimatization	Purohit et al. (2015)

(continued)

Table 5.3 (continued)

S. No.	Plant part	Medium	Hormone combination	Results	References
3	Shoot tip and axillary bud explants	MS medium	MS medium comprising Kn or BAP (5.0 mg/L) in combination with IAA (1.0 mg/L)	MS medium containing Kn or BAP (5.0 mg/L) in combination with IAA (1.0 mg/L) induced an optimal growth of shoots within 6–8 days from both apical and axillary bud explants. The roots developed on the same medium within 2–3 weeks. Hardening of in vitro grown plantlets in pots under glass-house conditions was dependent upon the temperature and humidity. A cold-temperate climate favored early establishment.	Mathur et al. (1988)

(continued)

Table 5.3 (continued)

S. No.	Plant part	Medium	Hormone combination	Results	References
4	Shoot buds	Solid medium	BA alone or in combination with IAA or NAA	Rapid and large-scale propagation of *V. jatamansi* by stimulation of shoot production from shoot buds was established. The sterilized explants were established on solid medium supplemented with BA alone or in combination with IAA or NAA. The buds cultured on nutrient medium supplemented with BA and IAA or NAA formed shoots, which after 3-4 weeks produced roots on the same medium. Survival of 100% was recorded on acclimatization and field establishment of well-rooted shoots	Kaur et al. (1999)

(continued)

Table 5.3 (continued)

S. No.	Plant part	Medium	Hormone combination	Results	References
5	Rhizomes, leaf	MS medium	Different amount of 2,4-D, NAA and IBA on callus stimulation and production of valepotriates	The callus stimulation frequency was detected to be optimum in rhizome explants on media supplemented with 0.5 mg/L 2,4-D. MS medium fortified with 0.75 mg/L thidiazuron in combination with 0.5 mg/l NAA exhibited the maximum regeneration frequency (88.6%) and generated the highest shoot buds number (15.20 ± 0.20). Vigorous callus observed from MS medium supplemented with diverse concentrations of 2,4-D, NAA and IBA were used for industrially important valepotriates (acevaltrate, valtrate and didrovaltrate) analysis. HPLC evaluation of callus showed that medium with 2, 4-D (1 mg/L) was increased acevaltrate and didrovaltrate yield, whereas valtrate production was higher in case of medium supplemented with NAA (1 mg/L).	Das et al. (2013)

be mixed in the soil. The soil was made friable using final plowing (NMPB 2008; Mukherjee 2015).

5.5.7.3 Transplantation and Geometry of the Plant

August month is observed appropriate for seedlings transplantation (height 8-10 cm) from nursery to the ground. Maximum rhizome yield can be obtained by keeping the spacing between rows about 40–45 cm while in a row it should be 30 cm amidst plants (Slathia 2005). Plantation of the seedlings of species into hills in mid of August demonstrated by higher growth and below ground biomass. Below-ground biomass production of *V. jatamansi* is influenced by distance as spacing requirement of a specific species can hamper its production in different stages (Mukherjee 2015). The findings from this research exhibited a suitable geometry of crop is vital throughout transfer, as it decreases plant competition for water, nutrient, requirement of space and light, and findings in best growth of biomass at its productive prospective.

5.5.7.4 Cultivation in Different Agroforestry System

V. jatamansi is medicinal plant found to have shade loving nature and detect to accompanying with forest trees in nature. Therefore, this species possess enormous possibilities in agroforestry. Various trees species like *Robinia pseudoacacia, Acacia mollissima*, (Singh et al. 2010), *Grevillea robusta, Jacaranda acutifolia, Bauhinia variegata* and *Morus alba* (Vats et al. 2002) are found to be associated with *V. jatamansi*. The plant is also observed to grow well under *Quarecus leucotricophora, Pinus roxiburgi and Rhododendron arboreum* canopy and open grassy habitat (Jugran et al. 2013a, 2018). Paquette et al. (2006) reported that the trees canopy in a forest create undergrowth microclimate for the improved growth of *V. jatamansi* (Paquette et al. 2006).

5.5.8 Nutrition

Soil fertility is an essential factor during development of *V. jatamansi*. Hence, use of FYM is recommended at different doses. It is generally found that Indian subcontinent soil has nitrogen deficiency than the other macronutrients. Thus, the use of inorganic fertilizer, i.e., N, P & K at the scale of 150, 75 and 75 kg/ha was suggested for proficient crop (Singh et al. 2000). In contrast, biofertilizer supplementation to the crop of *V. jatamansi* increases biomass by preserving soil nutrients and enhancing plant efficiency for more nutrient uptake (Slathia 2005). Different biofertilizers like phosphate solubilizing bacteria (PSB), azotobacter (Azoto) and VAM (Vesicular-arbuscular mycorrhiza) and their mixtures (10 kg/ha ratio) were used to treat the soil of *V. jatamansi* crop. Study revealed that plants treated with mixtures in the ratio

of 1:1:1 exhibited higher N, P, K. Similarly, underground rootstock, soil P and K exhibited positive corelationship with each other. Secondary metabolites (phenolics, flavonoids, tannins and antioxidants) development in control and mycorrhiza-treated *V. jatamansi* plants showed high level of these metabolites in treated plants (Jugran et al. 2015d).

5.5.9 Water Requirement

V. jatamansi crop does not need continuous irrigation practices. During summer season to obtain the optimum development and yield irrigation followed by day's break is suggested. However, appropriate moisture in soil is required immediately after transplantation for improved establishment. Slope and soil water holding capacity are most vital parameters to govern irrigation strategy which vary from 1 to 2 weeks. Herb from plantation to establishing period needed frequent irrigation. However, the irrigation in monsoon season is not required.

5.5.10 Plant Protection from Weeds

Prior to monsoon time, a lightly plowed field soil is highly beneficial to escape from weeds intrusion in both the years during plant development. Hence, uprooting annual grasses manually is beneficial for weeding beyond herbicides use. Studies suggested that weeding can be more appropriate within 30 days of field plantation of crop, and successive removal of weeds can be performed at a gap of 25–30 days (NMPB 2008). Before the field establishment of *V. jatamansi* plants, manual weeding needed to perform in the field plants to remove competition with invasive species. Species like *Ageratum conyzoides, Bidens pilosa, Cynodon dactylon, Plantago lanceolata*, other, grasses and sedges commonly observed are recorded in the crop of Indian Valerian at CSIR-IHBT, Palampur, India. However, in Uttarakhand at few places, *Azaratus adenophora* is also recorded (personal observation). Tissue culture-raised plantlets of *V. jatamansi* have been planted at Narayan Ashram, Pithoragarh, Uttarakhand, by G.B pant National Institute to promote and demonstrate the cultivation of *V. jatamansi* among farmers for their livelihood enhancement. Secondary metabolite of plant stimulates confrontation with pathogens and pests (Schmidt et al. 2008). The molds and pests are rarely caused any harm to the plants of the herb, for example, fungal infection, rhizome rot beneath water-logged situations. In case of such situation, 0.2% Dithane M-45 can be used to administer the plant soil, which prevents growth of the fungal spore (NMPB 2008).

5.5.11 Harvesting and Yield of Biomass

A study reported that during 1st year the yield of new root stock is 3.5–4.5 ton/hectare while it is nearly twofold in the 2nd year (7.0–7.5 ton/hectare). Thus, highest produce can be obtained by crop harvesting in 2nd year of plantation in month of July which is considered as favorable month to develop plant to attain highest length of shoots and root, else decrease in produce and quality is observed in July (Singh et al. 2010). Maximum volatile oil production was recorded in May month while it was lowest in October (Singh et al. 2010). On other hand, Rawat et al. (2017) observed highest volatile oil production in winter period and lowest in the spring period. Diverse developmental stages, namely preflowering, flowering and post-flowering samples from aerial and root parts of three natural populations located at an altitudinal gradient were evaluated Jugran et al. (2021). Qualitative and qualitative differences in essential oil obtained from rhizomes in diverse growth phenophases along the altitude were revealed. Maximum phenolics, flavonoids and antioxidant property in the root and aerial parts were recorded in post-flowering stage. The study concluded that post-flowering stage is suitable to produce maximum phytochemicals and antioxidant from *V. jatamansi*. Further, highest antioxidant property in flowering condition samples from higher altitude emphasized on the requirement of compound specific agroclimatic methods for commercial benefits from cultivation of this species. The production of enriched fraction of valepotriates is maximum in month of November or January of 2nd year (Singh et al. 2010). For *V. officinalis*, the optimum time for obtaining maximum valepotriates amount is February to March (Bos et al. 1998). This noticeably suggested the variations in the amount of chemical constituents in the context of season for specific species. Iridoids level in below-ground portion ranged from 2 to 5.6% in *V. jatamansi* while highest (8.0–12.0%) content of valepotriates in *V. edulis* of this genus was detected (Holzl 1975; Bos et al. 2002). These results also exhibited winter as the dormant period for *V. jatamansi* in which the amount of valepotriates demonstrated higher while the amount of essential oil increased throughout its dynamic growing period.

5.5.12 Management of Post-harvest Produce

Medicinal and aromatic plant (MAPs) crops management required suitable post-harvesting method. It involves handling, storage and other method of processing followed by mature crop harvesting helps to maintain the product quality for storage of a longer period. Some of the essential attributes determining superiority, e.g., color, moisture, active constituents and issues related to microbes are of serious concern with safety issue (Yahia 2006). Several parameters decreased the quality after crop harvesting if appropriate post-harvesting management practices are not followed. In case of storage conditions, most dominant post-harvest factors are relative humidity,

temperature, light, oxygen availability and atmospheric compositions which influence the quality of essential oil (Turek and Stintzing 2013). Among above-mentioned variables, most important factor is temperature which influence the products quality. Hence, the harvested material particularly rhizomes should be dried within 35–40 °C temperature. The options of deprivation of valepotriates constituents are higher above the mentioned temperature resulting in the formation of a yellow-colored compound known as baldrinals (Denee et al. 1979), which contain valtrate and acevaltrate isovaltrate (Hobbs 1989). In a study, valepotriates were not identified subsequently placing the sample at 36 °C for two weeks, as it degenerates rapidly in higher temperature and ultimately transformed into baldrinal, that comprises reactive constituents, namely acevaltrate and valtrate (Bos et al. 1996). The valtrate and acevaltrate are very responsive in nature and consequently can be utilized for polymers formation (Steinegger and Hansel 1992).

5.5.13 Genetic Diversity for Elite Identification

Studies are available on the genetic characterization, evaluation of genetic variations and elite identification of *V. jatamansi*. Genetic characterization of 7 morphotypes of the species was carried out using RAPD (Random amplified DNA polymorphic DNA) markers (Singh 2007). Similarly, analysis of six populations of Tagar was conducted by amplified fragment length polymorphism (AFLP) markers (Rajkumar et al. 2011) and displayed higher intra and low among population variations. Genetic variations of *V. jatamansi* gathered from 25 distant populations from Uttarakhand were investigated using ISSR & SSR markers (Jugran et al. 2013a, b, 2015a). The approaches using morphological, phytochemical and genetic variations data for elite identification and conservation of the elite *V. jatamansi* population and individuals were suggested (Jugran et al. 2016b, 2018). Likewise, several inter-simple sequence repeats (ISSR) makers associated with antioxidant property using analysis of molecular variance through locus-by-locus approach were identified (Jugran et al. 2013b). Similarly, ISSR markers linked with valerenic acid, phenolics and antioxidant properties were identified in this plant (Jugran et al. 2015b). Such studies are beneficial to identify suitable quality material for breeding as well as for large scale cultivation in the farmer's field.

5.6 Conclusions

In this review, we sought to project the clinical importance of *V. jatamansi* used traditionally in India and across the world. Researches on this herb highlighted particularly its pharmacological properties and secondary metabolites composition. Various disorders like diarrhea, stress, nervous complaints and gastrointestinal ailments are reported to be administered with *V. jatamansi*. However, authentication of the use of

species in such studies is needed to done by clinical trials for longer period. Further methods of cultivation and domestication of the species were also analyzed. As the herb is largely being utilized in multiple herbal mixture for formulation of diverse medicines and it is challenging to dedicate a specific remedial property is exclusively because of the constituents from this herb in the medicine, hence, it is important to measure bioguided isolation property to detect precise action of constituent. Besides, separation of metabolites and advancement in analytical tools of several in vivo and in vitro reports will carry several prospects to additionally decipher its potent biological properties. Moreover, existing research showed large intra- and inter-variations in the species or population; therefore, study should be focused on multiple locations/multi-populational samples to detect the effective molecule and new chemotypes. Moreover, the pharmacological investigations on this herb recommended its ability to be a potential source of drug for numerous diseases. Hence, strong clinical evidences, on this plant and constituents, would be critical for its protection and to assess the potential of the species to be used as a source of modern medicine. Although multiplication protocols for target species with 100% survival are reported, their genetic stability is also needed to be ensured not only comparing with few markers but in terms of their active constituents. For example, Himbala is a variety of *V. jatamansi* already developed by CSIR-IHBT Palampur which possesses high valeopotriates content. Recently, a new variety named as 'Him Surbhit' (CSIR-IHBT-VJ-05) has been developed by same institute comprises higher root biomass yield (3.40–4.50 tonnes/ha) and essential oil (0.29–0.31%) followed by two years of growth. Multi-location trials on species showed vigorous growth with higher adaptability in mid- and high hill regions (Pal et al. 2020). More new genotypes/population is needed to be explored for such varietal development. Further bisexual form of the species revealed higher biomass yield when cultivated in open environment. Therefore, emphasis is needed to be given on bisexual form for cultivation and breeding.

Acknowledgements We would like to thank Director G.B. Pant 'National Institute of Himalayan Environment' (NIHE) for providing the facilities and encouragement. AKJ is thankful to National Medicinal Plant Board (NMPB), New Delhi (File No. Z.18017/187/CSS/R&D/UK-01/2019-20-NMPB-IV A), and IDB is thankful to National Mission on Himalayan Studies (NMHS) for project grant (**Project ID** NMHS_ID_MG_34) for partial support of this work. All authors declare no conflicts of interest in publishing this manuscript.

References

Abraham J, Cheruvathur MK, Mani B, Thomas TD (2010) A rapid in vitro multiplication system for commercial propagation of pharmaceutically important Cycleapeltata (Lam) Hook & Thoms. based on enhanced axillary branching. Ind Crop Prod 31(1):92–98

Agnihotri S, Wakode S, Ali M (2011) Chemical composition, antimicrobial and topical anti-inflammatory activity of *Valeriana jatamansi* Jones. essential oil. J Essent Oil Bear Plant 14:417–422

Arora RB, Arora CK (1963) Hypotensive and tranquillizing activity of jatamansone (valeranone) a sesquiterpene from *Nardostachys jatamansi* DC. In: Chen KK, Mukerji B (eds) Pharmacology of oriental plants. Pergamon Press, Oxford, pp 51–60

Awan HMH (1990) Kitabulmufradat. GA Printers, Lahore, pp 178–179

Babu P, Verma SK, Mathur A (2015) Screening of solvent extracts of *valeriana jatamansi* for isolation of antimicrobial compound. Int J Pharm Sci Res 6:2641–2648

Baibado JT, Cheung HY (2011) Mini-Review on neuropsychiatric properties of the root extract of Valerian (*Valeriana officinalis* L.) Hong Kong Physiol J 18:70–81

Baquar SR (1989) Medicinal and poisonous plants of Pakistan. Karachi, Printas, p 461

Becker H, Chavadeoi S (1985) Valepotriate production of normal and colchicine-treated cell suspension cultures of *Valeriana wallichii*. J Nat Prod 48:17–21

Bhatt ID, Dauthal P, Rawat S, Gaira KS, Jugran A, Rawal RS, Dhar U (2012) Characterization of essential oil composition, phenolic content, and antioxidant properties in wild and planted individuals of *Valeriana jatamansi* Jones. Sci Horticult 136:61–68

Bhattacharjee SK (2008) Handbook of medicinal plants. Pointer Publisher, Jaipur, pp 364–365

Bhattacharya D, Jana U, Debnath PK, Sur TK (2007) Initial exploratory observational pharmacology of *Valeriana wallichii* on stress management: a clinical report. Indian J Exp Biol 45:764–769

Bos R, Hendriks H, Scheffer JJC, Woerdenbas HJ (1998) Cytotoxic potential of valerian constituents and valerian tinctures. Phytomedicine 5(3):219–225

Bos R, Woerdenbag HJ, Hendriks H, Smit HF, Wikström HV, Scheffer JJ (1997) Composition of essential oil from roots and rhizomes of valeriana wallichii DC. Flav Frag J 12(2):123–132

Bos R, Woerdenbag HJ, Hendriks H, Zwaving JH, De Smet PAGM, Tittel G, Wikstrom HV, Scheffer JJC (1996) Analytical aspects of phytotherapeutic valerian preparations. Phytochem Anal 7:143–151

Bos R, Woerdenbag HJ, Pras N (2002) Determination of valepotriates. J chromatograph A 967(1):131–146

Bounthanh C, Bergmann C, Beck JP, Hagg-Berrurier M, Anton R (1981) Valepotriates, a new class of cytotoxic and antitumor agents. Plant Med 41:21–28

Chen R, Zhang M, Lu J, Zhang X, Teixeira da Silva JA Ma, G (2014) Shoot organogenesis and somatic embryogenesis from leaf explants of *Valeriana jatamansi* Jones. Sci Horticult 165:392–397

Chevallier A (1996) The encyclopaedia of medicinal plants. Dorling Kindersley, London (seen at http://www.himalayahealthcare.com/aboutayurveda/cahv.html)

Chevallier A (1999) The encyclopedia of medicinal plants. Dorling Kindersley London (seen at http://www.diet-and-healthnet/neuropathy/valerian.html)

Cionga E (1961) Considerations on the root of valerian. Pharmazie1 6: 43–44

Das J, Mao AA, Handique PJ (2013) Callus-mediated organogenesis and effect of growth regulators on production of different valepotriates in Indian valerian (*Valeriana jatamansi* Jones.). Acta Physiol Plant 35(1):55–63

Denee R, Bos R, Hazelhoff B (1979) Isolation and structure elucidation of isovaltral, a decomposition product of isovaltrate. Planta Med 37(9):45–48

Dhiman B, Sharma P, Shivani, PK Pal (2020) Biology, chemical diversity, agronomy, conservation and industrial importance of *Valeriana jatamansi*: a natural sedative JARMAP 16:100243

Dinda B, Chowdhury DR, Mohanta BC (2009) Naturally occurring iridoids, secoiridoids and their bioactivity. An updated review, part 3. Chem Pharm Bull (Tokyo) 57:765–796

Dong FW, Yang L, Wu ZK, Wei-Gao Zi CT, Yang D, Luo HR, Zhou J, Hu JM (2015) Iridoids and sesquiterpenoids from the roots of *Valeriana jatamansi* Jones. Fitoterapia 102:27–34

Dunaev VV, Trzhetsinskii SD, Tishkin VS, Fursa NS, Linenko VI. (1987) Biological activity of the sum of the valepotriates isolated from *Valeriana alliariifolia*. Biologicheskaiaaktivnost' summy valepotriatov, vydelennykhi zvaleriany chesno chnikolistnoi. Farmakol Toksikol 50:33–37 (Published in Russian)

Fernandez S, Wasowski C, Paladini AC, Marder M (2004) Sedative and sleep enhancing properties of linarin, a flavonoids isolated from *Valeriana officinalis*. Pharmacol Biochem Behav 77:399–404

Ganta KK, Mandal A, Debnath S, Hazra B, Chaubey B (2017) Anti-HCV activity from semi-purified methanolic root extracts of *Valeriana wallichii*. Phyto Res 31:433–440

Gilani AH, Khan AU, Jabeena Q, Subhan F, Ghafar R (2005) Antispasmodic and blood pressure lowering effects of *Valeriana wallichii* are mediated through K+ channel activation. J Ethnopharmacol 100:347–352

Girgune JB, Jain NK, Garg BD (1980) Antimicorbial activity of the essential oil from *Valeriana wallichii* DC (Valerianaceae). Indian J Microbiol 20:142–143

He X, Wang S, Shi J, Sun Z, Lei Z, Yin Z, Qian Z, Tang H, Xie H (2018) Genotypic and environmental effects on the volatile Chemotype of *Valeriana jatamans*i Jones. Front Plant Sci 9:1003. https://doi.org/10.3389/fpls.2018.01003

Hendricks H, Geertsma HJ, Malingre TM (1981) The occurrence of valeranone and crypto-fauronol in the essential oil of *Valeriana offcinalis* L. s. l. collected in the northern part of The Netherlands. Pharmaceutisch Weekblad Sci Ed 116:1316–1320

Hobbs C (1989) Valerian: a literature review. Herbal Gram 21:19–34

Holzl J (1975) Signification of chemical races, origins, cultivars and species of the *in vivo* preparation of radioactively marked natural substances illustrated by valeriana. Planta Medica 28:301–314

Houghton PJ (1999) The scientific basis for the reputed activity of Valerian. J Pharm Pharmacol 51:505–512

Irshad M, Aziz S, Habib-ur-Rehman Hussain H (2012) GC-MS analysis and antifungal activity of essential oils of *Angelica glauca*, *Plectranthus rugosus* and *Valeriana wallichii*. J Essent Oil Bear Plant 15:15–21

IUCN (1993) Guidelines on the conservation of medicinal plants. The International Union for Conservation of Nature and Natural Resources (IUCN), Gland, Switzerland, p 38

Joseph L, Puthallath RE, Rao SN (2016) Acute and chronic toxicity study of *Valeriana wallichii* rhizome hydro-ethanolic extract in Swiss albino mice. Asian J Med Sci 7:49–54. https://doi.org/10.3126/ajms.v7i2.13326

Jugran A, Rawat S, Dauthal P, Mondal S, Bhatt ID, Rawal RS (2013a) Association of ISSR markers with some biochemical traits of *Valeriana jatamansi* Jones. Ind Crop Prod 44:671–676

Jugran AK, Rawat S, Bhatt ID, Rawal RS (2021) Essential oil composition, phenolics and antioxidant activities of *Valeriana jatamansi* at different phenological stages. Plant Biosyst 155:891–8981

Jugran AK, Bahukhandi A, Dhyani P, Bhatt ID, Rawal RS, Nandi SK (2016a) Impact of altitudes and habitats on valerenic acid, total phenolics, flavonoids, tannins, and antioxidant activity of *Valeriana jatamansi*. J Appl Biochem Biotechnol 179:911–926

Jugran AK, Bhatt ID, Mondal S, Rawal RS, Nandi SK (2015a) Genetic diversity of *Valeriana jatamansi* across habitat types and altitudinal range using nuclear and chloroplast microsatellite markers. Curr Sci 109:1273–1282

Jugran AK, Bhatt ID, Rawal RS (2015b) Identification of ISSR markers associated with valerenic acid and antioxidant activity in *Valeriana jatamansi* Jones in Western Himalaya. Mol Breed 35:1–14

Jugran AK, Bahukhandi A, Bhatt ID, Rawal RS, Nandi SK (2015c) H_2O_2 induced DNA damage preventive activity of selected *Valeriana* species from West Himalaya. Proc Natl Acad Sci India, Sect B Biol Sci. 10.1007/s40011-015-0559-0

Jugran AK, Bahukhandi A, Dhyani P, Bhatt ID, Rawal RS, Nandi SK, Palni LMS (2015d) The effect of inoculation with mycorrhiza: AM on growth, phenolics, tannins, phenolic composition and antioxidant activity in *Valeriana jatamansi* Jones. J Soil Sci Plant Nutri 15:1036–1049

Jugran AK, Bhatt ID, Rawal RS (2016b) Integrated approaches for conservation and effective utilization of *V. jatamansi* in Uttarakhand. Asia Pacific J Biodivers 9:152–159

Jugran AK, Bhatt ID, Rawal RS, Nandi SK, Pande V (2013b) Patterns of morphological and genetic diversity of *Valeriana jatamansi* Jones in different habitats and altitudinal range of West Himalaya, India. Flora 208:13–21

Jugran AK, Joshi RK, Bhatt ID, Rawal RS, Palni LMS (2018) The relationship of visiting insect diversity and density of *Valeriana jatamansi* with increasing altitude in Western Himalaya. Proc Natl Acad Sci India Sect B Biol Sci. https://doi.org/10.1007/s40011-017-0954-9

Jugran AK, Rawat S, Bhatt ID, Rawal RS (2019) *Valeriana jatamansi*: an herbaceous plant with multiple medicinal uses. Phytother Res. https://doi.org/10.1002/ptr.6245

Kalim MD, Bhattacharya D, Banerjee A, Chattopadhyay S (2010) Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine. BMC Compl Alt Med 10:77. http://www.biomedcentral.com/1472-6882/10/77

Kapoor LD (1990) CRC Handbook of ayurvedic medicinal plants. CRC Press, Boca Raton, p 330

Karnwal MK, Rai R, Singh VP (2012) Effect of sex form and environment on biomass yield in *Valeriana jatamansi* Jones. Progressive Horticult 44(1):55–59

Katoch O, Kaushik S, Kumar MSY, Agrawal PK, Misra K (2012) Radioprotective property of an aqueous extract from *Valeriana wallichii*. J Pharm Bioall Sci 4:327–332

Kaur R, Sood M, Chander S, Mahajan R, Kumar V, Sharma DR (1999) In vitro propagation of *Valeriana jatamansi*. Plant Cell, Tissue Organ Cult 59(3):227–229

Keochanthala-Bounthanh C, Haag- Berrurier M, Beck JP, Anton R (1993) Effects of two monoterpene esters, valtrate and didrovaltrate, isolated from *Valeriana wallichi*, on the ultrastructure of hepatoma cells in culture. Phyto Res 7:124–127

Khajuria A, Verma S, Sharma P (2011) Stylar movement in *Valeriana wallichii* DC.—A contrivance for reproductive assurance and species survival. Curr Sci 100:1143–1144

Khan AU, Gilani AH (2011) Pharmacological basis for the medicinal use of *Valeriana wallichii* in Constipation. Latin Am J Pharm 30:186–188

Khuda F, Iqbal Z, Khan A, Zakiullah Nasir F, Shah Y (2013) Anti-inflammatory activity of the topical preparation of *Valeriana wallichii* and *Achyranthes aspera* leaves. Pak J Pharm Sci 26:451–454

Khuda F, Iqbal Z, Khan A, Zakiullah Shah Y, Khan A (2014) Screening of selected medicinal plants for their enzyme inhibitory potential—a validation of their ethnopharmacological uses. Pak J Pharm Sci 27:593–596

Khuda F, Iqbal Z, Zakiullah KA, Nasir F (2012) Antimicrobial and anti-inflammatory activities of leaf extract of *Valeriana wallichii* DC. Pak J Pharm Sci 25:715–719

Leathwood PD, Chauffard F (1983) Quantifying the effects of mild sedatives. J Psychiatric Res 17:115–122

Leuschner J, Müller J, Rudman M (1993) Characterization of the central nervous depressant activity of a commercially available Valerian root extract. Arzneim Forschung 43:638–641

Li YD, Wu ZY, Li HM, Li HZ, Li RT (2013) Iridoids from the Roots of *Valeriana jatamansi*. Helve Chim Acta 96:424–430

Lin S, Chen T, Fu P, Ye J, Yang XW, Shan L, Li HL, Liu RH, Shen YH, Xu XK, Zhang WD (2015a) Three decomposition products of valepotriates from *Valeriana jatamansi* and their cytotoxic activity. J Asian Nat Prod Res 17:455–461

Lin S, Chen T, Liu XH, Shen YH, Li HL, Shan L, Liu RH, Xu XK, Zhang WD, Wang HJ (2010) Iridoids and lignans from *Valeriana jatamansi*. J Nat Prod 73:632–638

Lin S, Fu P, Chen T, Ye J, Su YQ, Yang XW, Zhang ZX, Zhang WD (2015b) Minor valepotriates from *Valeriana jatamansi* and their cytotoxicity against metastatic prostate cancer cells. Plant Med 81:56–61

Lin S, Shen YH, Li HL, Yang XW, Chen T, Lu LH, Huang ZS, Liu RH, Xu XK, Zhang WD, Wang H (2009) Acylated iridoids with cytotoxicity from *Valeriana jatamansi*. J Nat Prod 72:650–655

Lin S, Zhang ZX, Chen T, Ye J, Dai WX, Shan L, Su J, Shen YH, Li HL, Liu RH, Xu XK, Wang H, Zhang WD (2013) Characterization of chlorinated valepotriates from *Valeriana jatamansi*. Phytochemistry 85:185–193

Lin S, Fu P, Chen T, Ye J, Yang XW, Zhang WD (2017) Three minor valepotriate isomers from *Valeriana jatamansi* and their cytotoxicity. J Asian Nat Prod Res 19:15–21. https://doi.org/10.1080/10286020.2016.1258065

Liu XC, Zhou L, Liu ZL (2013) Identification of insecticidal constituents from the essential oil of *Valeriana jatamansi* Jones against *Liposcelis bostrychophila* Badonnel. J Chem Article ID 853912, http://dx.doi.org/10.1155/2013/853912

Marder M, Viola H, Wasowski C, Fernandez S, Medina JH, Paladini AC (2003) 6-methylapigenin and hesperidins: new Valeriana flavonoids with activity on the CNS. Pharmacol Biochem Behav 75:537–545

Mathela CS, Tiwari M, Sammal SS, Chanotiya CS (2005) *Valeriana wallichii* DC, a new chemotype from North-Western Himalaya. J Essent Oil Res 17(6):672–675

Mathur J, Ahuja PS (1991) Plant regeneration from callus cultures of *Valeriana wallichii* DC. Plant Cell Rep 9:523–526

Mathur J, Ahuja PS, Mathur A, Kukreja AK, Shah NC (1988) In vitro propagation of *Valeriana wallichii*. Plant Med 54(1):82–83

Morazzoni P, Bombardelli E (1995) *Valeriana officinalis* traditional use and recent evaluation of activity. Fitoterapia 66:99–112

Mukherjee D (2015) Studies on seed germination pattern and effect of sowing date with planting prototype on *Valeriana jatamansi* (Jones): a rare medicinal plant of Eastern Himalaya. J Crop Weed 11(2):9–13

Mukherjee D (2018) Effect of different seed priming treatments on germination and seedling establishment of two threatened endangered medicinal plants of Darjeeling Himalaya. In: Rakshit A, Singh HB (eds) Advances in seed priming. Springer Nature Singapore Pte Ltd., Singapore, p 241. https://doi.org/10.1007/978-981-13-0032-5_13

Mukherjee D, Chakraborty S (2014) Studies on ecology, habitats diversification and seed germination behavior of *Valeriana jatamansi* Jones: a critical endangered plant. Int J Agric Sci Res 4(5):203–209

Nadkarni KM (1976) Indian Medica, III edn. Popular Prakashan, Mumbai, pp 1260–1262

National Medicinal Plant Board (NMPB) (2008) Agro-techniques of selected medicinal plants. National Medicinal Plants Board Volume-I Department of AYUSH, Ministry of Health and Family Welfare, Government of India, New Delhi, 110001

Navarrete A, Avula B, Choi YW (2006) Chemical fingerprinting of *Valeriana* species: simultaneous determination of valerenic acids, flavonoids, and phenylpropanoids using liquid chromatography with ultraviolet detection. J AOAC Int 89:8–15

Pal PK, Sharma RK, Kumar D, Kumar S (2020). Indian Valerian (*Valeriana jatamansi*) variety Him Surbhit (CSIR-IHBT-VJ-05). Technical Folder: March, 2020

Pande A, Shukla YN (1993) Naphthoic acid derivative from *Valeriana wallichii*. Phytochemistry 32:1350–1351

Pandian DS, Nagarajan NS (2015) Comparison of chemical composition and antioxidant potential of hydrodistilled oil and supercritical fluid CO_2 extract of *Valeriana wallichi* DC. J Nat Prod Res J Nat Prod Res 1:25–30

Paquette A, Bouchard A, Cogliastro A (2006) Survival and growth of under-planted trees: a meta-analysis across four biomes. Ecol Appl 16(4):1575–1589

Phondani PC, Bhatt ID, Negi VS, Kothyari BP, Bhatt A, Maikhuri RK (2016) Promoting medicinal plants cultivation as a tool for biodiversity conservation and livelihood enhancement in Indian Himalaya. J Asia-Pacific Biodiver 9:39e46

Polunin O, Stainton A (1987) Concise flowers of the Himalaya. Oxford University Press, London

Prakash V (1999) Indian Valerianaceae: a monograph on medicinally important family. Scientific Publishers, Jodhpur, India, p 70

Prasad R, Naime M, Routray I, Mahmood A, Khan F, Ali S (2010) *Valeriana jatamansi* partially reverses liver cirrhosis and tissue hyperproliferative response in rat. Method Finding Exper Clin Pharm 32:713–719

Purohit S, Rawat V, Jugran AK, Singh RV, Bhatt ID, Nandi SK (2015) Micropropagation and genetic fidelity analysis in *Valeriana jatamansi* Jones. JARMAP 2(1):15–20

Raina AP, Negi KS (2015) Essential oil composition of *Valeriana jatamansi* Jones from Himalayan regions of India. Indian J Pharm Sci 77:218–222

Rajkumar S, Singh SK, Nag A, Ahuja PS (2011) Genetic structure of Indian valerian (*Valeriana jatamansi*) populations in Western Himalaya revealed by AFLP. Biochem Genet 49(9–10):674–681

Rather AM, Nawchoo IA, Wani AA, Ganie AH (2011) Effect of habitat diversification and temperature on *Valeriana jatamansi*. New York Sci J 4:57–59

Rehni AK, Pantlya HS, Rich S, Singh M (2007) Effect of chlorophyll and aqueous extracts of *Bacopa monniera* and *Valeriana wallichii* on ischaemia and reperfusion-induced cerebral injury in mice. Indian J Experim Biol 45:764–769

Ravichandran G, Bhardwaz VS, Kolhapure SA (2005) Evaluation of the efficacy and safety of antiwrinkle cream in the treatment of facial skin wrinkle: a prospective open phase II clinical trial. The Antiseptic 102:65–70

Rawat S, Jugran AK, Bhatt ID, Rawal RS, Andola HC, Dhar U (2017) Essential oil composition and antioxidant activity in Valeriana jatamansi Jones: Influence of seasons and growing sources. J Essent Oil Res 29:101–107

Riedel E, Hänsel R, Ehrke G (1982) Inhibition of γ-aminobutyric acid catabolism by valerenic acid derivatives. Plant Med 46:219–4220

Ron BH, Willis CV, Bone K, Morgan M (2000) Herbal products: active constituents, mode of action and quality control. Nutr Res Rev 13:47–77

Sah SP, Mathela CS, Chopra K (2010a) *Valeriana wallichii*: a phyto-pharmacological review. J Pharm Res 3:2337–2339

Sah SP, Mathela CS, Chopra K (2010b) Elucidation of possible mechanism of analgesic action of *Valeriana wallichii* DC chemotype(patchouli alcohol) in experimental animal models. Indian J Experim Bio 48:289–293

Sah SP, Mathela CS, Chopra K (2011) Antidepressant effect of *Valeriana wallichii* patchouli alcohol chemotype in mice: behavbiochem evid. J Ethnopharmacol 135:197–200

Sahu S, Ray K, Kumar YMS, Gupta S, Kauser H, Kumar S, Mishra K, Panjwani U (2012) *Valeriana wallichii* root extract improves sleep quality and modulates brain monoamine level in rats. Phytomedicine 19:924–929

Said HM (1970) Hamdard pharmacopoeia of eastern medicine. Times Press, Karachi, p 53

Samant SS, Dhar U, Palni LMS (1998) Medicinal Plants of Himalaya: Diversity, distribution Potential Values. Gyanodaya Prakashan, Nainital, India

Santos MS, Ferreira F, Faro C, Pires E, Carvalho AP, Cunha AP, Macedo T (1994) The amount of GABA present in aqueous extracts of Valerian is sufficient to account for [^{3}H] GABA release in synaptosomes. Plant Med 60:475–476

Sati S, Chanotiya CS, Mathela CS (2005) Comparative investigations on the leaf and root oils of *Valeriana wallichii* DC. from Northwestern Himalaya. J Essent Oil Res 17:408–409

Sati SC, Khulbe K, Joshi S (2011) Antimicrobial evaluation of the Himalayan medicinal plant *Valeriana wallichii*. Res J Microbiol 6:289–296

Schmidt B, Ribnicky DM, Poulev A, Logendra S, Cafalu WT, Raskin I (2008) A natural history of botanical therapeutics. Metabolism Clini Exper 57:3–9

Schneider G, Willems M (1982) Weitere Erkenntnisseuber die abbauprodukte der valepotriateaus Kentranthusruber (L.) DC (Further observations on the byproducts of Kentranthusruber valepotriates). Archive der Pharmazie 315:691–697

Sharma P, Kirar V, Meena DK, Suryakumar G, Misra K (2012a) Adaptogenic activity of *Valeriana wallichii* using cold, hypoxia and restraint multiple stress animal model. Biomed Aging Pathol 2:198–205

Sharma R (2003) Medicinal plants of India: An encyclopaedia. Daya Publishing House, New Delhi pp 253–254

Sharma S, Negi PS, Singh J (2012b) Vegetative propagation of *Picrorhiza kurrooa* Royle ex Benth and *Valeriana jatamansi* Jones through macroproliferation technique for large scale nursery production. Int J Farm Sci 2:91–97

Shi SN, Shi JL, Liu Y, Wang YL, Wang CG, Hou WH, Guo JY (2014) The anxiolytic effects of valtrate in rats involves changes of corticosterone levels. eCAM 325948. https://doi.org/10.1155/2014/325948

Singh N, Gupta AP, Singh B, Kaul VK (2006) Quantification of valerenic acid in *Valeriana jatamansi* and *Valeriana officinalis* by HPTLC. Chromatographia 63:209–213

Singh RD (2007) Evaluation of different variants of Muskbala (*Valeriana jatamansi*) for enhanced productivity and quality under mid hills of U.P. Final Technical Report, IHBT Palampur, Himanchal Pradesh, India

Singh RD, Ahuja PS, Nagar PK, Kaul VK, Singh B, Lal B, Vats SK, Yadav P, Mishra S (2000) Effect of manuring and shade on yield and quality of Valeriana wallichii. J Med Aroma Plant Sci 22(1B):669–670

Singh RD, Gopichand MRL, Sharma B, Bikram S, Kaul VK, Ahuja PS (2010) Seasonal variation of bioactive components in *Valeriana jatamansi* from Himachal Pradesh, India. Ind Crop Prod 32:292–296

Singh VK, Ali ZA (1998) Herbal drugs of Himalaya: medicinal plants of Garhwal and Kumaon regions of India. Today and Tomorrow's Printers and Publishers, New Delhi, pp 214

Slathia VS (2005) Studies on organic cultivation of *Valeriana jatamansi* Jones. Doctoral Dissertation, DYSP UHF Solan

Steinegger E, Hansel R (1992) Pharmakognosie, 5th edn. Springer, Berlin, pp 162–666

Subhan F, Karim N, Gilani AH, Sewell RDE (2010) Terpenoid content of *Valeriana wallichii* extracts and antidepressant-like response profiles. Photother Res 24:686–691

Subhan F, Karim N, Ibrar M (2007) Anti- inflammatory activity of methanolic and aqueous extracts of *Valeriana wallichi* DC rhizome. Pak J Plant Sci 13:103–108

Suri S, Thind TS (1978) Antibacterial activity of some essential oils. Indian Drug Pharm Ind 13:25–28

Tan YZ, Yong Y, Dong YH, Wang RJ, Li HX, Zhang H, Guo DL, Zhang SJ, Dong XP, Xie XF (2016) A new secoiridoid glycoside and a new sesquiterpenoid glycoside from *Valeriana jatamansi* with neuroprotective activity. Phytochem Lett 17:177–180

Thies PW (1968) Linarin-isovalerianate, a currently unknown flavonoid from *Valeriana wallichii*. Plant Med 16:363–371

Thies PW, Funke S (1966) On the active ingredients in baldrian 1. Detection and isolation of isovalerian acid esters with sedative effect from roots and rhizomes of various valerian and kentranthus species. Tetrahed Lett 11:1155–1162

Thind TS, Suri RK (1979) In vitro antifungal efficacy of four essential oils. Indian Perfum 23:138–140

Thusoo S, Gupta S, Sudan R, Kour J, Bhagat S, Hussain R, Bhagat M (2014) Antioxidant activity of essential oil and extracts of *Valeriana jatamansi* roots. BioMed Res Inter Article ID 614187. https://doi.org/10.1155/2014/614187

Turek C, Stintzing FC (2013) Stability of essential oils: a review. Comprehensive Rev Food Sci Saf 12:40–53. https://doi.org/10.1111/1541-4337.12006

Vats SK, Pandey S, Nagar PK (2002) Photosynthetic response to irradiance in *Valeriana jatamansi* Jones, a threatened understorey medicinal herb of Western Himalaya. Photosynthetica 40(4):625–628

Veith J, Schneider G, Lemmer B, Willems M (1986) The influence of some degradation products of valepotriates on the motor activity of light-dark synchronized mice (in German). Planta Med 52:179–183

Verma RS, Padalia RC, Chauhan A (2013) Chemical differentiation of rhizome and root essential oils of Indian Valerian (*Valeriana jatamansi* Jones). J Essent Oil Bear Plant 16:835–840

Vinegar R, Schreiber W, Hugo R (1969) Biphasic development of carrageenan oedema in rats. J Pharmacol Experim Therap 166:96–103

Vishwakarma S, Goyal R, Gupta V, Dhar KL (2016) GABAergic effect of valeric acid from *Valeriana wallichii* in amelioration of ICV STZ induced dementia in rats. Revista Brasileira de Farmacognosia 26:484–489

Wagner H, Jurcic K, Schaette R (1980) Comparative studies on the sedative action of Valeriana extracts, valepotriates and their degradation products. Plant Med 39:358–365

Wasowski C, Marder M, Viola H, Medina JH, Paladini AC (2002) Isolation and identification of 6-methylapigenin, a competitive ligand for the brain GABA (A) receptors, from *Valerianawallichii*. Plant Med 68:934–936

Xu J, Guo P, Guo Y, Fang L, Li Y, Sun Z, Gui L (2012a) Iridoids from the roots of *Valeriana jatamansi* and their biological activities. Nat Prod Res 26:1996–2001

Xu J, Guo Y, Xie C, Jin DQ, Gao J, Gui L (2012b) Isolation and neuroprotective activities of acylated iridoids from *Valeriana jatamansi*. Chem Biodivers 9:1382–1388

Xu J, Li Y, Guo Y, Guo P, Yamakuni T, Ohizumi Y (2012c) Isolation, structural elucidation, and neuroprotective effects of iridoids from *Valeriana jatamansi*. Biosci Biotechnol Biochem 76:1401–1403

Xu J, Yang B, Guo Y, Jin D, Guo P, Liu C, Hou W, Zhang T, Gui L, Sun Z (2011a) Neuroprotective bakkenolides from the roots of *V. jatamansi*. Fitoterapia 82:849–853

Xu J, Zhao P, Guo Y, Xie C, Jin DQ, Ma Y, Hou W, Zhang T (2011b) Iridoids from the roots of *Valeriana jatamansi* and their neuroprotective effects. Fitoterapia 82:1133–1136

Xu J, Guo P, Fang LZ, Li YS, Guo YQ (2012d) Iridoids from the roots of *Valeriana jatamansi*. J Asian Nat Prod Res 14:1–6

Yahia EM (2006) Postharvest handling of aromatic and medicinal plants

Yan ZY, Zhang TE, Pan LZ, Zuo CY (2011) Action of *Valeriana jatamansi* Jones on the apoptosis-related genes expression in the anxiety model of Rat. Proced Environ Sci 8:744–750

Yang B, Zhu R, Tian S, Wang Y, Lou S, Zhao H (2017) Jatamanvaltrate P induces cell cycle arrest, apoptosis and autophagy in human breast cancer cells in vitro and in vivo. Biomed Pharmacother 10:1027–1036

Zhang T, Chen C, Chen, C, Li S, Zuo C, Yan ZY (2012) Effect on TGF-beta signaling pathway of the total flavonoids from *Valeriana jatamansi* Jones in hepatocarcinoma 22-bearing mice. In: 2012 International conference on biomedical engineering and biotechnology. 10.1109/iCBEB.2012.172

Zhu J, Xu K, Zhang X, Cao J, Jia Z, Yang R, Ma C, Chen C, Zhang T, Yan Z (2016) Studies on the regulation of lipid metabolism and its mechanism of the iridoids rich fraction in *Valeriana jatamansi* Jones. Biomed Pharmacother 84:1891–1898

Chapter 6
Schisandra chinensis and *Schisandra sphenanthera*—From Traditional Far Eastern Medicine to International Utilization

Karolina Jafernik, Halina M. Ekiert, and Agnieszka Szopa

Abstract The fruit of *Schisandra chinensis* (Chinese magnolia vine) is one of the many raw materials derived from traditional Chinese medicine that is appreciated in modern phytotherapy, as well as in the cosmetics and food industries. Apart from this raw material, the lesser-known fruit of *S. sphenanthera* is also highly valued in Far Eastern medicine. This species is often confused with *S. chinensis*. In this chapter, comparative characteristics of both species are presented. Particular attention is paid to the differences in their occurrence, chemical compositions and possible applications. A broad review of the scientific literature has been made, and the most important information on the biological activity, industrial use and the progress in biotechnology research on the two species has been collected.

Keywords Chinese magnolia vine · Bei wu wei zi · Wu wei zi · Huazhong wu wei zi · Nan wu wei zi · Schisandra · Schisandra lignans · Utilization · Pharmaceutical use · Cosmetological application · Importance in food industry

6.1 Introduction

One of the most famous representatives of the genus *Schisandra* is *Schisandra chinensis* (Turcz.) Baill—Chinese magnolia vine ("bei wu wei zi" and "wu wei zi" in Chinese) (Chinese Pharmacopoeia Commission 2005; Szopa et al. 2017, 2018d, 2020). The raw material of *S. chinensis* is the fruit—*Schisandrae chinensis fructus*. Extracts of the fruit exhibit high biological activity and have been known for centuries in traditional Chinese medicine (TCM) (Hancke et al. 1999; Szopa et al. 2017, 2020). Monographs on the Schisandra fruit are listed in the pharmacopoeias of the Far East countries: Chinese (Chinese Pharmacopoeia Commission 2005), Korean

K. Jafernik · H. M. Ekiert · A. Szopa (✉)
Department of Pharmaceutical Botany, Jagiellonian University, Medical College Medyczna 9, 30-688 Kraków, Poland
e-mail: a.szopa@uj.edu.pl

H. M. Ekiert
e-mail: halina.ekiert@uj.edu.pl

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_6

(Central Pharmaceutical Affairs Council of Korea 2002), Japanese (Committee of the Japanese Pharmacopoeia Evaluation and Licensing Division Pharmaceuticals and Food Safety 2006) and also in the Russian Pharmacopoeia (Shaitan 2005). A monograph on the raw material can also be found in the American Pharmacopoeia (Upton et al. 2011). A monograph on *Schisandrae fructus* is also listed in the 10th European Pharmacopoeia (European Directorate for the Quality of Medicine 2010) and Polish Pharmacopoeia XI (Urząd Rejestracji Produktów Leczniczych Wyrobów Medycznych i Produktów Biobójczych 2018). Since 2008, this raw material has had a monograph in "Monographs on selected medicinal plants" published by WHO (World Health Organization) (World Health Organization 2007). Moreover, since 2010, *S. chinensis* fruit has had a positive opinion of European Food Safety Authority (EFSA). Additionally, this species has been positively evaluated by the European Union Inventory of Cosmetic Ingredients—CosIng (European Commission CosIng 2020).

Schisandra sphenanthera Rehder & E. H. Wilson ("huazhong wu wei zi" and "nan wu wei zi" in Chinese) has been confused with *S. chinensis* for years (Huyke et al. 2007; Sun et al. 2010). In 2000, the Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission 2005) separated these two species of raw materials by introducing two separate monographs entitled "*Fructus Schisandrae chinensis*" and "*Fructus Schisandrae sphenantherae.*" There are similarities, but also significant differences, in their chemical compositions and biological activities.

There is also the problem with the classification of *S. sphenanthera* because of the use of its name in Chinese—"nan wu wei zi," which is assigned to a completely different species, *Kadsura longipedunculata*, and for years has been incorrectly used also for *S. sphenanthera* (Zhu et al. 2007; Li et al. 2018). In the European countries and the USA, *S. sphenanthera* is a little-known species, there is no positive opinion on it from EFSA, and the raw material has no monograph in the WHO Pharmacopoeia. Only *S. sphenanthera* fruit extract is included in the CosIng database (European Commission CosIng 2020), which is synonymous with the possibility of using it in cosmetic preparations (European Commission CosIng 2020). Interestingly, this species is often used for medicinal purposes interchangeably with *S. chinensis,* which has been influenced by TCM applications. Nevertheless, modern scientific research has proved that these species are different in many ways, which is presented in this chapter.

6.2 Botanical and Ecological Characteristics

The genus *Schisandra* includes 25 officially accepted species that are naturally occurring in Southeast Asia (http://www.theplantlist.org/; Hancke et al. 1999; Saunders 2000). Twelve of them are endemic to China. *Schisandra glabra* (bay star-vine) is the only American species which is native to the southeastern USA and northern Mexico. Indeed, one of the first species of the genus *Schisandra* to be described was *S. glabra* in 1803, followed by *S. chinensis* and *S. propinqua* in 1868. The species of

this genus differ in the chemical composition of the fruit, leaves, flowers and stems, and also in their morphological structure (Saunders 2000).

The most important in terms of exploitation and utility is *S. chinensis*. In "The Plant List" database, there are listed nine synonymous Latin botanical names of this species: *Kadsura chinensis* Turcz., *Maximowiczia* amurensis Rupr., *M. chinensis* (Turcz.) Rupr., *M. japonica* (A. Gray) K. Koch, *M. sinensis* Rob., *S. chinensis* var. *glabrata* Nakai ex T. Mori, *S. chinensis* var. *leucocarpa* P. H. Huang & L. H. Zhuo, *S. viridicarpa* Y. N. Lee and *Sphaerostema japonicum* A. Gray (http://www.theplantlist.org/).

The natural habitats of *S. chinensis* are located in northeastern China, in Korea and Japan, as well as in eastern Russia, in Primorsk, on the Kuril Islands and in the southern part of Sakhalin Island (Saunders 2000). In China, *S. chinensis* grows naturally in the area of Hebei, Liaoning, Jilin, Shandong, Shanxi, Henan, Jiangsu and Anhui provinces (Saunders 2000). *S. chinensis* is also grown in the Baishilazi Nature Reserve, Fenghuang Mountain Nature Reserve, Tan Mountain Forest Garden and Laotuding in northeast China (Zhao et al. 2013b). In South Korea, *S. chinensis* grows in Mungyeong in Northern Gyeongsang Province, Jangsu in North Jeolla Province and Hoengseong in Gangwon Province (Lee et al. 2011).

This species usually occurs at the periphery of mixed forests, often near streams. *S. chinensis* is a dioecious climber vine with shoots up to 15 m in length. The leaves are arranged in a straight line on a woody stem. They take on various shapes, from oblong ovoid and ovoid to elliptical. There is a distinct, fine serration at their edges. The tip of the leaves is sharp or pointed. The leaves at the base are wedge-shaped or broadly wedge-shaped, 5–11 cm long and 2–7 cm wide. *S. chinensis* flowers are dioecious, 1.5–2 cm in diameter. They are set on long stalks. Usually, they are collected in a few leaf axils. The flowers can be white or cream in color and turn pale pink during the period of becoming mature. They have a slight fragrant scent. The flowering period in Europe is at the turn of May and June. The fruit—small red berries with a lemon scent, about 1 cm in diameter, is arranged in cluster-shaped infructescences about 10 cm long. Fruit maturation period is in September and October. Each fruit contains 1–2 yellow, kidney-shaped seeds (Hancke et al. 1999; Saunders 2000) (Table 6.1).

For *S. sphenanthera*, three synonymous Latin botanical names are recorded in The Plant List (http://www.theplantlist.org/.): *S. chinensis* var. *rubriflora* Franch., *S. flaccidiramosa* C. R. Sun and *S. grandiflora* var. *rubriflora* (Franch.) C. K. Schneid.

S. sphenanthera shares distinct natural sites of habitats with *S. chinensis*. This species is native to central and southern China. *S. sphenanthera* grows naturally in 13 provinces (or municipalities) from west to east: Anhui, Gansu, Guizhou, Guangdong, Henan, Hubei, Hunan, Jiangsu, Shaanxi, Shanxi, Sichuan, Yunnan and Zhejiang of China (Guo et al. 2016). *S. sphenanthera* also grows in the Jigong Mountain National Nature Reserve, which is located in Xinyang, Henan Province (Du and Xiao-Fan 2012), and in Ankang of the Shaanxi Province of China (Wei et al. 2020).

S. sphenanthera is a climber vine with shoots up to 7 m in length that looks like a dioecious plant, but is probably morphologically monoecious, which differs

Table 6.1 Comparison of morphological features of *S. chinensis* and *S. sphenanthera* species

		Schisandra chinensis	*Schisandra sphenanthera*
Name in Chinese		wu wei zi, bei wu wei zi	huazhong wu wei zi, nan wu wei zi
Morphological features	Length of shoots	Up to 15 m	Up to 7 m
	Distribution	Northern or northeastern China and eastern Asia	Middle and south China
	Leaves		
	Shape	Oblong ovoid, ovoid to elliptical	Elliptical to ovate
	Length	5–11 cm	5.5–11 cm
	Color	Green	Green
	Flowers		
	Color	White or cream	Yellow, orange or red
	Flowering period	May–June	April–May
	Fruits		
	Shape	Irregular round and oblatoid	Upside-down, oval
	Surface	Soft and glossy	Smooth
	Color	Red	Purple or dark red
	Diameter of single fruit	6–8 mm	5–9 mm
	Fruit maturation period	September–October	July–September

from *S. chinensis*. *S. sphenanthera* grows on rocks, shrubs and trees in evergreen deciduous forests or mixed coniferous forests (Kam Ming et al. 2015). The leaves are elliptical to ovate in shape. The leaf blades are 5.5–11 cm long and 2.5–6 cm wide (Saunders 2000). The flowers of *S. sphenanthera*, like those of *S. chinensis*, are dioecious. They grow several pieces in the leaf axils at the base of young shoots, 6–8 male flowers and 5–8 female flowers (Saunders 2000). They are yellow, orange or red in color, unlike the flowers of *S. chinensis*, which range in color from white to light pink, and they have a slight scent. Male flowers have an androecium consisting of 15–23 free stamens. The gynoecium of female flowers has 25–45 free carpels. The flowering period is at the turn of April and May. The petioles of the fruit are smooth, 3–6.5 cm long. The fruit is purple or dark red, with a smooth skin, 5–9 mm long and 4–8 mm wide, arranged in clusters. The plant bears fruit from July to September. Each fruit contains 1–2 flattened, kidney-shaped seeds (Saunders 2000) (Table 6.1).

6.3 Domestication and Cultivation

As previously mentioned, *S. chinensis* is a species widespread in the Far East. Currently, it is also cultivated, partially experimentally, in various areas outside Asia—most of all in Europe, for the purpose of scientific popularization as a valuable species with healing properties. In the Czech Republic, *S. chinensis* is cultivated by the Center of Medicinal Plants, Faculty of Medicine, Masaryk University in Brno, and at the Institute of Tropical and Subtropical Agriculture, Agricultural University in Prague (Czech Republic) (Slanina et al. 1997). In Germany, *S. chinensis* is grown in the Eberswalde Forest Botanical Garden in Eberswalde (Slanina et al. 1997). Also in Poland, *S. chinensis* is grown in the Botanical Gardens in Kraków and Lublin (Slanina et al. 1997). In Estonia, this species is cultivated at the Botanical Garden of Tartu University in Tartu and in the Botanical Garden of Tallinn (Slanina et al. 1997). In Ukraine, it is grown in the Botanical Garden of Kiev (Slanina et al. 1997). *S. chinensis* is also cultivated in the USA—in One Green World Nursery in Molalla, Oregon, in Fedco Trees Nursery in Clinton and in Maine, New England.

In Europe, a commercially available and successfully cultivated variety of *S. chinensis* is also a Ukrainian high-yielding cultivar—*S. chinensis* cv. Sadova No. 1 (Szopa et al. 2018b). It was selected in the Mikołaj Mikołajewicz Gryszko National Botanical Garden in Kiev (Национальный ботанический сад им. Н.Н. Гришко, M. M. Hryshko National Botanical Garden). The selector was Iwan Szajtan (1914–2002), Ph.D. in biological sciences, who in 1946–1996 dealt with acclimatization and selection of crop plants. In 1998, *S. chinensis* cv. Sadova No. 1 was entered into the State Register of Plants in Ukraine. The selection of this variety was based on the selection of the best seeds from the wild *S. chinensis* in Primorsk (Shaitan 2005).

The source of *S. chinensis* fruit has consisted mainly of naturally growing plants. In the vicinity of man-inhabited mountain areas have been severely damaged by forestry development, deforestation to prevent fires and collected by the population for therapeutic purposes. On the other hand, the natural resources of *S. chinensis* plants growing in remote mountain areas are not readily available, which has resulted in a decline in *S. chinensis* yield and quality, and rising prices. This has led to the establishment of protected areas where *S. chinensis* grows wild and to the development of artificially grown crops to meet the increasing market demand for *S. chinensis* fruit. Several *S. chinensis* crops have been established in the Liaoning and Heilongjiang provinces of China. The largest farm of *S. chinensis* fruit was built in the village of Dalishu in Fengcheng (Liaoning). In the Dandong city area (Liaoning), *S. chinensis* cultivation covers an area of up to 10,000 acres and has become the main source of *S. chinensis* fruit for a variety of medicinal products. Due to the short history of artificial cultivation and despite the development of several other places where *S. chinensis* is grown, such as "Hong-Zhen-Zhu," "Da-Li-Hong" and "Chang-Bai 1," it has not yet been possible to obtain an optimal variety with stable properties, yield and quality. In the future, standardized large-scale production should be carried out in accordance with the

requirements of the so-called good agricultural practice (GAP) (Kam Ming et al. 2015).

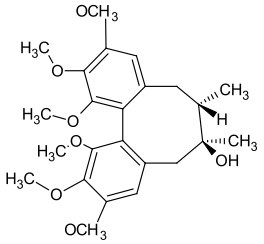
S. sphenanthera grows naturally mainly in the Qinling Mountains, which is the east–west mountain range that divides northern from southern China along the Huai River. It is an endangered species currently included in the Chinese list of key-protected wild medicinal species and as mentioned before, highly valued in traditional Chinese culture, which has led to a study on the future development of *S. sphenanthera* during climate change. The forecasting of the quality and distribution of *S. sphenanthera* has been made for the years 2020, 2050 and 2080, and covers three scenarios. The studies have shown that the natural position of *S. sphenanthera* will remain relatively stable despite the ongoing changes. The results also suggest that there would no longer be suitable habitat areas for *S. sphenanthera* in the study area if the average annual temperature there exceeds 20 °C or the annual rainfall exceeds 1200 mm (Guo et al. 2016).

S. sphenanthera has become the so-called *Cinderella Schisandra* due to the China's "Shaanxi" project. This strategy assumed saving the *S. sphenanthera* species and was developed for its utility value. The project for which this plant was selected in 2008 aimed to promote sustainable management of traditional medicinal plants in China's Upper Yangtze ecoregion. The cultivation of *S. sphenanthera* is expected to improve the income of the local population through sustainable harvesting outside nature reserves in the Upper Yangtze ecoregion (Cunningham and Brinckmann 2010). A winery has been set up in the "Shaanxi" project area to produce wine from the harvested wild *S. sphenanthera.* Annual production of wine from the plant is estimated at 100 tonnes. Currently, the annual demand for the fruit obtained from *S. sphenanthera* at this level of production is estimated at around 10,000 kg of dry matter. Through the expansion of the company, it is estimated that a tenfold scale-up will ultimately result in an annual demand of 100,000 kg of *S. sphenanthera* from the local harvesters involved in the project (Cunningham and Brinckmann 2010).

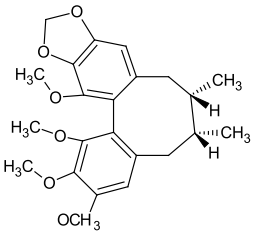
6.4 Chemical Composition

The major biologically active metabolites specific to the fruit of *S. chinensis* are dibenzocyclooctadiene lignans, otherwise known as "Schisandra lignans" (Opletal et al. 2004). Their concentration in the fruit ranges from 7.2 to 19.2 g% DW (dry weight). About 30 compounds classed as dibenzocyclooctadiene lignans have been isolated; the main compounds are: schisandrin, schisandrins B and C, γ-schisandrin, schisantherins A and B, schisanthenol, deoxyschisandrin, gomisins A and G (Kochetkov et al. 1961; Ikeya et al. 1979a; Opletal et al. 2004; Szopa et al. 2018a) (Fig. 6.1).

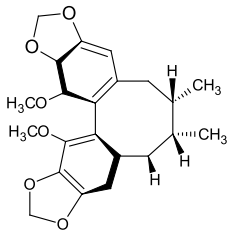
Recent studies have reported isolation of new structures named schisanchinins A–D (Hu et al. 2014), and the first example of a naturally occurring, N-containing lignan with the nicotinoyl group–nicotinoylgomisin Q (Shi et al. 2014). In addition, other types of lignans from *S. chinensis* fruit have been distinguished—dibenzylbutane lignans: schineolignans A–C, and tetrahydrofuran lignans: schinlignins A and



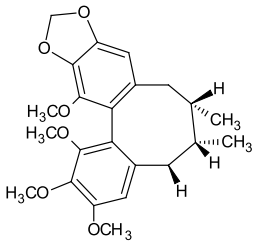
Schisandrin



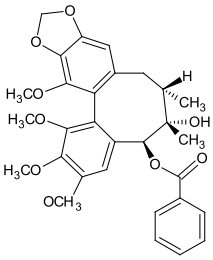
Schisandrin B



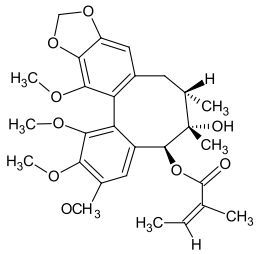
Schisandrin C



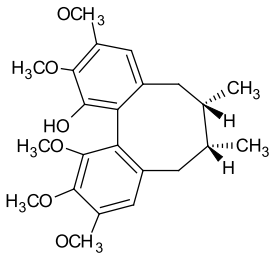
γ-Schisandrin



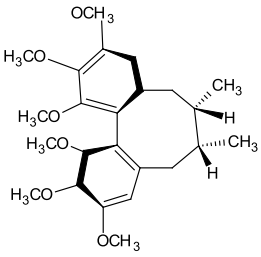
Schisantherin A



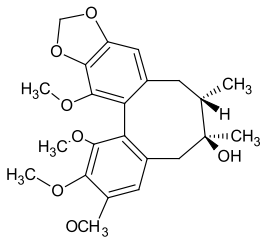
Schisantherin B



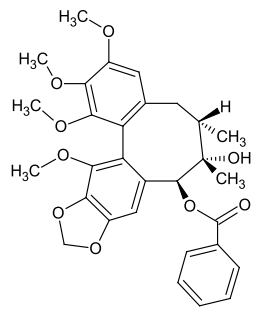
Schisanhenol



Deoxyschisandrin



Gomisin A



Gomisin G

Fig. 6.1 Chemical structures of the most important *S. chinensis* lignans

B (Xue et al. 2010; Zhang et al. 2014). *S. chinensis* berries turned out to contain compounds classified as triterpenoids, such as: preschisanartanes and schisanartanines B and 3,4-seco-21,26-olide-artane triterpenoid—wuweizilactone acid (Huang et al. 2007, 2008; Xue et al. 2010; Xia et al. 2015) (Table 6.2).

The fruit of *S. chinensis* also contains an essential oil, at around 3% DW, in which sesquiterpenes (sesquicarene, β-bisabolene, chamigrenal, α- and β-chamigren) are the predominant compounds. Oxygenated monoterpenoid, monoterpenoids (borneol, citral, 1,8-cineole, p-cymol, α- i β-pinene) and oxygenated sesquiterpenoids are present in smaller amounts (approximately 5%). Ylangen, β-himachalene, α-bergamotene and β-chamigrene are the main components, which make up about 75% of the oil (Chen et al. 2011) (Table 6.2).

Polysaccharides, monosaccharides (glucose, fructose, arabinose and galactose) and phytosterols (sitosterol and β-stigmasterol), next to vitamins (C and E) and bioelements (calcium, magnesium, manganese, iron, boron, chromium, zinc, nickel, cobalt and copper), are also a valuable fraction found in *S. chinensis* fruit (Miao et al. 2009; Chen et al. 2012; Szopa et al. 2017) (Table 6.2).

In addition, fruit extracts contain triterpenoid compounds: schintrilactones A and B, and wuweizidilactones C–F; flavonoids: rutoside, quercetin, hyperoside and isoquercitrin; organic acids: citric, fumaric, malic, tartaric and malonic; and phenolic acids: chlorogenic, gentisic, p-hydroxybenzoic, p-coumaric, protocatechuic, salicylic, syringic and salicylic (Hancke et al. 1999; Tong et al. 2012; Szopa and Ekiert 2015) (Table 6.2).

Scientific studies have proved the differences in the composition between the fruit and other plant organs. *S. chinensis* shoots and leaves are also a source of dibenzocyclooctadiene lignans, e.g., schisandrin, gomisins A and J, pregomisin, angeloylgomisin H. Leaf extracts have been found to contain: schisandrin, gomisin A, deoxyschisandrin B, gomisin G and schisantherin, in significant but smaller amounts than in the fruit. The results of analyses of the chemical composition of the leaves and stems of *S. chinensis* have shown a large number of compounds from the terpenoid group, especially the characteristic terpenoids from the cycloartane group, such as: schinchinenins A–H and schinchinenlactones A–C. These compounds are highly oxidized triterpenoids that are quite rare among the components isolated from the *Schisandra* species. Two schisandilactone-type nortriterpenoids have also been identified: schisandilactones A and B. In addition, 16,17-seco-preschisanartane nortriterpenoids: schisadilactones A–G (Song et al. 2013), and other triterpenoids: isoschicagenin C and schicagenin A–C, have also been identified. Besides the compounds mentioned above, the leaves have also been found to contain isoquercitrin as the main flavonoid, followed by hyperoside, rutoside, myricetin, quercitrin, quercetin and kaempferol. In addition to the flavonoids, there are glycosides in the leaves, such as: (+)—isoscarine and quercetin 3-O-β-L-rhamnopyranosyl(1 → 6)-β-D-glucopyranoside. Compared with the fruit, the leaves are a richer source of phenolic compounds, such as phenolic acids: chlorogenic, p-coumaric, p-hydroxybenzoic, protocatechuic, salicylic, syringic as well as gentisic, ferulic and cinnamic acids (Sovová et al. 2007; Szopa and Ekiert 2012; Mocan et al. 2014; Szopa et al. 2020).

Table 6.2 Chemical composition *S. chinensis* and *S. sphenanthera* fruits

Group of metabolites	Compounds		References
	Schisandra chinensis	*Schisandra sphenanthera*	
Dibenzocyclooctadiene lignans	Gomisin N, schisandrin C, (−)-gomisin K1, gomisin J, (−)-gomisin L2, (−)-gomisin L1, gomisin S, tigloylgomisin P, angeloylgomisin P, gomisin D, gomisin E, epigomisin O, gomisin O, 6-O-benzoylgomisin O, angeloylgomisin O, angeloylisogomisin O, benzoyl isogomisin O, schisandrene, benzoylgomisin Q, tigloyl gomisin Q, angeloylgomisin Q, gomisin F, gomisin G, schisantherin A (gomisin C), schisantherin B (gomisin B), schisantherin D, deoxyschisandrin (schisandrin A), (+)-gomisin K2, schisanhenol ((+)-gomisin K3), ɣ-schisandrin, schisandrin B, (+)-gomisin M1, (+)-gomisin M2, schisandrin, gomisin A, gomisin H, angeloylgomisin H, tigloylgomisin H, benzoylgomisin H, gomisin T, isoschisandrin, pregomisin, schisandrol A, schisandrol B, schisantherin C	Pregomisin, gomisin C, gomisin S, gomisin K3, gomisin U, gomisin J, epigomisin O, 6-O-benzoylgomisin U, methylgomisin, benzoyl gomisin P, tigloylgomisin P, angeloylgomisin P, tigloylgomisin O, benzoylgomisin Q, schisantherin A, schisantherin B, schisantherin C, schisantherin D, schisantherin E, deoxyschisandrin, schisanhenol, isochisandrin	Kochetkov et al. (1961), Ikeya et al. (1979b), Opletal et al. (2004), Zhu et al. (2007), Xue et al. (2010), Hu et al. (2014), Zhang et al. (2014)

(continued)

Table 6.2 (continued)

Group of metabolites	Compounds		References
	Schisandra chinensis	*Schisandra sphenanthera*	
Dibenzocyclooctadiene lignan derivatives	No data	Schisphenin C, schisphenin D, schisphenin E, schisphenin F, schisphenin G, schisphenone	Huang et al. (2011)
4-Aryltetralin lignans	No data	Schisandrone	Lu and Chen (2009), Ren et al. (2010), Huang et al. (2011), Chen et al. (2013)
Aryltetralone lignans	No data	Schisphentetralone A	Zhu et al. (2007), Lu and Chen (2009), Ren et al. (2010), Huang et al. (2011), Chen et al. (2013)
2,3-Dimethyl-1,4-diarylbutane lignans	No data	D, L-Anwulignan, (+)—anwulignan, sphenanlignan	Zhu et al. (2007), Lu and Chen (2009), Ren et al. (2010), Huang et al. (2011), Chen et al. (2013)
2,5-Diaryltetrahydrofuran lignans	No data	Chicane, D-epigalbacin, ganschisandrine	Zhu et al. (2007), Lu and Chen (2009), Ren et al. (2010), Huang et al. (2011), Chen et al. (2013)
Monoterpenoids	Borneol, citral, 1,8-cineole, p-cymol, α- and β-pinene	No data	Chen et al. (2011)
Sesquiterpenoids	Sesquicarene, β-bisabolene, chamigrenal, α- and β-chamigren	α-santalene, δ-cadinene, 2,4α, 5,6,7,8-hexanhydro-3,5,5,9-tetramethyl-benzocycloheptene, 6-methyl-2- (4-methyl-3-cyclohexen-1-yl) -1,5-heptadiene, β-chamigrene, γ-muurolene, α-copaene, isocaryophyllene	Song et al. (2007), Chen et al. (2011)

(continued)

Table 6.2 (continued)

Group of metabolites	Compounds		References
	Schisandra chinensis	*Schisandra sphenanthera*	
Triterpenoids	Schisandilactone A, schisandilactone B, wuweizidilactone C, wuweizidilactone D, wuweizidilactone E, wuweizidilactone F	Schizandronic acid, anwuweizic acid, kadsuric acid, coccinic acid, schinalactone A, schinlactone B, schinlactone C, schinlactone G, schisanol	Huang et al. (2007, 2008), Hill and Connolly (2013), Xia et al. (2015)
Nortriteprenoids	No data	Sphenalactone A, sphenalactone B. phenlactone C, sphenalactone D, sphendilactone C, sphenazine A	Xiao et al. (2007)
Flavonoids	Quercetin, hyperoside, isoquercitrin, rutoside	No data	Szopa et al. (2017, 2019b)
Phenolic acids	Chlorogenic acid, gentisic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, protocatechuic acid, salicylic acid, syringic acid	No data	Szopa et al. (2017, 2019a)
Organic acids	Citric acid, malic acid, tartaric acid, fumaric acid, malonic acid	No data	Szopa and Ekiert (2012)
Polysaccharides	BPS1-1, SCP, SCP-Ia, SCP-IIa, SCPP11, SFP, SCFP-1, SCP-0-1, ESCP, SCP-BII, WSLSCP, SC-2, SCPS-a, SCPS-b, SCPS-c, ASPS-a-1, ASPS-b-2, ASPS-b-3, SCPS-1-a, SCPS-1b, SCPS-2, SP	NPS1-1, SSPP11, SSPW1	Tong et al. (2012)
Vitamins	C, E	No data	Lu and Liu (1992)
Bioelements	Calcium, magnesium, manganese, iron, boron, chromium, zinc, nickel, cobalt, copper	No data	Lu and Chen (2009)

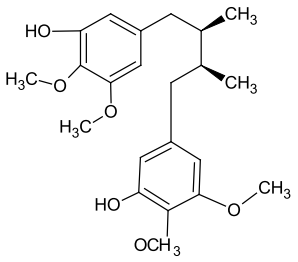
Chemical analyses of *S. sphenanthera* fruit have shown differences in composition between this species and *S. chinensis* (Table 6.2). The dominant group of secondary metabolites in *S. sphenanthera* fruit extracts are also dibenzocyclooctadiene lignans. However, *S. sphenanthera* fruit extracts contain other compounds from this group, such as: pregomisin, gomisin C, S, K3, U and J, epigomisin O, 6-O-benzoylgomisin, benzoylgomisin U, methylgomisin (Fig. 6.2), tigloylgomisin P, angeloylgomisin P, schisantherins A–D, deoxyschisandrin, schisanthenol and isoschisandrin. Dibenzocyclootadiene lignan derivatives—C–G schisphenins, and 6,7-seco-dibenzocyclooctadiene lignan-schisphenone have also been detected in *S. sphenanthera* fruit (Huang et al. 2011). In addition to dibenzocyclootadiene lignans, the fruit also contains other groups of lignans: 4-aryltetralin lignan (schisandrone), aryltetralone lignan (schisphentetralone A), 2,3-dimethyl-1,4-diarylbutane lignans (D, L-anwulignan, (+)—anwulignan and sphenanlignan) and 2,5-diaryltetrahydrofuran lignans (chicane, D-epigalbacin and ganschisandrine) (Zhu et al. 2007; Lu and Chen 2009; Ren et al. 2010; Huang et al. 2011; Chen et al. 2013; Liu et al. 2013) (Table 6.2).

An essential oil has been isolated from the dried fruits of *S. sphenanthera*. Twelve sesquiterpenoid compounds were found, which accounted for 82.4% of the total essential oil. The major sesquiterpenoids were α-santalene (10.1%), δ-cadinene (25.6%) and 2,4α, 5,6,7,8-hexanhydro-3,5,5,9-tetramethylbenzocycloheptene (19.8%). The other sesquiterpenoids were 6-methyl-2-(4-methyl-3-cyclohexen-1-yl)-1,5-heptadiene (1.4%), β-chamigrene (2.3%), γ-muurolene (1.5%), α-copaene (1.9%) and isocaryophyllene (5.6%) (Song et al. 2007) (Table 6.2).

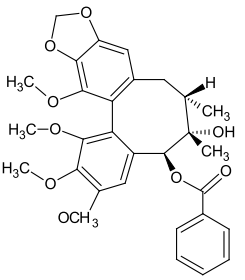
There are no literature data on the *S. sphenanthera* fruit composition with respect to, for example, polyphenolic compounds, organic acids, vitamins or bioelements.

Studies of the composition of *S. sphenanthera* stem extracts have revealed differences in comparison with fruit extracts. The stems of *S. sphenanthera* have been confirmed to contain: gomisins B, G and O, epigomisin O, schisantherins A and D, marlignan E and angeloylgomisin Q, as well as new dibenzocyclooctadiene lignans—schisphenlignans A–D (Liang et al. 2013). Apart from the lignans, there also appear the triterpenoids isolated from the fruit of *S. sphenanthera* and identified as: schisandronic acid (ganwuweizic acid), anwuweizic acid, kadsuric acid, coccinic acid, A–C and G schinalactones and schisanol. The fruit of *S. sphenanthera* is also a source of highly oxygenated nortriterpenoids: sphenalactones A–D and sphenadilactone C and sphenasin A (Xiao et al. 2007; Hill and Connolly 2013).

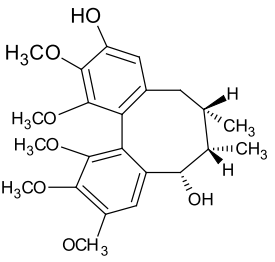
Analyses of the chemical composition of *S. sphenanthera* roots have confirmed the presence of butane-type lignans: schiglaucins A and B, epoxyzuonin, talaumidin, myristargenol A and eight compounds belonging to tetrahydrofuran lignans, which were previously unknown (Jiang et al. 2015a).



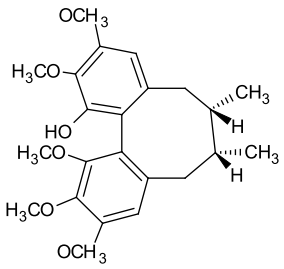
Pregomisin



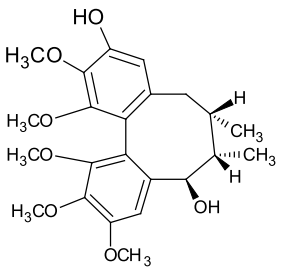
Gomisin C



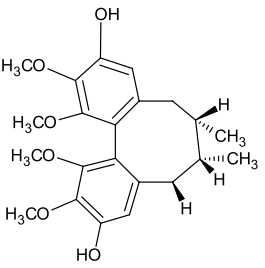
Gomisin S



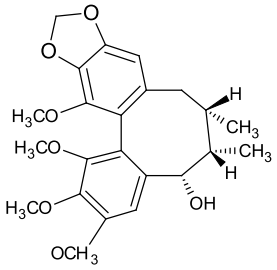
Gomisin K3



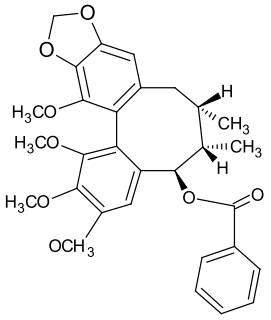
Gomisin U



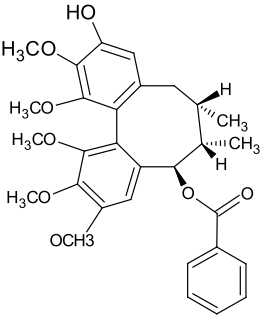
Gomisin J



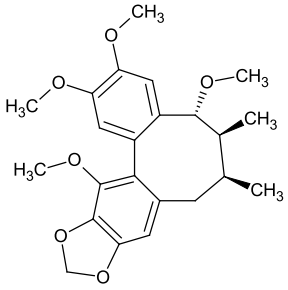
Epigomisin O



6-O-Benzoylgomisin



Benzoylgomisin U



Methylgomisin O

Fig. 6.2 Chemical structures of the most important *S. sphenanthera* lignans

6.5 Traditional Use

S. chinensis has been used in TCM for centuries, and for this reason it is now known and appreciated in modern phytotherapy. The first official mentions of this species were found in ancient Chinese medicine drafts written by Li Shih-Chen entitled "Peˆn T'shao Kang Mu" from 1596.

Schisandrae chinensis fructus (bei wu wei zi and wu wei zi) is called the "five-flavor fruit." In TCM, the specific flavors were assigned specific healing properties (Hancke et al. 1999; Szopa et al. 2017, 2018d). It was believed that the sour and salty taste had a positive effect on the functioning of the liver and male gonads, the bitter and tart taste had a positive effect on the heart and lungs, and the sweet taste affected the stomach. In TCM, the fruit was used to treat male sexual dysfunction, such as impotence or erectile dysfunction, frequent urination, involuntary and nocturnal enuresis, and gonorrhea. They were also used to treat diseases of the gastrointestinal tract, including diarrhea and dysentery. The fruit was thought to improve liver function and prevent inflammation. The Chinese used the fruit of *S. chinensis* in the treatment of respiratory failure, such as asthma or chronic cough, cardiovascular and gastrointestinal diseases, during body weakness and fatigue, excessive sweating and insomnia (Bensky and Gamble 1993).

Information about the healing properties of *S. chinensis* fruit is also found in traditional Russian medicine (Szopa et al. 2017, 2018d). They have been used as a tonic to reduce fatigue, hunger and thirst. According to traditional Russian use, *S. chinensis* is a plant that delays the aging process, prolongs life, increases vitality and improves mental health. The fruit has also been used in traditional Russian medicine as a tonic to reduce tiredness and hunger, delay aging and improve vitality and mental health (Xiao and Zhang 2012).

The fruit of *S. sphenanthera* (huazhong wu wei zi and nan wu wei zi) has been used in TCM for a very long time to lower nervous tension, strengthen the functioning of the kidneys, as an expectorant, and strengthen Qi, i.e., in Chinese medicine, the vital energy (Bensky and Gamble 1993).

According to Jiamo Chen of the Ming Dynasty, in the ancient book of herbal medicine, "Enlightening Primer Materia Medica" ("Ben Cao Meng Quan" in Chinese), *S. sphenanthera* fruit was used to treat, cold-wind cough, while *S. chinensis* fruit should treat, consumptive damage, according to TCM rules (Wei et al. 2020).

6.6 Current Therapeutic Applications

It is now known that the biological activity of *Schisandrae chinensis fructus* is mainly determined by the presence of dibenzocyclooctadiene lignans (Chinese Pharmacopoeia Commission 2005; Szopa et al. 2017, 2018d,2020). There have been many studies on the pharmacological action of *S. chinensis* fruit extracts and compounds isolated from them. Hepatoprotective activity is the best known activity profile of *S.*

chinensis fruit extract and of individual dibenzocyclooctadiene lignans (Table 6.3) (Zhu et al. 2000; Bi et al. 2013; Cheng et al. 2013; Jiang et al. 2015b).

Among individual lignans, the gomisin A mechanism of action has been explored. It has been shown that this compound increases the microsomal activity of cytochrome B5, P450, cytochrome C NADPH reductase, aminophenazone N-demethylase and 7-ethoxycoumarin O-deethylation, and also reduces the activity of 3,4-dibenzopyrene hydroxylase. It also accelerates the proliferation of hepatocytes, endoplasmic reticulum and hepatic flow (World Health Organization 2007; Panossian and Wikman 2008; So-Ra et al. 2011; Waiwut et al. 2012; Jiang et al. 2015b). It has also been shown that the mechanism of the hepatoprotective action of γ-schisandrin is to increase the concentration of mitochondrial glutathione (Lu and Liu 1992). There is also evidence that γ-schisandrin increases the concentration of vitamin C in the liver in the tested animals, which may also affect its protective effect on hepatocytes and lipid oxidation (Ip et al. 2001; Qiangrong et al. 2005; Jiang et al. 2006; Li et al. 2006). In addition, schisandrin B has also been shown to protect liver tissues from oxidative damage (Ip et al. 2001; Kwan et al. 2015; Thandavarayan et al. 2015). A study by Jiang et al. (Jiang et al. 2015b) demonstrated the hepatoprotective effects of six Schisandra lignans: gomisin A, schisandrin, deoxyschisandrin, schisandrin B, schisandrin C and schisantherin A, on acetaminophen-induced liver damage, showing that these effects were partly related to the inhibition of cytochrome bioactivation. The results of the morphological and biochemical evaluation in that study demonstrated the protective effect of all the lignans tested on paracetamol-induced liver damage. Among the tested compounds, schisandrin C and gomisin A had the strongest protective effects on the liver (Jiang et al. 2015b).

Lignans from *S. chinensis* fruit extracts also showed anti-inflammatory properties. Lignans inhibit the activity of nitric oxide (NO) and the production of prostaglandins by activating the release of cyclooxygenase-2 (COX-2) and inhibiting the expression of nitric oxide synthase (NOS) (Hu et al. 2014).

It has also been confirmed that the dibenzocyclooctadiene lignans isolated from *S. chinensis* inhibit the peroxidation of microsomal lipids and the oxidation of microsomal NADPH in hepatocytes, reduce the concentration of superoxide radicals and reduce the release of alanine aminotransferase (ALT) and lactate dehydrogenase, which increases membrane integrity and viability of hepatocytes (Yim et al. 2009).

The increase in the level of glutathione in the liver and the action of glutathione S-transferase and glutathione reductase is the mechanism of the protective, detoxifying and antioxidant action of lignans on liver cells (Miao et al. 2009). Researchers have shown that one of the lignans—schisandrin B—reduces the heart dysfunction caused by doxorubicin through its antioxidant and anti-inflammatory activity. Schisandrin B has also been proven to protect heart, brain and liver tissues in rodents from oxidative damage (Chiu et al. 2011; Thandavarayan et al. 2015).

Studies have shown that *S. chinensis* fruit extract has an anticancer effect on colorectal cancer and also stimulates cancer cells to apoptosis in liver cancer and leukemia (Zhao et al. 2013a). Scientists have identified the compounds that are responsible for this action and tracked its action. Gomisin A has been shown to inhibit the production of the placental form of glutathione S-transferase, which is

Table 6.3 Biological activities of *S. chinensis* and *S. sphenanthera* fruit extracts confirmed by scientific studies

Biological activity	Mechanism of action		References
	Schisandra chinensis	*Schisandra sphenanthera*	
Hepatoprotective and hepatoregenerative activities	– Increasing microsomal activity of: cytochrome B5, P450, NADPH, cytochrome C reductase, aminophenazone N-demethylase, O-deethylase-7-ethoxycoumarin – Reducing the activity of 3,4-dibenzopyrene hydroxylase – Acceleration of proliferation of hepatocytes, endoplasmic reticulum and hepatic flow – Increasing the concentration of mitochondrial glutathione – Increasing vitamin C concentration in the liver (may have a protective effect on hepatocytes and lipid oxidation) – Lowering the affinity of aflatoxins to DNA – Increasing levels of hepatic and mitochondrial glutathione – Increasing activity of glutathione reductase – Reducing enzyme activity: CYP2E1, CYP1A2, CYP3A11	– Preventing acetaminophen-induced liver injury by the inhibition of P450-mediated acetaminophen metabolic activation – Activation of the NRF2-ARE pathway to induce detoxification and antioxidation, and regulation of p53/p21-mediated cell cycle to facilitate liver regeneration after acetaminophen-induced liver injury	World Health Organization (2007), Panossian and Wikman (2008), Yim et al. (2009), Xiao and Zhang (2012), Kwan et al. (2015), Thandavarayan et al. (2015)

(continued)

Table 6.3 (continued)

Biological activity	Mechanism of action		References
	Schisandra chinensis	*Schisandra sphenanthera*	
Antioxidant activity	– Inhibition of microsomal lipid peroxidation – Reduction of the amount of superoxide free radicals in neutrophils – Reduction of the release of alanine aminotransferase (ALAT) and lactate dehydrogenase – Increase in hepatic glutathione concentration (GSH) and glutathione reductase (GRD) activity and glutathione S-transferase (GST)	– Inhibition of lipid peroxidation – Protective effects against oxidative degradation of protein	Miao et al. (2009), Chiu et al. (2011), Thandavarayan et al. (2015)
Ergogenic and adaptogenic activities	– Reducing the feeling of tiredness – Improving sensory organ perception – Increasing endurance for physical and mental effort	– No data	Pan et al. (2011)
Anti-inflammatory activity	– Reduction in prostaglandin production – Stimulating the release of cyclooxygenase 2 (COX-2) – Inhibition of the expression of nitric oxide synthase (NOS)	– Inhibition of the production of prostaglandin PGE2 catalyzed by COX-2 – Inhibition of PGE 2 prostaglandin production induced by ultraviolet light-UV-B – Inhibition of COX-2 expression in HaCaT keratinocytes – Prevention and treatment of hyperproliferative and inflammatory skin diseases – Significant promoting effect on macrophage phagocytosis	Yim et al. (2009)
Anti-ulcer activity	– Accelerating the regeneration of ulcer wounds	– No data	Zhang et al. (2020)

(continued)

Table 6.3 (continued)

Biological activity	Mechanism of action		References
	Schisandra chinensis	*Schisandra sphenanthera*	
Anticancer activity	– Inhibition of the production of the placental S form—glutathione—transferase—GST—P (tumor marker) in hepatocytes – Increase in carcinogen excretion – Reduction of neoplastic lesions in the liver – Inhibiting the development of skin cancer – Increase in the level of hepatic heat shock proteins Hsp70, preventing cell apoptosis induced by TNF-α – Induction of apoptosis in human U973 leukemia cells and liver cancer cells – Induction of apoptosis in colon adenocarcinoma cells	– No data	Hwang et al. (2008, 2011), Yim et al. (2009), Chen et al. (2012), Waiwut et al. (2012), Zhao et al. (2013a), Casarin et al. (2014)
Antiviral activity	– Inhibition of HIV multiplication – Inhibition of *Human papillomavirus* (HPV) multiplication	– Lowering the level of glutamine–pyruvate transaminase in the serum of people with chronic viral hepatitis	Xu et al. (2015)
Antibacterial activity	– Inhibition of the growth of Gram-positive bacteria: *Staphylococcus epidermidis, S. aureus*, *Bacillus subtilis* – Inhibition of the growth of Gram-negative bacteria: *Chlamydia pneumoniae*, *C. trachomatis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*	– No data	Chen et al. (2011), Mocan et al. (2014)

(continued)

Table 6.3 (continued)

Biological activity	Mechanism of action		References
	Schisandra chinensis	*Schisandra sphenanthera*	
Influence on central nervous system	– Increasing the level of neurotransmitters in the central nervous system – Improving learning abilities and remembering – Increased vigilance, improved concentration and mental performance – Treatment of neurasthenia and exhaustion – Supporting the treatment of Parkinson's disease, Meniere's disease, ADHD and depression – Enhancement of the action of barbiturates and the effect on sleep time induced by barbiturates	– No data	Choi et al. (2012)
Influence on cardiovascular system	– Preventing heart attacks – Reduction of high blood pressure	– No data	Alexander and Wang (2012), Park et al. (2012), Chun et al. (2014)
Influence on respiratory system	– Anti-asthmatic effect (reduction of lung hyperresponsiveness and immunoglobulin E levels, cough frequency and the likelihood of pneumonia)	– No data	Zhou et al. (2014)
Anti-osteoporotic action	– Induction of osteoblast proliferation	– No data	Li et al. (2014)

a tumor marker in liver cells, and by increasing the excretion of the carcinogen. Gomisin A also influences cytokinesis and reduces the number of focal neoplastic lesions in liver tissues. Other researchers confirmed the anti-tumor effect of gomisin A on colon cancer—it stimulated apoptosis by cleaving caspase-7 in colon cancer cell HTC-116 (Hwang et al. 2011). Another study showed that gomisin A has a significant effect on cancer cell proliferation and cell cycle arrest in HeLA cells. Gomisin A inhibited cell proliferation, especially in the presence of tumor necrosis factor-α (TNF-α) (Waiwut et al. 2012). In vitro studies were also carried out on human leukemic cells—U973, which showed that gomisin N—an isolated lignan from *S. chinensis*—induces their apoptosis. The same mechanism of gomisin N action has also been confirmed in hepatoma cells (Yim et al. 2009). Another lignan—deoxyschisandrin— was also tested on the mechanism of anti-tumor action on two human tumor cell lines (adenocarcinoma cells—2008 and colon adenocarcinoma cells—LoVo). Both lignans, deoxyschisandrin and gomisin N, inhibited cell growth in a dose-dependent manner in studied cell lines, but by inducing different types of cell death. In particular, deoxyschisandrin induced apoptosis in colon adenocarcinoma (LoVo) cells but not in ovarian adenocarcinoma cells, while gomisin N-induced apoptosis in both of the cell lines was present (Casarin et al. 2014).

It has been shown that not only dibenzocyclooctadiene lignans, but also their polysaccharide fraction are responsible for the anti-tumor activity of *S. chinensis* fruit extracts. Studies by Zhao et al. (2013a) and Chen et al. (2012) confirmed the anti-tumour and immunomodulatory activities of water-soluble low-molecular-weight polysaccharides from extract of *S. chinensis* fruits.

Furthermore, the immunostimulatory and immunomodulatory effects of polysaccharides isolated from *S. chinensis* fruit have also been demonstrated (Chen et al. 2012). It was confirmed that the polysaccharide fraction shows immunomodulatory activity by increasing the phagocytic activity of peritoneal macrophages and lymphocyte transformation, promoting the formation of hemolysin and improving the mass of the immune organs (Chen et al. 2012; Zhao et al. 2013a).

Fruit extracts of *S. chinensis* have shown a beneficial effect on the nervous system. They protect against the death of nerve cells and also increase the concentration of neurotransmitters; therefore, they can be used as an adjuvant, e.g., in Alzheimer's or Parkinson's disease (Kwan et al. 2015).

Another valuable pharmacological effect of fruit extracts is the inhibition of HIV multiplication (Shi et al. 2014). Assays have shown that schisandrin B and deoxyschisandrin selectively inhibited the HIV-1 reverse transcriptase-associated DNA polymerase activity. Schisandrin B was shown to be able to disrupt the early phases of HIV-1 replication in cellular assays. Additionally, schisandrin B impaired drug-resistant HIV-1 reverse transcriptase mutants. The structure–activity relationship revealed the importance of the cyclooctadiene ring substituents for effectiveness (Xu et al. 2015).

S. chinensis fruit extract and, in particular, the dibenzocyclooctadiene lignans contained in it have the potential to be used in the treatment of cardiovascular diseases—myocardial infarction and hypertension (Alexander and Wang 2012; Park et al. 2012; Chun et al. 2014), which is confirmed by observations of the use of

this species in traditional medicine. Scientific studies of the molecular mechanisms behind the observed phenomena have shown that *S. chinensis* fruit extract and its lignans exert a protective cardiovascular activity by controlling numerous signaling pathways that are involved in various biological processes such as fibrosis, inflammation, vascular contractility, oxidative stress and apoptosis (Chen et al. 2013; Chun et al. 2014).

In Europe, as well as in the rest of the world, Chinese magnolia vine fruits are available in pharmacies, herbal shops and online in dried form, as well as in the form of preserves: juices or teas. Both drugs and dietary supplements are available on the pharmaceutical market, which usually contain dried *S. chinensis* fruit extract. Among them, it is possible to distinguish preparations with an adaptogenic effect, stimulating the nervous, digestive and immune systems, hepatoprotective and hepatoregenerating, improving psychophysical fitness and memory processes, as well as toning and relieving the state of tension and stress (Table 6.4). Particularly noteworthy is the drug Bifendate (DDB, dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxybiphenyl-2,2'-dicarboxylate), which has been synthesized and tested by the team from the Chinese Academy of Medical Sciences and is produced by the Chinese company "Beijing Union Pharmaceutical Factory." This drug is approved only in China, Egypt, Indonesia, Vietnam and South Korea. Its main application is the treatment of liver diseases of various etiologies, including chronic hepatitis B. Bifendate is a synthetic derivative of the dibenzocyclooctadiene lignan—schisandrin C (Xie et al. 2010a), and although it is not as active as the natural lignan, its advantage is better bioavailability (Shuwei and Haidong 2000; Pan et al. 2011).

S. sphenanthera is a species used in East Asian countries as a tonic and restorative to improve the function of the liver and other organs (Fan et al. 2014) (Table 6.3). Hepatoprotective activity is closely related to the proven antiviral activity and protection activity against chemical hepatitis and various hepatotoxins (Zhu et al. 2000; Xie et al. 2010b). It was confirmed that isolated from *S. sphenanthera* fruit schisantherins A, B, C and D show a beneficial effect by reducing the level of glutamine–pyruvate transaminase in the serum of patients who suffered from chronic viral hepatitis. Additionally, the study showed that other compounds isolated from *S. sphenanthera* fruit—schisantherin E and deoxyschisandrin—were not active (Liu et al. 1978).

Researchers from the Sun Yat-sen University in Guangzhou (China) conducted significant research on *S. sphenanthera* fruit extracts, which were based on the interaction of herbs and drugs. Studies have shown a significant effect of *S. sphenanthera* fruit extracts contained in Wuzhi tablets on three important drugs: cyclosporine, tacrolimus and paclitaxel. Wuzhi tablets are a form of medicine that contains the standardized *S. sphenanthera* fruit extract containing 7.5 mg schisantherin A per tablet. Tets were manufactured by Fanglue Pharmaceutical Company (Guangxi, China). The study on rats showed that with the simultaneous administration of Wuzhi tablets, which contained the standardized extract of *S. sphenanthera* fruit and cyclosporin A, its concentration in the blood was significantly changed. With a low dose of cyclosporin A (1.89 mg/kg), the changes were significant, but only slight when the usual dose of cyclosporin A (37.8 mg/kg) was administered. Low-dose cyclosporine

Table 6.4 Chosen drugs and diet supplements, based on *S. chinensis* and *S. sphenanthera,* and their description in accordance with the data provided by the manufacturers

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
Schisandra chinensis			
Drugs			
China, Beijing Union Pharmaceutical Factory	Bifendate (pills)	**Bifendate, DDB**—dimetyl—4, 4'—dimethoxy—5, 6, 5', 6'—dimethylenodioxybifenyl—2, 2'—dicarboxylate	– Hepatoprotective – Hepatoregenerating
China, Sichuan Hezheng Pharmaceutical Company	Hezheng (capsules)	**Alcohol extract of *S. chinensis***	– Reduces serum alanine aminotransferase – Can be used for patients with chronic and persistent hepatitis with elevated alanine aminotransferase
Poland, Hasco-Lek	Penigra (capsules)	Dried extracts from: *Muira puama cortex*—40 mg, *Guarana*—30 mg, ***S. chinensis fructus*—40 mg**, zinc monomethionine—38 mg, Oil extract from *Serenoa repens fructus*—30 mg	– Improves sex drive in men
Diet supplements			
USA, Swanson	Full Spectrum Schisandra Berries (capsules)	**Dried extract from *S. chinensis fructus*—525 mg**	– Stimulates the central nervous system – Improves psychophysical fitness – Stimulates o the digestive system and metabolism – Reduces blood glucose level

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
Canada, Organic Traditions	Full spectrum Schisandra (powder)	***S. chinensis* berry certified powder—6:1**	– Adaptogenic
Canada, St Francis Company	Herb farm—Schisandra (drops)	***S. chinensis* berry—250 mg; quality crude equivalent (1:4)**	– Supports immune system – Adaptogen for mental and physical fatigue – Antioxidant – Hepatoprotective
China, Echeng	Hu Gan Pian—Liver Aid (pills)	Extracts from: *Bupleuri radix*, *Artemisia sinensis*, *Isatidis radix*, *Ilex mate radix*, *Simmondsia chinensis*, *Laburnum anagyroides*, ***S. chinensis fructus***	– Cleansing and detoxifying action of the body – Antibacterial, antiviral and antifungal – Astringent – Diuretic – Laxative – Improved digestion – Elimination of inflammation of the gallbladder and liver – Removes of intestinal worms
Czech Republic, Energia	Energia Vitaflorin (capsules)	Extracts from: *Hippophae rhamnoides*—200 mg, *Punica granatum*—50 mg, *Chaenomeles cathayensis*—20 mg, *Aloe vera*—6 mg; **powder of *S. chinensis* fruits—20 mg**, dired bee's milk—30 mg	– Adaptogenic – Anti-inflammatory activity – Increases vital energy

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
Czech Republic, Glenmark Pharmaceuticals	Eskeri (capsules)	*Ganoderma lucidum*—200 mg, *Hylotelephium spectabile*—1% salidrosides, ***S. chinensis***—80 g, *Rhodiola rosea*—120 mg, *Eleutherococcus senticosus*—40 mg (1% eleutherosides), zinc—10 mg	– Supports natural body's defenses – Supports the proper functioning of the liver – Provides natural comfort – Contributes to the proper synthesis of DNA – Contributes to the proper cognitive functions and the proper functioning of immune system – Protects cells from oxidative stress
Czech Republic, Phoenix Monopoly	Sayonara (tea)	Green tea—40%, black tea—33%, pu-er tea—24%, root of *Panax ginseng*—1%, **powder of fruits of *S. chinensis*—1%**	– Adaptogenic
Czech Republic, Phoenix Monopoly	Vilcacoran (tea)	Green tea—74%, black tea—14%, *Uncaria tomentosa*—10%**, powder of *S. chinensis* fruits—1.1%**	– Antiviral activity – Stimulates immune system – Antioxidant – Anti-inflammatory
Denmark, OmniVegan	Vital Adapt (capsules)	Extract from: *Siberian Ginseng*—1000 mg, ***S. chinensis***—500 mg, *Astragalus*—2000 mg, *Arctic Root*—300 mg	– Antioxidative – Adaptogenic – Stimulates of the immune system – Reduces tiredness and fatigue
Germany, Herba direkt	Schisandra Wu Wei Zi 500 mg (capsules)	**Extract from *S. chinensis* fruits—250 mg**	– Hepatoprotective

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
Germany, Fairvital	Olea Active Complex (capsules)	Vitamin B1—1.1 mg, vitamin B2—2.4 mg, vitamin B3—16 mg, pantothenic acid—6 mg, vitamin B6—1.4 mg, vitamin B12—10 μg, folic acid—200 μg, biotin—100 μg Olive leaf extract contains 250 mg, oleuropein—50 mg, ***S. chinensis* extract contains 111 mg, schisandrin—10 mg**, grape seed extract—150 mg, pomegranate extract—50 mg	– Adaptogenic – Supports normal heart function – Supports normal homocysteine metabolism
Germany, Fairvital	Schisandra (capsules)	***S. chinensis* fruit powder—350 mg, *S. chinensis* extract with 13.5 mg of schisandrin**	– Adaptogenic
Germany, Vitamintrend	Schisandra 500 mg (capsules)	**Extract from fruits of *S. chinensis* —150 mg (contains 9% schisandrin)**	– Hepatoprotective
Germany, ZeinPharma	Schisandra 500 mg, Wu Wei Zi (capsules)	**Extract from *S. chinensis*—505 mg**	– Antioxidant – Adaptogenic

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
Poland, Sanbios	Libidin (pills)	Extract from: *Tribulus terrestris fructus*—800 mg, *Muira puama cortex*—400 mg, *Siberian ginseng radix*—380 mg, ***S. chinensis fructus***—380 mg	– Toning – Strengthening the body's immune system – Improves psychophysical fitness – Improves sexual activity – Sedative – Improves memory processes – Protective effect on nerve cells
Poland, Axellus	Bodymax Vital (pills)	Standardized extract from *Panax ginseng radix* (8% of ginsenosides)—50 mg, **standardized extract from *S. chinensis fructus* (9% of schisandrin)—28 mg**, vitamin A—800 mcg, vitamin D—10 mcg, vitamin E—12 mg, vitamin C—80 mg, vitamin B1—1.1 mg, vitamin B2—1.4 mg, vitamin PP—16 mg, vitamin B6—1.4 mg, folic acid—200 μg, vitamin B12—2.5μg, biotin—50 μg, pantothenic acid—6 mg, iron—14 mg, magnesium—225 mg, zinc—10 mg, copper—1000 μg, manganese 2—mg, selenium—55 μg, chromium—40 μg, molybdenum—50 μg, iodine—150 μg	– Stimulates the immune system and an increase in the psychophysical efficiency of the body – Reduction of fatigue symptoms and supporting the body in stress – Positive influence on cardiovascular system – Antioxidant – Improves memory processes – Improving the vitality of the body – Toning

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
Poland, EkaMedica	Cytryniec chiński (capsules)	**Extract from S. *chinensis fructus*—510 mg**	– Stimulates the nervous system – Stimulates the digestive system and metabolism – Stimulates the immune system – Antioxidant – Improves psychophysical fitness
Poland, Yango	Ekstrakt *Schisandra* (capsules)	**Extract 10:1 from *S. chinensis*—900 mg**	– Hepatoprotective and hepatoregenerative – Action supporting the cleansing of the body of toxins – Stress reduction – Improves psychophysical fitness and memory processes
Poland, Mitra	Afra Cytryniec Chiński (tincture)	***S. chinensis folium*—0.006 g (in 90 drops),** water, ethanol (27%)	– Reduces the feeling of tiredness – Stimulates the respiratory system – Stimulates the digestive system and metabolism
Poland, Hasco-Lek	Hepa Balans (pills)	Extracts from: *Silybum marianum fructus*—35 mg (3.5 mg of silymarin), ***S. chinensis fructus*—35 mg**, L-ornithine L-aspartate—150 mg	– Hepatoprotective – Hepatoregenerating

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
Poland, Hasco-Lek	Liveran (capsules)	Extracts from: ***S. chinensis fructus*—80 mg**, *Cynara scolymus folium*—150 mg, *Silybum marianum fructus*—12.6 mg (silymarin—10 mg), vitamin B1—2 mg, vitamin B2—2 mg, cholin—165 mg	– Improves the functioning of the liver and fat metabolism – Helps to maintain the proper level of lipids in the blood – Supports the secretion of digestive juices – Hepatoprotective – Hepatoregenerating
Poland, Pharmovit	Schisandra cytryniec chiński (capsules)	**Extract 4:1 from *S. chinensis fructus*—300 mg**	– Improves physical and intellectual fitness – Supports the functioning of the immune system – Helps in maintaining the physiological cleansing functions of the liver
Poland, Hepatica	Hepatica Schisandra (capsules)	**Standardized extract from *S. chinensis fructus*(10% of schisandrin)—22 mg**	– Hepatoprotective – Positive influence on the kidneys, lungs, heart and brain – Simulates the production of antioxidants – Slightly calms and helps to cope with insomnia – Strengthens the entire body – Speeds up the metabolism – Influences on weight loss – Antidepressant

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
Sweden, Holistic	Holistic StressBalans (water ethanol extract)	Extracts from: *Glycyrrhiza glabra radix*—300 mg, *Withania somnifera radix*—200 mg, *Eleutherococcus senticosus radix*—200 mg, ***S. chinensis fructus***—150 mg, *Rhodiola rosea radix*—150 mg	– Improves psychophysical fitness and vitality – Improves memory processes and concentration – Stimulates the immune system – Sedative, hypnotic and tonic – Antioxidant – Reduces blood glucose level – Supports the proper functioning of the adrenal glands
Sweden, Vitalas AB	Red Power (pills)	Extracts from: ***S. chinensis fructus***—40 mg, *Holistic Rhodiola rosea radix*—125 mg, *Panax ginseng radix*—40 mg, vitamin B1—1.2 mg, pantothenic acid—6 mg, vitamin B12—1 μg, folic acid—200 μg	– Stress reduction – Improves vitality and psychophysical fitness – Improves memory processes and concentration – Stimulates the immune system – Reduces the feeling of tiredness
UK, Sash Vitality	Herb Pharm Ripe Berry Schisandra (capsules)	Turmeric, choline bitartrate, **Chinese magnolia vine,** amalaki, chicory, beetroot extract, bupleurum, chanca piedra, artichoke, dandelion, zinc citrate, acai extract, ginger root extract	– Hepatoprotective

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
UK, Sash Vitality	Liver Complex (capsules)	Turmeric—150 mg, artichoke—150 mg, dandelion—150 mg, ginger root—100 mg, choline—50 mg, **Chinese magnolia vine—**40 mg, amalaki—40 mg, chicory—40 mg, beetroot—40 mg, *Bupleurum* —40 mg, chanca piedra—40 mg, acai—40 mg, zinc—10 mg	– Hepatoprotective
UK, Na'vi Organics	Full Spectrum Wild Schisandra Berry (Dual Extraction) (Extract Powder)	**Extract from fruits of *S. chinensis* 10:1**	– Hepatoprotective
UK, Time Health	Schisandra (capsules)	**Extract from fruit of *S. chinensis*—300 mg—contains 9% schisandrin**	– Adaptogenic
UK, Simply Pure Organic	Organic Schisandra (capsules)	**Extract from *S. chinensis*—500 mg**	– Adaptogenic – Antioxidant
UK, GinSeng	Schisandra berry (tablets)	**Extract from *S. chinensis***	– Normalizes blood sugar – Improves concentration – Anti-aging properties
UK, Indigo Herbs	Pure Herbs, Schisandra (tincture)	**Extract from *S. chinensis***	– Adaptogenic
UK, Microingredients	Organic Schisandra extract (extract powder)	**Extract from fruit of *S. chinensis*—1 g**	– Supports liver function – Supports brain health and memory – Antioxidant – Improves memory conditions

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
UK, Irae	Schisandra Berry 1:1 oral spray (tincture)	***S. chinensis* berry**	– Promotes daily stress resistance – Supports liver function and detoxification – Supports blood cleansing – Detoxification effect – Reduces fatigue and stress – Neutralizes environmental toxins – Supports adrenal stress resistance – Supports respiratory health – Promotes mental clarity – Supports emotional centeredness – Supports digestive health
UK, Complementary Supplements	Ashwagandha Energy Complex (capsules)	Extracts from: *Ashwagandha* root—400 mg *Astragalus* root—350 mg, *Panax ginseng* root—200 mg, *Maca* root—200 mg, *Liquorice* root—100 mg, *Rhodiola rosea*—40 mg, *Reishi mushroom*—25 mg, ***S. chinensis***—25 mg, black pepper—5 mg, *Gotu kola* extract—5 mg, *turmeric*—5 mg; vitamin B3—20 mg, vitamin B5—10 mg, vitamin B6—2 mg, vitamin B12—25 μg	– Strengths immune system – Adaptogenic – Improves brain function – Strengths bones and joints – Supports heart function – Antioxidant – Improves energy level

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
UK, Health & Her	Natural Sleep Aid (capsules)	5-hydroxytryptophan—150 mg, inositol—25 mg, vitamin B6—1.5 mg, magnesium—10 mg, selenium—55 mcg, *Chamomilie*—100 mg, *Reishi* mushroom extract—50 mg, *Ashwagandha* extract—50 mg, *Hop* extract 4:1—50 mg, ***S. chinensis*berry extract—50 mg**, *Maca*—50 mg	– Hormone balancing and tiredness reduction – Improves the quality of sleep
USA, Nature's Way®	Nature's Way Schisandra Fruit (capsules)	**Extract from fruits of *S. chinensis*—580 mg**	– Hepatoprotective – Hepatoregenerating
USA, Nature's Answer	Nature's Answer Alcohol-Free Schisandra Berry (drops)	**Extract from fruits of *S. chinensis*—2 mg**	– Hepatoprotective
USA, Herb Pharm	Herb Pharm Ripe Berry Schisandra (liquid)	**Extract from fruits of *S. chinensis*—660 mg**	– Supports liver – Adaptogenic – Strengthens eye health
USA, Solaray	Schisandra berries (capsules)	**Extract from fruits of *S. chinensis*—580 mg**	– Anti-aging
USA, Canada, Honest Green	Kroeger Herb Schisandra (capsules)	***S. chinensis* berry standardized extracts—225 mg, *S. chinensis* berry an active base—325 mg**	– Adaptogenic – Antioxidant
Schisandra sphenanthera			
Drugs			

(continued)

Table 6.4 (continued)

Country of production and manufacturer	Trade name and form	Chemical composition (active compounds according to manufacturer)	Activity profile recommended by manufacturer
China, Fanglue Pharmaceutical Company	Wuzhi (tablets)	**Extract from fruits of *S. sphenanthera* which contains 7.5 mg of schisantherin A**	– Can be used in co-therapy with cyclosporine A, paclitaxel and tacrolimus – Antiviral
China, Sichuan Hygiene Pharmaceutical Company	Wu Zhi (tablets)	**Extract from fruits of *S. sphenanthera* which contains 11.25 mg of deoxyschisandrin**	– Adaptogenic – Hepatoprotective
Diet supplements			
China, Guangzhou Yuehua Pharmaceutical Company	Linuo Fu (capsules)	**Dried extract from fruits of *S. sphenanthera*—300 mg**	– Reduces serum alanine aminotransferase – Can be used for patients with elevated alanine aminotransferase in chronic and persistent hepatitis
China, Kangxian Duyiwei Bio-Pharmaceutical Company	Shengi Wuweizi (pills)	**Dried extract from fruits of *S. sphenanthera*—0.25 mg,** *Tangshen* roots and *Milkvetch* roots	– Protective effect on the liver – Excitement of the central nervous system – Inhibits peptic ulcer – Impacts on the cardiovascular system – Enhances the body's immune function
China, Guangzhou Zhongyi Company	Xiao Ke Wan (pills)	**Dried extract from fruits of *S. sphenanthera*—2.5 mg**	– Causes the long-term decrease in blood sugar levels

A may be more sensitive to the combined use of inhibitors or inducers of CYP3A/P-gp (Xue et al. 2013). The co-administration of Wuzhi tablets with paclitaxel was then investigated. A study in rats showed a significant increase in the blood level of paclitaxel after oral administration (AUC 0–24 h from 280.8 to 543.5 ng/ml). Pharmacokinetic data on viral administration of paclitaxel with Wuzhi tablets showed a relatively small but still significant increase in AUC 0–24 h (from 163.6 to 212.7 ng/ml) and a decrease in clearance (from 3.2 to 2.2 l/h/kg) (Jin et al. 2011; Xue et al. 2013). In conclusion, *S. sphenanthera* fruit extract significantly increased the concentration of paclitaxel in the blood of rats and thus increased the systemic exposure to this drug, which should be taken into account in clinical practice (Jin et al. 2011). Studies on the co-administration of Wuzhi tablets and tacrolimus to rats showed that most tissue concentrations of tacrolimus were slightly increased with the concomitant dose of Wuzhi tablets, but the concentration of tacrolimus in the whole blood was significantly increased (three-fold after co-administration of Wuzhi tablets) (Qin et al. 2010). In addition, the effect of *S. sphenanthera* fruit extract on absorption and first-pass metabolism in the intestines and liver was confirmed. The mechanism of action is based on the inhibition of tacrolimus mediated by P-gp and the metabolism of tacrolimus mediated by CYP3A, and the reduction of first-pass metabolism by fruit extract was the main reason for the increased oral bioavailability of tacrolimus (Qin et al. 2010).

The water-soluble polysaccharide—SSPP11—isolated from an extract of *S. sphenanthera*, shows an antioxidant effect. SSPP11 inhibits lipid peroxidation and has a protective effect on the oxidative degradation of protein (Zhao et al. 2014; Chen et al. 2019).

Through previous studies that confirmed the beneficial effects of *S. sphenanthera* fruit extracts on hepatocytes, a study was carried out on the effect of these extracts on hepatotoxicity induced by various drugs. The protective effect against acetaminophen was confirmed. Wuzhi tablets blocked the liver damage caused by acetaminophen by inhibiting the metabolic activity of acetaminophen mediated by P450, activating the NRF2-ARE pathway to induce detoxification and antioxidation, and regulating the p53/p21 mediated cell cycle to facilitate liver regeneration after induced liver damage (Fan et al. 2014; Qin et al. 2014).

6.7 Possibility of Cosmetic Use

Due to the high biological activity of *S. chinensis* extracts, this species has become the object of interest of cosmetic companies in recent years. The anti-aging and skin regenerating effects of fruit extracts have been used by TCM for centuries. Numerous modern scientific studies on *S. chinensis* fruit extracts are also of great importance because they confirm the potential use of this raw material in cosmetic preparations. Studies have confirmed antioxidant, anti-aging, anti-inflammatory and soothing activities, as well as anti-radiation, anti-allergic, whitening and brightening

properties (Chiu et al. 2011; Lam et al. 2011; Gopaul et al. 2012; Kang and Shin 2012; Park et al. 2013; Yan et al. 2015).

According to the CosIng database (European Commission CosIng 2020), which is a database that publishes details of cosmetic ingredients approved by the European Commission, *S. chinensis* can be used in as many as eleven forms, which are presented in Table 6.5. There are also extracts obtained from in vitro cultures. CosIng recommends *S. chinensis* for preparations with, for example, antioxidant, anti-sebum, humectant, skin and hair conditioning, emollient, skin protecting and fragrant properties.

The research on the antioxidant and sunscreen activities was carried out by administering *S. chinensis* fruit extract to the skin of rabbits whose skin was exposed to sunlight. It has been shown that schisandrin B contained in *S. chinensis* causes an increase in glutathione concentration and a decrease in malondialdehyde—a product of lipid peroxidation. In vitro studies on human fibroblasts exposed to UV rays have additionally demonstrated a reduction in MMP-1 production and elastase activity (Chiu et al. 2011; Park et al. 2013).

The anti-aging effect was tested with a combined fruit extract of *S. chinensis* and *Narcissus tatteta.* The tests were carried out in vitro on an epidermal skin equivalent. The combined extract showed increased expression of genes encoding collagen, elastin, hyaluronic acid and ceramides, and decreased expression of the gene encoding the protein responsible for collagen breakdown. Regulation of these

Table 6.5 Biological activity of extracts from various parts of plants obtained from *S. chinensis* and *S. sphenanthera* according to the CosIng base

Ingredient	Function
Schisandra chinensis	
Fruit	Anti-sebum, antioxidant, humectant, skin conditioning, emollient
Fruit ferment extract filtrate	Skin conditioning, emollient, antioxidant, humectant
Callus extract	Skin protecting
Fruit extract	Emulsion stabilizing, hair conditioning, humectant, skin conditioning, emollient, skin protecting
Fruit oil	Skin conditioning
Fruit powder	Skin conditioning
Fruit water	Fragrance, skin conditioning
Leaf extract	Antioxidant
Phytoplacenta extract	Anti-sebum, antimicrobial, antioxidant, hair conditioning, skin protecting
Seed extract	Hair and skin conditioning
Seed extract ferment filtrate	Antioxidant, skin protecting
Schisandra sphenanthera	
Fruit extract	Anti-sebum, antioxidant, skin conditioning, skin protecting

genes could have a major impact on improving the appearance of the skin as well as slowing down its aging (Lam et al. 2011; Gopaul et al. 2012).

The anti-allergic, soothing and anti-inflammatory effects of *S. chinensis* fruit extracts have been confirmed by showing that when applied topically, they reduced skin inflammation and decreased the levels of immunoglobulin E (IgE), immunoglobulin M (IgM) and histamine (Kang and Shin 2012).

The skin-lightening effect has been confirmed based on a study which isolated 1-*O*-methyl-fructofuranose from the fruit of *S. chinensis*. A study on melanoma cells from B16FO mice showed an inhibition of melanin synthesis and tyrosinase activity after the administration of 1-*O*-methyl-fructofuranose (Lam et al. 2011; Yan et al. 2015).

A fruit extract of *S. chinensis* was patented in 2004 by the German company Badische Anilin und Soda-Fabrik (BASF). The patent concerns the use of the extract in cosmetics and medical agents with the effect of inhibiting melanogenesis, protecting against UV-A radiation and anti-aging (Yan et al. 2015).

Cosmetic applications of *S. chinensis*, proven by scientific research, and traditional indications have made this species appear more and more often in the composition of various cosmetics. On the European market, these are mainly products of Asian (Korean) and Russian companies, based on *S. chinensis* fruit extracts (Szopa et al. 2016).

Scientific research has also already confirmed many valuable biological properties of *S. sphenanthera* fruit extracts, which also makes it interesting for cosmetic reasons. *S. sphenanthera* fruit extracts possess confirmed antioxidant, antiviral and anticancer properties (Huyke et al. 2007; Lu and Chen 2009).

Moreover, *S. sphenanthera* has been approved for use in cosmetology by the supervisory authorities of the CosIng (European Commission CosIng 2020) (Table 6.5). In the description of applications in the CosIng database, the main cosmetic activity profiles of *S. sphenanthera* fruit extracts are: antioxidant, anti-sebum, protecting and conditioning of the skin.

Nan-wuweizi (*S. sphenanthera fruit* extracts) extract has strong antioxidant properties and might be useful in the prevention and treatment of hyperproliferative and inflammatory skin diseases (Huyke et al. 2007; Lu and Chen 2009). This mechanism of action is currently protected by patents (Garnier et al. 2012; Garnier and Msika 2013).

Huyke et al. (Huyke et al. 2007) showed that the studied nonpolar *S. sphenanthera* fruit extract obtained by CO_2 extraction was the most active with a half-maximal inhibitory concentration of 20 μg/mL. In a cell-free enzyme inhibition assay with recombinant cyclooxygenase-2 (COX-2), this extract showed dose-dependent inhibition of COX-2 catalyzed prostaglandin PGE2 production (IC50 = 0.2 μg/mL). It also reduced the ultraviolet-B (UVB)-induced PGE2 production (IC50 = 4 μg/mL) and COX-2 expression in HaCaT keratinocytes.

6.8 Applications in the Food Industry

As food products, both *S. chinensis* and *S. sphenanthera* are used in China in fruit juices, soups, alcoholic and non-alcoholic beverages, among others. The fruit extracts of these species have the potential to be used as an ingredient in healthy beverages that are intended to supplement the diet, as so-called functional foods (European Food Safety Authority; Cunningham and Brinckmann 2010). Both *S. sphenanthera* and *S. chinensis* raw fruits or infusions made from dried fruit are also recommended for consumption. Both species are recognized as adaptogenic plants—they are species that are distinguished by a comprehensive, beneficial effect on health (Panossian and Wagner 2005). Adaptogens increase the mental and physical abilities to help adapt to negative external conditions. They affect the human immune system and nervous system, thanks to which the body responds better in a stressful situation and there is no significant impairment of their functions (Panossian and Wagner 2005).

6.9 Biotechnological Approaches

Plant biotechnology and its main tool—plant in vitro cultures, thanks to continuous improvements and the possibility of using new methods—allow increasing the production of secondary metabolites, which then allows them to be used, primarily, in the pharmaceutical, cosmetic and food industries (Verpoorte et al. 2002; Pietrosiuk and Furmanowa 2006; Kirakosyan and Kaufman 2009). In order to be able to increase the production of secondary metabolites in plant cultures in vitro, a number of "optimizing" experiments should be performed: selecting highly productive cell lines, testing the type of culture to be conducted (agar, agitated, bioreactors), optimizing the culture conditions (testing of the basal culture medium, composition of plant growth and development regulators), testing appropriate lighting and temperature conditions, and the elicitors (stressors) and genetic transformation used. Plant cultivation by culturing in vitro allows control and stimulation of the production of secondary metabolites (Karuppusamy 2007; Matkowski 2008; Akula and Ravishankar 2011; Dias et al. 2016). Moreover, micropropagation protocols create possibilities for effective multiplication of biomass of selected species (Chu and Kurtz 1990; Paula 2012).

In the case of *S. chinensis*, dibenzocyclooctadiene lignans are a particularly and intensively studied group of secondary metabolites arousing the interest of research centers specializing in plant biotechnology, due to its multidirectional and still not fully understood biological activity. So far, studies on in vitro cultures of *S. chinensis* in the areas of micro-reproduction and endogenous production of secondary metabolites have been carried out mainly in research centers in East Asian countries: in China, South Korea and Japan (Kim et al. 2004; Chen et al. 2010; Kohda et al. 2012). In Europe, such research has been recently undertaken by Czech (Havel et al. 2008; Březinová et al. 2010) and Polish institutions.

Endogenous accumulation of lignans in the biomass of *S. chinensis* cultures in vitro is a particularly interesting topic that has been explored by two research teams from academic centers in Brno: the Department of Plant Biology, Mendel University of Agriculture and Forestry, and the Department of Biochemistry, Medical Faculty of Masaryk University. As part of the first study, only a qualitative analysis was performed, which confirmed the presence of schisantherin A, deoxyschisandrin and γ-schisandrin. The next study involved analysis of selected lignans in terms of the quantitative content in the biomass of *S. chinensis* cultures in vitro. It determined the amounts of deoxyschisandrin, schisandrin, γ-schisandrin, schisandrin C, gomisin A and gomisin N in embryogenic agar cultures conducted in darkness, and in agitated cultures conducted under the same photoperiod. In the analyzed extracts from the biomass obtained from the agar cultures, gomisin N was the quantitatively dominant compound (max. 0.55 mg/100 g DW), while γ-schisandrin dominated in extracts from the agitated cultures (max. 0.54 mg/100 g DW). For comparative purposes, the concentrations of lignans in the seeds and leaves of the plant growing in vivo were also determined. In the analyzed seed extracts, the main lignans were: schisandrin—6.4 mg/100 g DW and deoxyschisandrin—4.4 mg/100 g DW. In contrast, gomisin A and schisandrin dominated in the leaf extracts: 0.8 mg/100 g DW and 0.6 mg/100 g DW, respectively (Havel et al. 2008; Březinová et al. 2010).

Thanks to the cooperation of three different research units from the Department of Pharmacognosy, Faculty of Pharmacy, the Experimental Station of Medicinal Plants, and the Department of Forensic Medicine, Graduate School of Biomedical Sciences (Hiroshima), the possibility of producing gomisin A and gomisin F has been investigated in *S. chinensis* callus cultures. The maximum amounts obtained (0.05 and 0.04 g%, respectively) were greater than in the fruit (0.04 and 0.01 g%, respectively) and the leaves (0.01 g% each) of *S. chinensis* (Havel et al. 2008; Březinová et al. 2010).

As part of the research work at the Department of Pharmaceutical Botany of the Jagiellonian University Medical College (Poland), extensive studies have been conducted on the optimization of the production of lignans in the biomass of *S. chinensis* cultures in vitro (Table 6.6) (Szopa and Ekiert 2012, 2013, 2015,2016,2018a; Szopa et al. 2015, 2016). The initial studies on the accumulation of metabolites in *S. chinensis* microshoot cultures tested variants of the Murashige and Skoog (MS) (Murashige and Skoog 1962) medium supplemented with different concentrations of plant growth regulators (PGRs): 6-BA (6-benzyladenine) and NAA (1-naphthaleneacetic acid), in the range 0 to 3 mg/l. The amounts of lignans in the analyzed methanolic extracts from biomass depended on the concentrations of the PGRs. The amounts of schisandrin (max. 71 mg/100 g DW) and gomisin A (max. 86 mg/100 g DW) were greater than their amounts in fruit and leaf extracts of the plant growing in vivo. As a result of the research, the MS medium supplemented with 3 mg/L BA and 1 mg/L NAA was selected as the best "production" medium (Szopa and Ekiert 2011, 2013). In subsequent studies on the accumulation of lignans in *S. chinensis* cultures in vitro, high levels of deoxyschisandrin (max. 310 mg/100 g DW), schisantherin A (max. 33 mg/100 g DW) and gomisin G (max. 22 mg/100 g DW) were also determined (Szopa and Ekiert 2013, 2014, 2015). The cooperation

Table 6.6 Maximal total contents (mg/100 g DW ± SD) of dibenzocyclooctadiene lignans reached in different types of *S. chinensis* microshoot cultures

Type of culture	Maximal total content	Special in vitro conditions
Agar	376	Blue light
Stationary liquid	275	*
Agitated	640	Elicitation with 1000 mg/L of YeE in 20th day of growth period
Bubble column bioreactor	303	*
Balloon bioreactor	227	*
Spray bioreactor	423	*
RITA® TIS	381	*
Plantform TIS	832	Elicitation with 1000 mg/L of YeE in 20th day of growth period

*Optimal culture condition was MS medium with 3 mg/L BA and 1 mg/L NAA, and 30 days of duration of growth period

of the Department of Pharmaceutical Botany of the Jagiellonian University Medical College with teams from the Medical University of Gdańsk and the Nicolaus Copernicus University in Toruń has included research on the influence of the type of in vitro cultures of *S. chinensis* on the accumulation of lignans. The research involved testing agar, liquid stationary and agitated microshoot cultures. Biomass extracts were analyzed for the concentrations of fourteen dibenzocyclooctadiene lignans: angeloyl-/tigloylgomisin H and Q, benzoylgomisin P, deoxyschisandrin, gomisins A and G, schisandrin, schisandrins B and C, ɣ-schisandrin, schisanthenol and schisantherins A, B and D. The quantitatively dominant compounds in all the types of culture were: schisandrin (max. 66 mg/100 g DW), gomisin A (max. 34 mg/100 g DW), angeloyl-/tigloylgomisin Q (max. 50 mg/100 g DW) and deoxyschisandrin (max. 44 mg/100 g DW). The concentration of the main metabolite—schisandrin—was 2.2 times higher than in the analyzed leaf extracts and 2 times lower than in the extracts obtained from the fruit of the mother plant. The maximum amounts of gomisin A and deoxyschisandrin were comparable with those in the leaf extracts, and 3.2 and 1.4 times lower, respectively, than in the fruit extracts (Szopa et al. 2016). The Department of Pharmaceutical Botany of the Jagiellonian University Medical College has also conducted a study on the influence of monochromatic light on the accumulation of dibenzocyclooctadiene lignans in *S. chinensis* microshoot cultures. Agar cultures were grown in the presence of monochromatic fluorescent light: under red light and blue light, and in the presence of UV-A and far-infrared radiation. The control group consisted of cultures grown in the dark and under white light. The total concentration of the determined lignans increased 1.7 times depending on the light conditions. Blue light proved to be the most conducive to the accumulation of secondary metabolites—the total concentration of the lignans was 376 mg/100 g DW (Table 6.6). By comparison, in the biomass extracts of cultures exposed to white light, the concentration of these compounds was 1.3 times lower. In quantitative terms, the dominant compounds

were: schisandrin (max. 68 mg/100 g DW), deoxyschisandrin (max. 55 mg/100 g DW) and gomisin A (max. 37 mg/100 g DW) (Szopa and Ekiert 2016).

As part of the cooperation between the team of the Department of Pharmaceutical Botany of the Jagiellonian University Medical College and the team from the Medical University of Gdańsk, research was conducted on the development of an efficient elicitation process of *S. chinensis* microshoot cultures in vitro, which would ensure high production of dibenzocyclooctadiene lignans. The in vitro cultures were maintained on variants of MS media that differed in the concentrations and times of supplementation of four elicitors: cadmium chloride ($CdCl_2$), yeast extract (YeE), chitosan (Ch), methyl jasmonate (MeJa), and a permeabilizing agent—dimethylsulfoxide (DMSO). The influence of the experimental factors on the accumulation of lignans in the analyzed biomass was confirmed, while the extracts obtained from the culture media showed trace amounts of the determined compounds (<5 mg/L). The best results were obtained after eliciting with 1000 mg/l YeE on day 20 of culture (640 mg/100 g DW), when the lignan content increased 1.8 times compared with non-elicited cultures. This elicitation method was also applied in temporary immersion system (TIS)—Plantform bioreactors (Plantform, Sweden). The total lignan content obtained as a result of elicitation in the TIS bioreactors was 830 mg/100 g DW (Table 6.6). The concentration of the analyzed compounds was 10% higher than in fruit extracts and as much as 40% higher than in extracts from the leaves of the mother plant (Szopa et al. 2018c).

Another upstream step in the biotechnological research on *S. chinensis* has been the optimization of culture growing in various types of bioreactors. Five bioreactor designs were tested as part of this research: balloon bioreactors, column bioreactors with a shoot immobilization system, spray bioreactors and two TIS bioreactors: Plantform and RITA® (Vitropic, France). The largest biomass increments of max. 18 g DW/l were obtained for cultures grown in RITA® bioreactors for a period of 60 days. On the other hand, the highest accumulation of the tested lignans was found in the extracts obtained from the biomass of cultures grown for 30 days in Plantform bioreactors (547 mg/100 g DW) (Table 6.6). A comparison of these results with those for extracts from the fruit and leaves of the mother plant showed that the total metabolite content was 1.7 times higher than in the leaf extracts and 1.4 times lower than in the fruit extracts. The dominant compounds were: schisandrin—max. 119 mg/100 g DW, deoxyschisandrin—78 mg/100 g DW and gomisin A—max. 68 mg/100 g DW (Szopa et al. 2017, 2018b, c).

In summary, the biotechnological research described above proves that the accumulation of dibenzocyclooctadiene lignans in *S. chinensis* cultures can be stimulated by the appropriate selection and concentration of plant growth and development regulators, and the selection of the type of culture (agar, agitated, liquid stationary, bioreactor), elicitation method and the light conditions under which the cultures are grown in vitro. Based on the results of that research, Plantform and RITA® TIS large laboratory structures were selected for the most efficient production of lignans. The results can potentially be used in the production of these compounds on an industrial scale (Szopa et al. 2020).

In vitro cultures of *S. sphenanthera* are currently the object of interest of research centers only in China (Wang and Xi 2009; Liang et al. 2011). This is probably related to the problems of obtaining raw material from *S. sphenanthera* by centers located outside Asian countries. Despite the rich chemical composition and broad biological activity of this species, only limited biotechnological studies are performed.

There are only three reports available, published by Chinese teams, dealing with biotechnological studies on *S. sphenanthera.* A research team from Central South University of Forestry and Technology (Hunan) has optimized the basic media and culture conditions in relation to callus growth and polysaccharide content. They tested MS (acc. to Murashige and Skoog), LS (acc. to Linsmaier and Skoog), B5 (acc. to Gamborg), N6 (acc. to Chu), 1/2 MS, WPM (acc. to Lloyd and McCown) and White media, adding 0.2 mg/L NAA and 0.5 mg/L 6-BA to each of them. The results proved that MS, LS and 1/2 MS media were optimal for callus culture growth, while 1/2 MS medium was the most beneficial for polysaccharide production. WPM and White media were neither suitable for callus growth nor conducive to polysaccharide accumulation (Liang et al. 2011). Another team from the same institution has tested the effects of different factors on callus induction from *S. sphenanthera* leaves as explants (Wu et al. 2007). The results showed that the MS medium supplemented with 0.5 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid), 0.1 mg/L NAA and 1.0 mg/L 6-BA was optimal for the light-green, dense texture, vigorous callus tissue growth, at an induction rate of 96.7%. A team from the College of Jilin Agriculture Science and Technology of Jilin (Jilin) has developed a protocol for shoot culture growth (Wang and Xi 2009). They tested the MS medium with different concentrations of NAA and 2,4-D. The medium with 0.1 mg/L of NAA and 0.5 mg/L of 2,4-D was chosen as the best for the highest stem induction survival rate of *S. sphenanthera.*

The very few works in the field of plant biotechnology on *S. sphenanthera* point to the fact that it is a new topic that will probably require exploration in the near future because this species is protected in China. At the same time, it is valued for its healing effects, which makes it worthy of interest also in this field of science.

6.10 Conclusions

The presented Chinese magnolia vine—*S. chinensis*, a species of East Asian origin—has a well-established position in both traditional Asian medicine—Chinese, Korean, Japanese and Russian—and an already established but still growing position in modern global medicine. Monographs of the fruit of this species are listed not only in the pharmacopoeias of East Asian countries, but also in the national pharmacopoeias of European Union countries, in the American Herbal Pharmacopoeia and in the International Pharmacopoeia published by WHO.

The raw material—the fruit—is obtained both from natural habitats and from crops successfully grown in the provinces of northeastern China. Cultivation of this species is currently conducted more experimentally than commercially in Central European countries (Ukraine, the Czech Republic, Germany and Poland), but it is gaining

popularity and may in the future constitute an alternative to uncertain imports from China. The hope for independence from imports is also created by the dynamically developing research on this species in the area of plant biotechnology conducted mainly by research centers in China, Japan, South Korea, the Czech Republic and Poland. This species has been making a career in Europe for several years not only as a medicinal plant, but also as a cosmetic plant and an important raw material for the production of dietary supplements.

The second of the presented species—*S. sphenanthera*—is a species less known outside of China. Its natural habitats are definitely poorer compared with those of *S. chinensis*. In TCM, this species is regarded as equivalent to *S. chinensis*. However, the latest professional scientific research has provided evidence of its different chemical compositions and slightly different directions of biological activity. A monograph of the raw material obtained from this species (the fruit) has so far been included only in the Chinese pharmacopoeia. The development of cultivation conditions for this species in China due to its "endangered species" status is an extremely valuable initiative. This will allow the needs of the pharmaceutical industry to be reconciled with the expectations related to species protection. The biotechnology research, currently being pursued only by Chinese research centers, is also starting to raise some hopes for ensuring the availability of the raw material. Perhaps, the results of these studies will increase the importance of *S. sphenanthera* as a valuable medicinal plant, also in non-Asian countries.

Acknowledgments Funding This study was supported by the financial support of the funds of the Ministry of Science and Higher Education Programs: N42/DBS/000136 and N42/DBS/00121 and National Science Centre, Poland (grant number 2016/23/D/NZ7/01316).

Author Contribution Statement All the authors read and approved the manuscript in its final form. All authors declare that they have no conflict of interest.

References

Akula R, Ravishankar GA (2011) Influence of abiotic stress signals on secondary metabolites in plants. Plant Signal Behav 6:1720–1731. https://doi.org/10.4161/psb.6.11.17613

Alexander J, Wang Y (2012) Therapeutic potential of *Schisandra chinensis* extracts for treatment of hypertension. Hypertens Res 35:892–893. https://doi.org/10.1038/hr.2012.101

Bensky D, Gamble A (1993) Chinese herbal medicine materia. Medica. https://doi.org/10.4236/eng.2013.510B001

Bi H, Li F, Krausz KW, et al (2013) Targeted metabolomics of serum acylcarnitines evaluates hepatoprotective effect of Wuzhi tablet (*Schisandra sphenanthera* extract) against acute acetaminophen toxicity. Evid Based Complement Altern Med 2013: ID 985257. https://doi.org/10.1155/2013/985257

Březinová L, Vlašínová H, Havel L et al (2010) Validated method for bioactive lignans in *Schisandra chinensis* in vitro cultures using a solid phase extraction and a monolithic column application. Biomed Chromatogr 24:954–960. https://doi.org/10.1002/bmc.1391

Casarin E, Dall'Acqua S, Smejkal K, Al E (2014) Molecular mechanisms of antiproliferative effects induced by Schisandra-derived dibenzocyclooctadiene lignanas (+)-deoxyschisandrin and (−)-gomisin N in human tumour cell lines. Fitoterapia 98:241–247.https://doi.org/10.1016/j.fitote.2014.08.001

Central Pharmaceutical Affairs Council of Korea (2002) Korean Pharmacopoeia. Seoul

Chen AH, Yang JL, Da NY et al (2010) High-frequency somatic embryogenesis from germinated zygotic embryos of *Schisandra chinensis* and evaluation of the effects of medium strength, sucrose, GA3, and BA on somatic embryo development. Plant Cell Tissue Organ Cult 102:357–364. https://doi.org/10.1007/s11240-010-9740-6

Chen X, Zhang Y, Zu Y, Al E (2011) Composition and biological activites of the essential oil from *Schisandra chinensis* obtained by solvent-free microwave extraction. LWT Food Sci Technol 44:2047–2052. https://doi.org/10.1016/j.lwt.2011.05.013

Chen Y, Tang J, Wang X, et al (2012) An immunostimulatory polysaccharide (SCP-IIa) from the fruit of *Schisandra chinensis* (Turcz.) Baill. Int J Biol Macromol 50:844–848. https://doi.org/10.1016/j.ijbiomac.2011.11.015

Chen YC, Liaw CC, Bin CY et al (2013) Anti-liver fibrotic lignans from the fruits of *Schisandra arisanensis* and *Schisandra sphenanthera*. Bioorg Med Chem Lett 23:880–885. https://doi.org/10.1016/j.bmcl.2012.11.040

Cheng N, Ren N, Gao H et al (2013) Antioxidant and hepatoprotective effects of *Schisandra chinensis* pollen extract on CCl4-induced acute liver damage in mice. Food Chem Toxicol 55:234–240. https://doi.org/10.1016/j.fct.2012.11.022

Chen X, Tang R, Liu T et al (2019) Physicochemical properties, antioxidant activity and immunological effects in vitro of polysaccharides from *Schisandra sphenanthera* and *Schisandra chinensis*. Int J Biol Macromol 131:744–751. https://doi.org/10.1016/j.ijbiomac.2019.03.129

Chinese Pharmacopoeia Commission (2005) Pharmacopoeia of the People's Republic of China. China Chemical Industry Press, Beijing

Chiu P, Lam P, Yan C, Ko K (2011) Schisandrin B protects against solar irradation-induced oxidative injury in BJ human fibroblasts. Fitoterapia 82:682–691. https://doi.org/10.1016/j.fitote.2010.11.018

Choi DY, Lee YJ, Hong JT, Lee HJ (2012) Antioxidant properties of natural polyphenols and their therapeutic potentials for Alzheimer's disease. Brain Res Bull 87:144–153. https://doi.org/10.1016/j.brainresbull.2011.11.014

Chu IYE, Kurtz SL (1990) Commercialization of plant micropropagation. Handbook of plant cell culture, vol 5. Ornamental species. McGraw-Hill Inc., New York, pp 126–164

Chun J, Cho M, So I, Al E (2014) The protective effects of Schisandra chinensis fruit extract and its lignans against cardiovascular disease: a review of the molecular mechanisms. Fitoterapia 97:224–233. https://doi.org/10.1016/j.fitote.2014.06.014

Committee of the Japanese Pharmacopoeia Evaluation and Licensing Division Pharmaceuticals and Food Safety (2006) Japanese Pharmacopoeia. Bureau Ministry of Health, Labour and Welfare, Tokyo

Cunningham A, Brinckmann J (2010) "Cinderella" Schisandra: a project linking conservation and local livelihoods in the upper yangtze ecoregion of China. J Am Bot Counc 28–39. https://doi.org/10.1016/j.jep.2018.05.042

Dias MI, Sousa MJ, Alves RC, Ferreira ICFR (2016) Exploring plant tissue culture to improve the production of phenolic compounds: a review. Ind Crops Prod 82(9), 22. https://doi.org/10.1016/j.indcrop.2015.12.016

Du W, Xiao-Fan W (2012) Intercarpellary growth of pollen tubes in the extragynoecial compitum and its contribution to fruit set in an apocarpous species, *Schisandra sphenanthera* (*Schisandraceae*). Am J Bot 99:961–966. https://doi.org/10.3732/ajb.1100165

European Commission Cosing (2020) CosIng—cosmetic Database

European Directorate for the Quality of Medicine (2010) European pharmacopoeia 10.0. Strasbourg

European Food Safety Authority Botanical Summary Report|European Food Safety Authority

Fan X, Jiang Y, WAng Y, Al E (2014) Wuzhi tablet (*Schisandra sphenanthera* extract) protects against acetaminophen-induced hepatotoxicity by inhibition of CYP-mediated bioactivation and regulation of NRF2-ARE and p35/p21 pathways. Drug Metab Dispos 42:1982–1990. https://doi.org/10.1016/j.apsb.2017.04.002

Garnier S, Msika P (2013) *Schisandra sphenanthera* fruit extract and cosmetic, dermatological and nutraceutical composition comprising, United States Patent, US 8,586,107 B2

Garnier S, Naaimi D, Baudouin C (2012) Cosmetic composition for the treatment of acne comprising a peptide extracts of *Schizandra, France, FR2948565B1*

Gopaul R, Kern D, Knaggs H, Lephart J (2012) *Narcissus tazetta* and *Schizandra chinensis* to regulate 'Youth Gene Clusters'—an in vitro analysis. Cosmet Toiletries 446–448

Guo Y, Wei H, Lu C, et al (2016) Predictions of potential geographical distribution and quality of *Schisandra sphenanthera* under climate change. PeerJ 1–21.https://doi.org/10.7717/peerj.2554

Hancke JL, Burgos RA, Ahumada F (1999) *Schisandra chinensis* (Turcz.) Baill. Fitoterapia 70:451–471. https://doi.org/10.1016/S0367-326X(99)00102-1

Havel L, Vlašínová H, Bohatcová I et al (2008) Dibenzocyclooctadiene lignan production in *Schisandra chinensis* embryogenic culture. J Biotechnol 136:S437. https://doi.org/10.1016/j.jbiotec.2008.07.1012

Hill RA, Connolly JD (2013) Triterpenoids. Nat Prod Rep 30:1028–1065.https://doi.org/10.1039/c3np70032a. http://www.theplantlist.org/

Hu D, Yang Z, Yao X et al (2014) Dibenzocyclooctadiene lignans from *Schisandra chinensis* and their inhibitory activity on NO production in lipopolysaccharide-activated microglia cells. Phytochemistry 104:72–78. https://doi.org/10.1016/j.phytochem.2014.04.014

Huang S, Yang J, Huang H, Al. E (2007) Structural characterization of schintrilactone, a new class of nortriterpenoids from *Schisandra chinensis*. Org Lett 9:4175–4178. https://doi.org/10.1021/ol701679n

Huang S, Han Q, Lei C, Al E (2008) Isolation and characterization of miscellaneous terpenoids of *Schisandra chinensis*. Tetrahedron 64:4260–4267. https://doi.org/10.1016/j.tet.2008.02.085

Huang HC, Lin YC, Fazary AE et al (2011) New and bioactive lignans from the fruits of *Schisandra sphenanthera*. Food Chem 128:348–357. https://doi.org/10.1016/j.foodchem.2011.03.030

Huyke C, Engel K, Simon-Haarhaus B et al (2007) Composition and biological activity of different extracts from *Schisandra sphenanthera* and *Schisandra chinensis*. Planta Med 73:1116–1126. https://doi.org/10.1055/s-2007-981559

Hwang H, Kim I, Kwon IC, Kim Y (2008) Tumor targetability and antitumor effect of docetaxel-loaded hydrophobically modified glycol chitosan nanoparticles. J Control Release 128:23–31. https://doi.org/10.1016/j.jconrel.2008.02.003

Hwang D, Shin SY, Lee Y et al (2011) A compound isolated from *Schisandra chinensis* induces apoptosis. Bioorg Med Chem Lett 21:6054–6057. https://doi.org/10.1016/j.bmcl.2011.08.065

Ikeya Y, Taguchi H, Yosioka I, Kobayashi H (1979a) The constituents of *Schizandra chinensis* Baill. The cleavage of the methylenedioxy moiety with lead tetraacetate in benzene, and the structure of angeloylgomisin. Q Chem Pharm Bull 27:2536–2538

Ikeya Y, Taguchi H, Yosioka I, Kobayashi H (1979b) The constituents of *Schizandra chinensis* Baill. III. The structures of four new lignans, gomisin H and its derivative, angeloyl-, tigloyl- and benzoylgomisin H. Chem Pharm Bull 27:1576–1582

Ip SP, Che CT, Kong YC, Ko KM (2001) Effects of schisandrin B pretreatment on tumor necrosis factor-alpha induced apoptosis and Hsp70 expression in mouse liver. Cell Stress Chaperones 6:44–48

Jiang ZT, Li R, Yu JC (2006) Pungent components from thioglucosides in *Armoracia rusticana* grown in China, obtained by enzymatic hydrolysis. Food Technol Biotechnol 44:41–45

Jiang K, Song QY, Peng SJ, et al (2015a) New lignans from the roots of *Schisandra sphenanthera*. Fitoterapia 103:63–70.https://doi.org/10.1016/j.fitote.2015.03.015

Jiang Y, Fan X, Wang Y, et al (2015b) Hepato-protective effects of six schisandra lignans on acetaminophen-induced liver injury are partially associated with the inhibition of CYP-mediated bioactivation. Chem Biol Interact 231:83–89.https://doi.org/10.1016/j.cbi.2015.02.022

Jin J, Bi H, Hu J, Al E (2011) Effects of Wuzhi tablet (*Schisandra sphenanthera* extract) on the pharmacokinetics of pacliaxtel in rats. Phytother Res 25:1250–1253. https://doi.org/10.1002/ptr.3407

Kam Ming K, Jun Y, Chuixin Q (2015) *Schisandra Chinensis*: an herb of North Eastern China origin. World Scientific Publishing, Singapore

Kang Y, Shin H (2012) Inhibitory effects of *Schizandra chinensis* extract on atopic dermatitis in NC/Nga mice. Immunopharmacol Immunotoxicol 34:292–298. https://doi.org/10.3109/08923973.2011.602689

Karuppusamy S (2007) A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. J Med Plants Res 3:1222–1239. https://doi.org/10.5897/JMPR

Kim SR, Lee MK, Koo KA et al (2004) Dibenzocyclooctadiene lignans from *Schisandra chinensis* protect primary cultures of rat cortical cells from glutamate-induced toxicity. J Neurosci Res 76:397–405. https://doi.org/10.1002/jnr.20089

Kirakosyan A, Kaufman PB (2009) Recent advances in plant biotechnology, 1-409. https://doi.org/10.1007/978-1-4419-0194-1

Kochetkov N, Khorlin A, Chizhov O, Al E (1961) Schizandrin—lignan of unusual structure. Tetrahedron Lett 2:730–734. https://doi.org/10.1016/S0040-4039(01)91684-3

Kohda H, Ozaki M, Namera A (2012) Production of lignans in calluses of *Schisandra chinensis*. J Nat Med 66:373–376. https://doi.org/10.1007/s11418-011-0586-y

Kwan HY, Niu X, Dai W et al (2015) Lipidomic-based investigation into the regulatory effect of Schisandrin B on palmitic acid level in non-alcoholic steatotic livers. Sci Rep 5:1–14. https://doi.org/10.1038/srep09114

Lam P, Yan C, Chiu P et al (2011) Schisandrin B protects against solar irradiation-induced oxidative stress in rat skin tissue. Fitoterapia 82:393–400. https://doi.org/10.1016/j.fitote.2011.02.010

Lee HJ, Cho IH, Lee KE, Kim YS (2011) The compositions of volatiles and aroma-active compounds in dried omija fruits (*Schisandra chinensis Baillon*) according to the cultivation areas. J Agric Food Chem 59:8338–8346. https://doi.org/10.1021/jf200762h

Li L, Lu Q, Shen Y, Hu X (2006) Schisandrin B enhances doxorubicin-induced apoptosis of cancer cells but not normal cells. Biochem Pharmacol 71:584–595. https://doi.org/10.1016/j.bcp.2005.11.026

Li W, Yan C, Wu Y et al (2014) Osteoblasts proliferation and differentiation stimulating activities of the main components of *Fructus Psoraleae corylifoliae*. Phytomedicine 21:400–405. https://doi.org/10.1016/j.phymed.2013.09.015

Liang C, Hu J, Shi Y, Al E (2013) Schisphenlignans A-E: five new dibenzocyclooctadiene lignans from *Schisandra sphenanthera*. Chem Pharm Bull 61:96–100. https://doi.org/10.1248/cpb.c12-00700

Li Z, He X, Liu F et al (2018) A review of polysaccharides from *Schisandra chinensis* and *Schisandra sphenanthera*: Properties, functions and applications. Carbohyd Polym 184:178–190. https://doi.org/10.1016/j.carbpol.2017.12.058

Liang W, Deng B, Xiao J, Wang S (2011) Effects of basic media and culture conditions on callus growth and polysaccharide content in *Schisandra sphenanthera*. J Central South Univ For Technol

Liu C, Fang S, Huang M, Al E (1978) Studies on the active principles of *Schisandra sphenanthera* Rehd. et Wils. The structures of schisantherin A, B, C, D, E, and the related compounds. Sci Sinica 21:483–502

Liu HW, Yu XZ, Padula D et al (2013) Lignans from *Schisandra sphenathera* Rehd. et Wils. and semisynthetic schisantherin A analogues: absolute configuration, and their estrogenic and anti-proliferative activity. Eur J Med Chem 59:265–273. https://doi.org/10.1016/j.ejmech.2012.11.003

Lu H, Liu GT (1992) Anti-oxidant activity of dibenzocyclooctene lignans isolated from *Schisandraceae*. Planta Med 58:311–313. https://doi.org/10.1055/s-2006-961473

Lu Y, Chen DF (2009) Analysis of *Schisandra chinensis* and *Schisandra sphenanthera*. J Chromatogr A 1216:1980–1990. https://doi.org/10.1016/j.chroma.2008.09.070

Paula MW (2012) The status of temporary immersion system (TIS) technology for plant micropropagation. Afr J Biotech 11:14025–14035.https://doi.org/10.5897/ajb12.1693
Matkowski A (2008) Plant in vitro culture for the production of antioxidants—a review. Biotechnol Adv 26:548–560. https://doi.org/10.1016/j.biotechadv.2008.07.001
Miao M, Gao J, Zhang G, et al (2009) Effect of *Schisandra chinensis* polysaccharide on intracerebral acetylcholinesterase and monoamine neurotransmitters in a D-galactose-induced aging brain mouse model. Neural Regeneration Res 4:687–693. https://doi.org/10.3969/j.issn.1673-5374.2009.09.009
Mocan A, Crişan G, Vlase L et al (2014) Comparative studies on polyphenolic composition, antioxidant and antimicrobial activities of *schisandra chinensis* leaves and fruits. Molecules 19:15162–15179. https://doi.org/10.3390/molecules190915162
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tabacco tissue cultures. Physiol Plant 15:473–497. https://doi.org/10.1016/S0031-9422(01)00179-0
Opletal L, Sovova H, Bartlova M (2004) Dibenzo [a, c] cyclooctadiene lignans of the genus Schisandra: importance, isolation and determination. J Chromatogr B 812:357–371. https://doi.org/10.1016/j.jchromb.2004.07.040
Pan S-Y, Chen S-B, Dong H-G, et al (2011) New perspectives on chinense herbal medicine (zhongyao) research and development. Evid Based Complement Altern Med 1–11.https://doi.org/10.1093/ecam/neq056
Panossian A, Wagner H (2005) Stimulating effect of adaptogens: an overview with particular reference to their efficacy following single dose administration. Phytother Res 19:819–838. https://doi.org/10.1002/ptr.1751
Panossian A, Wikman G (2008) Pharmocology of *Schisandra chinensis* Bail: an overview of Russian research and uses in medicine. J Ethnopharmacol 118:183–212
Park E, Chun J, Kim S, Al E (2012) Schisandrin B supresses TGF- β1 signaling by inhibiting Smad2/3 and MAPK pathways. Biochem Pharmacol 83:378–384. https://doi.org/10.1016/j.bcp.2011.11.002
Park S, Kim J, Jeong K et al (2013) Antioxidant activity and inhibition of MMP-1 expression of *Schizandrae fructus* (*Schizandra chinensis*) extract. Korean J Pharmacognosy 1:47–52
Pietrosiuk A, Furmanowa M (2006) Plant biotechnology in human healthcare. Biotechnologia 116–123
Qiangrong P, Wang T, Lu Q, Hu X (2005) Schisandrin B—a novel inhibitor of P-glycoprotein. Biochem Biophys Res Commun 335:406–411. https://doi.org/10.1016/j.bbrc.2005.07.097
Qin X, Bi H, Wang X, Al E (2010) Mechanistic understanding of the different effects of Wuzhi tablet (*Schisandra sphenanthera* extract) on the absorption and first-pass intestinal and hepatic metabolism of Tacrolimus (FK 506). Int J Pharm 389:114–121. https://doi.org/10.1016/j.ijpharm.2010.01.025
Qin XL, Chen X, Wang Y et al (2014) In vivo to in vitro effects of six bioactive lignans of wuzhi tablet (*Schisandra sphenanthera* extract) on the CYP3A/pglycoprotein-mediated absorption and metabolism of tacrolimus. Drug Metab Dispos 42:193–199. https://doi.org/10.1124/dmd.113.053892
Ren R, Ci X, Lia H, Al E (2010) New dibenzocyclooctadiene lignans from *Schisandra sphenanthera* and thier proinflammatory cytokine inhibitory activites. Z Naturforsch 65:211–218. https://doi.org/10.1016/j.bmc.2004.12.020
Saunders RM (2000) Monograph of Schisandra (*Schisandraceae*). Am Soc Plant Taxonomists 58:1–146
Shaitan I (2005) Колбасина ЕИ: Актиндия и лимонник (Actinidia and Chinese Magnolia vine). In: Издательский Дом МСП. Moscow
Shi Y, Zhong W, Chen H, Al E (2014) New lignans form the leaves and stems of *Schisandra chinensis* an their anti-HIV activites. Chin J Chem 32:734–740. https://doi.org/10.1002/cjoc.201400001
Shuwei L, Haidong C (2000) On the indications of bifendate. Adverse Drug React J 2:225–228. https://doi.org/10.1016/s0140-6736(00)02219-4

Slanina J, Táborská E, Lojková L (1997) Lignans in the seeds and fruits of *Schisandra chinensis* cultured in Europe. Planta Med 63:277–280. https://doi.org/10.1055/s-2006-957676

So-Ra C, Chang-Su K, Jong-Yeob K et al (2011) Changes of antioxidant activity and lignan contents in *Schisandra chinensis* by harvesting times. Korean J Med Crop Sci 19:414–420

Song L, Ding JY, Tang C, Yin CH (2007) Compositions and biological activities of essential oils of *Kadsura longepedunculata* and *Schisandra sphenanthera.* Am J Chin Med 35:353–364. https://doi.org/10.1142/S0192415X07004874

Song QY, Zhang CJ, Li Y et al (2013) Lignans from the fruit of *Schisandra sphenanthera*, and their inhibition of HSV-2 and adenovirus. Phytochem Lett 6:174–178. https://doi.org/10.1016/j.phytol.2012.12.008

Sovová H, Opletal L, Bártlová M et al (2007) Supercritical fluid extraction of lignans and cinnamic acid from *Schisandra chinensis.* J Supercrit Fluids 42:88–95. https://doi.org/10.1016/j.supflu.2007.01.008

Sun Y, Wen X, Huang H (2010) Population genetic differentiation of *Schisandra chinensis* and *Schisandra sphenanthera* as revealed by ISSR analysis. Biochem Syst Ecol 38:257–263. https://doi.org/10.1016/j.bse.2010.01.005

Szopa A, Ekiert H (2011) Lignans in *Schisandra chinensis* in vitro cultures. Pharmazie 66:633–634

Szopa A, Ekiert H (2012) In vitro cultures of *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine)—a potential biotechnological rich source of therapeutically important phenolic acids. Appl Biochem Biotechnol 166:1941–1948. https://doi.org/10.1007/s12010-012-9622-y

Szopa A, Ekiert H (2013) Production of deoxyschizandrin and γ-schizandrin in shoot-differentiating and undifferentiating callus cultures of *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine). J Biotechnol 165:209–213. https://doi.org/10.1016/j.jbiotec.2013.03.010

Szopa A, Ekiert H (2014) *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine) in vitro cultures. In: Govil JN (ed) Recent progress in medicinal plants. Biotechnology and genetic engineering II, 39th edn. Studium Press LLC, USA, pp 405–434

Szopa A, Ekiert H (2015) Production of schisantherin A and gomisin G in in vitro cultures of *Schisandra chinensis*. Phytochem Lett 11:440–444. https://doi.org/10.1016/j.phytol.2014.12.022

Szopa A, Ekiert H (2016) The importance of applied light quality on the production of lignans and phenolic acids in *Schisandra chinensis* (Turcz.) Baill. cultures in vitro. Plant Cell, Tissue Organ Cult 127:115–121. https://doi.org/10.1007/s11240-016-1034-1

Szopa A, Kisiel W, Ekiert H, Szewczyk A (2015) Isolation of three dibenzocyclooctadiene lignans from in vitro cultures of *Schisandra chinensis* (Turcz.) Baill.—the first report. Pharmazie 70:337–339. https://doi.org/10.1691/ph.2015.4779

Szopa A, Kokotkiewicz A, Marzec-Wróblewska U et al (2016) Accumulation of dibenzocyclooctadiene lignans in agar cultures and in stationary and agitated liquid cultures of *Schisandra chinensis* (Turcz.) Baill. Appl Microbiol Biotechnol 100:3965–3977. https://doi.org/10.1007/s00253-015-7230-9

Szopa A, Ekiert R, Ekiert H (2017) Current knowledge of *Schisandra chinensis* (Turcz.) Baill. (Chinese magnolia vine) as a medicinal plant species: a review on the bioactive components, pharmacological properties, analytical and biotechnological studies. Phytochem Rev 16:195–218. https://doi.org/10.1007/s11101-016-9470-4

Szopa A, Dziurka M, Warzecha A, Kubica P, Klimek-Szczykutowicz M, Ekiert H (2018a) Targeted lignan profiling and anti-inflammatory properties of *Schisandra rubriflora* and *Schisandra chinensis* extracts. Molecules 23:3103.https://doi.org/10.3390/molecules23123103

Szopa A, Klimek-Szczykutowicz M, Kokotkiewicz A, et al (2018b) Phytochemical and biotechnological studies on *Schisandra chinensis* cultivar Sadova No. 1—a high utility medicinal plant. Appl Microbiol Biotechnol 102:5105–5120.https://doi.org/10.1007/s00253-018-8981-x

Szopa A, Kokotkiewicz A, Król A, et al (2018c) Improved production of dibenzocyclooctadiene lignans in the elicited microshoot cultures of *Schisandra chinensis* (Chinese magnolia vine). Appl Microbiol Biotechnol 102:945–959.https://doi.org/10.1007/s00253-017-8640-7

Szopa A, Warzecha A, Klimek-Szczykutowicz M, Stańczyk K (2018d) Cytryniec chiński (*Schisandra chinensis* (Turcz.) Baill.)—z tradycyjnej medycyny chińskiej do współczesnej fitoterapii. Herbalism 1:101–119

Szopa A, Klimek-Szczykutowicz M, Kokotkiewicz A, et al (2019a) Phenolic acid and flavonoid production in agar, agitated and bioreactor-grown microshoot cultures of *Schisandra chinensis* cv. Sadova No. 1—a valuable medicinal plant. J Biotechnol 305:61–70.https://doi.org/10.1016/j.jbiotec.2019.08.021

Szopa A, Kokotkiewicz A, Bednarz M, et al (2019b) Bioreactor type affects the accumulation of phenolic acids and flavonoids in microshoot cultures of *Schisandra chinensis* (Turcz.) Baill. Plant Cell, Tissue Organ Cult 139:199–206.https://doi.org/10.1007/s11240-019-01676-6

Szopa A, Kokotkiewicz A, Klimek-Szczykutowicz M, et al (2020) Different Types of In vitro cultures of *Schisandra chinensis* and its cultivar (*S. chinensis* cv. Sadova): a rich potential source of specific lignans and phenolic compounds. In: Ramawat K, Ekiert H, Goyal S (eds) Plant cell and tissue differentiation and secondary metabolites. Reference series in phytochemistry. Springer, Cham, pp 1–28. https://doi.org/10.1007/978-3-030-11253-0_10-1

Thandavarayan R, Giridharan V, Arumugam S, Al E (2015) Schisandrin B prevents doxorubcin induced cardiac dysfunction by modulation of DNA damage, oxidative stress and inflammation through inhibition of MAPK/p53 signaling. PLoS ONE 10:119–214. https://doi.org/10.1371/journal.pone.0119214

Tong H, Zhao B, Du F, Al E (2012) Isolation and physicochemical characterization of polysaccharide fraction isolated from *Schisandra chinensis*. Chem Nat Compd 47:969–970. https://doi.org/10.1007/s10600-012-0116-5

Upton R, Graff A, Jolliffe G, et al (2011) Botanical pharmacognosy—microscopic characterization of botanical medicines. In: American herbal pharmacopoeia. p CRC Press

Urząd Rejestracji Produktów Leczniczych Wyrobów Medycznych i Produktów Biobójczych (2018) *Schisandrae chinensis* fructus. In: Farmakopea Polska XI. Rzeczpospolita Polska

Verpoorte R, Contin A, Memelink J (2002) Biotechnology for the production of plant secondary metabolites. Phytochem Rev 1:13–25. https://doi.org/10.1023/A:1015871916833

Waiwut P, Shin M, Yokoyama S, Al E (2012) Gomisin A enhances tumor necrosis factor-α-induced G1 cell cycle arrest via signal transducer and activator of transcription 1-mediated phosphotylation of retinoblastoma protein. Biol Pharm Bull 35:1997–2003. https://doi.org/10.1248/bpb.b12-00450

Wang Y, Xi G (2009) Screening culture medium of *Schisandra sphenanthera* Rehd.et Wils's induction and differentiation. Northern Horticulture

Wei X, Li H, Che P et al (2020) Comparing chloroplast genomes of traditional Chinese herbs *Schisandra sphenanthera* and *S. chinensis*. Chinese Herbal Medicines 12:247–256. https://doi.org/10.1016/j.chmed.2019.09.009

World Health Organization (2007) Monographs on selected medicinal plants, vol 3. Fructus Schisandrae, Geneva, pp 296–313

Wu L, Xie B, Deng B, et al (2007) Callus induction in *Schisandra sphenanthera* Rehd. Et Wils. Nonwood Forest Research

Xia Y, Yang B, Kuang X (2015) *Schisandraceae* triterpenoids: a review. Phytochem Rev 14:155–187. https://doi.org/10.1007/s11101-014-9343-7

Xiao Y, Zhang H (2012) Homogeneous ionic liquid microextraction of the active constituents from fruits of *Schisandra chinensis* and *Schisandra sphenanther*a. Anal Chim Acta 712:78–84. https://doi.org/10.1016/j.aca.2011.11.033

Xiao W, Yang L, Li L, Al E (2007) Sphenalactones A-D, a new class of high oxygenated trinotritrtpenoids from *Schisandra sphenanthera*. Tetrahedron Lett 48:5543–5546. https://doi.org/10.1016/j.tetlet.2007.05.152

Xie J, Lee S, Chen X (2010a) Nanoparticle-based theranostic agents. Adv Drug Deliv Rev 62:1064–1079.https://doi.org/10.1016/j.addr.2010.07.009

Xie y, Hao H, Kang A, et al (2010b) Integral pharmacokinetics of multiple lignan components in normal, CCl4-induced hepatic injury and hepatoprotective agents pretreated rats and correlations with hepatic injury biomarkers. J Ethnopharmacol 131:290–299.https://doi.org/10.1016/j.jep.2010.06.038

Xu L, Grandi N, Del Vecchio C, Al E (2015) From the traditional Chinese medicine plant *Schisandra chinensis* new scaffolds effective on HIV-1 reverse transcriptase resistant to non-nucleoside inhibitors. J Microbiol 53:288–293. https://doi.org/10.1007/s12275-015-4652-0

Xue YB, Zhang YL, Yang JH et al (2010) Nortriterpenoids and lignans from the fruit of *Schisandra chinensis*. Chem Pharm Bull 58:1606–1611. https://doi.org/10.1248/cpb.58.1606

Xue XP, Qin XL, Xu C et al (2013) Effect of Wuzhi tablet (*Schisandra sphenanthera* extract) on the pharmacokinetics of cyclosporin A in rats. Phytother Res 27:1255–1259. https://doi.org/10.1002/ptr.4849

Yan ZF, Guo J, Tian FH et al (2015) Active compounds from *Schisandra chinensis* exhibiting tyrosinase activity and melanin content inhibition in B16 melanoma cells. Biotechnol Bioprocess Eng 20:814–823. https://doi.org/10.1007/s12257-014-0867-x

Yim S, Lee Y, Lee Y, Al E (2009) Gomisin N isolated from *Schisandra chinensis* significantly induces anti-proliferative and pro-apoptopic effects in hepatic carcinoma. Mol Med Rep 2:725–732. https://doi.org/10.3892/mmr_00000163

Zhang J, Chen J, Liang Z, Al E (2014) New lignans and their biological activites. Chem Biodivers 11:1–54. https://doi.org/10.1002/cbdv.201100433

Zhang SW, Xin HuX, Shen C et al (2020) Protective effect of *Schisandra chinensis* extract against ethanol-induced gastric ulcers in mice by promoting anti-inflammatory and mucosal defense mechanisms. Rev Bras. https://doi.org/10.1007/s43450-020-00096-4

Zhao T, Mao G, Mao R, et al (2013a) Antitumor and immunomodulatory activity of a water-soluble low molecular weight poly saccharide from *Schisandra chinensis* (Turcz.) Baill. Food Chem Toxicol 55:609–616.https://doi.org/10.1016/j.fct.2013.01.041

Zhao X-N, Huang S, Zhao J, Zhang Y (2013b) Gender variation in a monoecious woody vine *Schisandra chinensis* (*Schisandraceae*) in northeast China. Annales Botanici Fennici 50:209–219. https://doi.org/10.5735/086.050.0402

Zhao T, Mao G, Feng W et al (2014) Isolation, characterization and antioxidant activity of polysaccharide from *Schisandra sphenanthera*. Carbohyd Polym 105:26–33. https://doi.org/10.1016/j.carbpol.2014.01.059

Zhou E, Li Y, Wei Z et al (2014) Schisantherin A protects lipopolysaccharide-induced acute respiratory distress syndrome in mice through inhibiting NF-κB and MAPKs signaling pathways. Int Immunopharmacol 22:133–140. https://doi.org/10.1016/j.intimp.2014.06.004

Zhu M, Chen XS, Wang KX (2007) Variation of the lignan content of *Schisandra chinensis* (Turcz.) Baill. and *Schisandra sphenanthera* Rehd. et Wils. Chromatographia 66:125–128. https://doi.org/10.1365/s10337-007-0261-3

Zhu M, Yeung RY, Lin KF, Li RC (2000) Improvement of phase I drug metabolism with *Schisandra chinensis* against CCl4 hepatotoxicity in a rat model. Planta Med 66:521–525. https://doi.org/10.1055/s-2000-11202

Chapter 7
Cultivation and Utilization of *Coleus* Species

Nikhila Reddy Reddymalla, Sushanth Pureti, and Viswanatha Chaitanya Kolluru

Abstract *Coleus* is a genus comprising of a vast amount of medicinal plants that are being easily grown in house and cultivated in the fields for various economic purposes. This genus comprises around 325 medicinal plants that are used in Ayurveda and folk medicine. Among them, the most economically important species are *C. aromaticus, C. zeylanicus, C. amboinicus, C. amboinicus variegatus, C. canninus, C. vettiveroides, C. barbatus, and C. forskohlii*. Morphology, medicinal properties, phytochemistry, geographical distribution, and cultivation of the above *Coleus* species were discussed in detail in this chapter, with special emphasis on *C. forskohlii*. The conditions for the in vitro culture of *Coleus forskohlii* and its propagation and the medicinal importance of forskolinextracted from the tuberous roots of *Coleus forskohlii* were also discussed.

Keywords Coleus species · Phytochemistry · Medicinal properties · Cultivation · In vitro propagation

7.1 Introduction

Genus *Coleus* is a perennial, branched aromatic herb belonging to the family "*Lamiaceae*" consists of more than 500 species that are cultivated worldwide. The word *Coleus* was derived from the Greek word *Koleos*, the meaning sheath around style as described by De Loureiro in 1970. *Coleus* plants can adapt to semi-dry conditions and are capable of tolerating low levels of light, warm, and dry environmental conditions, which provides an additional advantage of cultivating this plant with much ease (Rice et al. 2011). They can grow up to a height of 30–60 cm with thick, tuberous roots that are aromatic, and the colour of the flowers varies from blue to pale lavender (Himesh and Singh 2012). *Coleus* plants can be propagated using seeds as well as by the means of a vegetative method (Prajapati et al. 2003). The whole plant *Coleus* is being used since ancient times due to

N. R. Reddymalla · S. Pureti · V. C. Kolluru (✉)
Department of Biotechnology, GITAM Institute of Technology, GITAM Deemed to be University, Visakhapatnam, Andhra Pradesh 530045, India

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_7

their medicinal value as anticancer, antispasmodic, antidepressant, anti-aggregant, antidiuretic, antimetastatic, antiglaucomic, vasodilator, bronchodilator, cardiotonic, cAMP-genic, bronchospasmolytic, central nervous system depressant, immunosuppressant, hypotensive, glycogenolytic, gluconeogenic, gastrostimulant, myorelaxant, neurogenic, secretagogue, sialagogue, thyrotropic, lipolytic, positive inotropic, and pancreostimulant properties (Duke et al. 2002). Species of *Coleus* that are grown indoors as well as outdoors possess medicinal properties, which exhibits biological activities against various dreadful diseases. These properties are due to the presence of a variety of bioactive compounds such as phenols, flavonoids, tannins, and terpenes. *Coleus* species are used in the preparations of Ayurvedic medicine and folk medicine against worms, skin problems, heart diseases, abdominal colic, respiratory disorders, painful micturition, insomnia, and convulsions. A large number of *Coleus* species are used in Southern Africa for the preparation of traditional medicine as they possess a potency of curing several diseases (Gaspar-Marques et al. 2006). Leaves of *Coleus* are used against several health-related conditions including malarial fever, epilepsy, cough, dyspepsia, otalgia, cephalalgia, flatulence, diarrhoea, cholera, convulsions, hepatopathy, bronchitis, hiccough, halitosis, and strangury. The antibacterial, antifungal, antiplasmodial, antitumoural, and insecticidal properties of *Coleus* are due to the presence of diterpenoids particularly of abietane diterpenes (Grayer et al. 2010). The major phytoconstituents of *Coleus* reported so far are flavonoids, glycosides, phenolic, and volatile compounds. The presence of these bioactive compounds with potential pharmacological properties makes this genus an important medicinal plant for the development of novel drugs.

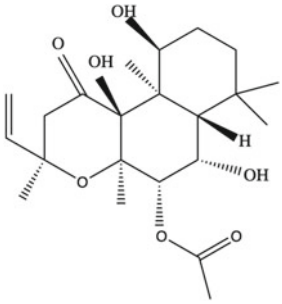
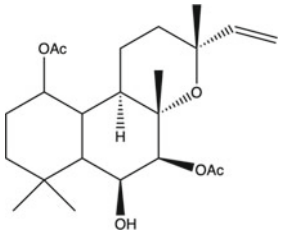
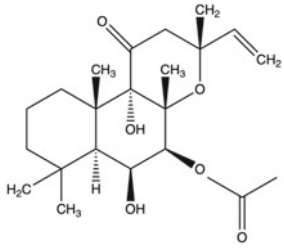
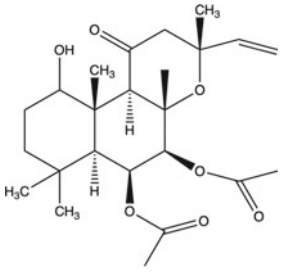
The medicinal properties of different *Coleus* species were displayed in Table 7.1. It has been established that the medicinal use of this plant is up to 85% (Lukhoba et al. 2006). Tissues of different *Coleus* species like leaf, root, stem, and tubers have

Table 7.1 Medicinal properties of different *Coleus* species

Species name	Medicinal use
Coleus ambiguous	Used to treat respiratory disorders (Neuwinger 2000)
Coleus barbatus	Used to cure digestive, liver complaints and to treat skin, genito-urinary, respiratory infections, fever, pain, inflammation, poison treatment (Lukhoba et al. 2006)
Coleus amboinicus	Used to treat respiratory infections (Albuquerque 2001)
Coleus hadiensis	Used to treat respiratory disorders (Pooley 1998)
Coleus esculentus	Used to treat pain and digestive system disorders (Neuwinger 2000)
Coleus ecklonii	Used for headache, hay fever and skin infections (Pooley 1998)
Coleus laxiflorus	Used against skin infections, inflammation, pain, gastro-urinary disorders (Neuwinger 2000), digestive, respiratory disorders (Hutchings et al. 1996), fever, and also used as mouth wash (Pooley 1998)
Coleus madagascariensis	Used to treat skin and respiratory infections (Pooley 1998; Neuwinger 2000)

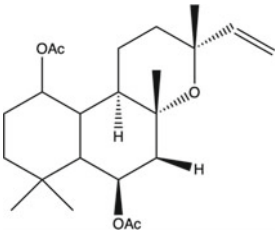
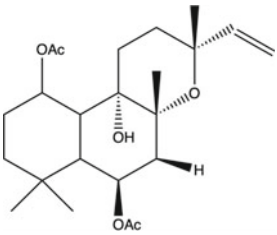
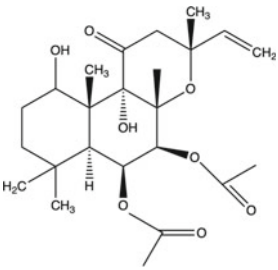
a potential role in treating several ailments mentioned in the Economic Botany Data Collection Standard (Cook 1995). Apart from their medicinal use, they are also used as insect repellents (Pooley 1998) and culinary herbs (Lukhoba et al. 2006) (Table 7.2).

Table 7.2 Diterpenoids obtained from the roots of *C. forskohlii*

S. No	Compound	Structure	References
1	Forskolin		Shah et al. (1980)
2	Forskolin E		Jin and He (1998)
3	Forskolin F		Jin and He (1998)
4	Forskolin G		Xu and Kong (2004)

(continued)

Table 7.2 (continued)

S. No	Compound	Structure	References
5	Forskolin H		Xu and Kong (2004)
6	Forskolin I		Xu and Kong (2004)
7	Forskolin J		Xu and Kong (2004)

7.2 Different Species of *Coleus* and Their Significance

Coleus aromaticus (Fig. 7.1) is a succulent perennial herb that grows up to a height of 50 cm with leathery heart-shaped, thick, and juicy aromatic leaves, used to enhance the flavour of meat dishes. It has fleshy, tomentose stems of about 30–90 cm, and the flowers are pale purplish in colour. It is commonly known as "*Karpurvalli"* in South India and as "*Parnayavani*" in Sanskrit, and it is also popularly known as "Indian Oregano". *C. aromaticus* leaves contain volatile oils, carvacrol, thymol, quercetin, luteolin, apigenin, and triterpenoids (Chatterjee and Pakrashi 2001). It exhibits anti-tumour and cytotoxic activities used against cancer. The leaves are more commonly used to control diarrhoea in developing countries like India. *Coleus aromaticus* is found effective against mycobacteria, the causative agent of tuberculosis. This herb is popularly known as "Karpuravalli" as it can remove stones formed in kidneys. The major compound responsible for the radical scavenging activity of *C. aromaticus* was found to be rosmarinic acid.

Fig. 7.1 *Coleus aromaticus*

Coleus zeylanicus (Fig. 7.2) is an aromatic herbaceous plant popularly known as "Iruveli", grown in tropical countries. It grows up to the height of 1 m with thin, slender, aromatic fibrous roots of 30–90 cm long. Leaves are simple, sub-succulent, and petiolate. It is used as a hair tonic and to treat fever, hyperpiesia, skin diseases, leprosy, ulcers, leucoderma, and burning sensation (Sharma 1997). More than 75 Ayurvedic drugs currently available in the local and global markets are prepared by extracting the essential oil from the root of *Coleus zeylanicus*. Urinal disorders and the problem of indigestion can be cured using this plant preparation marketed in the name of Snana Choornam, Devashtagandha, and Kashayam. The National Medicinal Plants Board (NMPB), Government of India, has recognized the importance of this plant and promoted its cultivation, research, and development (Nisheeda et al. 2016). *C. zeylanicus* possesses antimicrobial, anticancer, antidiabetic, antioxidant, and hepatoprotective properties (Sundara Ganapathy et al. 2015). This plant is used

Fig. 7.2 *Coleus zeylanicus*

Fig. 7.3 *Coleus ambonicus*

against several health-related conditions like vomiting, nausea, strangury, genito-urinary diseases, insanity, giddiness, and thirst. The essential oil obtained from the root of *Coleus zeylanicus* can be used as a hand sanitizer (Das et al. 2005).

Coleus amboinicus (Fig. 7.3) is a succulent herb that grows up to the height of 30–90 cm with simple, broad leaves, and hairy roots. It is used to treat indigestion, fever, epilepsy, headache, dyspepsia, diarrhoea, toothache, insect bites, nervous tension, earache, rheumatism, bronchitis, and whooping cough. It is well known as an effective expectorant used commonly in India and Southeast Asia to treat sore throat and cough. The herbal tea made from the leaves of *Coleus amboinicus* is used to treat pain, insomnia, asthma, and flatulence.

Coleus barbatus (Fig. 7.4) is a perennial, succulent-branched fleshy herb that grows up to the height of 15–40 cm between 1000–2600 m altitudes above sea level (Ryding 2006). This plant is used as a stimulant in the treatment of cough. The aerial parts of the plant have cytotoxic, anti-tumour, and diuretic activities, also used in the treatment of gums and teeth disorders. The major active compounds present in this plant are diterpenes, triterpenes, tormentic acid, α-amyrin, and the flavones such as 3,7 dimethyl quercetin, sitosterol, and kumatakinin.

Coleus vettiveroides (Fig. 7.5) is a small profusely branched bushy shrub that

Fig. 7.4 *Coleus barbatus*

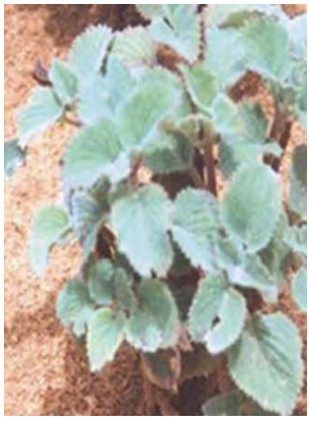


Fig. 7.5 *Coleus vettiveroides*

grows to a height of 1 m, with clusters of adventitious roots reaching up to 50 cm in length and 1 mM in thickness (Saraswathy et al. 2011). This *Coleus* species is a native of Sri Lanka and India grown widely in sandy loams. In India, this plant is cultivated in Tamil Nadu for its medicinal properties. Dried roots of *Coleus vettiveroides* are known as Hrivera in Sanskrit, used for curing gastrointestinal disorders such as diarrhoea, dysentery, and fever due to these disorders.

Coleus caninus Roth (Fig. 7.6) also known as *Coleus spicatus* Benth. is an annual herb that grows to a length of 0.3 m. Stems are erected fleshy, hairy, and moderately pubescent. Leaves are subsessile or with short petioles with fleshy, obovate, moderately rounded, margin dentate, and oblanceolate blade. Similar to remaining *Plectranthus* species, inflorescence is spike-like with flowered cymes, pedicles suberect with the recurved apical part. Fruiting calyx is broad, with the densely villous throat, posing broad, concave, decurrent upper lip, and long lateral lower lobes. Corolla is violet in colour and with a long lower lip with embedded long stamens. This plant has been fabled to be scaring the canine fauna especially cats, hence the name *caninus*. This species is endemic to India (Valdes et al. 1987).

Coleus atropurpureus Benth. commonly known as *C. blumei* Benth. (Fig. 7.7) is grown as an ornamental plant due to different colours and shades of leaves. Stems of this plant are erect which was finely pubescent to glabrous. Typical characteristics of this plant are its decorative leaves which are petiolate with brightly coloured or blotched, generally ovate-deltoid to broadly ovate, and attenuate at base, acute to acuminate at apex, crenate to laciniate at the margin. Flowers are in verticils, with oblique calyx, and the corolla is infundibular with abruptly recurved upper lip and deeply concave lower lip. Stamens are filamentous and united at base and are grown both in sun and shade with large pointed reddish pink leaves (Eggli 2004).

Coleus amboinicus variegatus (Fig. 7.8) is a succulent herb with fleshy 30–90 cm quadrangular stems with either hispidly villous or tomentose hairy structures. Leaves are broad, simple, undivided, egg, or oval-shaped with a tapering tip and are very thick with a pleasant aromatic refreshing odour. *Coleus amboinicus variegatus* leaf has white creamy colour striated line decorating the borders of the leaf giving a

Fig. 7.6 *Coleus caninus*

Fig. 7.7 *Coleus blumei*

typical significant appearance (Kaliappan and Viswanathan 2008). Roots are hairy, slender with white colour arising from the nodes exposed to soil or water. The flower is violet in colour with illiac corolla which is bilobed as the upper lip and long concave boat like a lower lip. Four stamens are observed of which two are uneven and attached to the ovary.

7.3 Phytochemistry of *Coleus* Species

C. aromaticus contains a large number of bioactive compounds with antimicrobial and antioxidant properties. The presence of compounds like carvacrol (13.25%), γ-terpinolene (3.75%), pinene (2.50%), β-caryophyllene (4.20%), methyl eugenol (2.10%), 1,8-cineole (5.45%), eugenol (4.40%), phellandrene (1.90%), and thymol

Fig. 7.8 *Coleus amboinicus variegatus*

(41.3%) were reported in the essential oil of *C. aromaticus* (Baslas and Kuma 1981). Important compounds such as 3-hexadiene (0.1%), (Z)-3-hexenol (0.6%), (E,Z) farnesene (0.2%), (E,E) farnesene (0.2%), and murolene (0.2%)and (Z)-1 were identified in this plant (Prudent et al. 1995). Twenty-six different compounds were identified by GC-MS analysis from the leaf extracts of *C. aromaticus* extracted using hexane, steam distillation, and supercritical fluid extraction (Pino et al. 1996). *C. aromaticus* contains a major compound called rosmarinic acid responsible for the radical scavenging activity (Kumaran and Karunakaran 2007). The compound eucalyptol was isolated by the method of solid-phase microextraction and steam distillation from the leaves of *C. aromaticus*. The antioxidant activity of *c. aromaticus* is due to the presence of rosmarinic acid, caffeic acid, chlorogenic acid, flavonoids, and carvacrol (Palani et al. 2010). The presence of compounds, namely Eudesma-4 (14),11-diene, Squalene, phytol, and 1,2-Benzene diol 4-(1,1 dimethyl ethyl), was reported from the acetone extracts of *C. aromaticus* responsible for the antimicrobial activity (Jasmine and Selvi 2013).

Diterpenoids such as forskolin, deactylforskolin, 9–deoxyforskolin, 1,9-deoxyforskolin, and 1,9-dideoxy-7-deacetylforskol were identified in the root extracts of *Coleus forskohlii* (Saleem et al. 2006). 14-deoxycoleon U, demethylcryptojaponol, α-amyrin, betulic acid, α-cedrol, and β-sitosterol compounds were identified from the root extracts of *C. forskohlii* (Xu et al. 2005). Shen and Xu (2005) identified two new compounds, namely forskolin I and J, which are diterpenoids. The major components responsible for the biological properties were identified to be β-cadinene, citronellal, two labdane derivatives, ß-citronellol, and α-cedrene (Murugesan et al. 2012).

By using the techniques of GC–MS and GLC, 13 terpene hydrocarbons and seven oxygenated compounds were identified in the essential oil of *c. amboinicus*. The major compounds like thymol, carvacrol, 1,8-cineole, spathulenol, terpine-4-ol, and p-cymene were identified in the essential oil of *C. amboinicus* (Singh et al. 2002). Three flavones, namely crisimaritin, salvigenin, and chrysoeriol, were isolated from the leaves of *C. amboinicus* using silica gel chromatography (Ragasa et al. 1999). The presence of phytochemicals used for the traditional medicine preparation was also reported in other *Coleus* species: *Coleus barbatus* and *Coleus zeylanicus*. The main constituents reported in the essential oil of *Coleus zeylanicus* were δ-cadinene, α-Terpineol, geraniol, hexane, octane, and nerol derivatives (Jirovetz et al. 1998). Flavonoids and diterpenes were some of the compounds identified in the species *Coleus barbatus* (Jaslin and Padmaja 2011).

7.4 Biological Activities of *Coleus* Species

The free radical scavenging activity was reported in three *Coleus* species: *Coleus zeylanicus*, *Coleus forskohlii,* and in *Coleus aromaticus* (Rasineni et al. 2008). The content of polyphenols and the antioxidant activity were high in *c. forskohlii* compared to *C. zeylanicus* and *c. aromaticus*. The antioxidant activity of leaf, stem, tubers, and roots of *c. forskohlii* was reported (Khatun et al. 2011). The enzymatic antioxidants like catalase, polyphenol oxidase, peroxidase, and superoxide dismutase were found to be high in the *c. forskohlii* tuber compared to the other parts of the plant. Apart from the antioxidant potential, *Coleus* species extracts and volatile oils also exhibit antimicrobial potential against several pathogenic microbes. The essential oils of *C. zeylanicus* and *c. aromaticus* were tested against the strains of *Fusarium solani, Candida albicans, Alternaria brassicicola, Aspergillus niger, A. parasiticus, Rhizoctonia oryzar-sativae, R. oryzae, Colletotrichum musae, Pseudomonas aeruginosa, Xanthomonas campestris, Bacillus megaterium, B. subtilis, Escherichia coli, and Proteus vulgaris* in which *C. zeylanicus* showed higher antimicrobial activity compared to *c. aromaticus* (Deena et al. 2002). The presence of compounds carvacrol and β caryophyllene-4, 5-oxide in *Coleus amboinicus* was found to be responsible for the antimicrobial activity against the strains of *Cladosporium cucumerinum, Pseudomonas fluorescens, and Bacillus subtilis* (Vasquez et al. 2004). The antifungal activity of *Coleus barbatus* and *Coleus forskohlii* extracts against the strains of *Candida albicans*, *Proteus vulgaris, Aspergillus fumigatus,* and *Aspergillus niger* was reported (Nilani et al. 2006). The anti-inflammatory activity of aqueous leaf extracts of *Coleus aromaticus* and the methanol, chloroform, and hexane extracts of *Coleus forskohlii* were reported (Menon and Latha 2011). The leaves of *Coleus aromaticus* possess antiurolithiatic activity effective in reducing the deposition of calcium oxalate stones in the kidney and urinary tract (Venkatesh et al. 2010). The anthelmintic activity and the antioxidant activity of stem, leaf, and root alcoholic

extracts of *Coleus amboinicus* were reported (Prasenjit et al. 2011). The anticonvulsant and antiepileptic activity of leaf, stem, and root extracts of *Coleus amboinicus* was reported (Kumari et al. 2012).

7.5 *Coleus forskohlii*

Coleus forskohlii Briq. (Fig. 7.9) is an essential member of family Lamiaceae with a vast range of medicinal properties. *Coleus forskohlii* is commonly called as "pashanbhedi" or "Makandi" in Sanskrit. This plant grows up to a height of 1–2 feet and contains teardrop-shaped leaves and thick brown-coloured tuberous roots. The thick, golden brown, fibrous, and tuberous roots were seen only in the species of *c. forskohlii,* whereas not seen in the other species of *Coleus*.

7.5.1 Geographic Distribution

Coleus forskohlii is distributed in the hilly regions with subtropical warm temperatures. Globally, this plant is grown in countries such as India, Burma, Nepal, Thailand, and Sri Lanka (Bhowal and Mehta 2017), and it is also said to be distributed to other countries like Egypt, Ethiopia, Brazil, tropical East Africa, and Arabia (Fig. 7.10). This plant is believed to be originated from the Indian subcontinent. In India, it is grown in Karnataka, Tamil Nadu, Rajasthan, Maharashtra, Gujarat, Garhwal, and Kumaon region of Himachal Pradesh, Parasnath hills in Bihar (Fig. 7.11). The areas of Maharashtra, Rajasthan, Tamil Nadu, and Karnataka were popular for growing this plant commercially.

Fig. 7.9 *Coleus forskohlii*

Fig. 7.10 Global distribution of *Coleus forskohlii*

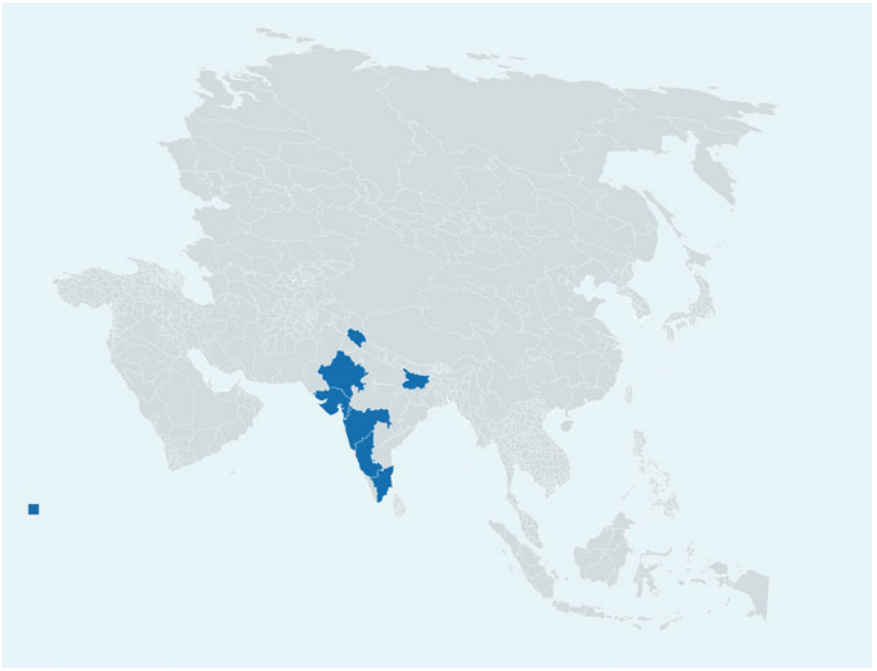


Fig. 7.11 Distribution of *Coleus forskohlii* in India

7.5.2 Phytochemistry of C. forskohlii

C. forskohlii plants are rich in a compound known as forskolin, which is considered as a drug with potential anti-algal, antibacterial, anti-protozoan, antifungal, and anti-inflammatory properties. Forskolin a labdane diterpene possesses therapeutic value used to treat cancer, psoriasis, asthma, cardiovascular diseases, and hypertension (Kavitha et al. 2010). It is also used to treat eczema, insomnia, painful urination, congestive heart failure, convulsions, and respiratory disorders. It possesses anti-inflammatory properties used to treat tumour metastases and thromboembolic platelet disorders of certain cancers. It prevents cancer and relaxes smooth muscles by activating the enzyme adenylate cyclase and by increasing the intracellular cAMP levels, prevents the release of histamine, platelet aggregation, degranulation of mast cells and basophils, lowers intraocular pressure and blood pressure, stimulates the breakdown of lipids in fat cells, and promotes the secretion of thyroid hormone, bronchodilation, and vasodilation. Forskolin stimulates cAMP, regulates hormones, enzymes, biological activities and also acts as a secondary messenger for intracellular signal transduction. The decrease in the level of cyclic AMP leads to the development of disease (Reddy et al. 2005) as it plays a major role in maintaining the body's basal metabolic rate, regulates the body's thermogenic response to food, and also increases the body's fat utilization. Body fat can be controlled when forskolin is used in combination with hydroxycitric acid.

The compound forskolin can activate all the nine different types of adenylate cyclase enzymes present in humans except the type 9 enzyme present in spermatozoa (Iwatsubo et al. 2003). The compound forskolin present in the roots of *Coleus forskohlii* was first discovered in the year 1974 initially termed as "coleonol". After further studies, the name coleonol changed to forskolin due to the identification of diterpenoids along with the other coleonols (Dubey et al. 1981, Saksena et al. 1985). The absence of forskolin in the roots of other species of *Coleus* like *C. blumei*, *C. canisus*, *C.spicatus*, *C.parviflorus*, *c. amboinicus*, and *C.malabaricus* was reported (Shah et al. 1980). Forskolin inhibits the transport of glucose in various cells, platelets, adipocytes, and in erythrocytes (Mills et al. 1984) and produces independent cyclic AMP effects by modulating the receptor channel of nicotinic acetylcholine, voltage-dependent potassium channels, and the reversal of resistance against multiple drugs (Morris et al. 1991). The root extracts of *Coleus forskohlii* are used to treat skin infections, also used to kill worms in the stomach. *c. forskohlii* is used widely for curing several disorders like intestinal disorders, respiratory disorders, heart diseases, asthma, bronchitis, convulsions, insomnia, burning sensation, epilepsy, and constipation (Ammon and Muller 1985). *c. forskohlii* is found to be effective in treating obesity, congestive heart failure, hypertension, psoriasis, glaucoma, asthma, depression, and cancer metastasis. Apart from the medicinal value of this plant, *forskohlii* also contains essential oils used in the food industries as flavouring agents and as an antimicrobial compound (Chowdhary and Sharma 1998). Oil extracted from *Coleus forskohlii* possesses antimicrobial property used to treat acne effective against the causative organism *propionibacterium acne* (Barkat et al.

2012). Many researchers reported the effective antimicrobial activity against several microbes, effective in inhibiting the growth of pathogens responsible for skin infections and eruptions. *c. forskohlii* is found to be effective against yeast (Majeed and Prakash 2007). The high antioxidant activity and high amounts of flavones, flavanols, and polyphenols were reported in the leaf extracts of *Coleus forskohlii* compared to other species (Rasineni et al. 2008). The major constituents reported in the essential oil of *c. forskohlii* root are sesquiterpene hydrocarbon (7.5%), β-sesquiphellandrene (13.15%), γ-eudesmol (12.5%), 3-decanone (7%), and bornyl acetate (15%). α-pinene, α-copaene, β-phellandrene, caryophyllene oxide, limonene, α-humulene, and β-caryophyllene are the major compounds identified in the stem of *c. forskohlii* (Kerntopf et al. 2002).

7.5.3 *Medicinal Properties of* Coleus forskohli *and Mechanism of Action in Different Diseases*

C. forskohlii is used to treat various disorders in many countries around the world. It is used as a stomach aid and also used for treating intestinal disorders in Brazil. Its leaves are used as an expectorant, emmenagogue, and diuretic in Egypt and Africa (Valdes et al. 1987). In India, this plant is used to treat dysentery and digestive disorders. *C. forskohlii* has been used for the treatment of heart disease, abdominal colic, respiratory disorders, insomnia, convulsions, asthma, bronchitis, intestinal disorders, burning sensation, constipation, epilepsy, and angina in traditional Ayurvedic systems (Desouza and Shah 1988). Its roots are used in worm cure and in festering boils to relieve burns. The root extract is used to treat eczema and skin infections when combined with mustard oil. The plant also has various veterinary uses. Forskolin is employed in the preparation of medicine, which halts the greying of hair and repairs grey hair to return to its normal colour. Though grouped as a medicinal plant, *C. forskohlii* tubers contain essential oils that have a very attractive and delicate fragrance with a spicy note. The essential oils found in its tuber have potential applications in the food industry, and it can also be used as an antimicrobial agent (Misra et al. 1994).

Dubey et al. (1974) reported the lowering of blood pressure and antispasmodic effects of *C. forskohlii* root extracts based on the extensive screening of Indian plants for biological activity. De Souza et al. (1983) found that root tuber extracted methanol from *C. forskohlii* helps to lower blood pressure and exhibited positive inotropic activity in animal models. The key mechanism by which forskolin exerts its hypotensive activity is by inducing adenylate cyclase and thus increasing the cell concentrations of the second messenger cyclic AMP (cAMP). Forskolin stimulates nearly all hormone-sensitive adenylate cyclases directly in intact cells, tissues, and even adenylate cyclase preparation. Adenylate cyclase stimulation is thought to be the mechanism by which forskolin relaxes a range of smooth muscles. This forskolin behaviour has proved its potential use not only as an important research tool for

understanding cyclical processes—AMP-dependent physiological processes—but also as a potential therapeutic agent for diseases such as heart failure, hypertension, glaucoma, thrombosis, asthma, and metastatic conditions (Seamon 1984).

cAMP levels are increased by forskolin, which obstructs basophil and mast cell degranulation and histamine release, decreases blood pressure and intraocular pressure, hinders platelet aggregation, develops vasodilation bronchodilation and thyroid hormone secretion, and triggers lipolysis in fat cells. Besides its cAMP-stimulating activity, forskolin hinders the binding of platelet-activating factor (PAF), independently of cAMP formation. This may be a result of forskolin's direct effect on PAF or via obstruction with PAF binding to receptor sites. Forskolin is also said to affect several membrane transport proteins and prohibits glucose transport in erythrocytes, adipocytes, platelets, and other cells.

7.5.3.1 Heart Disorder

Forskolin exerts positive inotropic activity on cardiac tissue by increased levels of cAMP. Detailed pharmacological studies showed that forskolin reduced normal or raised blood pressure in various animal species through a vasodilatory effect and had a strong inotropic effect on the cardiac muscle.

7.5.3.2 Glaucoma

Caprioli and Sears (1983) first described the effect of forskolin on aqueous humour dynamics and intraocular pressure. Forskolin's topical application decreased intraocular pressure in rabbits, monkeys, and healthy human volunteers and was associated with a decrease in aqueous inflow and no improvement in outflow facilities suggesting the potential of forskolin as a therapeutic agent in glaucoma care (Caprioli et al. 1984).

7.5.3.3 Asthma

Forskolin has been investigated for its possible role as a bronchodilator in treating asthma. It blocked bronchospasm, the chief characteristic of guinea pigs asthma and bronchitis caused by histamine and leukotriene C-4 (Kreutner et al. 1985). Forskolin blocked the release of histamine and leukotriene C-4 in human basophils and mast cells. Research involving humans showed that formulations of forskolin inhaled powder were able to induce bronchodilation in patients with asthma (Bauer et al. 1993). Forskolin appears to be a promising drug if used for the treatment of patients with congestive heart failure, glaucoma, and asthma in an acceptable dose (Bauer et al. 1993).

7.5.3.4 Antithrombotic Effect

Forskolin inhibits platelet aggregation by stimulating adenylate cyclase, increasing prostaglandin effects (Rupp et al. 1986). Cerebral vasodilation may enhance its antithrombotic properties, and it has been observed in rabbits. Adenosine did not potentiate this vasodilatation. It has been suggested that crude *C. forskohlii* extract be used as a logical antithrombotic phytotherapeutic (Desouza 1993).

7.5.3.5 Cancer Metastases

Most tumour cell lines that metastasize cause platelet aggregation, both in vitro and in vivo. Platelets release substances, which promote tumour growth upon aggregation. Researchers have demonstrated the ability of forskolin to block platelet aggregation through its platelet adenylate cyclase stimulation and intracellular cAMP increase (Seigl et al. 1982). Agarwal and Parks had given mice 82 μgforskolin 30–60 min before injection with a highly metastatic cell line of melanoma (B16-F10). Forskolin decreased lung tumour colonization by 70% (Agarwal and Parks Jr 1983).

7.5.3.6 Psoriasis

Like asthma, psoriasis is another disease, characterized by decreased cAMP levels in the skin. This imbalance results in a much higher cell division rate—1000 times higher than average, leading to psoriatic outbreaks. While specifics of the study are not available, Ammon et al. reported an improvement in psoriasis symptoms in four patients with forskolin supplementation. It has been shown that the forskolin's ability to regulate cAMP levels in skin cells has therapeutic benefit for psoriasis sufferers (Kavitha et al. 2010).

7.5.3.7 Anti-obesity

Henderson indicated that *C. forskohlii* does not seem to encourage weight loss but can help reduce weight gain in females with apparently no side effects of clinically relevant significance. Forskohlii's anti-obesity effects were studied in ovariectomized rats, and the administration of *C. forskohlii* extracts in those rats decreased body weight, food consumption, and fat accumulation, indicating that *C. forskohlii* could be useful in the treatment of obesity (Han et al. 2005).

7.5.4 Cultivation and Domestication of Coleus forskohlii

Conservation of the genus *Coleus* is a very important aspect of the ongoing scenario over the globe. Along with the protection of the plants, the quick growth of the plant parts is very much needed to revive new plants through various cultivation techniques. Not every plant can give rise to seeds, rather few plants can grow their vegetative parts. *Coleus forskohlii* (wild) Briq. can produce seeds for the growth of new plants. *Coleus* plants also acclimatize to the regeneration method through stem cutting. This plant has the ability for a variety of propagations, both through asexual and sexual methods (Patel 2016). *C. forskohlii* grows and prospers well in red, sandy loam soils with pH between 5.5 and 7. Optimum temperature range is 10–25 °C. Relative humidity of 83–95% is best suitable for the growth of this plant. The rainfall needed for better plant growth is 100–160 cm rain per year, usually from June to September. The propagation of *Coleus forskohlii* through seeds is tedious and is a little slow. Hence, it is economical to propagate this plant through terminal stem cutting. To encourage rooting, 10–12-cm long-terminal cuttings of 3–4 pairs of leaves are planted into nursery beds. When the terminal cuttings are one-month-old and adequate roots have been developed, then the roots are shifted to the land for cultivation. The best planting time is during June/July and September/October, and the rooted cuttings must be planted at 60 cm intervals. Daily care should be taken about watering, weeding, and plant protection (Rajamani and Vadivel 2009). The crop matches organic and inorganic fertilizers well. Organic manure is needed on the 30th day and 45th day of planting to a level of 140 kg. The combination of 40 kg N, 60 kg P_2O_5, and 50 kg K_2O per ha is ideal for getting the full tuber yield of fresh (120 t/ha) and dry (3982 t/ha). Half the dose of N, all of P, and all of K can be applied as the basal dose and the left half of N, after 30 days of the top dressing (Veeraragavathatham et al. 1985). *Coleus* plants are grown in the presence of *Glomus bagyarajii* arbuscular mycorrhizal fungi, depicted for improvement in plant growth and forskolin amount compared to those grown without AM fungi (Sailo and Bagyaraj 2005).The caterpillars that feed on leaves, mealybugs, and root-knot nematodes are the main pests that target the coleus plant. Wilt caused due to *Fusarium chlamydosporum* is a very severe soil-borne disease, but inoculation with *Trichoderma viride* and *Glomus mosseae* can provide a good outcome in control of the disease-causing pests. *Macrophomina phaseolina* that causes root rot affects up to 100% tuber yield. Bioformulation, viz. *Trichoderma harzianum* and zinc sulphate will lead to complete eradication of root rot disease (Sailo and Bagyaraj 2005). The crop can be harvested within 4½–5 months of planting. The plants are uprooted, and the tubers are removed, washed, and then sun-dried. On average, dry tubers can yield between 800 and 1000 kg/ha. If proper cultivation practices are used, dry tuber yields of up to 2000–2200 kg/ha can be produced simply (Rajamani and Vadivel 2009).

In India, *C. forskohlii* is primarily cultivated under the contract agriculture scheme. A study conducted by Agila et al. (2006) infers that minimal risk in farming practices, guaranteed price for harvested goods, reduced price risk, eliminating intermediaries, sure income and accessibility of financial assistance, company technical

guidance, timely convenience of inputs, and knowledge of correct technology are the key important components for enhancing *Coleus* contract farming output.

7.5.5 Biotechnological Approaches

The study of tissue culture techniques for forskolin synthesis was conducted as it was comparatively adequate content of forskolin in the plant, and its development as a drug has been limited (Mukherjee et al. 2000). Forskolin was detected by TLC and HPLC in shoot culture differentiation, micro-propagated plants, and suspension of root organs. Forskolin developed by shoot differentiating culture was close to that of micro-propagated plants, caused the suspension of root organ depicted only in minute quantity of forskolin (Sen et al. 1992). Krombholz et al. (1992) documented the root cultivation of *C. forskohlii* originated from primary callus or IBA-treated suspension cultures and maintained on a Gamborg B5 medium containing 1 mg/L of IBA derived forskolin and its derivatives in quantities between 500 and 1300 mg/kg dry weight, corresponding to approximately 4–5 mg/L. Suspension cultures from gall calli that were obtained after *Agrobacterium tumefaciens* (C58) infection were developed in *C. forskohlii*. Cell line selection experiments were performed using single-cell cloning or cell aggregate cloning to select cell lines that had ability of rapid growth and high forskolin development. Molecular cloning and functional expression of geranylgeranyl pyrophosphate synthase from *C. forskohlii* has been illustrated. Engprasert et al. (2004) suggested that forskolin should be synthesized through a non-mevalonate pathway from isopentenyl diphosphate (IPP), a common biosynthetic precursor. It is believed that GGPP synthase is involved in forskolin biosynthesis, which is mainly synthesized in the leaves and the accumulated in the stems and roots. Forskolin is isolated from tuber. The tubers are collected on a wet basis at a moisture level of 75–85% and processed after drying at less than 12% moisture. Sun drying required more time than mechanical drying and registered the lowest forskolin recovery. Mechanically, dried tubers at 40 °C with a tuber slice thickness of 0.5 cm and packed in polyethylene lined gunny bag conserved maximum quantity of forskolin.

Various chromatographic techniques are used for forskolin quantification, and the earliest technique developed is the gas–liquid chromatography (GLC) (Inamdar et al. 1980). Later, the techniques used were TLC and HPLC, respectively. The HPLC approach is found to be quicker and less sensitive than GLC and used to track differences in the quality of forskolin in different germplasms. A forskolin-specific monoclonal antibody has been identified for forskolin affinity isolation and used for the especially sensitive quantification of forskolin in plant tissues at various stages of progress (Yanagihara et al. 1996). For quantification of forskolin, both nuclear magnetic resonance (NMR) and gas chromatography mass spectral techniques are being utilized. Reversed-phase liquid chromatography with a 210 nm photodiode array detector is effective in the qualitative and quantitative examination of forskolin in plant material and forskolin in consumer products (Schaneberg and

Khan 2003).To extract high-purity forskolin, a clear, effective, quick, and economical reverse-phase high-performance liquid chromatography (RP-HPLC) technique using activated charcoal as a column adsorbent is developed (Saleem et al. 2006).

7.6 Conclusions

Plants are the remarkable source of a range of products including food, fodder, fuel, medicine, etc. Due to the presence of secondary metabolites, a few plants are being used in medicine for the treatment of specific disorders. The group of plants consisting of these compounds used for the medical treatments is referred to as medicinal and aromatic plants. Demand for these plants in society is increasing day by day due to their rich potential of treatment, low cost, and minimum side effect. Genus *Coleus* plant is an important member of the family Lamiaceae, commonly known as the mint family. Traditionally, many species of the genus *Coleus* are used for the treatment because of its medicinal properties. The present study provides the biodiversity, importance of *Coleus* in health care, and the benefits of *Coleus* cultivation with special emphasis on *Coleus forskohlii*. Screening of different *Coleus* species and their extracts is necessary for the isolation, identification, and characterization of bioactive compounds responsible for various biological activities. The study of their mechanism of action gives valuable information for the development of drugs to enhance the treatment against dreadful diseases.

Acknowledgements The research laboratory of K. V. Chaitanya is funded by grants from the Department of Science and Technology and University Grants Commission (UGC), Government of India.

References

Agarwal KC, Jr Parks RE (1983) Forskolin: a potential antimetastatic agent. Inter J Cancer 32:801–804

Agila R, Manoharan M, Ravi KC (2006) Performance of *Coleus* under Contract farming. In: Proceedings of international conference on globalization of traditional, complementary and alternative systems of medicine held at TNAU, Coimbatore, p 24

Ammon HP, Muller AB (1985) Forskolin: from an ayurvedic remedy to a modern agent. Planta Med 6:473–477

Barkat AK, Naveed A, Masood A, Tariq M, Haroon K, Irshad H, Kamran AK (2012) Botanical description of *Coleus forskohlii*: a review. J Med Plants Res 6:4832–4835

Baslas RK, Kuma P (1981) Chemical examination of essential oil of *Coleus aromaticus* Benth. J Indian Chem Soc 58:103–104

Bauer K, Dietersdorder F, Sertl K, Kaik B, Kaik G (1993) Pharmaco- dynamic effects of inhaled dry powder formulations of fenoterol and colforsin in asthma. Clin Pharmacol Therapeu 53:76–83

Bhowal M, Mehta DM (2017) *Coleus forskholii*: Phytochemical and pharmacological profile. Inter J Pharmaceu Sci Res 8(9):3599–3618. https://doi.org/10.13040/IJPSR.0975-8232.8(9).3599-18

Caprioli J, Sears M (1983) Forskolin lowers intraocular pressure in rabbits, monkeys and man. Lancet 8331:958–960
Caprioli J, Sears M, Bausher L (1984) Forskolin lowers intraocular pressure by reducing aqueous inflow. Investigative Ophthalmol Visual Sci 25:268–277
Chatterjee A, Pakrashi SC (2001) The treatise of Indian medicinal plants. Counc Indus Sci Res New Delhi, India 5:8–9
Chowdhary AR, Sharma ML (1998) GC-MS investigations on the essential oil from *Coleus forskohlii* Briq. Indian Perfumer 42:15–16
Cook FEM (1995) Economic Botany data collection standard. In: International working group on taxonomic databases for plant sciences (TDWG), Royal Botanic Gardens Kew, UK
Das JKL, Sharma SN, Prasad MK, Tiwari S, Singh UN, Kollhapure SA (2005) Evaluation of the efficiency and safety of Pure hands in hand hygiene: a prospective, randomized, double blind, placebo-controlled, phase III clinical trial. Indian Pract 58:275–282
De Souza NJ (1993) Industrial development of traditional drugs: the forskolin example. A mini-review. J Ethnopharmacol 38:177–180
De Souza NJ, Dohadwalla AN, Reden J (1983) Forskolin: a labdane diterpenoid with antihypertensive, positive inotropic, platelet aggregation inhibitory, and adenylate cyclase activating properties. Med Res Rev 3:201–219
De Souza NJ, Shah V (1988) Forskolin—an adenylate cyclase activating drug from an Indian herb. Eco Med Plant Res 2:1–16
Deena MJ, Sreeranjini K, Thoppil JE (2002) Antimicrobial screening of essential oils of *Coleus aromaticus* and *Coleus zeylanicus*. Indian J Aromatherapy 12:105–107
Dubey MP, Srimal RC, Patnaik GK, Dhawan BN (1974) Blood pressure lowering and antispasmodic effects of C. forskohlii Briq. Indian J Pharm 6:15
Dubey MP, Srimal RC, Nityanand S, Dhawan BN (1981) Pharmacological studies on coleonol, a hypotensive diterpene from Coleus forskohlii. J Ethnopharma 3:1–13
Duke JA, Godwin M J, Cellier DUJ, Duke PAK (2002) Handbook of medicinal herbs, CRC Press, Boca Raton USA, pp 210–215
Eggli U (2004) Illustrated handbook of succulent plants: dicotyledons. Springer, Berlin, Germany
Engprasert S, Taura F, Kawamukai M, Shoyama Y (2004) Molecular cloning and functional expression of geranylgeranyl pyrophosphate synthase from *Coleus forskohlii* Briq. BMC Plant Biol 18:18
Gaspar-Marques C, Rijo P, Simões MF, Duarte MA, Rodriguez B (2006) Abietanes from *Plectranthus grandidentatus* and *P. hereroensis* against methicillin- and vancomycin-resistant bacteria. Phytomedicine 13:267–271
Grayer RJ, Eckert MR, Lever A, Veitch NC, Kite GC, Paton AJ (2010) Distribution of exudate flavonoids in the genus *Plectranthus*. Biochem Syst Ecol 38:335–341
Han LK, Morimoto C, Yu RH, Okuda H (2005) Effects of *Coleus forskohlii* on fat storage in ovariectomized rats. Yakugaku Zasshi 125:449–453
Himesh S, Singh AK (2012) Recent updates on the genus *Coleus*: a review. Asian J Pharmaceu Clin Res 5:12–17
Inamdar PK, Dornauer H, de Souza NJ (1980) GLC method for assay of forskolin, a novel positive inotropic and blood pressure-lowering agent. J Pharmaceu Sci 69:1449–1451
Iwatsubo K, Tsunematsu T, Ishikawa Y (2003) Isoform-specific regulation of adenylyl cyclase: a potential target in future pharmacotherapy. Expert Opin Ther Targets 7:441–451
Jaslin EJ, Padmaja V (2011) Antioxidant potential of ethanolic extract of aerial parts of *Coleus spicatus* Benth. Afr J Biotech 10:12054–12057
Jasmine R, Selvi JS (2013) Analysis of antibacterial activity of the bioactive constituents from the acetone extract of *Coleus aromaticus* (Benth) leaves by gas chromatography-mass spectrometry. World J Pharm Pharmaceu Sci 2:3088–3093
Jin QD, He BH (1998) Minor constituents from Coleus forskohlii. Acta Bot Yunnanica 20:469–73

Jirovetz L, Jirovetz K, Buchbauer G, Fleischhacker W, Shafi PM, Saidutty A (1998) Analyses of the essential oil of the leaves of the medicinal plant *Coleus zeylanicus* from India. Sci Pharmaceu 66:223–229

Kaliappan ND, Viswanathan PK (2008) Pharmacognostical studies on the leaves of *Plectranthus amboinicus* (Lour) Spreng. Inter J Green Pharm 2:182–184

Kavitha C, Rajamani K, Vadivel E (2010) *Coleus forskohlii*: a comprehensive review on morphology, phytochemistry and pharmacological aspects. J Med Plants Res 4:278–285

Kerntopf MR, de Albuquerque RL, Machado MIL, Matos FJA, Craveiro AA (2002) Essential oils from leaves, stems and roots of *Plectranthus barbatus* Andr. (Labiatae) grown in Brazi. J Ess Oil Res 14:101–102

Khatun S, Cakilcioglu U, Chatterjee NC (2011) Antioxidant activity of the medicinal plant *Coleus forskohlii* Briq. Afr J Biotech 10:2530–2535

Kreutner W, Chapman RW, Gulbenkian A, Tozzi S (1985) Broncho-dilator and antiallergy activity of forskolin. Euro J Pharmacol 111:1–8

Krombholz R, Mersinger R, Kreis W, Reinhard E (1992) Production of forskolin by axenic Coleus forskohlii roots cultivated in shake flasks and 20-l glass jar bioreactors. Planta Med 58:328–333

Kumaran A, Karunakaran RJ (2007) Activity guided isolation and identification of free radical—scavenging components from an aqueous extract of *Coleus aromaticus*. Food Chem 100:356–361

Kumari BP, Sujatha D, Chand CG, Divya K, Malleswari I, Ranganayakulu D (2012) Evaluation of antiepileptic activity and probable mechanism of action of *Coleus amboinicus* in MES and PTZ models. J Pharm Res 5:1587–1591

Lukhoba CW, Simmonds MSJ, Paton AJ (2006) *Plectranthus*: a review of ethnobotanical uses. J Ethnopharmacol 103:1–24

Majeed M, Prakash L (2007) Fighting acne and more effective natural approaches to skincare. Cosmetics & Toiletries, USA, pp 215–219

Menon D, Latha K (2011) Phytochemical screening and in vitro anti-inflammatory activity of the stem of *Coleus forskohlii*. Pharmacognosy J 3:75–79

Mills I, Moreno FJ, Fain JN (1984) Forskolin inhibition of glucose metabolism in rat adipocytes independent of adenosine 3', 5'-monophosphate accumulation and lipolysis. Endocrinology 115:1066–1069

Misra LN, Tyagi BR, Ahmad A, Bahl JR (1994) Variability in the chemical composition of the essential oil of Coleus forskohlii genotypes. J Essential Oil Res 6:243–247

Morris DI, Speicher LA, Ruoha AE, Tew KD, Seamon KB (1991) Interaction of forskolin with the p-glycoprotein multi-drug transporter. Biochemistry 30:8371–8377

Mukherjee S, Ghosh B, Jha S (2000) Establishment of forskolin yielding transformed cell suspension cultures of *Coleus forskohlii* as controlled by different factors. J Biotechnol 76:73–81

Murugesan S, Rajeshkannan C, Sumathi R, Manivachakam P, Suresh Babu D (2012) Bioactivity of root hexane extract of *Coleus forskohlii* Briq. *Labiatae*: GC/MS/MS characterization and identification. Eur J Exp Biol 2:1469–1473

Neuwinger HD (2000) African traditional medicine: a dictionary of plant use and applications. Medpharm Scientific Publishers, Germany, pp 1–589

Nilani P, Duraisamy B, Dhanabal PS, Khan S, Suresh B (2006) Antifungal activity of some *Coleus* species growing in Nilgiris. Anc Sci Life 26:81–84

Nisheeda BA, Safeer PM, Sreekumar S, Biju CK, Seeja G, Manivannan C (2016) A review on *Plectranthus vettiveroides*: an endemic to South Indian high value aromatic medicinal plant. IOSR J Pharm Biol Sci 11:1–11

Palani S, Raja S, Naresh R (2010) Evaluation of nephroprotective, diuretic, and antioxidant activities of Plectranthus amboinicus on acetaminophen-induced nephrotoxic rats. Toxicol Mech Methods 20:213–312. https://doi.org/10.3109/15376511003736787

Patel DK (2016) Vegetative Propagation of *Coleus forskohlii* (Wild) Briq using their stem cutting for ex-situ conservation in herbal garden. Med Aromat Plants (los Angeles) 5:261–263

Pino JA, Garcia J, Martinez MA (1996) Comparative chemical composition of the volatiles of Coleus aromaticus produced by steam distillation, solvent extraction and supercritical carbon dioxide extraction. J Essent Oil Res 8:373–375

Pooley E (1998) A field guide to wildflowers of KwaZulu-Natal and the eastern region. Natal Flora Publications Trust, Durban

Prajapati ND, Purohit SS, Sharma AK, Kumar T (2003) A handbook of medicinal plants. Agrobios Publishers, Jodhpur, India, p 162

Prasenjit B, Hullatti KK, Kumar VML (2011) Antihelmintic and antioxidant activity of alcoholic extracts of different parts of *Coleus amboinicus* Lour. Inter J Res Ayurveda Pharm 2:181–185

Prudent D, Perineau F, Bessiere JM, Michel GM, Baccou JC (1995) Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus* Benth.). J Essent Oil Res 7:165–173

Ragasa CY, Sagalang V, Pendon Z, Rideout JA (1999) Antimicrobial flavones from *Coleus amboinicus*. Philippine J Sci 128:347–351

Rajamani K, Vadivel E (2009) Marunthu Kurkan—medicinal coleus. Naveena mulikai sagupaddi thozhil nuttpangal. Tamil Nadu Agricultural University, Coimbatore, pp 17–22

Rasineni GK, Siddavattam D, Reddy AR (2008) Free radical quenching activity and polyphenols in three species of *Coleus*. J Med Plants Res 2:285–291

Reddy CS, Desireddy RB, Ciddi V (2005) A review on forskolin: a cyclic AMP modulator from tissue cultures of *Coleus forskohlii*. Pharmaco Mag 1:85–88

Rice LJ, Brits GJ, Potgieter CJ, Van Staden J (2011) *Plectranthus*: a plant for the future? South African J Bot 77:947–959

Rupp RH, De Souza NJ, Dohadwalla AN (1986) Proceedings of the international symposium on Forskolin: its chemical, biological and medical potential. Hoechst India Limited, Bombay, pp 19–30

Ryding O (2006) Lamiaceae. In: Hedberg I, Kelbessa E, Edwards S, Demissew S, Persson E (eds), Flora of Ethiopia and Eritrea, Gentianaceae to Cyclocheilaceae. The National Herbarium, vol 55. Addis Ababa University, Addis Ababa, Ethiopia, p 588

Sailo GL, Bagyaraj DJ (2005) Influence of different AM-fungi on the growth, nutrition and forskolin content of *Coleus forskohlii*. Mycol Res 109:795–798

Saksena AK, Green MJ, Shue HJ (1985) Identity of coleonol with forskolin: structure revision of a base-catalysed rearrangement product. Tetrahedron Lett 26:551–554

Saleem AM, Dhasan PB, Rafiullah MR (2006) Simple and rapid method for the isolation of forskolin from Coleus forskohlii by charcoal column chromatography. J Chromato 1101:313–314

Saraswathy A, Amala K, Arunmozhi D (2011) *Coleus vettiveroides* K.C. Jacob: Botany and pharmacognosy. Indian J Tradition Know 10:636–642

Schaneberg BT, Khan IA (2003) Quantitative analysis of forskolin in *Coleus forskohlii* (Lamiaceae) by reversed-phase liquid chromatography. J AOAC Inter 86:467–470

Seamon KB (1984) Forskolin and adenylate cyclase: new opportunities in drug design. In: Bailey DM (ed) Ann Rep medical chemistry, vol 19. Academic Press, New York, pp 293–302

Sen J, Sharma AK, Sahu NP, Mahato SB (1992) Production of forskohlin in in vitro cultures of *Coleus forskohlii*. Planta Med 58:324–327

Shah VC, Bhat SV, Bajwa BS, Dornaeur H, De Souza NJ (1980) The occurrence of forskolin in *Labiatae*. Planta Med 39:183–185

Sharma PV (1997) Dravyaguna Vigyan, 2nd edn. Chowkambha Bharti Academy, Varanasi, pp 120–123

Shen YH, Xu YL (2005) Two new diterpenoids from *Coleus forskohlii*. J Asian Nat Prod Res 7:811–815

Siegl AM, Daly JW, Smith JB (1982) Inhibition of aggregation and stimulation of cyclic AMP generation in intact human platelets by the diterpene forskolin. Mol Pharmacol 21:680–687

Singh G, Singh OP, Prasad YR, de-Lampasona MP, Catalan CC (2002) Studies on essential oils, part 33: chemical and insecticidal investigations of leaf oil of *Coleus amboinicus* Lour. Flavour Fragrance J 17:440–442

Sundara Ganapathy R, Mohan S, Kameshwaran S, Dhanapal C (2015) Hepatoprotective activity of *Plectranthus vettiveroides* against paracetamol and D-galactosamine induced hepatic toxicity. Inter J Pharm Therapeu 6:242–246

Valdes LJ, Mislankar SG, Paul AG (1987) *Coleus barbatus* (*C. forskohlii*) (Lamiaceae) and the potential new drug forskolin (Coleonol). Eco Bot 44:474–483

Vasquez EA, Kraus W, Conrad J, Volger C, Zebit Z, Rejesus BM (2004) Bioassay-guided isolation of the antimicrobial compounds of *Coleus amboinicus* Loureiro (*Lamiaceae*). Ann Tropical Res 26:167–183

Veeraragavathatham D, Venkatachalm R, Sundararajan S (1985) Performance of two varieties of *Coleus forskohlii* under different spacing levels. South Indian Hortic 33:389–392

Venkatesh G, Baburao K, Rajesh BM, Dhanalakshmi S, Indira PDG (2010) The *Coleus aromaticus* activity. Inter J Phytomed 2:284–291

Xu LL, Kong LY (2004) Isolation and identification of labdane diterpenoids from the roots of Coleus forskohlii. Chin J Nat Med 2:344–347

Xu LL, Lu J, Li WJ, Kong LY (2005) Studies on the chemical constituents in root of *Coleus forskohlii*. Zhongguo Zhong Yao Za Zhi 30:1753–1755

Yanagihara H, Sakata R, Shoyama Y, Murakami H (1996) Rapid analysis of small samples containing forskolin using monoclonal antibodies. Planta Med 62:169–172

Chapter 8
Cultivation of *Hypericum perforatum* (St. John's Wort) and Biotechnological Approaches for Improvement of Plant Raw Material Quality

Inga Kwiecień, Noemi Nicosia, and Halina M. Ekiert

Abstract *Hypericum perforatum* L. is a species long used in traditional medicine of Central and Eastern Europe, including Poland. Since many years, it possesses the important position in official medicine in EU countries as a medicinal species for allopathic and homeopathic use. *Hypericum perforatum* herb, which is the raw material derived from this species, has a very rich chemical composition, containing naphtodianthrone derivatives (dimeric anthraquinones), flavonoids, catechins and their derivatives, phenolic acids, xanthones, phloroglucinol derivatives and essential oil. The generous chemical composition of this raw material determines its numerous possible therapeutic applications. This raw material is used in allopathy to treat digestive tract ailments, and in particular gallbladder dysfunction. It is also applied in the treatment of depressive disorders. The newest lines of biological activity documented by professional studies include neuroprotective, antibacterial, antiviral, anti-inflammatory and anticancer actions. *H. perforatum* can be found in plentiful natural locations in Europe; however, its natural resources are quickly depleted due to a very large demand for the raw material. For this reason, it is commercially cultivated on a large scale and with great success not only in European countries. Cultivation obviously ensures control and high quality of the raw material. The article reviews basic information on the morphology, ecology and distribution of this plant species. Taxonomic problems are also signaled. Chemical composition, traditional medicinal

I. Kwiecień (✉) · H. M. Ekiert (✉)
Department of Pharmaceutical Botany, Jagiellonian University, Collegium Medicum, Medyczna 9, 30-688 Kraków, Poland
e-mail: inga.kwiecien@uj.edu.pl

H. M. Ekiert
e-mail: mfekiert@cyf-kr.edu.pl

N. Nicosia
Foundation "Prof. Antonio Imbesi", University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy

Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Polo Universitario dell'Annunziata, 98168 Messina, Italy

N. Nicosia
e-mail: noemi.nicosia92@gmail.com

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_8

uses and new directions of biological activity confirmed by scientific researches are presented in detail, and cultivation requirements were thoroughly discussed. Moreover, numerous biotechnological studies of this species have been characterized. They are mostly related to the development of micropropagation protocols and procedures for endogenous production of secondary metabolites in various types of in vitro cultures. Some studies have also focused on elucidation of biogenetic pathways of different groups of secondary metabolites under in vitro conditions. A single study has explored the biotransformation potential of cells cultured in vitro.

Keywords Biological characteristic · *Hyperici herba* · Chemical composition · Traditional use · Official therapeutical application · Commercial cultivation · Biotechnological studies

8.1 Introduction

Hypericum perforatum L. (St. John's wort)—*Hypericaceae* is a widely distributed species in many regions all over the world across the eastern and western hemisphere. This plant is a very popular and highly valued medicinal species in Central Europe and also in Poland. It is a famous medicinal plant species in traditional European medicine. For many years, the herb of this plant has enjoyed the status of a pharmacopoeial plant raw material in European countries. It is authorized for use in allopathy and also in homeopathy.

The natural resources of this plant in Central Europe and also in Poland are rich; however, demand for this raw material (*Hyperici herba*) is so high that natural supplies have to be supported by cultivation. Cultivation ensures an appropriate quality of the raw material for pharmaceutical and/or cosmetic use. European growers have been successful in producing several cultivars possessing various beneficial features, of which 'Topas' is the most popular.

The popularity of this plant as a medicinal plant species stems from its very rich chemical composition. The herb of this plant is comprised mostly of naphtodianthrone compounds (e.g., hypericin, pseudohypericin, protohypericin, cyclopseudohypericin, isohypericin), flavonoids (e.g., quercetin, hyperoside, rutoside), biflavonoids—dimers (e.g., amentoflavone and 3,8-biapigenin) and also procyanidins. The important ingredients also include phenolic compounds: tannins (polymers of catechins) and phenolic acids (e.g., caffeic acid and chlorogenic acid) as well as also xanthones (derivatives of γ-pyrone). Fresh flowers of the plant contain hyperforin (a phloroglucinol derivative). These constituents are accompanied by essential oil. An extraordinary abundance of valuable ingredients in this plant species is decisive for a very wide range of its therapeutic applications. *H. perforatum* herb exhibits antidepressant properties, accelerates healing of burns and wounds, shows astringent and spasmolytic effects and is efficient in alleviation of digestive tract ailments. Hyperforin has antibiotic actions. In turn, hypericin present in St. John's

wort herb is a UV radiation sensitizer for the human skin and is used in limited amounts in the cosmetics industry.

The aim of this chapter is to review the basic information on the biology, ecology and geographical distribution of this plant species. Special attention was paid to the rich chemical composition of *H. perforatum* herb, which is a raw material derived from this medicinal plant, and the resulting numerous therapeutic applications in traditional and official phytotherapy in Europe. We also focus on natural biodiversity and successfully cultivation of various cultivars of St. John's wort. Additionally, the growing importance of a biotechnological approach to improving the quality of the herbal material was highlighted. The possibility of controlling and stimulating the production of secondary metabolites, in particular those, which are valuable pharmaceuticals, in in vitro culture conditions inspired, research into the biosynthetic potential of *Hypericum perforatum* cells cultured in different in vitro systems.

8.2 Botanical Characteristics

The genus *Hypericum* is divided into 36 sections comprising a total of over 500 species. The most important classification subdivides the genus into two subgenera based on the presence or absence of hypericin-containing glands. Eurasian species are distinguished by the presence of hypericin while species of the so-called New World lack it (Dauncey et al. 2019).

Hypericum perforatum is a widely known species and is referred to by many common names. In various countries, it is named after its blooming season during the summer solstice or after its morphology (the presence of essential oil or hypericin glands) (Barnes et al. 2001), e.g., millepertuis, herbe à mille trous (fr.); Johanniskraut, Tüpfel-Hartheu (ger.); iperico, erba di San Giovanni (it.); corazoncillo, hierba de San Juan (esp.).

Hypericum perforatum is a perennial plant, 30–100 cm tall. The stem of this species has two ridges; it is reddish and woody at the base and profusely branching near the top. The leaves are stalkless, arranged in opposite pairs, elliptical or lance-shaped, entire margin, 2–3 cm long. Leaf blades bear small translucent oil glands, seen especially clearly when observed against the light. Black-colored secretory glands containing hypericin can be found on the leaf edges (Fig. 8.1).

H. perforatum develops a complex root system with rhizome. Rhizomes and adventitious roots spread just below the soil surface, and long taproots, reaching down even to a depth of 1.5 m, make this species drought tolerant.

The plant flowers in June developing numerous radially symmetric flowers collected in flattened helicoid cymes (Fig. 8.1) with ca. 25–100 flowers on one stem. The flowers are up to ca. 3 cm across, pentamerous, with yellow or orange-yellow petals of corolla. As in the case of leaves, petals have black hypericin-containing secretory glands. These glands can also occur on filaments of numerous stamens. Its fruit is a tri-locular capsule which turns brown when ripe. Fruit opening depends on

Fig. 8.1 *Hypericum perforatum:* overground parts of plant with flowers (raw material) (left), leaf with hypericin and essential oil glands (center), and flowers (right)

air humidity. St. John's wort produces abundant cylindrical, dark, tiny (1 mm) seeds, with characteristic smell of turpentine. The thousand-seed weight ranges from 0.10 to 0.15 g (Crockett and Robson 2011; Rizzo et al. 2020; Szafer et al. 1988).

8.3 Geographical Distribution and Ecology

Hypericum perforatum is a relatively widespread medicinal plant. It is associated mostly with the temperate climate zone. This species is naturally distributed across Europe and Northern Africa in such countries as Morocco, Algeria and Tunisia. St. John's wort is also found in Asia, especially in the Arabian Peninsula, Caucasus, Turkey, Mongolia and in the countries south of Kazakhstan reaching as far as India and China (Global Biodiversity Information Facility 2020; McCutcheon 2017; Rumińska 1991).

St. John's wort inhabits diverse mostly sunny sites, such as meadows, glades, fields and light forests in the highlands and lowlands. In the mountains and rocky habitats, it grows in dry sunny, grasslands, meadows, forests and banks of water bodies. In Poland, this plant is encountered in the lowlands and at lower elevations in the mountains. It is increasingly found in wastelands and ruderal areas. This species does not have high soil requirements, but prefers fertile, light, permeable soils rich in mineral nutrients (calcium, nitrogen) (Kołodziej 2018; Szafer et al. 1988).

In other temperate regions, especially in North America, Australia and New Zealand, South Africa and Japan, this species was introduced accidentally or purposefully. It occurs as a naturalized weed also in Brazil, Argentina, Chile, Uruguay, Cuba and Haiti. Due to its widespread occurrence, this plant species is a serious problem in some countries as a weed or invasive species (Crushers and Zhou 2016; Germplasm

Resources Information Network 2020; McCutcheon 2017). Studies have been carried out in order to check the differences in capability of interspecies competition between St. John's wort in North America and Europe. It was thought that plants transferred to other countries and cultivated therein for years will be more competitive. However, investigations did not show any significant differences between examined populations (Vilà et al. 2003).

8.4 Taxonomy

The taxonomic position of St. John's wort is well established, though synonymic names of this species can still be found, such as *Hypericum vulgare* Lam. or *Hypericum assurgens* Peterm. However, the name St. John's wort is very often used in popular literature, web sources and scientific literature to refer also to other *Hypericum* species (Dauncey et al. 2019).

Robson (2003) described *H. perforatum* as an allotetraploid hybrid of *Hypericum maculatum* subsp. *immaculatum* (Murb.) A. Fröhl. and *Hypericum attenuatum* C.E.C.Fisch. ex Choisy. However, the present genetic research suggests that *H. perforatum* does not contain genetic admixture of *H. attenuatum* but rather it possesses a polyploid genome and is derived from *H. maculatum.* A majority of wild-type *H. perforatum* plants are tetraploid ($2n = 32$) but the presence of other forms, such as diploids, hexaploids and other polyploids, has also been confirmed (Dauncey et al. 2019).

H. perforatum is the most common species in *Hypericum* genus occurring in Poland. Another species found in Poland—*Hypericum humifusum* L. (trailing St. John's wort) is similar to *H. perforatum* and is capable of hybridization with it (Szafer et al. 1988). There are also numerous reports from various regions of Europe on hybridization between *H. perforatum* and *H. maculatum* when both species occur in the same area. For this reason, at present, St. John's wort population is heterogeneous (Dauncey et al. 2019).

Depending on the sources, from four to six subspecies of *H. perforatum* have been distinguished, apart from several varieties of unspecified taxonomic status (Dauncey et al. 2019; Global Biodiversity Information Facility 2020).

Hypericum perforatum subsp. *perforatum* L. is characterized by short oval or elliptic green leaves of low color intensity. Inflorescences are not abundant in flowers which have light yellow elongated petals. In natural locations, this plant has been found in Northern and Northwestern Europe and in central to eastern Siberia. It also occurs in southeastern China where it is gradually transformed into the subspecies *chinese.*

Hypericum perforatum subsp. *chinense* N. Robson is characterized by short narrow leaves and abundant flowers. This subspecies originates from China wherefrom it was introduced to Japan.

H. perforatum subsp. *songaricum* (Ledeb. ex Rchb.) N. Robson is a subspecies with elongated or ovate, pale green, leathery leaves. Flowers are light yellow in color with pointed sepals. This subspecies occurs in Kyrgyzstan and Xinjiang, a province of China, and also in Russia and on the Crimean Peninsula.

Hypericum perforatum subsp. *veronense* H. Lindb. has narrow, lance-shaped, rounded at the base leaves. Flowers have pointed sepals and light yellow petals. This subspecies is naturally distributed in Tajikistan and northwestern India reaching to Turkey. It also occurs in the Mediterranean region, Micronesia and Azores.

Hypericum perforatum subsp. *latifolium* Froehlich is found in Germany and on the Balkan Peninsula.

Hypericum perforatum subsp. *veronense* Froehl is distributed in Central Europe (reaching as far as to the coast of Black Sea) and in Southern Australia.

H. perforatum varieties: *Hypericum perforatum* var. *angustifolium* DC, *H. perforatum* var. *latifolium* Gaudin, *H. perforatum* var. *microphyllum* DC, *H. perforatum* var. *perforatum* L. grow in natural locations throughout Europe. Some of them, e.g., var. *angustifolium* have been included into the subspecies *H. perforatum* subsp. *veronense* H. Lindb. (Dauncey et al. 2019; Global Biodiversity Information Facility 2020).

8.5 Phytochemistry of the Species

Hypericum perforatum herb is a raw material with very rich chemical composition (Table 8.1) (Barnes et al. 2007; ESCOP 2018; Turek 2005). Plentiful secondary metabolites belong to the groups of naphthodianthrones (dimeric anthraquinones), flavonoids, tannins, phenolic acids, xanthones, phloroglucinol derivatives and essential oil (monoterpenes, sesquiterpenes) of which the best known are hypericin and hyperforin and common in the plant kingdom—hyperoside (Fig. 8.2).

Protohypericin and pseudoprotohypericin were the first compounds of the naphthodianthrone (dimeric anthraquinone) class isolated from St. John's wort. They are transformed under the influence of light into more stable products, such as hypericin and pseudohypericin. On the other hand, pseudohypericin is the main compound from this group occurring in St. John's wort. Its content is 2–4 times as high as hypericin. In some instances, the weight ratio of these compounds can reach even 10:1. Depending on the stage of plant development, percent content of hypericin ranges from 0.03 to 0.3% of dry weight. Usually, the highest content of metabolites from this group is found in flowers. Another naphtodianthrone compound, cyclopseudohypericin, is formed by oxidation of pseudohypericin. *Hypericum perforatum* contains also isohypericin (Barnes et al. 2001; Nahrstedt and Butterweck 1997). It is the presence of hypericins that makes St. John's wort juice turn red (ESCOP 2018; Turek 2005).

Hypericum perforatum was shown to contain also flavonols, flavones, their glycosides and biflavonoids. Their total content in the raw material reaches ca. 5%. The group of flavonols is represented by kaempferol and quercetin (0.3%) while the group of flavones by luteolin and its ether derivative: luteolin 5,3′-dimethyl

Table 8.1 Phytochemical composition of *Hypericum perforatum* L. (Barnes et al. 2007; ESCOP 2018; Mir et al. 2019; Jürgenliemk and Nahrstedt 2002; Asgarpanah 2012; Patočka 2003; Pirbalouti et al. 2014)

Group of metabolites	Compounds
Naphthodianthrones (dimeric anthraquinones)	Hypericin, pseudohypericin, isohypericin, protohypericin, protopseudohypericin, cyclopseudohypericin
Monomeric anthraquinones	Emodin
Flavonoids	Flavonols (kaempferol, quercetin, myricetin), Flavones (luteolin), Glycosides (hyperoside, izoquercitrin, quercitrin, rutoside, isoorientin, miquelianin, guaijaverin, astilbin), Biflavonoids (I3,II8-biapigenin, I3',II8-biapigenin, amentoflavone)
Catechins	Catechin, epicatechin
Procyanidins	Procyanidins A2, B1, B2, B3, B5, B7, C1
Phenolic acids and their derivatives	Caffeic acid, chlorogenic acid, *p*-coumaric acid, cryptochlorogenic acid, ferulic acid, *p*-hydroxybenzoic acid, neochlorogenic acid, protocatechuic acid, vanillic acid, cafeoylquinic esters, *p*-coumaorylquinic esters
Xanthones	1,3,6,7-tetrahydroxyxanthone, mangiferin, kielcorin, paxanthone, 1,6-dihydroxy-5-methoxy-4′,5′-dihydro-4′,4′,5′-trimethylfurano-(2′,3′:3,4)-xanthone; 4,6-dihydroxy-2,3-dimethoxyxanthone
Prenylated phloroglucinols	Hyperforin, adhyperforin and oxidated forms
Essential oil	α-amorphene, aromadendrene, bicyclogermacrene, bicyclosesquiphellandrene, β-bourbonene, cadalene, 1,4-cadinadiene, γ-cadinene, Δ-cadinene, δ-3-carene, carvacrol, β-caryophyllene, caryophyllene oxide, α-cedrene, α-copaene, α-cubebene, β-cubebene, p-cymene, decane, 2-methyl-decane, 1-dodecanol, α-farnesene, β-farnesene, geraniol, germacrene B, germacrene D, α-humulene, limonene, β-myrcene, n-nonanal, nonane, 3-methyl-nonane, β-ocimene, 2-methyl-octane, 1-octanol, α-pinene, β-pinene, α-phellandrene, pulegone, α-selinene, β-selinene, spathulenol, α-terpinene, γ-terpinene, α-terpineol, terpinolene, tridecane, undecane, 5-methyl-undecane, viridiflorol, ylangene
Others	Amino acids (cysteine, glutamine, leucine, lysine, ornithine, proline, threonine) pectins, fatty acids (myristic, palmitic, stearic), β-sitosterol, vitamins (A, C, PP), isovaleric acid, γ-aminobutyric acid, lutein, choline, long-chain alkanes and alcohols

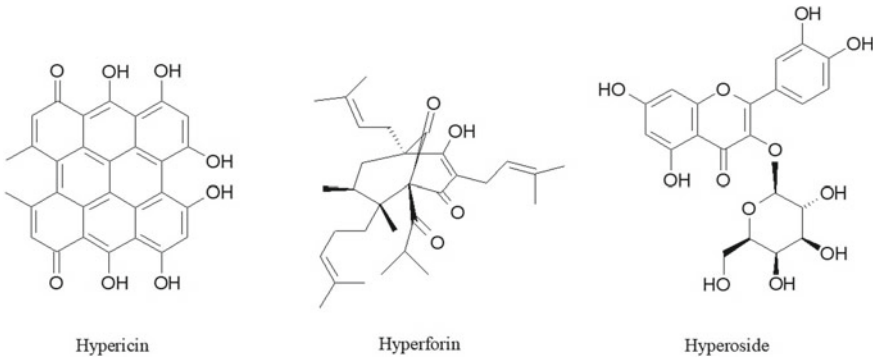


Fig. 8.2 Secondary metabolites characteristic for *H. perforatum* named after the species name

ether. However, these are flavonoid glycosides that are the most abundant group of compounds with dominating quercetin glycosides, such as hyperoside (0.5–2%), rutoside (0.3–1.6%), quercitrin and isoquercitrin (0.3%). Small amounts of luteolin glycosides were also identified. Biflavonoid compounds present in *Hyperici herba* comprise amentoflavone and 3,8-biapigenin occurring in flower buds and flowers (Barnes et al. 2001; ESCOP 2018).

Proanthocyanidins occur in *H. perforatum* in the fraction of tannins at a concentration of 6.2–12.1%. Their highest content in the plant is observed during flowering. Tannin's fraction includes principally catechin and epicatechin compounds, both dimers, trimers, tetramers and polymers (Patočka 2003; Ploss et al. 2001; Turek 2005).

The main metabolite from the group of phenolic acids in *Hypericum perforatum* was identified as chlorogenic acid (with the content below 1%). Moreover, the following compounds were isolated: caffeic acid, *p*-coumaric acid, ferulic acid, isoferulic acid, *p*-hydroxybenzoic acid and vanillic acid (Barnes et al. 2001; Jürgenliemk and Nahrstedt 2002; Nahrstedt and Butterweck 1997).

Many species of the genus *Hypericum* were confirmed to contain compounds of the xanthone group. Specifically, kielcorin (0.01%) was isolated from roots and 1,3,6,7-tetrahydroxyxanthone from stems and leaves (Mir et al. 2019; Nahrstedt and Butterweck 1997).

Phloroglucinol derivatives are highly prevalent in *Hypericum* species with hyperforin being the most characteristic of St. John's wort. Its derivative adhyperforin contains an additional methyl group. Both these compounds are present primarily in flowers and fruits. Hyperforin contents range from 2% in flowers to 4.5% in unripe fruits. Furohyperforin is the third derivative while other polar phloroglucinol derivatives were estimated to be present at range from 0.005 to 0.3%. Hyperforin is a relatively unstable compound and is oxidized under the influence of light and at higher temperatures; thus, it can be found mostly in fresh plant material (Barnes et al. 2001; Patočka 2003; Turek 2005).

The leaves and flower petals contain oil glands, from which essential oil can be extracted by steam distillation in the concentration range from 0.1 to 0.25% of raw material weight. Its main constituent—unsaturated hydrocarbon, methyl-2-octane accounts for 30% of its weight. Besides, the following compounds were identified: n-nonane, methyl-2-decane and n-undecane, α-pinen, β-pinen, α-terpineol, geraniol, trace amounts of other monoterpenes: myrcene and limonene and sesquiterpenes: caryophyllene and humulene (Asgarpanah 2012; Pirbalouti et al. 2014).

St. John's wort was also found to contain small amounts of amino acids, pectins, fatty acids such as myristic, palmitic and stearic acids, as well as β-sitosterol, vitamins (A, C, PP), lutein, choline and saturated hydrocarbons and alcohols (Barnes et al. 2007).

The contents of individual metabolites in the plant fluctuate during vegetation. Certain groups of compounds are characteristic of specific organs. Differences in metabolic profiles also result, most of all from individual genetic variability in population, origin of plants, the growing season, sun exposure, the type and humidity of soil.

8.6 Medicinal and Paramedical Applications of *Hyperici Herba* and Biological Activities of Its Metabolites

8.6.1 Traditional Use and Official Pharmacopoeial Monographs

Hypericum perforatum was recognized as a magical and powerful herb during the medieval period, but history of its use as a medicinal plant has been documented in scientific literature to date back to ancient times. By 400 BC, the great Greek physicians such as Galen, Pliny and Hippocrates described herb of this plant species, considerable wound healing property, as well as its usage in the treatment of women's disorders, hemorrhoids and kidney stones (Istikoglou et al. 2010).

In traditional treatment, infusions and decoctions of *Hypericum perforatum* herb (*Hyperici herba*) have been used in treating indigestion, and inflammatory and spastic conditions of bile ducts and digestive tract. It is due to the presence of flavonoids, tannins and phenolic acids, which show spasmolytic, cholagogic, cholepoietic and astringent actions. Flavonoid-rich aqueous extracts of *Hypericum perforatum* herb exhibit diuretic action. They facilitate removal of metabolic waste products and are used to prevent urolithiasis (Barnes et al. 2007; European Medicines Agency 2009; Jakovljevic et al. 2000).

St. John's worth as always been considered one of the most significant sources of biologically active and high-value metabolites which have drawn particular attention among researchers worldwide for wide range of their pharmaceutical applications, especially in the treatment of psychological disorders (Apaydin et al. 2016). Several studies confirmed *H. perforatum* effectiveness as an alternative treatment for mild

to moderate depression comparable to currently used synthetic antidepressants. Its valuable pharmacological activities, due to a complex phytochemical profile, make this plant one of the best-selling phytomedicines and dietary supplements in the world (Booker et al. 2018; Chauhan et al. 2011). In particular, a wide range of various pharmacological properties, such as neuroprotective, antibacterial, antiviral, anti-inflammatory and anticancer activities, have been highlighted (Barnes et al. 2001; Scotti et al. 2019).

The most common commercial St. John's wort preparations are as follows: *Succus* and *Intractum,* prepared from fresh material, tablets and capsules based on its dry extracts or aqueous and ethanolic extracts. The oily extract of the flower, crude herb and herbal mixtures containing St. John's wort herb are also available on the market. They differ in composition because compounds with distinct chemical structure are extracted by particular solvents, thus showing different biological activity. Aqueous extracts contain water-soluble active ingredients such as phenolic acids or flavonoid glycosides and consequently are used mostly as spasmolytic and astringent agents. St. John's wort oil can be efficient in healing wounds, ulcers and inflammatory states. On the other hand, ethanolic preparations—like tinctures and alcoholatures act as antidepressants owing to the presence of hypericin and hyperforin (Barnes et al. 2007; Turek 2005).

Due to well-established medicinal use resulting from chemical composition of St. John's wort herb, *Hypericum perforatum* is a pharmacopoeial plant. The current European Pharmacopoeia 10.0 (2020) lists three St. John's wort-derived raw materials: St. John's wort herb (*Hyperici herba*), St. John's wort dry extract, quantified (*Hyperici herbae extractum siccum quantificatum*) and *Hypericum* for homeopathic preparations (*Hypericum perforatum ad praeparationes homoeopathicas*) prepared from whole, fresh plant of *Hypericum perforatum* L., collecting at the beginning of the flowering period. St. John's wort herb is mentioned in the International Pharmacopoeia monographs edited by the World Health Organization (WHO 2004) too. Information on this medicinal raw material can also be found in the ESCOP (European Scientific Cooperative on Phytotherapy) monograph of 2018. Both documents provide information on actions, dosage and safety of St. John's wort herb. The American Herbal Pharmacopoeia (AHP) also developed the monograph on St. John's wort (Upton et al. 1997). The European Medicines Agency (EMEA) (2009) provides a detailed description of this raw material, as well. Apart from the typical monograph of *Hyperici herba*, EMEA issued also information on the use of this raw material as a veterinary homeopathic medicine. St. John's wort oil is one of preparations listing in this document. Different plant oils could be used to prepare, such as olive, maize or sunflower oil. According to EMEA data, St. John's wort oil is used in animals orally and topically. Moreover, St. John's wort herb is the subject of monographs in national pharmacopoeias of many countries, where it is defined as: aerial parts with flowers, aerial parts and flowering top (Dauncey et al. 2019).

8.6.2 Biological Activities Confirmed by Scientific Studies

Antidepressant activity

Nowadays, among the most specific uses of St. John's wort, its antidepressant efficacy has been amply proven by numerous studies. In fact, *H. perforatum* extracts are well known to be effective in treating mild and moderate depression compared to placebo, and its effects were comparable to antidepressants (Marrelli et al. 2020). Hypericin is active substance considered responsible for the antidepressant properties. However, other compounds contained in *Hypericum* extracts such as hyperforin, xanthones, flavonoids (especially amentoflavone) and tannins synergistically contribute to antidepressant activity. The mechanism of action of these compounds is partially related to that of classical antidepressant: selective serotonin reuptake inhibitors (SSRIs) block the activity of serotonin transporter SerT (active for a Na^+/Cl^- gradient) by competitive inhibition, whereas hyperforin has been found to reduce SerT activity by increasing the intracellular sodium and calcium gradient as a consequence of the alteration of the Na^+/Cl^- pump (Zirak et al. 2019). Hypericin action on some neurotransmitter transporters, such as dopamine, glutamate, noradrenaline and GABA with consequent inhibition of their reuptake, has also been proven. The specific mechanism of action of hypericin is thought to be related to the activation of the transient receptor potential channel protein 6 (TRPC6), an ion channel capable of regulating the cellular movement of cations, such as Na^+ and Ca^{2+}. It results in an increase in sodium uptake by the neuron followed by a decrease in its concentration in the synaptic cleft and, therefore, its unavailability for the monoamine transporter proteins (Teufel-Mayer and Gleitz 1997; Zirak et al. 2019). On the other hand, hypericin also strongly contributes to the antidepressant properties thanks to its high affinity for sigma receptors, which influence the concentration of dopamine. Furthermore, hypericin acts as an antagonist of adenosine receptors, GABA-A, GABA-B and inositol triphosphates, which regulate the action potentials caused by neurotransmitters. The inhibitory activity of hypericin on monoamine oxidase enzymes (MAOs), that have been used as a pharmacological target in the development of some antidepressant drugs called MAOI, is also of significant interest.

In some countries, *Hypericum* is administered as an alternative antidepressant treatment in adolescent patients even before a pharmacological intervention due to an efficacy similar to classic antidepressant drugs but with significantly fewer side effects. In addition, studies conducted on *Hypericum* extracts have shown a significantly superior activity vs. placebo in the treatment of major depression. It is commonly used in herbal medicine to treat some forms of anxiety in association with other products (Apaydin et al. 2016).

Neuroprotective activity

Recent evidence highlighted the application of *H. perforatum* in the treatment of neurodegenerative diseases. With regard to Alzheimer's disease (AD), Cao et al. (2017) found an improvement in cognitive performance, an increase in glutamic acid levels and acetylcholinesterase activity and norepinephrine levels in the aluminum

chloride-induced AD rat model after 60 days of treatment with *H. perforatum*. In another study, Brenn et al. (2014) discovered significant reductions of parenchymal Aβ accumulation and a moderate increase in cerebrovascular P-glycoprotein expression following a 60–120-day diet with the addition of *H. perforatum*. Hofrichte et al. (2013) found an alleviation of memory impairment in the transgenic amyloidosis mouse model. Furthermore, reductions in amyloid plaque formation, microglial activation and improvement of cognitive functions were found. However, it should be noted that these preclinical studies need to be supported by clinical evidence.

Vecchia et al. (2015) studied the effects of *H. perforatum* in an animal model of Parkinson's disease using different dosages for 35 consecutive days. All doses elicited improvement in contralateral turning behavior, probably due to counteracting the overexpression of dopaminergic receptors in the lesioned striatum. Using two standardized extracts of *H. perforatum* (6 and 0.2%) in the rotenone-induced Parkinson's disease rat model, Gomez del Rio et al. (2013) found that the usage of the extracts 60 min before the rotenone injection for 45 days efficiently ameliorated the neurochemical parameters as well as normalized catalepsy. The 6% extract was also capable of reducing neuronal damage and inhibiting the apoptotic cascade by decreasing Bax protein levels. According to current evidence from preclinical studies, *H. perforatum* could be applied in the treatment of Parkinson's disease; although clinical trials are needed before, conclusions can be drawn.

Antimicrobial activity

Studies on extracts of aerial parts have shown an interesting antibacterial activity of St. John's wort. Hyperforin is the main compound involved in the antimicrobial activity against *Staphylococcus aureus*, multidrug-resistant *S. aureus* and Gram-positive bacteria, such as *Streptococcus pyogenes* and *Corynebacterium diphtheriaea* (Brondz et al. 1982; Mullaicharam and Halligudi 2019). According to Lyles et al. research, samples of extracts of *H. perforatum* aerial parts examined for minimum inhibitory concentration (MIC) values and reveled high-growth inhibitory activity against *S. aureus* strains (Marrelli et al. 2020). In a recent study, Okmen and Balpinar (2017) observed the high efficacy of *H. perforatum* flower extracts against five coagulase-negative *Staphylococcus* strains and two *S. aureus* isolated from cow mastitis. On the other hand, a growth inhibitory effect has never been detected against Gram-negative bacteria, such as *E. coli, Enterococcus faecalis* and *Pseudomonas aeruginosa* (Schempp et al. 1999).

Although hyperforin is the major constituent related to the antimicrobial activity, new compounds such as imanine and novoimanine from *H. perforatum* have been described. Moreover, further antimicrobial activity against a clinical isolate of methicillin-resistant *S. aureus* has been emphasized for several other species of *Hypericum* (Barnes et al. 2007).

Knowledge of antifungal activity of St. John's wort is limited. One of the studies demonstrated diverse efficacy of hypericin against *Candida albicans, Exophiala dermatitidis, Microsporum canis, Fusarium oxysporum, Trichophyton rubrum, Pichia fermentans, Kluyveromyces marxianus* and *Saccharomyces cerevisiae* (Sytar

et al. 2016). In addition, aqueous extracts of St. John's wort are capable of growth inhibition of *Microsporum gypseum* and *Trichophyton rubrum* (Wölfle et al. 2014).

Antiviral activity

H. perforatum is also involved in antiviral effects due to flavonoid- and catechin-containing fractions, which exhibited the ability to inhibit the influenza virus by 83–100% (Barnes et al. 2001). Moreover, several studies reported the in vitro inhibiting activity of hypericin and pseudohypericin against encapsulated viruses, such as herpes simplex virus types 1 and 2 (Weber et al. 1994; Wood et al. 1990), human immunodeficiency virus (HIV)-1 (Lavie et al. 1989; Meruelo et al. 1988), varicella zoster-virus and hepatitis C (Barnes et al. 2007; Hudson et al. 1991). According to Hudson et al. (1991) experiment, hypericin has been proven to be helpful in murine cytomegalovirus (MCMV) and Sindbis virus inactivation. Indeed, hypericin is a well-known photosensitizing agent which appears to act, on the one hand, probably directly on the virion membrane and on the other hand, on the virus-infected cells, and these actions are enhanced by the light (Barnes et al. 2001; Hudson et al. 1991).

The newest studies have reported that characteristic St. John's wort metabolites of the flavonoid group (kaempferol, quercetin and its glycosides, hypericin, rutoside, isoquercetin) and other phenolic compounds show antiviral activity against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). They act via different mechanisms, from inhibition of its entering the cell to blockade of polymerase activity (Khan et al. 2021).

Anti-inflammatory activity

Anti-inflammatory properties have been investigated using several extracts of *H. perforatum* during studies conducted on the mouse ear model with consequent reduction of inflammation. The compounds isolated from St. John's wort extract: amentoflavone, hypericin and adhyperforin are responsible for the anti-inflammatory properties comparable to the activity of the synthetic product indomethacin. No particular anti-inflammatory activities were found for flavonoid compounds, such as hyperoside and isoquercitrin (Wölfle et al. 2014).

Hyperforins are considered the most important anti-inflammatory components in St. John's wort extracts due to their high concentrations and strong anti-inflammatory potency (Sosa et al. 2007; Wölfle et al. 2014). Hypericin was found both to inhibit interleukin-12 (IL-12) production and to activate the IL-12 gene promoter, negatively regulating IL-12 production at the transcriptional level (Kang et al. 2001). Anti-inflammatory mechanisms of hyperforin action have also been documented as an inhibition of cyclooxygenase-1 (but not cyclooxygenase-2) and 5-lipoxygenase at low concentrations (Albert et al. 2002; Hammer et al. 2007; Koeberle et al. 2011). It would appear that hyperforin has a high-efficiency 5-lipoxygenase inhibiting function in vivo (Feißt et al. 2009), and in vivo capability of suppressing oxidative bursts in polymorphonuclear cells (Heilmann et al. 2003). Inhibition of interferon-γ production, downregulation of chemokine receptor—CXCR3 expression on activated T cells and downregulation of expression of matrix metalloproteinase-9 was documented in the rat model of experimental allergic encephalomyelitis (Cabrelle et al.

2007). Due to its ability to relieve symptoms, hyperforin has been considered as a possible therapeutic molecule for Th1-lymphocyte mediated inflammatory autoimmune diseases. With specific reference to dermatological applications, Schempp et al. (2000) investigated the antigen-presenting function of human epidermal cells exposed to *Hypericum* ointment containing hypericin and hyperforin in a mixed EC (epidermal cells) lymphocyte reaction in vivo. Compared to untreated skin, *Hypericum* ointment suppressed the mixed EC lymphocyte reaction in vivo ($p < 0.001$) which was comparable to the effect simulated by solar radiation. A similar effect was achieved with the use of an ointment with the same hyperforin content but devoid of hypericin. The same research group demonstrated that hyperforin inhibited the proliferation of blood mononuclear cells via a dose-dependent mechanism without showing a high toxic effect. These results made it possible to consider the treatment of inflammatory skin disorders with the use of *Hypericum* extracts containing hyperforin.

In a mouse model, St. John's wort appeared to suppress the inflammation and leukocyte infiltration induced by carrageenan and prostaglandin E_1 (PGE_1) (Shipochliev 1981). In in vitro studies, hypericin apparently inhibited TNF-mediated activation of NF-kB (Bork et al. 1999), growth factor specifically regulated protein kinase (Agostinis et al. 1995; De Witte et al. 1993; Takahashi et al. 1989) and the release of arachidonic acid and leukotriene B4 (Panossian et al. 1996). Furthermore, it seems that since protein kinase C is important in cellular reactions that occur in PVR, hypericin-mediated modulation of protein kinase C may be a factor in this system.

The anti-inflammatory action of St. John's wort is linked with its antioxidant properties. Some studies proved high free radical scavenging ability of ethanolic *H. perforatum* extracts. Their antioxidant potency results mostly from a high content of flavonoid compounds. Apart from the latter, also hyperforin exhibited such potential in vitro (Wölfle et al. 2014). Antioxidant activity of St. John's wort herb infusions was also documented (European Medicines Agency 2009).

Anticancer activity

The anticancer effects of St. John's wort are divided into light-dependent mechanisms, linked to the photoactivation of hypericins and light-independent mechanisms related to hyperforin. Antitumor effects of *H. perforatum* have been revealed through animal and in vitro studies. Schempp et al. (2002b) demonstrated the phototoxic and apoptosis-inducing capacity of hypericin with human leukemic lymphoma cells (Jurkat) due to a dose-dependent inhibition and DNA fragmentation. Schempp et al. (2002a) evaluated the antiproliferative potential in vitro of hypericin against six cancer cell lines. Hypericin exhibited a better or equal anti-growth inhibitory activity compared to other cytostatic drugs (e.g., camptothecin, paclitaxel and vincristine). The inhibitory activity of hypericin revealed the best effects against cancerous mammary cells, although good results were obtained against human squamous cell carcinoma, malignant melanoma and lymphoma cell lines. According to data, hyperforin would induce apoptosis of tumor cells via mitochondrial activation, cytochrome C release and caspase activation, resulting in the activation of the cell death pathway

(Schempp et al. 2002a). Neem oil (from *Azadirachta indica*) and *H. perforatum* flowers extracts proved, respectively, their cicatrizing and anti-inflammatory activities against acute skin toxicity in patients with head and neck cancer undergoing radio- or chemo-radiotherapy. Furthermore, it appears to have a prophylactic effect in moist desquamation. However, the effect of this combination has yet to be tested in a controlled study (Franco et al. 2014).

Over the years, researchers have studied the photosensitizing effect of hypericin and its application in the field of photodynamic therapy in cancer patients. Various in vitro studies on a number of cell lines have all demonstrated the cytotoxic effect on cancer cells following photosensitization (Mullaicharam and Halligudi 2019). The photoactivating effect has also been studied in vitro on various cell lines and animal models in vivo, such as in human prostate adenocarcinoma cells and in human prostate metastatic cell lines (Colasanti et al. 2000), in human urinary bladder carcinoma cells (Kamuhabwa et al. 2000) and in pancreatic cancer cell lines (Liu et al. 2000). Furthermore, intratumoral laser photodynamic therapy associated with the use of hypericin showed a significant induction of tumor necrosis in mice following human squamous carcinoma cell transplantation when compared to laser treatment alone (Chung et al. 2000).

Supportive treatment of addiction

Most studies on the use of St. John's wort extract in addiction treatment have focused on alcoholism. Serotonergic deficiency can play a significant role in the development of addiction and withdrawal syndrome. *H. perforatum* acts as a serotonin reuptake inhibitor; thus, it can alleviate unpleasant withdrawal symptoms. The efficiency of St. John's wort in the treatment of such disturbances can also be connected with the blockade of nitric oxide synthase. Specifically, St. John's wort extract at a dose of 50–200 mg/kg reduced hyperactivity and tremor (Barnes et al. 2007; Wright et al. 2003).

In addition, methanolic St. John's wort extract containing a minimum of 2% hyperforin and 900 μg hypericin was tested in nicotine addiction, and also in this case, it was demonstrated to improve mood in patients. It is possible that it will be efficient also in addiction to other psychoactive substances: amphetamine and cocaine (Uzbay 2008).

Dermatological diseases

Aqueous and methanolic St. John's wort extracts exhibit anti-inflammatory and astringent actions and are used in the treatment of slow-healing wounds, first and second degree burns, ulcers and frostbites (European Medicines Agency 2009; Quave 2018). St. John's wort oil demonstrates regenerative actions and promotes healing in difficult-to-treat wounds and blunt injuries, myalgia and first-degree burns (European Medicines Agency 2009).

Hypericin is an inhibitor of protein kinase II and epidermal growth factor receptor (EGFR) tyrosine kinase. These kinases are implicated in the pathomechanism of psoriasis. Hence, hypericin is used to treat vitiligo in combination with photodynamic therapy. Local application of *Hypericum perforatum* extracts or isolated hypericin

produced, depending on the intensity of UV-A radiation, either irritation of the skin or phototoxicity (Schempp et al. 2003). This property is also used in therapeutic strategies for basal cell carcinoma and psoriasis (Bodeker et al. 2017; Colasanti et al. 2000; Kiesslich et al. 2006). Studies in mice demonstrated that hypericin acetate more easily penetrated though the epidermis and caused a stronger damage to pathologically changed cells than hypericin (De Witte et al. 1993; Dyrała et al. 2015). Owning to the action of hypericin on the skin, St. John's wort has been found to be a useful ingredient of preparations for treatment of discoloration of the skin, including vitiligo, and for darkening of the skin tone, especially in sunless tanning preparations (Cosmetic Ingredient Database 2021).

Other activities and actions

Due to anxiolytic effects, St. John's wort can be used to relieve anxiety, irritation, premenstrual syndrome and even in bedwetting in children (Barnes et al. 2007; Pirbalouti et al. 2014). *H. perforatum* is also applied in the therapy of peripheral nerve damage accompanied with pain and migraine headaches (Galeotti and Ghelardini 2013; European Medicines Agency 2009). Moreover, ophthalmologic applications of hypericin were tested to cure macular degeneration (Dyrała et al. 2015).

Usage in homeopathy

St. John's wort is used in homeopathy. The European Pharmacopoeia 10.0 (2020) lists *Hypericum perforatum ad praeparationes homoeopathicas* and describes it as the whole fresh plant of *Hypericum perforatum* L., at the beginning of the flowering period. The mother tincture of *Hypericum perforatum* L. is prepared by maceration using alcohol of a suitable concentration. The final product is a dark red to brownish red liquid. *H. perforatum* is useful for injures to densely innervated areas, like the spine or fingers. It also relieves pain after dental treatment and post-surgery. St. John's wort is helpful in treating painful scars and phantom pains (Phatak 2001).

8.6.3 Cosmetic Applications

According to Cosmetic Ingredient Database (2021), extracts from *Hypericum perforatum* can be used in cosmetics mainly for skin conditioning. This function is ascribed to four *H. perforatum* extracts: leaf extract, flower extract, flower/leaf extract and flower/leaf/stem extract. *H. perforatum* oil is used as a skin conditioning emollient. Apart from skin conditioning, *H. perforatum* flower/twig extract shows antimicrobial and antiperspirant action. Another extract prepared from the whole aerial part of *H. perforatum* exhibits antimicrobial, astringent, fragrant, skin conditioning, skin protecting, soothing and tonic properties. Two types of extracts from in vitro culture of *H. perforatum* are also available: *H. perforatum* callus culture extract and *H. perforatum* phytoplacenta culture extract; both of them are used as antioxidant, hair conditioning and humectant ingredients.

8.6.4 Interactions and Side Effects

H. perforatum phytoconstituents including hypericin, hyperforin and flavonoids may be interacting molecules involved in the decrease in the bioavailability of cytochrome P450/P-glycoprotein (CYP3A4/Pgp) substrates (Chrubasik-Hausmann et al. 2019).

In detail, St. John's wort activates the pregnane X-receptor mediated upregulation of intestinal cytochrome—P450, specifically CYP3A4 and CYP1A2 isoforms. Induction of this enzyme causes an increase in metabolism of some drugs, with consequent decrease in plasma concentration and potential clinical effect (Wenk et al. 2004). Moreover, some St. John's wort compounds may modulate CYP isoenzymes, such as 2E1, 2C9 and 2C19 (Mannel 2004; Nowack 2008; Rahimi and Abdollahi 2012; Šemeláková et al. 2016).

Moreover, there is strong evidence that *H. perforatum* is responsible for the P-glycoprotein efflux transporter induction (Velingkar et al. 2017). This mechanism leads to decreased plasma concentrations and reduced clinical efficacy of some drugs due to lower absorption and greater clearance (Gurley et al. 2008).

Various interferences with the effects of some drugs have been documented. Therefore, coadministration of St. John's wort with antiepileptics, antidepressants, anticancer drugs (e.g., irinotecan) and coumarin anticoagulants (e.g., warfarin) is highly discouraged. In psychiatric and neurologic settings, its use is not recommended in patients with schizophrenia, cognitive impairment or bipolar disorder (Drugs.com 2020; NCCIH 2020). The possible presence of serotonin syndrome has been proven following the combination of St. John's wort and antidepressants, as an increase in serotonin levels has been documented (Borrelli and Izzo 2009).

Interactions can also occur between St. John's wort and immunosuppressive drugs, such as cyclosporin or non-nucleoside HIV reverse transcriptase inhibitors, e.g., nevirapine or HIV protease inhibitors, such as indinavir. Dangerous effects can develop due to pharmacodynamic interactions between *Hypericum* and cardiac glycosides (digoxin) when *Hypericum* doses exceed 1 g/day. *H. perforatum,* acting as CYP3A4 isoenzyme inductor, drastically reduces the plasma concentration of ranolazine as well as its therapeutic effect as antianginal drug. Therefore, combination of *Hypericum* and ranolazine is strongly discouraged (US National Library of Medicine 2020). In cases of unplanned pregnancies, the decreased efficacy of oral contraceptives containing estrogen used in combination with St. John's wort has been reported (Russo et al. 2013). Although most of the interactions were highlighted during concomitant and chronic administration of *H. perforatum* herb and drugs, further clinically relevant interactions were detected between *H. perforatum* phytocompounds and food, spices and other herbs (Borrelli and Izzo 2009).

The use in therapy of *H. perforatum* is known to be well tolerated, and some side effects (2%), even life-threatening ones, have been reported in the literature as a consequence of the coadministration of St. John's wort and a variety of prescribed medications (NCCIH 2020).

The administration of *H. perforatum* may generate allergic reactions (0.5% of cases) as well as photosensitization reactions due to the presence of hypericin that

induces sensitivity to visible and ultraviolet light. Therefore, exposure to UV radiation is not recommended after the administration of high doses of St. John's wort (Ernst et al. 1998). In addition, 0.6% of adverse reactions including gastrointestinal discomfort (nausea, abdominal pain, loss of appetite and diarrhea), dizziness, confusion, fatigue, sedation, dry mouth, restlessness and headache were found to be related to ingestion of *H. perforatum* (Barnes et al. 2007; Ernst et al. 1998; Greeson et al. 2001; Parker et al. 2001). Concomitant administration of St. John's wort and contraceptive drugs is strongly discouraged due to the accelerated metabolism of estrogens, which leads to the decreased efficacy of the latter (NCCIH 2020). Moreover, additional adverse reactions could arise due to the similar profile between *H. perforatum* and SSRI drugs, such as fluoxetine (Hoban et al. 2015). Both SSRI and St. John's wort act on the central nervous system; therefore, their simultaneous administration could induce additive effects and exceeding the toxic dose.

H. perforatum has been demonstrated to be a safe phytotherapeutic drug during pregnancy and lactation. Nevertheless, caution is always advised in the simultaneous use of *Hypericum* during pharmacological therapies due to the possibility of herb-drug interactions.

8.7 Natural Diversity and Cultivation of St. John's Wort

8.7.1 Natural Locations

Since *Hypericum perforatum* is a medicinal plant containing a wide variety of chemical constituents, it presents a broad spectrum of biological activities, which have been described in the previous subchapters. However, the chemical composition of St. John's wort herb and its preparations can vary depending on genotypic and phenotypic variability in a population and on external conditions to which plants were exposed during their growth. The climate, type of soil, water supply, sunlight exposure and temperature can contribute to its variable phytochemical composition (Agapouda et al. 2019; Bruni and Sacchetti 2009; Turek 2005).

Hypericum perforatum is a perennial plant, which propagates generatively and vegetatively. Above-ground parts of the plant die back before winter. In spring, St. John's wort produces many new shoots from underground rhizomes. In temperate climate of Europe, also in Poland, it is cold hardy (Osińska and Rosłon 2016; Senderski 2004).

St. John's wort is naturally distributed in Asia, Northern Africa and practically in all of Europe. Only locations in the far north of the European continent are the exception (Germplasm Resources Information Network 2020; Global Biodiversity Information Facility 2020). In all these countries, the raw material can be harvested from the wild.

Due to such a wide natural distribution range of *H. perforatum*, it is morphologically diverse and differs in blooming period. However, differences in their phytochemical profile have not been fully established, yet. Natural hybridization between *H. perforatum* subspecies is also possible (Dauncey et al. 2019).

This species was naturalized in Africa (Sudan, Republic of South Africa, Lesotho, east cost of the Indian Ocean), Asia (Japan), Australia and New Zealand, North America, Hawaii, Cuba, Haiti, South America (Brazil, Argentina, Chile, Uruguay). In many of these countries, this species has spread very extensively by generative and vegetative propagation and is considered to be an invasive plant (Crushers and Zhou 2016; Germplasm Resources Information Network 2020; McCutcheon 2017).

8.7.2 Cultivation

Due to a wide use of St. John's wort herb in medicine, the demand for this plant material is constantly on the rise. Although *H. perforatum* is harvested from the wild in large quantities, it was also brought into cultivation systems. The raw material from natural habitats is delivered to the European market by Central and Eastern European countries, including Albania, Austria, Bosnia and Herzegovina, Bulgaria, Croatia, Germany, Hungary, Kosovo, Poland, Romania, Serbia and Macedonia. Plant material from commercial cultivation is able to fully meet the demand from the herbal industry. The largest St. John's wort plantation growing areas are in European countries, such as Poland, Germany, Italy, Belarus, Switzerland and in Asia (Siberia) (Germplasm Resources Information Network 2020; McCutcheon 2017). In the US, the plant raw material is acquired from naturalized plants; in addition, it is imported from China (McCutcheon 2017).

The raw material traded on the world market is also sourced from other *Hypericum* species, which is a serious problem. Sometimes, it results from deliberate adulterations of the herbal material, while in other cases, it is caused by different understanding of the common name of St. John's wort. Most often the following species are used: *H. barbatum, H. maculatum, H. montanum, H. hirsutum, H. tetrapterum, H. patulum* and *H. crux-andreae* (Dauncey et al. 2019; McCutcheon 2017). At the same time, wide-ranging studies of the chemical composition of the herb of *Hypericum perforatum* and *H. maculatum* from over 100 natural locations in Poland have demonstrated that *H. maculatum* is an equally valuable species. Therefore, its harvest for medicinal purposes could be authorized in case of insufficient supplies of *H. perforatum* (Brunarska et al. 1984).

In natural habitats, like it was mentioned previously, St. John's wort grows best in warm sunny locations. It tolerates various soil types, mostly fertile, well-drained with admixture of sand. It does not thrive in compact wet soils (Senderski 2004).

In Poland, climate conditions are favorable for St. John's wort cultivation. The plantations are established in locations with appropriate humidity, sun exposure and protected from the wind. The best results are achieved on fields where rape, legumes or root crops were earlier grown. Fields in which monocots were planted in the

previous year are not advisable due to soil depletion. Sites with a high level of weed infestation are also not recommended for St. John's wort cultivation (Rumińska 1991).

The most suitable soils have slightly acid to neutral pH values and good structure, preferably in the second or third year after fertilization with animal manure. If mineral fertilization is used, it is important to apply N rates of 60–80 kg/ha, P rates of 25–60 kg/ha and K rates of 80–100 kg/ha before sowing. Although St. John's wort requires a high supply of nutrients, it does not tolerate excess of nitrogen and phosphorus in the soil; therefore, soil condition should be monitored. In the second year of cultivation, apply of potassium salts should be reduced. Nitrogen fertilizer input is often split into three applications (before sawing, at the beginning of vegetation and after the first harvest of plant material). Autumn tillage includes pre-sowing deep plowing, and then, soil should be left for 3–4 weeks. Thereafter, before commencement of sowing, precise land leveling should be carried out. Harrowing followed by rolling is recommended. Spring sowing should be preceded by land leveling and rolling (Kołodziej 2018; Osińska and Rosłon 2016).

St. John's wort is propagated for field crop production mostly by direct seeding either in the autumn or in the spring. Seeds increase their germination capacity during storage and must be stratified. Prior to seeding, the seeds should be appropriately prepared, namely they should be mixed with wet sand and kept at a temperature from 0 to −3 °C for 2–3 months. Subsequently, partially dried seeds are sown directly in the ground. In the case of autumn sowing, cold stratification occurs in a natural way for temperate climate. The most suitable time for sowing the *Hypericum perforatum* seeds is in October or just before frosts. Seeds are planted in rows ca. 30–40 cm apart and gently pressed into soil. The sowing rate is between 3 and 4 kg/ha. If plantations are established in the spring, seeds have to be sown under shelter (0.5 kg/ha). Seedlings obtained under glass are transplanted outside at the beginning of May. The latter method is more labor-intensive but lower seed rates are required. St. John's wort can be also propagated vegetatively by cutting larger clumps in the late autumn or by division of rhizomes in the spring. Seeds for own use can be harvested at the end of September (Rumińska 1991; Senderski 2004).

If necessary, depending on soil and atmospheric conditions, the soil should be loosened between rows. During the growing season, the area has to be weeded manually or mechanically because weed infestation is dangerous for slow-growing young plants. Weeding before harvest time is also advisable.

The above-ground parts of the plant are cut at the onset of blooming, always in the afternoon on sunny dry days (plants contain higher levels of constituents at that time). In the first year of cultivation, one harvest is carried out in the middle of August while in the next years, plant material is harvested twice: at the end of June and in the middle of August (Senderski 2004).

Hyperici herba consists of densely leaved upper parts of stems (25–35 cm) without woody parts. In contrast to manual harvest at natural locations, with large plantings, mechanical harvesting is carried out. The harvested plant material is immediately dried in shady and well-ventilated places or in hot air dryers. Too high drying temperature and exposure to sunlight may reduce hypericin content in the raw material. The

plant material can be dried at a temperature of 60 °C but the application of a lower temperature (up to 40 °C) is recommended so as not to compromise the quality of raw material (Osińska and Rosłon 2016).

The average yield in the first year of cultivation is ca. 1–2 t/ha. In the subsequent years, when plants are well established, 3–4 tons of dried herb can be obtained per hectare. The contents of hypericin and hyperoside in the raw material also rise by 50% and 30%, respectively. One ton of herbal raw material is obtained from 4 tons of fresh herbage (Kołodziej 2018; Rumińska 1991).

Monoculture cropping systems can be affected by pests and spread of diseases; thus, appropriate plant protection products have to be used to avoid plant pest infestation. *H. perforatum* plantings should not be planned in a direct vicinity of croplands already existing for several years. Pests that feed on St. John's wort include beetles and their larvae (*Chrysomela hyperici* Forst), various species of tortrix moths (Tortricidae), e.g., *Tortrix viridana* Forst and St. John's wort aphids (*Aphis chloris* Koch.). *H. perforatum* plantations are also at risk of fungal infestation caused by *Verticillium album–atrum* Reike et Berthold, *Septoria hyperici* Desm. and *Erysiphe hyperici (*Wallr.) ex Blumer. Most of infections lead to considerable plant damage and leaf fall. In Poland, many diseases are caused by fungal pathogens of roots of the genera *Fusarium* and *Sclerotinia* as well as *Rhizoctonia solani* J.G. Kühn., *Phoma exiqua* var. *exiqua* Sacc. and *Botrytis cinerea* Pers.. *Seimatosporium hypericinum*, a new species in Poland (Greater Poland), causes necrosis of St. John's wort stems. *H. perforatum* plantations in Switzerland, Germany and Hungary were observed to be infected by *Colletotrichum gloeosporioides* Penz. and Sacc., inducing anthracnose (Kołodziej 2018; Osińska and Rosłon 2016; Rumińska 1991; Zimowska and Machowicz-Stefaniak 2004).

8.7.3 St. John's Wort Cultivars

St. John's wort cultivars with various properties have been developed in order to improve crop production, unify plant material, increase plant yield and enhance immunity to diseases and resistance to external conditions.

The most commonly used *H. perforatum* cv. 'Topas' is a Polish cultivar created in 1960–1982 at the Institute of Plants and Herbal Products in Poznań as a result of radiation-induced mutation. It is suitable for cultivation and herbal product processing. These plants have thin stems and abundant flowers. The plant raw material contains 0.15% hypericin and 2.5% hyperoside (Osińska and Rosłon 2016; Rumińska 1991; Seidler-Lozykowska and Dąbrowska 1996). The cultivar 'Elixir' ('Medizinal') was produced in Denmark and is characterized by a higher content of hypericin. Another Danish cultivar 'Helos' is highly anthracnose-tolerant. The German cultivar 'New Stem' ('Anthos') is specifically useful for mechanical

harvest and is distinguished by resistance to pathogens. The literature contains also reports on Russian cultivars 'Zveroboy' and 'Zlotodolynsky' (Germplasm Resources Information Network 2020).

8.7.4 Quality of Raw Material

Apart from genetic and environmental factors, there are many circumstances that influence the crop yield and phytochemical quality of plant raw materials. Seed quality, sowing practices, fertilization schemes and crop protection methods, which are largely at the discretion of the farmer, contribute to a high crop quality. In the case of *H. perforatum,* the harvest time depends on pharmacopoeial requirements (Bruni and Sacchetti 2009; Poutaraud and Girardin 2005). In Europe, it is unified by the European Pharmacopoeia

The outer appearance of St. John's wort herb after drying should show leaves naturally green in color and yellow flowers. According to the EU regulations, raw material containing seeds is of sub-standard quality. However, in conformity with the AHP monograph (Upton et al. 1997) valid in the US, the presence of seeds is permissible. In accordance with the European Pharmacopoeia (2020) and WHO monographs (2004), *Hyperici herba* should contain not less than 0.08% total hypericins while AHP requires it to be not less than 0.04%. On the other hand, the monograph of *H. perforatum* presented by the United States Pharmacopeial Convention (2015) states that *Hyperici herba* should contain not less than 0.04% hypericin and pseudohypericin and at least 0.06% hyperforin.

The plant raw material should be kept in paper packages in a dark and well-ventilated place to assure its uncompromised quality. Many secondary metabolites are volatile or unstable and undergo degradation by enzymatic and non-enzymatic processes of oxidation or hydrolysis. Hypericins are stored in special glands which may disintegrate upon drying, causing metabolite loss (Poutaraud and Girardin 2005; Turek 2005). During drying, light may slightly reduce the content of protohypericin and increase the amounts of hypericin and pseudohypericin (Poutaraud et al. 2001a). Noteworthy, dry plant material exposed to light for 2 h loses 20% of hyperforin (Poutaraud et al. 2001b).

A wide range of methods have been used for assessment of the herbal raw material identity, quality and for detection of possible adulterations. Confirmation of botanical identity of the dried material is not simple and should be performed by an expert. The material identity can be determined by organoleptic methods and by microscopic and thin-layer chromatography techniques (TLC or high-performance TLC (HP-TLC)). State-of-the-art genetic methods of identity testing can also be used. DNA barcoding and comparison of species-specific DNA markers using PCR allow for control of fresh and dry plant material. However, DNA in extracts and some finished products can be too degraded to obtain reliable results (Agapouda et al. 2019).

In the case of chromatographical analytical methods, reproducibility of the used procedures and the reference standards is also very important. According to the literature data, flavonoid profile in St. John's wort is relatively invariable and can be used for determination of the identity of raw material and finished products prepare from them. In accordance with pharmacopoeial recommendations (European Pharmacopoeia 2020), analysis should focus on hypericins, hyperforin and flavonoids to confirm quality of herbal material by HP-TLC and column chromatography. Apart from the above-mentioned simple chromatographic methods (TLC, HP-TLC), high-performance liquid chromatography (HPLC) technique with DAD- (diode array), UV- or MS- (mass spectrometry) detection is often used. Other spectroscopic methods can also be applied, if necessary, including mid-infrared (MIR) and near-infrared (NIR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy (Agapouda et al. 2019; Poutaraud and Girardin 2005).

8.8 Biotechnological Approaches for Improving the Quality of Herbal Material

Plant raw material for the herbal industry could also originate from wild harvest plants from natural locations and from cultivation (different cultivars and cultivated populations). As already mentioned, its quality is very diverse, which constitutes a significant obstacle to obtaining standardized products. This problem can be solved by plant biotechnology techniques.

Plant biotechnology is based on in vitro cultures. They include cell, tissue and organ cultures on appropriately selected media under sterile conditions. In vitro cultures are maintained under strictly controlled conditions both in smaller laboratories and industrial scale production plants. Basic nutrients in culture media, plant growth and development regulators (PGRs), light conditions and temperature are established and reproducible.

Undoubtedly, in vitro cultures have an advantage of fast cultivation of a large number of plants. In addition, they enable the propagation of species without the need to use seeds, and in cases when there is a little chance for germination and growth of plants in the natural environment. In vitro cultures prove useful for propagation of genetically modified plants and specimens obtained by selection. Biotechnology is helpful in increasing crop yield, reducing problems caused by diseases and pests, boosting resistance to disadvantageous environmental conditions and also in enhancing the concentration of valuable metabolites produced by the plants.

8.8.1 St. John's Wort Micropropagation

The production of *H. perforatum* plantlets in in vitro cultures is an alternative to traditional propagation of this plant material. It allows for obtaining the industrial raw material that is uniform in terms of genetic information and consequently also in terms of chemical composition. Micropropagation is a type of propagation of plants under in vitro conditions. It can replace long-lasting breeding and selection of cultivars. It allows for attaining high yields of plants in relatively short time. There are two paths leading to regeneration of new plants from explants in in vitro conditions: organogenesis and somatic embryogenesis (the process by which an embryo is derived directly from somatic cells). If any of these processes involves formation of the callus tissue, they are defined as indirect regeneration.

Micropropagation of *Hypericum perforatum* is a method of fast multiplication of plantlets derived from stock plants showing the desired characteristics, for industrial production. Basically, regeneration of *H. perforatum* plants in vitro, either by somatic embryogenesis or organogenesis is relatively simple and quick (Franklin and Dias 2006). Preservation of genetic integrity of plants micropropagated from basic genotypes is the most important factor for maintaining agronomic traits. Since organized meristems are thought to be resistant to genetic changes during division (somaclonal variation) and differentiation of cells under in vitro conditions. It is believed that this method of multiplication produces genetically uniform plants identical with the genome of mother plant (Rani and Raina 2000).

In vitro regeneration of *H. perforatum* was successfully attained with the use of many types of explants, including whole seedlings (Cellarova et al. 1992), leaves (Pasqua et al. 2003; Pretto and Santarem 2000; Wójcik and Podstolski 2007), nodal segments (Savio et al. 2012), petals (Palmer and Keller 2011), hypocotyls (Murch et al. 2000a), shoots and shoot apices (Alan et al. 2015; Zobayed and Saxena 2004), root explants (Pawełczak 2010; Zobayed and Saxena 2003), isolated anthers (Kirakosyan et al. 2000) and organogenic aggregates from cell suspension (Franklin et al. 2007).

The development of plants can be stimulated on the medium enriched in different combinations of PGRs. Although generally in a majority of species, shoot regeneration requires a high cytokinin/auxin ratio; in *H. perforatum,* efficient regeneration was achieved when this ratio was low (Palmer and Keller 2011; Pasqua et al. 2003). Interestingly, these plants can be readily regenerated from root explants on medium supplemented with indoleacetic acid (Zobayed and Saxena 2003).

Many studies proved the influence of age of St. John's wort parental plant on regeneration and potential of shoot explants. However, such effect on morphogenetic potential of root explants was not observed. It could be caused by a high metabolic activity and faster divisions of root cells due to the constant meristematic activity of the root apex (Hou et al. 2016). The root explants of *H. perforatum* are a very good material for shoot regeneration compared with *H. maculatum*. They develop abundant adventitious shoots in a relatively short time (Pawełczak 2010). The root explants respond to cytokinin–thidiazuron with intense organogenesis and shoot development

(Murch et al. 2000b; Zobayed and Saxena 2004). Within the framework of those studies, a number of St. John's wort micropropagation protocols have been developed. One of the techniques of St. John's wort micropropagation involves in vitro culture of adventitious roots in liquid medium in GrowTek vessels (Goel et al. 2009).

8.8.2 In Vitro Production of Secondary Metabolites

Secondary metabolites show a multitude of pharmacological activities and can be used to treat many diseases, and unfortunately, they are usually present in plant material in low amounts. St. John's wort is a species distinguished by a very rich chemical composition, and its structurally diverse metabolites have a wide spectrum of actions. In the case of *H. perforatum*, the biogenesis and accumulation of individual compounds can be enhanced by the appropriate choice of in vitro culture parameters. Plant biotechnology techniques can generate the material containing increased amounts of only one metabolite/group of metabolites, i.e., that which has a defined composition, and, consequently, a targeted action.

In vitro culture systems have many advantages over traditional field cultivation because they are free of contaminants and bacterial and fungal infections typical of plants. Species of the genus *Hypericum* has been a frequent focus of biotechnological studies but most in vitro systems were developed for *H. perforatum.* Due to a high therapeutic potential of hypericin and hyperforin, factors influencing the contents of these metabolites have been most widely studied (Danova 2014; Kirakosyan et al. 2008). Culture type and maintenance procedure are the basic factors having a fundamental impact on the obtained results. Apart from typical shoot cultures, cell suspensions, root cultures and transformed root cultures have also been used. They are carried out as static cultures on agar or liquid media, agitated or bioreactors cultures (Fig. 8.3). Even so simple procedure as the choice of type of culture can result in the rise in metabolite contents. It has been demonstrated that basic medium composition (Linsmaier & Skoog and Murashige & Skoog) and the concentrations of PGRs (6-benzylaminopurine and 1-naphthaleneacetic acid) affect substantially for growth and accumulation of phenolic acids and flavonoids in agitated cultures of St. John's wort three cultivars (Kwiecień et al. 2015, 2018).

In addition to testing the above-mentioned basic parameters of culture, a number of more advanced strategies have been examined. The procedures such as immobilization, selection of high-productive cell lines, precursor feeding, genetic transformation or elicitation have been applied for stimulation of in vitro production of metabolites. Immobilization is used in cell culture and is not applicable to organ culture; thus, its usefulness is limited. Supplementation of biosynthetic precursors, i.e., substrates for a particular stage of biogenetic pathway for a given group of metabolites is a good strategy but relatively cost-intensive. In St. John's wort cultures, the administration of amino acids and emodin brought a moderate success (Coste et al. 2021; Liu et al. 2007).

Fig. 8.3 *Hypericum perforatum* in vitro shoot cultures: agitated culture (left), culture in PlantForm temporary immersion bioreactor (right)

Selection of high-productive cell lines is another approach useful as well in micropropagation as in metabolite production in in vitro cultures. It involves initially the choice of a plant with the highest contents of the desired secondary metabolite (the so-called parent strain), and then, via primary callus cultures, secondary cultures are established from which cell cultures or other culture types are derived. They could be the source of metabolites or the material for plant regeneration (Danova 2014; Shakya et al. 2017).

The elicitation process of in vitro cultures is a broad research issue. Elicitors are stress factors inducing an amplified defense response in plants. Stress stimulates the enhanced synthesis of secondary metabolites. Stress factors encompass a very wide range of agents. Elicitors are classified into the following groups: biotic elicitors (of biological origin), abiotic elicitors (chemical and physical factors) and constitutive and endogenous elicitors (mediators of plant response to a pathogen). Biotic elicitors include bacterial (e.g., *Stenotrophomonas maltophilia, Agrobacterium* sp.) or fungal pathogens and mycorrhizal fungi (e.g., *Aspergillus niger*, *A. flavus*, *Botrytis cinerea, Colletotrichum gloeosporioides, Funneliformis* sp., *Fusarium oxysporum*, *Phoma exigua, Phytophthora* sp., *Rhizophagus intraradices*) and polysaccharides (chitosan, dextran, mannan, β-1,3-glucan, pectins, yeast extract). The group of abiotic elicitors is very abundant. It comprises chemical compounds, such as heavy metal ions, polyethylene glycol and nanoparticles of different compounds. Physical elicitations are defined as all changes in physical conditions of culture growth: light conditions, gamma and UV radiation, osmotic potential of the medium, changes in atmosphere composition (ozone, carbon dioxide), thermal shock and even intensive shaking. The third group comprises hormonal elicitors referred to also as signaling

molecules, which include jasmonic acid, methyl jasmonate, salicylic acid and nitric oxide (Namdeo 2007; Thakur et al. 2019).

Hypericum perforatum seedlings as well as shoot, root, callus and suspension cultures and transformed root cultures were treated with a number of elicitors to induce biosynthesis of secondary metabolites. It has been shown that the culture type has a much greater impact on determining which groups of compounds are induced than the kind of elicitor. Besides the type of culture, elicitation success depends on several other factors, including elicitor concentration, incubation conditions and elicitation duration. The elicited cultures were confirmed to contain the elevated contents of secondary metabolites. In particular, callus cultures produced more hypericin and pseudohypericin, root cultures showed greater amounts of hypericin and xanthones, shoot cultures accumulated higher contents of hypericin, pseudohypericin and hyperforin, while seedlings produced higher levels of protopseudohypericin, hypericin and pseudohypericin. Cell suspension model proved to contain the most comprehensive range of metabolites. These cultures after elicitation with different agents were demonstrated to contain large amounts of total phenols, flavonoids (flavonols, flavanols, flavones) anthocyanins, hypericin, pseudohypericin, hyperforin, xanthones and lignin. However, this type of culture is morphologically most remote from the plant model, while shoot and root cultures are the closest.

Another biotechnological method, namely genetic transformation, is slightly different albeit the aim assumed by researchers is similar, i.e., to increase production of metabolites. Although tools for genome modification are available, genetic improvement of *H. perforatum* by genetic engineering still remains a challenge because it is very complicated to acquire full knowledge of metabolic pathways at the protein and gene level. Genetically modified plants can be obtained by vectorless transfer or using vectors. Frequently used vectors include bacteria of the genus *Rhizobium*, *Agrobacterium rhizogenes* (plasmid pRi) and *Agrobacterium tumefaciens* (plasmid pTi), and this is the method that is the focus of research.

A. tumefaciens is useful in metabolic engineering and functional genomic studies in *H. perforatum* although resistance of plants to transformation via *A. tumefaciens* is a serious problem. Recently, this resistance has been attributed to the induction of defensive responses in plants (Hou et al. 2016).

The use of the second species -*Agrobacterium rhizogenes* leads to induction of 'hairy root' culture at the site of infection. 'Hairy roots' are characterized by quick growth, independent of PGRs, genetic stability and the absence of geotropism. Efficacy of 'hairy root' induction depends on *A. rhizogenes* strain used. On the other hand, the type of explant does not have a significant impact on transformation success (Hou et al. 2016).

'Hairy root' cultures of *H. perforatum* can synthesize larger amounts of secondary metabolites compared with non-transformed cultures and may be a promising tool for production of new metabolites (Tusevski et al. 2013a). They revealed biosynthetic potential for producing such metabolites as quinic acid, quercetin 6-C-glucoside, quercetin 3-O-rutinoside, isorhamnetin O-hexoside, kaempferol, catechin and epicatechin. In addition, 'hairy root' cultures synthesize and accumulate considerable amounts of xanthones. Therefore, they constitute a good experimental system for

studying the regulation of xanthone synthesis and also for their production (Tusevski et al. 2013b).

Agrobacterium strains exerts also an eliciting effect and was confirmed to increase hypericin levels in shoot cultures of *H. perforatum* (Hou et al. 2016). Likewise, suspension cell culture of *H. perforatum* subjected to combined treatment with *A. tumefaciens* and *A. rhizogenes* produced greater amounts of secondary metabolites (Tusevski et al. 2015). Some 'hairy root' cultures exhibit significant potential to regenerate whole transgenic plants (Vinterhalter et al. 2006).

Table 8.2 presents examples of increased production of secondary metabolites in various *Hypericum perforatum* in vitro cultures. The currently used strategies and obtained results have been described in detail in several newest review articles (Coste et al. 2021; Mir et al. 2019; Shakya et al. 2017). Biological activity of biomass cultured in vitro established on the basis of chemical composition of the extracts can be much higher compared to the plants grown in open air. In addition, the targeted action can be expected due to elevated contents of particular metabolites.

Table 8.2 Examples of secondary metabolites production enhancing in different type of *Hypericum perforatum* in vitro cultures

Metabolite	Type of culture	Supplementation/transformation	References
Hypericins	Cell suspension	Heat shock	Xu et al. (2008)
		Zn and Fe nano-oxide	Sharafi et al. (2013)
		Jasmonic acid	Walker et al. (2002)
		Salicylic acid	Gadzovska et al. (2013)
		Chitin; pectin	Gadzovska-Simic et al. (2015a)
		Fusarium oxysporum extract; *Phoma exigua* extract; *Botrytis cinerea* extract	Gadzovska-Simic et al. (2015b)
	Callus culture	Salicylic acid	Gadzovska et al. (2013)
	Agar shoot culture	Pectin	Gadzovska-Simic et al. (2014)
		Saccharose + methyl jasmonate	Pavlik et al. (2007)
		Photoperiod	Sood et al. (2015)
	Liquid shoot culture	L-Phenylalanine; emodin; methyl jasmonate	Liu et al. (2007)
	Agitated shoot culture	Mannan, β-glucan	Kirakosyan et al. (2000)
	Micropropagated shoots	Method and time of maintaining	Savio et al. (2012)

(continued)

Table 8.2 (continued)

Metabolite	Type of culture	Supplementation/transformation	References
	Bioreactor's shoot culture	Sucrose and CO_2	Zobayed et al. (2003)
	Seedlings	Cr ions	Tirillini et al. (2006)
	Transgenic plants	*A. tumefaciens* transformation	Khan et al. (2018)
	Adventitious roots in bioreactors	None	Cui et al. (2014)
Flavonoids	Cell suspension	Methyl jasmonate	Wang et al. (2015)
		Jasmonic acid	Gadzovska et al. (2007)
		Salicylic acid	Gadzovska et al. (2013)
		Chitin; pectin; dextran	Gadzovska-Simic et al. (2015a)
		Fusarium oxysporum extract; *Phoma exigua* extract; *Botrytis cinerea* extract	Gadzovska-Simic et al. (2015b)
	Callus culture	Salicylic acid	Gadzovska et al. (2013)
	Agitated shoot culture	Different amounts of PGRs	Kwiecień et al. (2018)
	Adventitious roots in bioreactors	None	Cui et al. (2014)
	'Hairy roots'	Transformation	Tusevski et al. (2019)
Anthocyanidins	Cell suspension	*Aspergillus flavus* extract	Gadzovska-Simic et al. (2012)
		Chitin; pectin; dextran	Gadzovska-Simic et al. (2015a)
		Fusarium oxysporum extract; *Phoma exigua* extract; *Botrytis cinerea* extract	Gadzovska-Simic et al. (2015b)
Phenolic acids and their derivatives	Cell suspension	Chitin; pectin; dextran	Gadzovska-Simic et al. (2015a)
		Fusarium oxysporum extract; *Phoma exigua* extract; *Botrytis cinerea* extract	Gadzovska-Simic et al. (2015b)
		Salicylic acid	Gadzovska et al. (2013)
	Callus culture	Salicylic acid	Gadzovska et al. (2013)

(continued)

Table 8.2 (continued)

Metabolite	Type of culture	Supplementation/transformation	References
	Agitated shoot culture	Different amounts of PGRs	Kwiecień et al. (2015)
	'Hairy roots'	Transformation	Tusevski et al. (2019)
Xanthones	Cell suspension	*Colletotrichum gloeosporioides* extract; Methyl jasmonate + *C. gloeosporioides*; Salicylic acid + *C. gloeosporioides*	Conceição et al. (2006)
		A. tumefaciens, A. rhizogenes	Tusevski et al. (2015)
	Callus culture	None	Tusevski et al. (2016)
	Root culture	Chitosan	Brasili et al. (2014)
		Acetic acid; chitosan	Valletta et al. (2016)
Hyperforin	Cell suspension	Zn and Fe nano-oxide	Sharafi et al. (2013)
	Agar shoot culture	Saccharose, Saccharose + methyl jasmonate; Saccharose + polyethylene glycol; Saccharose + *A. tumefaciens*	Pavlik et al. (2007)
	Liquid shoot culture	L-Tryptophane + methyl jasmonate; Cinnamic acid	Liu et al. (2007)
	Bioreactor's shoot culture	Sucrose and CO_2	Zobayed et al. (2003)
Adhyperforin	Agitated shoot culture	L-Threonine, L-Isoleucine	Karppinen et al. (2007)

Many biochemical and biotechnological investigations are carried out to explain secondary metabolite biogenetic pathways. These studies are very often conducted on in vitro cultures using advanced methods of genetic engineering. This is also the case with *Hypericum* metabolites. Due to the widespread occurrence of flavonoids, the biogenesis of this group is studied the most thoroughly. A number of inter-species differences in biosynthetic routes of this group of compounds have been documented, but the basic stages are well understood (Mir et al. 2019). Similar studies are in progress for other metabolites specific for St. John's wort, such as hypericin, xanthones and hyperforin (Adam et al. 2002; Bais et al. 2003; Nagia et al. 2019). Considering the therapeutic potential of these compounds as well as the demand for them, these investigations can enable the targeted stimulation of production of a given desired metabolite in in vitro cultures.

8.8.3 The Biotransformational Potential of Cells Cultured in Vitro

Enzymatic potential of *H. perforatum* cells in in vitro cultures enables their use in biotransformation processes of exogenous substrates. Optimization of conditions for hydroquinone biotransformation into its β-D-glucoside, arbutin, in agitated shoot cultures of *Hypericum perforatum* resulted in maximum content of this therapeutic and cosmetic product of 7.2% (dry weight) (Piekoszewska et al. 2010). This content is higher than the respective values required for standardization of known arbutin-containing plant raw materials according to the European Pharmacopoeia (7% in monograph of *Arctostaphylos uva-ursi and* and 4% in the Polish national monograph of *Vaccinium vitis-idea*).

8.9 Conclusion and Prospects

Hypericum perforatum is one of the most often used medicinal plants in Europe, including Poland. As demonstrated by professional studies, the herb of this species shows astringent, spasmolytic, cholagogic, metabolic stimulant, antidepressant, neuroprotective, antibacterial, antiviral, anti-inflammatory and anticancer actions. The increasing depletion of natural resources (locations) of this species caused by overexploitation to meet the demand from the pharmaceutical industry forces more extensive cultivation of this species.

St. John's wort is successfully cultivated in many Southern, Eastern and Central European countries. In Europe, the crop can be harvested twice per one vegetative period. Cultivation ensures a decidedly greater control over growth conditions for plants and a high quality of raw material compared with wild harvest.

Plant biotechnology methods are becoming increasingly important. The use of high-productive plant material obtained by micropropagation in in vitro cultures for crop production is gaining in popularity.

Studies on endogenous accumulation of secondary metabolites in various types of in vitro cultures (cell suspension, shoots, roots, bioreactors cultures) are carried out in parallel. They have proven a high production most of all of hypericins, flavonoids and xanthones under in vitro conditions. It can be expected that in the future, it will be possible to obtain valuable active ingredients of *Hypericum perforatum* in large amounts from high-productive biomass grown in bioreactors.

Much basic research in plant biotechnology aims to elucidate the biogenetic pathways of secondary metabolites of St. John's wort in in vitro cultures. Practical results of these studies (knowledge of biosynthetic precursors, isolation of enzymes participating in biogenesis, etc.) could also in the future contribute to the expected result, namely ample production of pharmacologically valuable compounds.

References

Adam P, Arigoni D, Bacher A et al (2002) Biosynthesis of hyperforin in *Hypericum perforatum*. J Med Chem 45:4786–4793

Agapouda A, Booker A, Kiss T et al (2019) Quality control of *Hypericum perforatum* L. analytical challenges and recent progress. J Pharm Pharmacol 71:15–37

Agostinis P, Vandenbogaerde A, Donella-Deana A et al (1995) Photosensitized inhibition of growth factor-regulated protein kinases by hypericin. Biochem Pharmacol 49:1615–1622

Alan AR, Murch SJ, Saxena PK (2015) Evaluation of ploidy variations in *Hypericum perforatum* L. (St. John's wort) germplasm from seeds, in vitro germplasm collection, and regenerants from floral cultures. Vitro Cel Dev Biol Plant 51:1–11

Albert D, Zündorf I, Dingermann T et al (2002) Hyperforin is a dual inhibitor of cyclooxygenase-1 and 5-lipoxygenase. Biochem Pharmacol 64:1767–1775

Apaydin EA, Maher AR, Shanman R et al (2016) A systematic review of St. John's wort for major depressive disorder. Syst Rev 5:148

Asgarpanah J (2012) Phytochemistry, pharmacology and medicinal properties of *Hypericum perforatum* L. Afr J Pharm Pharmacol 6:1387–1394

Bais HP, Vepachedu R, Lawrence CB et al (2003) Molecular and biochemical characterization of an enzyme responsible for the formation of hypericin in St. John's Wort (*Hypericum perforatum* L.). J Biol Chem 278:32413–32422

Barnes J, Anderson LA, Phillipson JD (2001) St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. J Pharm Pharmacol 53:583–600

Barnes J, Anderson LA, Phillipson D (eds) (2007) Herbal medicines. Pharmaceutical Press, London, Chicago

Bodeker G, Ryan TJ, Volk A et al (2017) Integrative skin care: dermatology and traditional and complementary medicine. J Altern Complement Med 23:479–486

Booker A, Agapouda A, Frommenwiler DA et al (2018) St John's wort (*Hypericum perforatum*) products—an assessment of their authenticity and quality. Phytomedicine 40:158–164

Bork PM, Bacher S, Schmitz ML et al (1999) Hypericin as a non-antioxidant inhibitor of NF-κB. Planta Med 65:297–300

Borrelli F, Izzo AA (2009) Herb–drug interactions with St John's wort (*Hypericum perforatum*): an update on clinical observations. AAPS J 11:710–727

Brasili E, Giulia Praticó G, Marini F et al (2014) A non-targeted metabolomics approach to evaluate the effects of biomass growth and chitosan elicitation on primary and secondary metabolism of *Hypericum perforatum* in vitro roots. Metabolomics 10:1186–1196

Brenn A, Grube M, Jedlitschky G et al (2014) St. John's wort reduces beta-amyloid accumulation in a double transgenic Alzheimer's disease mouse model—role of p-glycoprotein. Brain Pathol 24:18–24

Brondz I, Greibrokk T, Groth PA et al (1982) The relative stereochemistry of hyperforin-an antibiotic from *Hypericum perforatum* L. Tetrahedron Lett 23:1299–1300

Brunarska Z, Węgiel J, Wiatr E et al (1984) Możliwości ochrony zasobów dziurawca zwyczajnego *Hypericum perforatum* L. jako wartościowego surowca farmaceutycznego. Studia Nat A 25:51–66

Bruni R, Sacchetti G (2009) Factors affecting polyphenol biosynthesis in wild and filed grown St John's Wort (*Hypericum perforatum* L. Hypericaceae/Guttiferae). Molecules 14:682–725

Cabrelle A, Dell'Aica I, Melchiori L et al (2007) Hyperforin down-regulates effector function of activated T lymphocytes and shows efficacy against Th1-triggered CNS inflammatory-demyelinating disease. J Leukoc Biol 83:212–219

Cao Z, Wang F, Xiu C et al (2017) *Hypericum perforatum* extract attenuates behavioral, biochemical, and neurochemical abnormalities in aluminum chloride-induced Alzheimer's disease rats. Biomed Pharmacother 91:931–937

Cellarova E, Kimakova K, Brutovska R (1992) Multiple shoot formation and phenotypic changes of R0 regenerants in *Hypericum perforatum* L. Acta Biotechnol 12:445–452

Chauhan RS, Vashistha RK, Nautiyal MC et al (2011) Essential oil composition of *Hypericum perforatum* L. from cultivated source. J Essent Oil Res 23:20–25
Chrubasik-Hausmann S, Vlachojannis J, McLachlan AJ (2019) Understanding drug interactions with St John's wort (*Hypericum perforatum* L.): impact of hyperforin content. J Pharm Pharmacol 71:129–138
Chung PS, Rhee CK, Kim KH et al (2000) Intratumoral hypericin and KTP laser therapy for transplanted squamous cell carcinoma. Laryngoscope 110:1312–1316
Colasanti A, Kisslinger A, Liuzzi R et al (2000) Hypericin photosensitization of tumor and metastatic cell lines of human prostate. J Photochem Photobiol B 54:103–107
Conceição L, Ferrares F, Tavares R et al (2006) Induction of phenolic compounds in *Hypericum perforatum* L. cells by *Colletotrichum gloeosporioides* elicitation. Phytochem 67:149–155
Cosmetic Ingredient database (2021) https://ec.europa.eu/growth/sectors/cosmetics/cosing_en. Accessed on 10 Jan 2021
Coste A, Pop C, Halmagyi A et al (2021) Secondary metabolites in shoot cultures of *Hypericum*. In: Ramawat KG, Ekiert HM, Goyal S (eds) Plant cell and tissue differentiation and secondary metabolites. Reference series in phytochemistry. Springer, Cham, pp 273–307
Crockett SL, Robson NKB (2011) Taxonomy and chemotaxonomy of the genus *Hypericum*. Med Aromat Plant Sci Biotechnol 5:1–13
Crushes S, Zhou Y (2016) Invasive plant risk assessment: St John's wort (*Hypericum perforatum)*. Department of Agriculture and Fisheries, State of Queensland, Australia
Cui XH, Murthy HN, Paek KY (2014) Pilot-scale culture of *Hypericum perforatum* L. adventitious roots in airlift bioreactors for the production of bioactive compounds. Appl Biochem Biotechnol 174:784–792
Danova K (2014) Biotechnological utilization of the indigenous biosynthetic capacity of medicinal and aromatic plants. Experience in genera *Hypericum*, *Pulsatilla* and essential oil bearing *Artemisia alba* characteristic for the Balcan Region. In: Govil JN, Kumar PA (eds) Recent progress in medicinal plants, vol 39 Studium Press LLC, USA, pp 355–391
Dauncey EA, Irving JTW, Allkin R (2019) A review of issues of nomenclature and taxonomy of *Hypericum perforatum* L. and Kew's medicinal plant names services. J Pharm Pharmacol 71:4–14
De Witte P, Agostinis P, Van Lint J et al (1993) Inhibition of epidermal growth factor receptor tyrosine kinase activity by hypericin. Biochem Pharmacol 46:1929–1936
Drugs.com (2020) St. John's wort uses, side effects & warnings—Drugs.com. https://www.drugs.com/mtm/st-john-s-wort.html. Accessed on 20 Dec 2020
Dyrała A, Kaleta-Richter M, Cylupa K et al (2015) Zastosowanie hiperycyny w terapii fotodynamicznej—przegląd badań przedklinicznych. Acta Bio-Opt Inform Med 21:209–215
Ernst E, Rand JI, Barnes J et al (1998) Adverse effects profile of the herbal antidepressant St. John's wort (*Hypericum perforatum* L.). Eur J Clin Pharmacol 54:589–594
European Medicines Agency (2009) Community herbal monograph on *Hypericum perforatum* L. herba (traditional use). Committee on Herbal Medicinal Products, London
European Pharmacopoeia 10.0 (2020) European directorate for the quality of medicines, Strasburg
European Scientific Cooperative on Phytotherapy Monographs (2018) Hyperici Herba—St. John's Wort. ESCOP, Exeter
Feißt C, Pergola C, Rakonjac M et al (2009) Hyperforin is a novel type of 5-lipoxygenase inhibitor with high efficacy in vivo. Cell Mol Life Sci 66:2759–2771
Franco P, Potenza I, Moretto F et al (2014) *Hypericum perforatum* and neem oil for the management of acute skin toxicity in head and neck cancer patients undergoing radiation or chemo-radiation: a single-arm prospective observational study. Radiat Oncol 9:297
Franklin G, Dias ACP (2006) Organogenesis and embryogenesis in several *Hypericum perforatum* genotypes. Vitro Cell Dev Biol Plant 42:324–330
Franklin G, Oliveira M, Dias ACP (2007) Production of transgenic *Hypericum perforatum* plants via particle bombardment-mediated transformation of novel organogenic cell suspension cultures. Plant Sci 172:1193–1203

Gadzovska S, Maury S, Delaunay A et al (2007) Jasmonic acid elicitation of *Hypericum perforatum* L. cell suspensions and effects on the production of phenylpropanoids and naphtodianthrones. Plant Cell Tiss Org 89:1–13

Gadzovska S, Maury S, Delaunay A et al (2013) The influence of salicylic acid elicitation of shoots, callus, and cell suspension cultures on production of naphtodianthrones and phenylpropanoids in *Hypericum perforatum* L. Plant Cell Tiss Org 113:25–39

Gadzovska-Simic S, Tusevski O, Antevski S et al (2012) Secondary metabolite production in *Hypericum perforatum* L. cell suspensions upon elicitation with fungal mycelia from *Aspergillus flavus*. Arch Biol Sci 64:113–121

Gadzovska-Simic S, Tusevski O, Delaunay A et al (2014) Effects of polysaccharide elicitors on secondary metabolite production and antioxidant response in *Hypericum perforatum* L. shoot cultures. Sci World J. https://doi.org/10.1155/2014/609649

Gadzovska-Simic S, Tusevski O, Maury S et al (2015a) Polysaccharide elicitors enhance phenylpropanoid and naphtodianthrone production in cell suspension cultures of *Hypericum perforatum*. Plant Cell Tiss Org 122:649–663

Gadzovska-Simic S, Tusevski O, Maury S et al (2015b) Fungal elicitor mediated enhancement in phenylpropanoid and naphtodianthrone contents of *Hypericum perforatum* L. cell cultures. Plant Cell Tiss Org 122: 213–226

Galeotti N, Ghelardini C (2013) St. John's wort reversal of meningeal nociception: a natural therapeutic perspective for migraine pain. Phytomedicine 20:930–938

Germplasm Resources Information Network (2020) https://npgsweb.ars-grin.gov/gringlobal/search.aspx. Accessed on 05 Apr 2020

Global Biodiversity Information Facility (2020) https://www.gbif.org/species/3189486. Accessed on 10 Apr 2020

Goel M, Bisht NS, Kukreja AK (2009) In vitro manipulations in St. John's wort (*Hypericum perforatum* L.) for incessant and scale up micropropagation using adventitious roots in liquid medium and assessment of clonal fidelity using RAPD analysis. Plant Cell Tiss Org 96:1–9

Gomez del Rio MA, Sánchez-Reus MI, Iglesias I et al (2013) Neuroprotective properties of standardized extracts of *Hypericum perforatum* on rotenone model of Parkinson's disease. CNS Neurol Disord Drug Targets 12:665–679

Greeson JM, Sanford B, Monti DA (2001) St. John's wort (*Hypericum perforatum*): a review of the current pharmacological, toxicological, and clinical literature. Psychopharmacology 153:402–414

Gurley BJ, Swain A, Williams DK et al (2008) Gauging the clinical significance of P-glycoprotein-mediated herb-drug interactions: comparative effects of St. John's wort, *Echinacea*, clarithromycin, and rifampin on digoxin pharmacokinetics. Mol Nutr Food Res 52:772–779

Hammer KD, Hillwig ML, Solco AK et al (2007) Inhibition of prostaglandin E2 production by anti-inflammatory *Hypericum perforatum* extracts and constituents in RAW264 7 mouse macrophage cells. J Agric Food Chem 55:7323–7331

Heilmann J, Winkelmann K, Sticher O (2003) Studies on the antioxidative activity of phloroglucinol derivatives isolated from *Hypericum* species. Planta Med 69:202–206

Hoban CL, Byard RW, Musgrave IF (2015) A comparison of patterns of spontaneous adverse drug reaction reporting with St. John's Wort and fluoxetine during the period 2000–2013. Clin Exp Pharmacol Physiol 42:747–751

Hofrichte J, Krohn M, Schumacher T et al (2013) Reduced Alzheimer's disease pathology by St. John's Wort treatment is independent of hyperforin and facilitated by ABCC1 and microglia activation in mice. Curr Alzheimer Res 10:1057–1069

Hou W, Shakya P, Franklin G (2016) A perspective on *Hypericum perforatum* genetic transformation. Front Plant Sci 7:879. https://doi.org/10.3389/fpls.2016.00879

Hudson JB, Lopez-Bazzocchi I, Towers GHN (1991) Antiviral activities of hypericin. Antivir Res 15:101–112

Istikoglou CI, Mavreas V, Geroulanos G (2010) History and therapeutic properties of *Hypericum Perforatum* from antiquity until today. Psychiatriki 21:332–338

Jakovljevic V, Popovic M, Mimica-Dukic N et al (2000) Pharmacodynamic study of *Hypericum perforatum* L. Phytomedicine 7:449–453
Jürgenliemk C, Nahrstedt A (2002) Phenolic compounds from *Hypericum perforatum*. Planta Med 68:88–91
Kamuhabwa AR, Agostinis P, D'Hallewin MA et al (2000) Photodynamic activity of hypericin in human urinary bladder carcinoma cells. Anticancer Res 20:2579–2584
Kang BY, Chung SW, Kim TS (2001) Inhibition of interleukin-12 production in lipopolysaccharide-activated mouse macrophages by parthenolide, a predominant sesquiterpene lactone in *Tanacetum parthenium*: involvement of nuclear factor-κB. Immunol Lett 77:159–163
Karppinen K, Hokkanen J, Tolonen A (2007) Biosynthesis of hyperforin and adhyperforin from amino acid precursors in shoot cultures of *Hypericum perforatum*. Phytochemistry 68:1038–1045
Khan SA, Verma P, Akshata Arbat A et al (2018) Development of enhanced hypericin yielding transgenic plants and somaclones: high throughput direct organogenesis from leaf and callus explants of *Hypericum perforatum*. Ind Crops Prod 111:544–554
Khan T, Khan MA, Mashwani ZUR et al (2021) Therapeutic potential of medicinal plants against COVID-19: the role of antiviral medicinal metabolites. Biocatal Agric Biotechnol 31:101890. https://doi.org/10.1016/j.bcab.2020.101890
Kiesslich T, Krammer B, Plaetzer K (2006) Cellular mechanisms and prospective applications of hypericin in photodynamic therapy. Curr Med Chem 13:2189–2204
Kirakosyan A, Hayashi H, Inoue K et al (2000) Stimulation of the production of hypericins by mannan in *Hypericum perforatum* shoot cultures. Phytochemistry 53:345–348
Kirakosyan A, Gibson DM, Kaufman PB (2008) The production of dianthrones and phloroglucinol derivatives in St. John's wort. In: Ramawat KG, Mérillon JM (eds) Bioactive molecules and medicinal plants. Springer, Berlin, pp 149–164
Koeberle A, Rossi A, Bauer J et al (2011) Hyperforin, an anti-inflammatory constituent from St. John's wort, inhibits microsomal prostaglandin E2 synthase-1 and suppresses prostaglandin E2 formation in vivo. Front Pharmacol 2. https://doi.org/10.3389/fphar.2011.00007
Kołodziej B (ed) (2018) Poradnik dla plantatorów. PWRiL, Warszawa
Kwiecień I, Szydłowska A, Kawka B et al (2015) Accumulation of biologically active phenolic acids in agitated shoot cultures of three *Hypericum perforatum* cultivars: 'Elixir', 'Helos' and 'Topas.' Plant Cell Tiss Org 123:273–281
Kwiecień I, Smolin J, Beerhues L et al (2018) The impact of media composition on production of flavonoids in agitated shoot cultures of the three *Hypericum perforatum* L. cultivars 'Elixir', 'Helos', and 'Topas.' Vitro Cell Dev Biol-Plant 54:332–340
Lavie G, Valentine F, Levin B et al (1989) Studies of the mechanisms of action of the antiretroviral agents hypericin and pseudohypericin. Proc Natl Acad Sci 86:5963–5967
Liu CD, Kwan D, Saxton RE et al (2000) Hypericin and photodynamic therapy decreases human pancreatic cancer in vitro and in vivo. J Surg Res 93:137–143
Liu XN, Zhang XQ, Zhang SX et al (2007) Regulation of metabolite production by precursors and elicitors in liquid cultures of *Hypericum perforatum*. Plant Cell Tiss Org 91:1–7
Mannel M (2004) Drug interactions with St John's wort. Drug Saf 27:773–797
Marrelli M, Statti G, Conforti F (2020) *Hypericum* spp.: an update on the biological activities and metabolic profiles. Mini-Rev Med Chem 20:66–87
McCutcheon A (2017) On adulteration of St. John's wort (*Hypericum perforatum*). Bot Adulterans Bull. http://www.botanicaladulerants.com Accesed on 20 Jan 2020
Meruelo D, Lavie G, Lavie D (1988) Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: aromatic polycyclic diones hypericin and pseudohypericin. Proc Natl Acad Sci 85:5230–5234
Mir MY, Hamid S, Kamili AN et al (2019) Sneak peek of *Hypericum perforatum* L.: phytochemistry, phytochemical efficacy and biotechnological interventions. J Plant Biochem Biot 28:357–373
Mullaicharam AR, Halligudi N (2019) St John's wort (*Hypericum perforatum* L.): a review of its chemistry, pharmacology and clinical properties. Int J Res Phytochem Pharmacol 1:5–11

Murch SJ, Choffe KL, Victor JMR et al (2000a) Thidiazuron-induced plant regeneration from hypocotyl cultures of St. John's wort (*Hypericum perforatum*, cv 'Anthos'). Plant Cell Rep 19:576–581

Murch SJ, KrshnaiRaj S, Saxena PK et al (2000b) Tryptophan is a precursor for melatonin and serotonin biosynthesis in in vitro regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. Plant Cell Rep 19:698–704

Nagia M, Gaid M, Biedermann E et al (2019) Sequential regiospecific *gem*-diprenylation of tetrahydroxyxanthone by prenyltransferases from *Hypericum* sp. New Phytol 222:318–334

Nahrstedt A, Butterweck V (1997) Biologically active and other chemical constituents of the herb *Hypericum perforatum* L. Pharmacopsychiatry 30:129–134

Namdeo AG (2007) Plant cell elicitation for production of secondary metabolites: a review. Pharmacogn Rev 1:69–79

NCCIH (National Center for Complementary and Integrative Health) (2020) US National Institutes of Health https://www.nccih.nih.gov. Accessed on 12 Dec 2020

Nowack R (2008) Cytochrome P450 enzyme, and transport protein mediated herb–drug interactions in renal transplant patients: grapefruit juice, St John's Wort and beyond! Nephrology 13:337–347

Okmen G, Balpınar N (2017) The biological activities of *Hypericum perforatum* L. Afr J Tradit Complement Altern Med 14:213–218

Osińska E, Rosłon W (2016) Zioła. Uprawa i zastosowanie. Hortpress Sp. Z o.o, Warszawa

Palmer CD, Keller WA (2011) Plant regeneration from petal explants of *Hypericum perforatum* L. Plant Cell Tiss Org 105:129–134

Panossian AG, Gabrielian E, Manvelian V et al (1996) Immunosuppressive effects of hypericin on stimulated human leukocytes: inhibition of the arachidonic acid release, leukotriene B4 and interleukin-Iα production, and activation of nitric oxide formation. Phytomedicine 3:19–28

Parker V, Wong ABH, Boon HS et al (2001) Adverse reactions to St John's wort. Can J Psychiatry 46:77–79

Pasqua G, Avato P, Monacelli B et al (2003) Metabolites in cell suspension cultures, calli, and in vitro regenerated organs of *Hypericum perforatum* cv. Topas. Plant Sci 165:977–982

Patočka J (2003) The chemistry, pharmacology, and toxicology of the biologically active constituents of the herb *Hypericum perforatum* L. J Appl Biomed 1:55–59

Pavlik M, Vacek J, Klejdus B et al (2007) Hypericin and hyperforin production in St. John's wort in vitro culture: influence of saccharose, polyethylene glycol, methyl jasmonate, and *Agrobacterium tumefaciens*. J Agric Food Chem 55:6147–6153

Pawełczak A (2010) Plonowanie i jakość generatywnie i wegetatywnie rozmnażanych form dziurawca zwyczajnego i czterobocznego—*Hypericum perforatum* L. i *H. maculatum* Cr. PhD dissertation, SGGW, Warszawa

Phatak RS (2001) Materia medica leków homeopatycznych. Similimum, Nowy Sącz

Piekoszewska A, Ekiert H, Zubek S (2010) Arbutin production in *Ruta graveolens* L. and *Hypericum perforatum* L. in vitro cultures. Acta Physiol Plant 32:223–229

Pirbalouti GA, Fatahi-Vanani M, Craker L et al (2014) Chemical composition and bioactivity of essential oils of *Hypericum helianthemoides*, *Hypericum perforatum* and *Hypericum scabrum*. Pharm Biol 52:175–181

Ploss O, Petereit F, Nahrstedt A (2001) Procyanidins from the herb of *Hypericum perforatum*. Pharmazie 56:509–511

Poutaraud A, Girardin P (2005) Improvement of medicinal plant quality: a *Hypericum perforatum* literature review as an example. Plant Genet Resour 3:178–189

Poutaraud A, Di Gregorio F, Tin VC et al (2001a) Effect of light on hypericins contents in fresh flowering top parts and in an extract of St John's Wort (*Hypericum perforatum*). Planta Medica 67:254–259

Poutaraud A, Lobstein A, Girardin P et al (2001b) Improved procedure for the quality control of *Hypericum perforatum* L. Phytochem Anal 12:355–362

Pretto FR, Santarém ER (2000) Callus formation and plant regeneration from *Hypericum perforatum* leaves. Plant Cell Tissue Organ Cult 62:107–113

Quave CL (2018) Wound healing with botanicals: a review and future perspectives. Curr Dermatol Rep 7:287–295
Rahimi R, Abdollahi M (2012) An update on the ability of St. John's wort to affect the metabolism of other drugs. Expert Opin Drug Metab Toxicol 8:691–708
Rani V, Raina SN (2000) Genetic fidelity of organized meristem-derived micropropagated plants: a critical reappraisal. Vitro Cell Dev Biol Plant 36:319–330
Rizzo P, Altschmied L, Ravindran BM et al (2020) The biochemical and genetic basis for the biosynthesis of bioactive compounds in *Hypericum perforatum* L., one of the largest medicinal crops in Europe. Genes 11:1210. https://doi.org/10.3390/genes11101210
Robson NKB (2003) *Hypericum* botany. In: Ernst E (ed) *Hypericum*: the Genus *Hypericum*. Medicinal and Aromatic Plants-Industrial Profiles. Taylor and Francis, London, pp 1–22
Rumińska A (ed) (1991) Poradnik plantatora ziół. PWRiL, Poznań
Russo E, Scicchitano F, Whalley B et al (2013) *Hypericum perforatum*: pharmacokinetic, mechanism of action, tolerability, and clinical drug-drug interactions. Phytother Res 28:643–655
Savio LEB, Astarita LV, Santarém ER (2012) Secondary metabolism in micropropagated *Hypericum perforatum* L. grown in nonaerated liquid medium. Plant Cell Tiss Org 108:465–472
Schempp CM, Pelz K, Wittmer A et al (1999) Antibacterial activity of hyperforin from St John's wort, against multiresistant *Staphylococcus aureus* and gram-positive bacteria. Lancet 353:2129
Schempp C, Winghofer B, Lüdtke R et al (2000) Topical application of St John's wort (*Hypericum perforatum* L.) and of its metabolite hyperforin inhibits the allostimulatory capacity of epidermal cells. Br J Dermatol 142:979–984
Schempp CM, Kirkin V, Simon-Haarhaus B et al (2002a) Inhibition of tumour cell growth by hyperforin, a novel anticancer drug from St. John's wort that acts by induction of apoptosis. Oncogene 21:1242–1250
Schempp C, Simon-Haarhaus B, Simon J (2002b) Phototoxic and Apoptosis-Inducing Capacity of Pseudohypericin. Planta Med 68:171–173
Schempp CM, Winghofer B, Müller K et al (2003) Effect of oral administration of *Hypericum perforatum* extract (St. John's wort) on skin erythema and pigmentation induced by UVB, UVA, visible light and solar simulated radiation. Phytother Res 17:141–146
Scotti F, Löbel K, Booker A et al (2019) St. John's wort (*Hypericum perforatum*) products—how variable is the primary material? Front Plant Sci 9:1973
Seidler-Lozykowska K, Dąbrowska J (1996) Topaz—polska odmiana dziurawca zwyczajnego (*Hypericum perforatum* L.). Herba Pol 43:143–143
Šemeláková M, Jendželovský R, Fedoročko P (2016) Drug membrane transporters and CYP3A4 are affected by hypericin, hyperforin or aristoforin in colon adenocarcinoma cells. Biomed Pharmacother 81:38–47
Senderski ME (2004) Prawie wszystko o ziołach. MOMAG S.A, Podkowa Leśna
Shakya P, Marslin G, Siram K (2017) Elicitation as a tool to improve the profiles of high-value secondary metabolites and pharmacological properties of *Hypericum perforatum*. J Pharm Pharmacol 71:70–82
Sharafi E, Nekoei SM, Fotokian MH et al (2013) Improvement of hypericin and hyperforin production using zinc and iron nano-oxides as elicitors in cell suspension culture of St John's wort (*Hypericum perforatum* L.). J Med Plants by-Prod 2:177–184
Shipochliev T (1981) Extracts from a group of medicinal plants enhancing uterine tonus. Vet Med Nauki 18:94–98
Sood H, Shitiz K, Sharma N (2015) Rapid method for in vitro multiplication of hypericin rich shoots of *Hypericum perforatum*. J Plant Sci 3:279–284
Sosa S, Pace R, Bornanciny A, Morazzoni P et al (2007) Topical anti-inflammatory activity of extracts and compounds from *Hypericum perforatum* L. J Pharm Pharmacol 59:703–709
Sytar O, Švedienė J, Ložienė K et al (2016) Antifungal properties of hypericin, hypericin tetrasulphonic acid and fagopyrin on pathogenic fungi and spoilage yeasts. Pharm Biol 54:3121–3125

Szafer W, Kulczyński S, Pawłowski B (1988) Rośliny polskie: opisy i klucze do oznaczania wszystkich gatunków roślin naczyniowych rosnących w Polsce bądź dziko, bądź też zdziczałych lub częściej hodowanych. PWN Warszawa

Takahashi I, Nakanishi S, Kobayashi E et al (1989) Hypericin and pseudohypericin specifically inhibit protein kinase C: possible relation to their antiretroviral activity. Biochem Biophys Res Commun 165:1207–1212

Teufel-Mayer R, Gleitz J (1997) Effects of long-term administration of *Hypericum* extracts on the affinity and density of the central serotonergic 5-HT1 A and 5-HT2 A receptors. Pharmacopsychiatry 30:113–116

Thakur M, Sujata Bhattacharya S, Khosla PK et al (2019) Improving production of plant secondary metabolites through biotic and abiotic elicitation. J Appl Res Med Aroma 12:1–12. https://doi.org/10.1016/j.jarmap.2018.11.004

Tirillini B, Ricci A, Pintore G et al (2006) Induction of hypericins in *Hypericum perforatum* in response to chromium. Fitoterapia 77:164–170

Turek S (2005) Ziele dziurawca zwyczajnego—składniki czynne i potencjalne zastosowanie lecznicze. Postępy Fitoter 6:80–86

Tusevski O, Stanoeva JP, Stefova M et al (2013a) Phenolic profile of dark-grown and photoperiod-exposed *Hypericum perforatum* L. hairy root cultures. Sci World J. https://doi.org/10.1155/2013/602752

Tusevski O, Stanoeva JP, Stefova M et al (2013b) Hairy roots of *Hypericum perforatum* L.: a promising system for xanthone production. Cent Eur J Biol 8:1010–1022

Tusevski O, Stanoeva JP, Stefova M et al (2015) *Agrobacterium* enhances xanthone production in *Hypericum perforatum* cell suspensions. Plant Growth Regul 76:199–210

Tusevski O, Stanoeva JP, Markoska E et al (2016) Callus cultures of *Hypericum perforatum* L. a novel and efficient source for xanthone production. Plant Cell Tiss Org 125:309–319

Tusevski O, Petreska-Stanoeva J, Stefova M et al (2019) State of antioxidant systems and phenolic compounds' production in *Hypericum perforatum* L. hairy roots. Acta Physiol Plant 41:132. https://doi.org/10.1007/s11738-019-2919-5

U.S. National Library of Medicine (2020) Ranolazine: medlineplus drug information. https://medlineplus.gov/druginfo/meds/a606015.html. Accessed on 20 Dec 2020

United States Pharmacopeial Convention (2015) St. John's wort, St. John's wort powder, and St. John's wort powdered extract. In: United States Pharmacopeia 38 and National Formulary 33. United States Pharmacopeial Convention, Rockville, MD, USA

Upton R, Graff A, Swisher D (1997) American herbal pharmacopoeia and therapeutic compendium: St. John's wort. *Hypericum perforatum* L. quality control, analytical, and therapeutic monograph. American Herbal Pharmacopoeia, Santa Cruz, CA, USA

Uzbay TI (2008) *Hypericum perforatum* and substance dependence: a review. Phytother Res 22:578–582

Valletta A, De Angelis G, Badiali C et al (2016) Acetic acid acts as an elicitor exerting a chitosan-like effect on xanthone biosynthesis in *Hypericum perforatum* L. root cultures. Plant Cell Rep 35:1009–1020

Vecchia DD, Schamne MG, Ferro MM et al (2015) Effects of *Hypericum perforatum* on turning behavior in an animal model of Parkinson's disease. Braz J Pharm Sci 51:111–115

Velingkar VS, Gupta GL, Hegde NB (2017) A current update on phytochemistry, pharmacology and herb-drug interactions of *Hypericum perforatum.* Phytochem Rev 16:725–744

Vilà M, Gómez A, Maron JL (2003) Are alien plants more competitive than their native conspecifics? A test using Hypericum perforatum L. Oecologia 137:211–215

Vinterhalter B, Ninkovic S, Cingel A (2006) Shoot and root culture of *Hypericum perforatum* L. transformed with *Agrobacterium rhizogenes* A4M70GUS. Biol Plant 50:767–770

Walker TS, Bais HP, Vivanco JM (2002) Jasmonic acid-induced hypericin production in cell suspension cultures of *Hypericum perforatum* L. (St. John's wort). Phytochemistry 60:289–293

Wang J, Qian J, Yao L et al (2015) Enhanced production of flavonoids by methyl jasmonate elicitation in cell suspension culture of *Hypericum perforatum*. Bioresour Bioprocess 2:5. https://doi.org/10.1186/s40643-014-0033-5

Weber ND, Murray BK, North JA et al (1994) The antiviral agent hypericin has in vitro activity against HSV-1 through non-specific association with viral and cellular membranes. Antivir Chem Chemother 5:83–90

Wenk M, Todesco L, Krähenbühl S (2004) Effect of St John's wort on the activities of CYP1A2, CYP3A4, CYP2D6, N-acetyltransferase 2, and xanthine oxidase in healthy males and females. Br J Clin Pharmacol 57:495–499

WHO (2004) Who monographs on selected medicinal plants, vol 2, Herba Hyperici. World Health Organization, Geneva

Wójcik A, Podstolski A (2007) Leaf explant response in in vitro culture of St. John's wort (*Hypericum perforatum* L.). Acta Physiol Plant 29:151–156

Wölfle U, Seelinger G, Schempp CM (2014) Topical application of St. John's wort (*Hypericum perforatum*). Planta Med 80:109–120

Wood S, Huffman J, Weber N et al (1990) Antiviral activity of naturally occurring anthraquinones and anthraquinone derivatives. Planta Med 56:651–652

Wright CW, Gott M, Grayson B, Smith AG et al (2003) Correlation of hyperforin content of *Hypericum perforatum* (St John's wort) extracts with their effects on alcohol drinking in C57Bl/6J mice: a preliminary study. J Psychopharmacol 17:403–408

Xu M, Dong J, Zhang X (2008) Signal interaction between nitric oxide and hydrogen peroxide in heat shock-induced hypericin production of *Hypericum perforatum* suspension cells. Sci China Ser C 51:676–686

Zimowska B, Machowicz-Stefaniak Z (2004) Grzyby zagrażające uprawie Dziurawca zwyczajnego (*Hypericum perforatum* L.) w województwie lubelskim. Acta Sci Pol Hortorum Cultus 3:61–74

Zirak N, Shafiee M, Soltani G et al (2019) *Hypericum perforatum* in the treatment of psychiatric and neurodegenerative disorders: current evidence and potential mechanisms of action. J Cell Physiol 234:8496–8508

Zobayed SMA, Saxena PK (2003) In vitro grown roots: a superior explant for prolific shoot regeneration of St. John's wort (*Hypericum perforatum* L. cv 'New Stem') in a temporary immersion bioreactor. Plant Sci 165:463–470

Zobayed S, Saxena PK (2004) Production of St. John's wort plants under controlled environment for maximizing biomass and secondary metabolites. Vitro Cel Dev Biol Plant 40:108–114

Zobayed SMA, Murch SJ, Rupasinghe HPV (2003) Elevated carbon supply altered hypericin and hyperforin contents of St. John's wort (*Hypericum perforatum*) grown in bioreactors. Plant Cell Tiss Org 75:143–149

Chapter 9
Mango Ginger: Prospects for Domestication and Utilization

Ajit Arun Waman, Kalyan P. Kadbhane, and Gourish R. Karanjalker

Abstract Zingiberaceae is a botanical family to which a number of medicinally important taxa belong. Mango ginger is a term used to describe two species, viz. *Curcuma amada* and *C. manga*, of this family. These species are characterized by the presence of raw mango like aroma in their rhizomes and hence the name. Both these species have been traditionally employed in the tropical regions for food, flavoring and medicinal purposes. In recent past, a number of pharmaceutical and clinical studies have been taken up which have opened up avenues for their utilization. The present chapter is a systematic compilation about botanical description, distribution, phytochemistry, medicinal properties, importance and applications in various industries, agro-techniques, biotechnological approaches, etc., in these species. This review will serve as a guide for various stakeholders including researchers, processors, cultivators and others.

Keywords *Curcuma amada* · *Curcuma mangga* · Indian system of medicines · Spice · Tropical plant

9.1 Introduction

Zingiberaceae is a family of rhizomatous plants, members of which are known for their diversified applications in food and pharmaceutical sectors. Turmeric (*Curcuma longa* L.) is the most important species of this family. Owing to high medicinal properties, it is being cultivated on commercial scale in various parts of the world (Syamkumar and Sasikumar 2007). The genus *Curcuma* is represented by ca. 100

A. A. Waman (✉)
Division of Horticulture and Forestry, ICAR—Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands 744105, India

K. P. Kadbhane
AYUSH Hospital, Directorate of Health Services, Port Blair, Andaman and Nicobar Islands 744103, India

G. R. Karanjalker
Don Bosco College of Agriculture, Quepem, Goa 403705, India

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_9

known species, of which ca. 41 are distributed in India, and ten are considered as endemic to the Indian subcontinent (Nair 2013). However, considering taxonomic complexities in the genus (Skornickova et al. 2010), number of species in India could be reduced to 30 (Nair 2013). Of the *Curcuma* species, a number of lesser-known and neglected species are being valued locally in the regions of their natural distribution (Waman et al. 2018).

'Mango ginger' is a term used to describe two species *viz. Curcuma amada* Roxb. and *C. mangga* Val. et Zijp., which are distributed in tropical parts of the world. The term implies to the fact that rhizomes of these species bear morphological resemblance with ginger (*Zingiber officinale*), while they smell like unripe mango (*Mangifera indica* L.) when cut open. Both the species have been utilized in regions of their natural distribution for variety of purposes, and numerous studies have been taken up in the recent past pertaining to their pharmacological properties, phytochemical constituents, essential oil composition, etc. (Policegoudra et al. 2011; Liu and Nair 2012; Srirod and Tewtrakul 2019), while limited studies are available on their cultivation and value addition aspects (Ayodele et al. 2018; Priyanka and Bhoomika 2018; Waman et al. 2018). Considering their importance and potential as raw materials for various industries, the present report provides an overview of these two species.

Curcuma amada is believed to be originated in India and is widely distributed here, mainly in the plains (Velayudhan et al. 1999). The species name '*amada'* has been originated from the Bengali word, meaning 'mango-like flavour'. It is herbaceous perennial growing up to 90 cm in height with broad leaves. It produces stout aromatic rhizomes, and at the end of crop cycle, foliage dries back and rhizomes undergo dormancy during winters. Inflorescence sprouts from the rhizome base and peduncle can grow up to 20–25 cm (Nair 2013). Karyotyping suggested chromosome numbers of $2n = 40$ for *C. amada* (Das et al. 1999), while other report suggests it to be 42 (Mohanty et al. 2014a).

C. mangga is known to produce flowers laterally and produce rhizomes of bold type (Ravindran et al. 2007). It is native to Java and was first reported in India from the Andaman Islands, and since then, it was often mistaken as *C. amada* owing to the similarity of raw mango flavour (Skornickova et al. 2010). It has been used in the Andaman Islands as well as other South East Asian countries for variety of purposes (Neamsuvan et al. 2012; Silalahi et al. 2015). Plants grow up to 90–120 cm in height with about 60 cm long and 14 cm wide narrowly ovate or elliptic leaves and green to reddish midrib (Aminah 2008). Coma bracts in the species are pink/white and a pink blotch is present at the centre (Sirirugosa et al. 2007). Chromosome number has been reported to be $2n = 42$ and 63 (Skornickova et al. 2007).

9.2 Geographic Distribution

After turmeric, *C. amada* is the second most commonly grown species of the genus (Syamkumar and Sasikumar 2007). It is a species native to Eastern India (Singh 2017)

and is distributed throughout the country (Nair 2013) with small and scattered cultivation pockets in states of Odisha, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh, etc.; however, owing to limited and scattered cultivation pattern, systematic data on area, production and productivity are not available (Priyanka and Bhoomika 2018). Apart from India, it is also grown in Thailand, Malaysia and Myanmar (Sirirugosa et al. 2007; Jatoi et al. 2010; Priyanka and Bhoomika 2018).

The species has vernacular names in almost all the major languages of India, which suggests that the species is commonly used in these regions for one or the other purposes. Different vernacular names are as followed: Sanskrit: *Amragandhi Haridra*, *Darvidbheda*, *Asragandha, Surabhidaru*, *Daru, Karpura, Padmapatra, Surimat, Suratarka*; Hindi: *Aam Haldi*; Marathi: *Ambe halad*; Gujarati: *Amba Haldar*; Bengali: *Aam Ada*; Farsi: *Darchobah*; Tamil: *Mankayinchi*; Telugu: *Mamidi allam*; Malayalam: *Mangainchi*; Kannada: *Huli Arasin* (Sharma 2009; Pande 2010; Samant 2012; Gogte 2017).

C. mangga has been considered to be native of Java, while it is naturally distributed in tropical countries such as Thailand, Malaysia, Indonesia and Andaman and Nicobar Islands of India (Sirirugosa et al. 2007; Skornickova et al. 2010; Singh et al. 2016; Singh 2017). It is locally known as '*aam haldi*' or '*aam adrak*' in the Andaman Islands, while it is called '*temu mangga*' in Indonesia, '*temu pauh'* in Malaysia and '*khamin khao'* in Thailand (Hong et al. 2016).

9.3 Brief Phytochemistry

Both mango ginger species are known to have medicinal properties, and hence, research work on isolation and identification of various phytochemicals from leaves and rhizomes has been carried out (Wong et al. 1999; Policegoudra et al. 2007a, b; Wahab et al. 2011; Sajitha and Sasikumar 2015). The literature survey on various species of *Curcuma* (Sun et al. 2016) suggested that *C. amada* ranks third among the *Curcuma* species in terms of number of reported compounds, while *C. mangga* ranks 13th in the same category. Sun et al. (2016) have elaborated diversity of phytochemical constituents present in different *Curcuma* species. They have emphasized that various species of the genus, including mango ginger, have great potential for utilization in different sectors due to their richness in various compounds. Diphenylalkanoids, phenylpropene derivatives, monoterpenes, sesquiterpenes, diterpeness, sesterterpenoids, triterpenoids, alkaloids, flavonoids, steroids, etc., are the most important classes of compounds obtained from these species. Important compounds reported in mango ginger are presented in Table 9.1.

Interestingly, the composition of volatiles from different types of rhizomes viz. mother rhizomes, primary rhizomes and secondary rhizomes showed some similarities as well as differences (Waman et al. 2018). They have also reported variations in the essential oil content, curcumin content and total phenolic content among these tissues. Some compounds were found to be present only in specific plant parts. This suggests that for better recovery of targeted compounds, specific rhizomes could

Table 9.1 Phytochemical constituents reported from mango ginger

Species	Group of compounds	Particulars	References
C. amada	Curcuminoids	Curcumin (0.21%), demethoxy curcumin and bis-demethoxy curcumin	Gupta et al. (1999), Kharade et al. (2017)
	Phenolic compounds	Caffeic acid (195 mg/g), gentisic acid (180 mg/g), ferulic acid (150 mg/g), gallic acid (75 mg/g), cinnamic acid (52.5 mg/g), protocatechuic acid (52.5 mg/g), syringic acid (30 mg/g) and p-coumaric acid (15 mg/g)	Siddaraju and Dharmesh (2007)
	Volatile constituents	Car-3-ene, cis-ocimene, cis- hydro ocimene, ocimene, trans hydro ocimene, myrcene, turmerone	Rao et al. (1989); Policegoudra et al. (2011)
	Other compounds	Difurocumenonol (sesquiterpene dimer); amadannulen (substituted sesquiterpene); amadaldehyde; (E)-Labda-8(17),12-diene-15,16-dial (1.7%); (E)-Labda-8(17),13-diene-15,16-olide; Coronarin B; Coronarin D; Zerumin A and Zerumin B (Labdane type diterpenes); starch (45–48.5% with 43% amylose); β-caryophyllene epoxide	Policegoudra et al. (2007a, b, 2010, 2011); Singh et al. (2010); Sajitha and Sasikumar (2015); Sheeja and Nair (2012); Santhoshkumar and Yusuf (2019)
C. mangga	Curcuminoids	Mother rhizome (0.35%), primary rhizome (0.34%) and secondary rhizome (0.45%)	Waman et al. (2018)
	Volatile constituents	β-Myrcene (46.5–79.0%) varies with rhizome type, i.e. 52.4% (mother rhizome), 58.5% (primary rhizome) and 65.3% (secondary rhizome) Cyclofenchene (6.4–10.7%), 3-Bornanol (2.0–5.1%); trans-Ocimene (2.5–3.6%); α-Pinene (1.4–2.4%); α-Himachalene (1.0–3.2%), Androstane-3,16-diol (0.7–1.8%); β-Pinene (0.7–2.4%)	Waman et al. (2018); Wahab et al. (2011), Wong et al. (1999)
	Other compounds	Alkaloids, flavonoids, tannins	Rachkeeree et al. (2020)

be utilized rather than bulking up the produce. Efficacies of different solvents in extracting these compounds also vary (Awin et al. 2016), and detailed studies could help in fine-tuning the extraction protocols for scale up.

Curcumin is a valuable resource derived from a number of *Curcuma* species, the major source being turmeric (*C. longa*). Curcumin is referred as 'Indian Solid Gold'. It is known to have manifold uses in food (as natural colourant/preservative) and pharmaceutical industries owing to its antioxidant, wound healing, anticancer, antiviral, anti-allergic, cardio-protectant and anti-fungal properties (Aggarwal et al.

2007). Rhizomes of both *C. amada* and *C. mangga* have been reported to contain curcumin (Gupta et al. 1999; Kharade et al. 2017; Waman et al. 2018), and hence, both these species could be valuable for these industries.

There have been confusions in the correct identification of *C. amada* and *C. mangga* owing to similarities in their morphology and aroma (Skornickova et al. 2010). Hence, use of biochemical compounds as markers could help in ascertaining the identity of such species. Labdane-type diterpenes viz. (E)-Labda-8(17),12-diene-15,16-dial (1.7%), Coronarin B, Coronarin D and Zerumin A have been identified as chemotaxonomic markers for identification of *C. amada* (Sheeja and Nair 2012). Similarly, essential oils obtained from rhizomes of *C. mangga* are known to contain β-Myrcene as dominant compound, and it has been considered as infra-generic chemotaxonomical marker for identification of this species (Wahab et al. 2011).

9.4 Medicinal Properties and Usage

9.4.1 Utilization in Traditional and Folk Medicines

C. amada, being more common in India, has widely been employed in the Indian System of Medicines, especially in Ayurveda rather than *C. mangga*. In Ayurveda, juice of fresh rhizomes (10–20 ml) and powder of dried rhizomes (1–4 g) are reported to be used internally for various indications (Sharma 2009; Pande 2010). Rhizomes are reported to be sweet (*Madhur*) and bitter (*Tikta*) in taste (*Rasa*); cool (*Sheeta*) in potency (*Veerya*) and pungent (*Katu*) in end result (*Vipaka*). They are pleasantly fragrant (*Sugandhi*); pacify *pitta,* aggravate *vata,* improve appetite (*Deepan*), help in digestion (*Paachan*), correct bowel motility (*Graahi*), facilitate anti-carminative action (*Vatanulomak)* and are especially of importance in skin disorders and allergies (*Sarvakanduvinashini*) (Sharma 2009; Pandey 2010; Gogte 2017).

Ayurvedic classics report that *C. amada* possesses therapeutic properties similar to the well-known *Haridra* (*C. longa*). Apart from curing skin disorders (*Kushthaghna*) and anaemia (*Pandughna*), the species is known to have anti-melanin (*Varnya*), anti-diabetic (*Pramehahara*), wound healing (*Vranaropan*), analgesic (*Vedanasthapan*), galactogogue (*Stanyashodhan*), uterus cleansing (*Garbhashayshodhan*), semen quality improving (*Shukrashodhan*), anti-pyretic (*Jwaraghna*) and hepato-protective (*Kamalahar*) properties (Sharma 2009; Pande 2010; Samant 2012; Gogte 2017).

In '*Dhanwantary Nighantu*', it has been reported to be useful in poisoning (*Vishaghna*), curing skin disorders (*Kushthaghna*), treating pruritus and skin allergy (*Kandughna*), detoxifying and cleansing body (*Vishodhani*), anthelminthic (*Kramihar*), curing sinusitis (*Peenasa*) and treating tastelessness of mouth (*Aruchi*). According to other Ayurvedic classic '*Kaiyadev Nighantu*' (Sharma 2009; Pande 2010; Gogte 2017), the species has been reported to be useful in treatment of hiccup (*Hikka*), cough, dyspnea, asthma, bronchitis and pneumonia (*Shvasa* and *Kasa*).

As external application, *C. amada* has been reported as anti-inflammatory and analgesic and is useful for cleaning and healing of open wounds, external piles, conjunctivitis (as paste) and eye drop (as filtered decoction) (Pande 2010; Gogte 2017). Fumigation with powder is traditionally reported for unconsciousness (*murcha*), hiccups, respiratory distress and scorpion bite (Pande 2010). Therapeutic uses have also been reported in Unani system of medicine (Samant 2012). Tribal inhabitants of Odisha commonly employ paste of rhizomes for treating piles (Panda 2014), while tribal from Western India is known to use powder for treating snakebite (Swarnkar and Katewa 2008).

Rhizomes of *C. mangga* have been used as a component of traditional Thai formulations which are commonly employed to treat dyspepsia and gastritis (Neamsuvan et al. 2012). In North Sumatra, rhizomes are used in the *Oukup* which is a type of sauna in which aromatic spices are used (Silalahi et al. 2015). Thai Yuan ethnic group of Thailand use the rhizomes for treatment of flatulence (Panyadee et al. 2019), whereas in folk medicines of Thailand, *C. mangga* rhizomes are components of formulations used for treatment of gastritis and dyspepsia (Neamsuvan et al. 2012). In Malaysia, rhizomes are used for treating allergies (Harun et al. 2015).

9.4.2 Utilization in Modern Medicines

A large number of studies have been conducted on both the species to know their potential medicinal properties. Various in vitro models/animal and microbial models have been used for these purposes. As multiple bioactive chemicals have been reported from these species, various solvent systems have been employed for improved recovery of desirable bioactive molecules from the plant parts. Details of such studies in *C. amada* and *C. mangga* have been presented in Tables 9.2 and 9.3, respectively. In general, these studies indicate that both mango ginger species possess valuable clinically tested properties and are candidates for commercial-scale cultivation.

9.4.3 Utilization in the Food, Aroma and Other Industries

Mango ginger species have been a part of traditional cuisines in their native regions. Due to their raw mango like aroma, rhizomes have been deliberately incorporated to impart exotic flavour to the dishes. Rhizomes of *C. amada* are commonly used in culinary preparations in India and other regions of Asia in the form of pickles, sauce and scented sweets (Samant 2012; Gogte 2017). Products such as *Kondaikadalai pachadi*, mango ginger gravy, grilled pan chicken with mango ginger salsa, gingerbread cupcakes with mango ginger icing, hot grilled shrimps with mango ginger sauce, couscous cake with fresh mango ginger chutney, grilled Thai chicken salad

Table 9.2 Established pharmacological activities of *C. amada*

Plant part/extract used	Type of experiment and animal/tissue used (clinical/animal/in vitro/cell line, etc.)	Dose/conc./route/duration of administration	Activity observed	References
Hexane and chloroform extracts of rhizomes	In vitro	IC_{50}—92 to 158 μg	Anti-oxidant	Policegoudra et al. (2010)
Ethanolic extract of rhizomes	Male albino vistar rats (with CCl4 induced hepatotoxity)	200 mg/kg BW, twice a week for 28 d	Hepato-protective with no toxicity in normal control	Varadrajan et al. (2018)
Methanolic extract of rhizomes	In vitro i. Respiratory burst of whole blood of healthy human subjects ii. Isolated human polymorphonuclear leaucocytes (PMN) iii. Isolated mice peritoneal macrophages	IC_{50}—0.9 to 1.5 μg/ml	Immuno-modulatory (strong inhibition of oxidative burst of PMN; chemiluminescence and chemotactic activity of phagocytes)	Jantan et al. (2011)
Acetone extract of rhizomes	Rats	100 and 300 mg/kg for 21 days	Augmented the memory by inhibiting acetylcholinesterase activity Controlled the level of dopamine and serotonin; and reduced oxidative stress and neuro-degeneration	Nissankara Rao et al. (2019)
Acetone extract of rhizomes	Rats	100 and 300 mg/kg for 21 days	Anti-obesity	Nissankara Rao et al. (2019)

(continued)

Table 9.2 (continued)

Plant part/extract used	Type of experiment and animal/tissue used (clinical/animal/in vitro/cell line, etc.)	Dose/conc./route/duration of administration	Activity observed	References
Crude extracts of rhizomes, diterpenes and its derivatives	Rat intestinal α-glucosidase and porcine pancreatic lipase	–	Anti-obesity (inhibition of α—Glucosidase and pancreatic Lipase)	Yoshioka et al. (2019)
Rhizome extract (10%) and isolated curcumin	Female albino rats (Normal and hyperlipidemic)	4 weeks orally administered	Hypo-triglyceridemic	Srinivasan and Chandrashekharan (1992)
Amadaldehyde isolate of hexane and chloroform extracts of rhizomes	In vitro (platelet-rich plasma of healthy human volunteers)	IC_{50}—113 μg	Platelet aggregation inhibitory/anti-thrombotic	Policegoudra et al. (2010)
Fraction of hydro-methanolic extract from rhizomes	Streptozotocin-induced diabetic male albino rats	10 mg/100 g BW/day for 4 w	Anti-diabetic and antioxidant	Mitra et al. (2019)
Methanolic extract of rhizomes	Mice (normal and diabetic)	Up to 650 mg/kg BW	Hypoglycemic and anti-hyperglycemic	Syiem et al (2010)
Rhizome extract	Placebo-controlled clinical trial on Human subjects	15 d orally administered	Anti-allergic (in allergic rhinitis)	Bhaskaran et al. (2012)
Ethanolic and aqueous extracts of rhizomes	Rats	250–500 mg/Kg BW	Dose-dependent anti-pyretic activity	Kaur et al. (2011)
Rhizome extract	Normal and arthritic (Collagen-induced RA) female vistar albino rats	Orally administered	Anti-arthritic	Karata et al. (2020)
Hydro-methanolic extract of rhizomes	Albino rats (with diabetes-induced testicular dysfunction)	20–80 mg/100 g BW/day for 28 d	Improvement in reproductive parameters	Sarkar et al (2019)

(continued)

Table 9.2 (continued)

Plant part/extract used	Type of experiment and animal/tissue used (clinical/animal/in vitro/cell line, etc.)	Dose/conc./route/duration of administration	Activity observed	References
Hydro-alcoholic extract of rhizomes	Normal and infertile rats (cell phone radiation induced)	100–300 mg kg/BW	Reversal of radiation-induced infertility (dose-dependent improvement in sperm count, sperm motility and testosterone levels)	Siddappa et al. (2015)
Chloroform extract and isolate-amadaldehyde of rhizomes	In vitro culture	MBC—100 to 180 mg/L	Bactericidal against Gram +ve and Gram −ve organisms	Policegoudra et al. (2010)
Methanolic extract of rhizomes	In vitro (Nine species of MDR uropathogenic bacteria isolated from clinical samples)	MIC—3.41 mg/mL MBC—4.27 mg/mL	Moderately antibacterial against six uropathogenic bacteria	Rath and Padhy (2014)
Isolate of chloroform extract (Labdane terpenoid dialdehyde and its semi-synthetic analogues	*Mycobacterium tuberculosis* H(37)Rv strain in BACTEC-460 assay	MIC—500 μg/mL	Anti-tubercular activity	Singh et al. (2010)
Ethanolic and DCM extract of rhizomes	Earthworms	150 mg/mL	Anti-helminthic (death of earthworms)	Gill et al. (2011)
Methanolic extract of rhizomes	Larvae of *Anopheles stephensi* mosquitos	LC_{50}—0.045 mg/L; at 60–72 h	Larvicidal activity in dose-dependent manner	Jegajeevanram et al. (2016)

(continued)

Table 9.2 (continued)

Plant part/extract used	Type of experiment and animal/tissue used (clinical/animal/in vitro/cell line, etc.)	Dose/conc./route/duration of administration	Activity observed	References
Hexane and chloroform extracts of rhizomes and isolate-amadaldehyde	i. Vero (Normal African green monkey kidneys) cell culture; ii. Human small cell lung cancer CL (A-549)	CTC_{50}—102 μg for A-459; and 118 μg for NC	Cytotoxicity (More toxicity towards cancerous cells compared to normal cells)	Policegoudra et al. (2010)
Super critical CO_2 extract of rhizomes	i. Human alveolar (SJRH30) and embryonal (RD) rhabdomyosarcoma CL; ii. Nude mice with SJRH30 tumours (Xenograft studies)	IC_{50}—7.133 μg/mL for SJRH30; and 7.501 μg/mL for RD CL	– Cytotoxicity Superior to *C. longa* and *C. xanthorrhiza* – Synergistic effects with Vinblastin and Cyclophosphamide – Inhibited tumour growth rate with and without Vinblastin and increased the survival rate significantly in xenografts	Ramachandran et al. (2015)
Hydro-methanolic extract	In vitro	MBC—15.6 to 62.5 μg/mL	Significant bactericidal activity against *Helicobacter pylori*	Zaidi et al. (2009)

Table 9.3 Established pharmacological activities of *C. mangga*

Plant part/extract used	Type of experiment and animal/tissue used (clinical/animal/in vitro/cell line, etc.)	Dose/conc./route /duration of administration	Activity observed	References
Rhizome extract	Cows	0.06% BW/day, orally administered	Chelation of Pb in milk	Nurdin et al. (2013)
Aqueous extract of rhizome	RBL-2H3 cell line	$IC_{50} = 36.1$ μg/mL	Anti-allergic	Tewtrakul and Subhadhirasakul (2007)
Ethanolic extract of rhizome and fractions of aqueous, chloroform, ethyl acetate and hexane extracts	i. Nociceptive responses in mice ii. Rat paw oedema and mouse ear oedema	i. 200 mg/kg, orally administered as analgesic; ii. 150 mg/kg, orally administered as anti-inflammatory	Centrally acting analgesic as well as anti-inflammatory effects	Ruangsang et al. (2010)
Cream containing rhizome extract (10% w/w)	Models of inflammation	IC_{50} —34.1 μg/ml and 37.9 μg/mL	Anti-inflammatory and wound healing effect (superior than Diclofenac gel-$IC_{50} = 54.3$ μg/mL)	Srirod and Tewtrakul (2019)
Hexane, dichloromethane, methanolic and aqueous extracts of rhizomes	i. In vitro culture of asexual blood stage of chloroquine resistant *P. falciparum*; ii. normal Madin-Darby Bovine Kidney cell lines	$EC_{50} < 10$ μg/mL	Anti-parasitic (Antimalarial); with negligible toxicity against normal cell lines	Razak et al. (2014)
Methanolic extracts and fractions from hexane, ethyl acetate and aqueous extracts	In vitro using cancer cell lines: breast (MCF-7), nasopharyngeal epidermoid (KB), lung (A549), cervical (Ca Ski) and colon (HCT 116 and HT-29)	MIC varied with type of extract, fraction and cell line	Cytotoxicity against human cancerous cell lines but less toxicity to normal cells	Malek et al. (2011)

with mango ginger, spicy mango ginger tofu, mango ginger sorbet, etc., are prepared in various parts of the world (Ravindran et al. 2007).

Mango ginger holds good potential for use in food industries as well. Possibility of using *C. amada* powder as a substitute to wheat flour was explored in the preparation of soup sticks (Crassina and Sudha 2015). Replacement with the powder at 10% level showed acceptable sensory attributes of the sticks, improved nutritional properties, texture and breaking strength. Due to inclusion of mango ginger powder, total dietary fibre content (8.64%) and antioxidant activity (48.06%) of the soup sticks showed significant improvement, when compared with control sticks (3.31 and 26.83%).

In processing industries, *C. amada* could be a useful ingredient for blending with variety of horticultural crops. For example, pummelo (*Citrus grandis*) is a medicinally important fruit crop, juice of which turns bitter just after extraction due to the presence of alkaloids. Use of *C. amada* helped in masking the bitterness and improved the overall acceptability of blended products such as nectar and syrup (Bohra et al. 2012; Bohra and Srinivas 2015). This suggests that mango ginger could be a very good material for developing novel processed products. Mango ginger is known to be a source of starch with high amounts of amylose and limited solubility. These attributes could be helpful in preparation of nutraceutical products (Policegoudra et al. 2011). Further, this starch has high swelling power, and hence, it could be a novel natural substitute for use in food industry (Sajitha and Sasikumar 2015). Apart from rhizomes, functional food properties possessed by the leaves of *C. mangga* are also worth exploring for commercial-scale utilization (Liu and Nair 2012).

Essential oil is an important part of any aromatic spice, and its content determines quality of the spice produce (Waman 2020). Rhizomes of *C. amada* are known to contain about 0.6–2.1% of volatile oils (Sajitha et al. 2014; Priyanka et al. 2018). This oil contains a number of compounds, which are known to have high demand in fragrance industries worldwide. India is the largest producer of mango ginger oil, and it produces about 50% of the world's mango ginger volatile oil production (Al-Qudah et al. 2017), which depicts its large export potential. Primary, secondary and mother rhizomes of *C. mangga* are known to contain volatile oils to the tune of 0.12–0.37% (Kamazeri et al. 2012; Waman et al. 2018). Myrcene and cyclofenchene are the major compounds of this species, which are used in aroma industries.

Even though mango ginger is cultivated mainly for the rhizomes, flowers of *C. amada* have also been valued for ornamental purpose as cut flower due to their showy nature. Each flower has a stalk of 10–12 cm and rachis of 10–15 cm, and such flower stalks are harvested when 3/4th flowers in the inflorescence are opened. Flowers have a vase life of six days (Nair 2013).

9.5 Agro-Technology, Cultivation and Domestication

Rhizomatous species are herbaceous perennials in which the underground part is perennial, while the above-ground pseudostem exhibits seasonal growth habit. Due

to this, most of the rhizomatous species are grown as annual crops, and mango ginger species are no exception to it (Sasikumar 2008).

Most rhizomatous species can tolerate sunlight as well as partial shade, thereby making them amenable for cultivation in open condition as well as intercrops in existing plantations. Both the discussed mango ginger species also perform well as sole crop and as intercrop (Waman et al. 2018; Priyanka and Bhoomika 2018). Rainfed cultivation is a rule in these species as most of the crucial period of crop growth receives sufficient rainfall in the areas of their natural distribution. However, as the species are in semi-domesticated condition, studies on effect of irrigation during critical growth phases on growth, yield and phytochemical composition could be a matter of investigation.

Friable soils support development of underground rhizomes. To provide optimum soil aeration, planting is generally done on raised beds of variable size or on ridges and furrows. Beds are prepared after thorough working of soil to get fine tilth, and well-decomposed farmyard manure (30–40 t/ha) could be incorporated (Sasikumar 2008). In *C. mangga*, use of beds of 2 m × 1.2 m × 0.15 m has been reported (Waman et al. 2018). In *C. amada*, beds of 3 m × 1–1.2 m beds were used (Mridula and Jayachandran 2001; Priyanka and Bhoomika 2018). Dimensions of these beds could vary based on ease of operations, availability of space, growing conditions, intercultural operations, etc. Spacing of 30 cm × 30 cm in *C. mangga* (Waman et al. 2018) and 30 cm × 25–30 cm in *C. amada* (Mridula and Jayachandran 2001; Chatterjee et al. 2012; Priyanka and Bhoomika 2018) has been employed.

Even though improved varieties play a key role in successful cultivation of any commercial crop, only one variety 'Amba' has been developed in *C. amada* in India by Pottangi Centre of the Orissa University of Agriculture and Technology, Bhubaneswar (Sasikumar 2008). Germplasm evaluation studies under West Bengal conditions suggested considerable diversity for growth, yield and quality parameters among the nine local collections studied (Chatterjee et al. 2012). No improved germplasm has been identified in *C. mangga* so far.

Rhizome pieces are the most common propagules used for planting, which are also the economic parts in both the species. Use of larger size rhizomes would increase planting material requirement, whereas undersized rhizomes would not produce sufficient plant growth for getting desired yield (Hailemichael and Tesfaye 2008). Hence, use of optimum-sized propagules is required. For planting in *C. amada*, rhizomes of 20–60 g size have been recommended in different regions of growing (Sasikumar 2008; Priyanka and Bhoomika 2018). In *C. mangga*, use of rhizomes of 20–25 g was recommended for growing the crop meant for aroma and pharmaceutical industries; while if the produce is intended for processing industries, smaller rhizome pieces of 15–20 g were advised (Waman et al. 2018). Considering the value of this species, it has been identified as a potential crop for the Andaman Islands, India (Waman et al. 2018).

Organic manures have been found to support plant growth and yields in both the species (Waman et al. 2018; Pariari et al. 2019). In *C. amada*, highest yields of 33 t/ha were obtained with application of poultry manure @ 2 t/ha (Pariari et al. 2019). Use of inorganic nutrients has been reported to promote plant growth and

yields in *C. amada*, and amount of such inputs required varies with soil conditions. Under Karnataka condition, dose of 180:150:300 kg/ha of N_2: P_2O_5:K_2O was found optimum (Priyanka and Bhoomika 2018), while it was much lower (30:30:60 kg/ha) in Kerala (Sasikumar 2008). Split application of nitrogen into two equal doses and basal application of phosphorus and potassium are advantageous. Considering exhaustive nature of rhizomatous crops, region-specific nutrient management studies could be useful.

As the rhizomes start developing, it is commonly seen that the clump pushes the plants upwards, thereby exposing the developing rhizomes. Earthing up is thus required as a major intercultural operation, apart from two to three manual weedings (Waman et al. 2018). Maturity of the crop is indicated by drying of above ground pseudostem part. The rhizomes are then carefully lifted by using a spade and by removing the surrounding soil. Rhizomes should be cleaned and cured in shade to facilitate better storability. It is necessary to store them in cool and dry place, as the required quantity of products would also be used as planting material for next year.

As these species are not systematically studied, very limited information is available on plant protection aspects. *C. amada* grown under Nigerian condition revealed that leaf blight (c.o. *Alternaria alternata*, *Rhizoctonia solani* and *Colletotrichum gloeosporioides*) severely affected older leaves causing their death. *Pythium aphanidermatum*, *Rhizoctonia solani* and *Fusarium solani* caused rhizome rot, which significantly reduced the yields by 3.02–3.25% (Ayodele et al. 2018). Interestingly, *C. amada* has been reported to be resistant to bacterial wilt disease caused by *Ralstonia solanacearum,* which damages ginger cultivation drastically (Prasath et al. 2014). Microbial load on dried produce meant for pharmaceutical industries is undesirable as per good manufacturing practices, and hence, their decontamination with irradiation of Gamma rays (5 kGy) has been recommended (Rahayu et al. 2016). A lepidopteran pest *Conogethes punctiferalis* Guen., which also causes considerable damage in turmeric, has been reported to infest *C. amada* under Indian conditions (Nair 2013).

9.5.1 Biotechnological Approaches

Both the species are multiplied using pieces of rhizomes, which is a slow means of propagation, and hence, attempts have been made to standardize micropropagation protocols. Successful adventitious plantlet development from rhizome and leaf sheath explants has been demonstrated by some researchers (Prakash et al. 2004; Das et al. 2010; Banerjee et al. 2012). For in vitro multiplication of *C. amada*, Murashige and Skoog's (MS) medium supplemented with 6-benzyladenine (2 mg/L), and indole acetic acid (0.5 mg/L) was optimum as it has been resulted in development of 3.8 ± 0.2 shoots per culture (Mohanty et al. 2014a). Later, report by Bhattacharya and Chakraborty (2015) suggested combined use of 6-benzyladenine (3 mg/L) and Kinetin (3 mg/L) for improving multiplication to 6.06 ± 0.23 shoots per culture. About 92% and 98% success was reported during polyhouse and field

acclimatization, respectively. Use of half-strength MS medium supplemented with 6-benzyladenine (2 mg/L) and 30 g/l sucrose +10 g/L maltose facilitated slow growth culture in this species, and the cultures could be conserved for 10 months without subculturing, while 24 months with subculturing (Mohanty et al. 2014a).

In Malaysia, an attempt was made to develop micropropagation protocol for *C. mangga* which suggested use of high concentrations (9 mg/L) of 6-benzyladenine for optimum shoot multiplication (3.3 ± 0.9 shoots/ explant) and NAA (1 mg/L) for successful rooting (Rihana et al. 2011). To address the issue of this low multiplication rate, a high-efficiency methodology was developed at senior author's institute (CIARI 2019), in which use of MS medium supplemented with novel cytokinin- *meta* topolin (1 mg/L) and dextrose (3%) as carbon source with two bud inoculum size was found to be optimum. As high as 13.3 to 15.2 shoots/explants were obtained during subculture-V. Auxin-free concurrent ex vitro rooting cum hardening was developed which could reduce the time as well as inputs/cost to a great extent.

In order to carry out genetic engineering, somatic embryogenesis is desirable (Raju et al. 2014). The process of indirect somatic embryogenesis through cell suspension culture has been standardized (Raju et al. 2013). However, involvement of callus phase in the regeneration process has been considered undesirable as it may result into unwanted changes in the regenerated plants. Considering this, Raju et al. (2014) developed a two-step system for obtaining somatic embryogenesis using leaf sheath explants. First step involved culturing the explants onto a medium containing 2,4-dichlorophenoxyacetic acid (2.24 μM) and 6-benzyladenine (1.11 μM). After two weeks, explants were subcultured onto the medium supplemented with thidiazuron (9.10 μM) and naphthaleneacetic acid (1.33 μM). Plantlet development (87%) was achieved by culturing the embryos in dark on half strength MS medium containing gibberellic acid.

In case of *C. mangga*, combined use of 2,4-dichlorophenoxyacetic acid and naphthalene acetic acid (5 mg/L each) could induce somatic embryogenesis with 96% success. Shoot development occurred after subculturing the embryos onto medium containing 6-benzyladenine (3.0 mg/L), naphthalene acetic acid (0.5 mg/L) and maltose (3%) as carbon source. Agrobacterium-mediated transformation was also successfully demonstrated (Pikulthong et al. 2016).

As emphasized earlier, *C. amada* is a source of resistance to *Ralstonia solanacearum*, a devastating pathogen of ginger. In order to identify genes responsible for this resistance, trasncriptome of ginger and mango ginger was sequenced using the Illumina sequencing technology (Prasath et al. 2014). This resulted in identification of candidate genes for further exploitation and development of a web resource—ginger transcriptome database.

A study was carried out in ten species of Zingiberaceae family, and it was observed that for RAPD analysis, the primer OPA4 was identified to the best for segregation of species at nuclear DNA level (Mohanty et al. 2014b). The Jaccard's coefficient suggested that *C. amada* and *C. aromatica* had close similarities amongst the species studied with a similarity value of 0.29 using RAPD and a value of 0.40 in combined markers analysis (RAPD, ISSR and SSR).

In order to characterize the genetic structure of *C. amada* in Myanmar, neutral and functional genomic markers were employed (Jatoi et al. 2010). Results revealed high degree of polymorphism of more than 91%, suggesting presence of significant genetic variability in the germplasm investigated. Further, amplification of source-specific alleles suggested that the neutral regions were more variable than functional regions. They also reported higher genetic diversity in gene bank collections than that in farmers' accessions. It was recommended to employ concurrent use of different molecular markers to understand the existing variability in the germplasm.

9.6 Perspectives

Rhizomatous species are known for their medicinal and nutritional properties, and mango ginger species are among the most potential ones. Though these species have been used traditionally by the local masses including native tribes and several advanced trials have been taken up in both the species, their commercial-scale exploitation has not been undertaken yet. Considering multifaceted applications of these species in food, fragrance, pharmaceutical, floriculture sectors, systematic cultivation could not only ensure livelihood security to the growers of tropical regions but would also help in developing diversified range of health and wellness products.

References

Aggarwal BB, Sundaram C, Malani N, Ichikawa H (2007) Curcumin: the Indian solid gold. In: Aggarwal BB, Surh YJ, Shishodia S (eds) The molecular targets and therapeutic uses of curcumin in health and disease. Advances in experimental medicine and biology, vol 595. Springer, Boston, MA. https://doi.org/10.1007/978-0-387-46401-5_1

Al-Qudah TS, Malloh SA, Nawaz A, Ayub MA, Nisar S, Jilani MI, Al-Qudah TS (2017) Mango ginger (*Curcuma amada* Roxb.): a phytochemical mini review. Intl J Chem Biochem Sci 11:51–57

Aminah A (2008) Yield and growth performance of selected *curcuma* species grown on bris sandy soil. Acta Hortic 786:246–252

Awin T, Mediani A, Maulidiani SK, Faudzi SMM, Sukari MAH, Lajis NH, Abas F (2016) Phytochemical profiles and biological activities of *Curcuma* species subjected to different drying methods and solvent systems: NMR-based metabolomics approach. Ind Crop Prod 94:342–352

Ayodele VO, Olowea OM, Afolabib CG, Kehinde IA (2018) Identification, assessment of diseases and agronomic parameters of *Curcuma amada* Roxb (Mango ginger). Curr Plant Biol 15:51–57. https://doi.org/10.1016/j.cpb.2018.10.001

Banerjee S, Singh S, Pandey H, Pandey P, Rahman L (2012) Conservation and storage of *Curcuma amada* Roxb. synseeds on *Luffa* sponge matrix and RAPD analysis of the converted plantlets. Ind Crop Prod 36:383–388

Bhaskaran A, Rao SR, Subramanya P, Mahesh TS, Krishnamurthy MS (2012) Clinical evaluation of *Amragandha Haridra* (*Curcuma amada* Roxb.) in Pratishaya WSR to allergic rhinitis: a folklore claim. Intl J Res Ayur Pharm 3(1):85–89

Bhattacharya M, Chakraborty S (2015) Efficient in vitro regeneration of pathogen free mango ginger (*Curcuma amada* Roxb.) from rhizome bud explants. Res Plant Biol 5(6):9–13

Bohra P, Srinivas KN (2015) Development of nectar from pummelo (*Citrus grandis*), an underutilized fruit crop, by blending with kokum (*Garcinia indica*) and mango ginger (*Curcuma amada*). J Andaman Sci Assoc 19(1):70–74

Bohra P, Sreenivas KN, Sreeramu BS (2012) Development of a cost-effective, palatable and shelf stable blended beverage of pummelo (*Citrus grandis* Linn.). Fruits 67:249–256

Chatterjee R, Chattopadhyay PK, Hnamte V, Chongtham T, Datta Ray SK (2012) Assessment of quality characteristics of mango ginger (*Curcuma amada* Roxb.) germplasm. Intl J Bio-resource Stress Mgt 3(3):380–382

CIARI (2019) Annual report for 2018–19 and 2019 of ICAR-central island agricultural research institute. Port Blair, India

Crassina K, Sudha ML (2015) Evaluation of rheological, bioactives and baking characteristics of mango ginger (*Curcuma amada*) enriched soup sticks. J Food Sci Technol 52:5922–5929. https://doi.org/10.1007/s13197-014-1548-7

Das AB, Rai S, Das P (1999) Karyotype analysis and cytophotometric estimation of nuclear DNA content in some members of the Zingiberaceae. Cytobios 97:23–33

Das A, Kesari V, Rangan L (2010) Plant regeneration in *Curcuma* species and assessment of genetic stability of regenerated plants. Biol Plant 54(3):423–429

Gill R, Kalsi V, Singh A (2011) Phytochemical investigation and evaluation of anthelmintic activity of *Curcuma amada* and *Curcuma caesia*: a comparative study. Inventi impact: ethnopharmacol, Article ID-Inventi: ep/412/11

Gogte VM (2017) Dravyaguna Vijnyan, Edition III, Vaidyamitra Publication, Pune, India, p 279

Gupta AP, Gupta MM, Kumar S (1999) Simultaneous determination of curcuminoids in *Curcuma* samples using high performance thin layer chromatography. J Liq Chromatogr Relat Technol 22(10):1561–1569

Hailemichael G, Tesfaye K (2008) The effects of seed rhizome size on growth, yield and economic return of ginger (*Zingiber officinale* Rosc). Asian J Pl Sci 7(2):213–217

Harun NH, Septama AW, Jantan I (2015) Immuno-modulatory effects of selected Malaysian plants on the CD18/11a expression and phagocytosis activities of leukocytes. Asian Pac J Trop Biomed 5(1):48–53

Hong GW, Hong SL, Lee GS, Yaacob H, Malek SNA (2016) Non-aqueous extracts of *Curcuma mangga* rhizomes induced cell death in human colorectal adenocarcinoma cell line (HT29) via induction of apoptosis and cell cycle arrest at G0/G1 phase. Asian Pacific J Trop Med 9(1):8–18

Jantan I, Harun NH, Septama AW, Murad S, Mesaik MA (2011) Inhibition of chemiluminescence and chemotactic activity of phagocytes in vitro by the extracts of selected medicinal plants. J Nat Med 65(2):400–405. https://doi.org/10.1007/s11418-010-0492-8

Jatoi SA, Kikuchi A, Ahmad D, Watanabe KN (2010) Characterization of the genetic structure of mango ginger (*Curcuma amada* Roxb.) from Myanmar in farm and genebank collection by the neutral and functional genomic markers. Electronic J Biotechnol 13(6). https://doi.org/10.2225/vol13-issue6-fulltext-10

Jegajeevanram P, Alhaji NMI, Velavan S (2016) Larvicidal activity of *Curcuma amada* rhizome and *Tagetes erecta* flower against *Anapheles stephensi*. Asian J Innov Res 1(3):48–52

Kamazeri TSAT, Samah OA, Taher M, Susanti D, Qaralleh H (2012) Antimicrobial activity and essential oils of *Curcuma aeruginosa*, *Curcuma mangga* and *Zingiber cassumunar* from Malaysia. Asian Pac J Trop Med 202–209

Karata ŞA, Orhan C, Tuzcu M et al (2020) Mango ginger (*Curcuma amada*) inhibits collagen-induced arthritis by modulating inflammatory cytokine levels in rats. Turk J Med Sci. https://doi.org/10.3906/sag-2004-105

Kaur R, Satija S, Kalsi V, Mehta M, Gupta P (2011) Comparative study of analgesic and antipyretic activity of *Curcuma caesia* and *Curcuma amada Roxb.* Rhizomes-Ethnopharmacol Inventi: ep/441/11

Kharade SS, Samal KC, Rout GR (2017) High performance thin layer chromatography fingerprint profile of rhizome extracts of five important *Curcuma* species. Proc Natl Acad Sci India Sect B Biol Sci 87(4):1335–1341. https://doi.org/10.1007/s40011-016-0709-z

Liu Y, Nair MG (2012) *Curcuma longa* and *Curcuma mangga* leaves exhibit functional food property. Food Chem 135:634–640

Malek SNA, Lee GS, Hong SL, Yaacob H, Wahab NA, Weber JFF, Shah SA (2011) Phytochemical and cytotoxic investigations of *Curcuma mangga* rhizomes. Molecules 16(6):4539–4548. https://doi.org/10.3390/molecules16064539

Mitra D, Sarkar R, Ghosh D (2019) Antidiabetic and antioxidative properties of the hydro-methanolic extract (60:40) of rhizomes of *Curcuma amada* roxb. (Zingiberaceae) in streptozotocin-induced diabetic male albino rat: a dose-dependent study through biochemical and genomic approaches. J Complement Integr Med. https://doi.org/10.1515/jcim-2017-0182

Mohanty S, Parida R, Sahoo S, Nayak S (2014a) In vitro conservation of nine medicinally and economically important species of zingiberaceae from Eastern India. Proc Natl Acad Sci India Sect B Biol Sci 84(3):799–803.https://doi.org/10.1007/s40011-013-0251-1

Mohanty S, Panda MK, Acharya L, Nayak S (2014b) Genetic diversity and gene differentiation among ten species of Zingiberaceae from Eastern India. 3 Biotech 4:383–390. https://doi.org/10.1007/s13205-013-0166-9.

Mridula KR, Jayachandran BK (2001) Quality of mango-ginger (*Curcuma amada* Roxb.) as influenced by mineral nutrition. J Trop Agri 39:182–183

Nair KPP (2013) The agronomy and economy of turmeric and ginger. Elsevier. https://doi.org/10.1016/B978-0-12-394801-4.00002-8

Neamsuvan O, Tuwaeyah T, Fatin B, Asma A, Kholeel M (2012) A survey of folk remedies for gastrointestinal tract diseases from Thailand's three southern border provinces. J Ethnopharmacol 144:11–21

Nissankara Rao LS, Kilari EK, Kola PK (2019) Protective effect of *Curcuma amada* acetone extract against high-fat and high-sugar diet-induced obesity and memory impairment. Nutr Neurosci 1–14.https://doi.org/10.1080/1028415X.2019.1616436

Nurdin E, Putra DP, Amelia T (2013) Analysis of heavy metal lead (Pb) levels with Aas in cow's milk by giving cumin (*Cuminum cyminum* L.), white turmeric (*Curcuma zedoaria* Rosc.) and mango turmeric (*Curcuma mangga* Val.). Pak J Biol Sci 16(21):1373–1377. https://doi.org/10.3923/pjbs.2013.1373.1377

Panda SK (2014) Ethno-medicinal uses and screening of plants for antibacterial activity from Similipal Biosphere Reserve, Odisha, India. J Ethnopharmacol 151:158–175

Pande GS (2010) Bhavmishra *Bhavprakash* Hindi commentary by Chunekar KC. Chaukhamba Bharti Academy. Varanasi, India, p 113

Panyadee P, Balslev H, Wangpakapattanawong P, Inta A (2019) Medicinal plants in homegardens of four ethnic groups in Thailand. J Ethnopharmacol 239:111927

Pariari A, Karthik CS, Bhattacharya S (2019) Effect of organic manures on growth, yield and quality of mango ginger (*Curcuma amada* Roxb.) in the Gangetic Alluvial Plains of West Bengal. Int J Curr Microbiol App Sci 8(11):1030–1034

Pikulthong V, Teerakathiti T, Thamchaipenet A, Peyachoknagul S (2016) Development of somatic embryos for genetic transformation in *Curcuma longa* L. and *Curcuma mangga* Valeton & Zijp. Agric Nat Resour 50(4):276–285

Policegoudra RS, Divakar S, Aradhya SM (2007) Identification of Difurocumenonol, a new antimicrobial compound isolated from mango ginger (*Curcuma amada* Roxb.) rhizome. J Appl Microbiol 102:1594–1602

Policegoudra RS, Swaroop Kumar HM, Aradhya SM (2007) Accumulation of bioactive compounds and their bioactivity during developmental stages of mango ginger (Curcuma amada Roxb.) rhizome. J Agric Food Chem 55:8105–8111

Policegoudra RS, Rehna K, Rao LJ, Aradhya SM (2010) Antimicrobial, antioxidant, cytotoxicity and platelet aggregation inhibitory activity of a novel molecule isolated and characterized from mango ginger (*Curcuma amada* Roxb.) rhizome. J Biosci 35(2):231–240 https://doi.org/10.1007/s12038-010-0027-1

Policegoudra RS, Aradhya SM, Singh L (2011) Mango ginger (Curcuma amada Roxb.)—a promising spice for phytochemicals and biological activities. J Biosci 36(4):739–748

Prakash S, Elangomathavan R, Seshadri S, Kathiravan K, Ignacimuthu S (2004) Efficient regeneration of *Curcuma amada* Roxb. Plantlets from rhizome and leaf sheath explants. Plant Cell Tissue Org Cult 78:159–165

Prasath D, Karthika R, Habeeba NT, Suraby EJ, Rosana OB, Shaji A, Eapen SJ, Deshpande U, Anandaraj M (2014) Comparison of the transcriptomes of ginger (*Zingiber officinale* Rosc.) and mango ginger (*Curcuma amada* Roxb.) in response to the bacterial wilt infection. PLoS One 9(6):e99731. https://doi.org/10.1371/journal.pone.0099731

Priyanka BM, Bhoomika HR (2018) Effect of rhizome weight and nutrition on plant growth and yield in mango ginger (*Curcuma amada* Roxb.) under arecanut cropping system. J Pharmacog Phytochem SP3:308–310

Priyanka BM, Bhoomika HR, Sannidi HS, Shivaprasad M, Hanumanthraya L, Nadukeri S (2018) Effect of different seed rhizome weight and nutrition levels on yield, quality and economics of mango ginger (Curcuma amada Roxb.) cultivation. Int J Curr Microbiol Appl Sci 7(8):1213–1218

Rachkeeree A, Kantadoung K, Puangpradub R, Suksathan R (2020) Phytochemicals, antioxidants and anti-tyrosinase analyses of selected Ginger plants. Pharmacogn J 12(4):872–883

Rahayu DP, Saputri FC, Darwis D (2016) The effect of gamma radiation on microbial content and curcuminoids of *Curcuma amada* Roxb. Rhizomes. Atom Indonesia 42(2):53–58

Raihana R, Faridah QZ, Julia AA, Abdelmageed AHA, Kadir MA (2011) In vitro culture of *Curcuma mangga* from rhizome bud. J Med Plants Res 5(28):6418–6422

Raju SC, Kathiravan K, Aslam A, Shajahan A (2013) An efficient regeneration system via somatic embryogenesis in mango ginger (*Curcuma amada* Roxb.). Plant Cell Tiss Org Cult 112:387–393

Raju CS, Aslam A, Kathiravan K, Palani P, Shajahan A et al (2014) Direct somatic embryogenesis and plant regeneration from leaf sheath explants of mango ginger (*Curcuma amada* Roxb.). Vitro Cell Dev Bio Plant 50:752–759. https://doi.org/10.1007/s11627-014-9653-x

Ramachandran C, Quirin KW, Escalon EA, Lollett IV, Melnick SJ (2015) Therapeutic effect of supercritical CO_2 extracts of *Curcuma* species with cancer drugs in rhabdomyosarcoma cell lines. Phytother Res 29(8):1152–1160. https://doi.org/10.1002/ptr.5360

Rao AS, Rajanikanth B, Seshadri R (1989) Volatile aroma components of *Curcuma amada* Roxb. J Agric Food Chem 37:740–743

Rath S, Padhy RN (2014) Monitoring in vitro antibacterial efficacy of 26 Indian spices against multidrug resistant urinary tract infecting bacteria. Integr Med Res 3(3):133–141. https://doi.org/10.1016/j.imr.2014.04.002

Ravindran PN, Nirmal Bau K, Shiva KN (2007) Botany and crop improvement of turmeric. In: Ravindran PN, Nirmal Babu K, Sivaraman K (eds) Turmeric—the gebus Curcuma. CRC Press, Boca Raton, pp 15–70

Razak MAMR, Afzan A, Ali R, Jalaluddin NFA, Wasiman MI, Zahari SHS, Abdullah NR, Ismail Z (2014) Effect of selected local medicinal plants on the asexual blood stage of chloroquine resistant *Plasmodium falciparum*. BMC Complement Altern Med 14:492. https://doi.org/10.1186/1472-6882-14-492

Ruangsang P, Tewtrakul S, Reanmongkol W (2010) Evaluation of the analgesic and anti-inflammatory activities of *Curcuma mangga* Val and Zijp rhizomes. J Nat Med 64(1):36–41. https://doi.org/10.1007/s11418-009-0365-1

Sajitha PK, Sasikumar B (2015) Qualitative and quantitative variation in starch from four species of *Curcuma*. Cytologia 80(1):45–50

Sajitha PK, Prasath D, Sasikumar B (2014) Phenological variation in two species of *Curcuma*. J Plant Crops 42(2):252–255

Samant LR (2012) *Curcuma amada* Roxb.: a phytopharmalogical review. J Pharm Res 5(4):1992–1993

Santhoshkumar R, Yusuf A (2019) Chemotaxonomic studies on rhizome extract compositions of twenty *Curcuma* species from South India. Biochem Systematics Ecol 84:21–25

Sarkar R, Ghosh P, Tripathy A, Ghosh D (2019) Correction of diabetes-induced testicular dysfunction by a hydro-methanol (60:40) extract of *Curcuma amada* rhizomes: a dose-dependent study. J Food Biochem 43(5):e12829. https://doi.org/10.1111/jfbc.12829

Sasikumar B (2008) Other economically important zingiberaceous species. In: Krishnamurthy KS, Prasath D, Kandiannan K, Suseela Bhai R, Johnson George K, Parthasarathy VA (eds) National workshop on zingiberaceous spices-meeting the growing demand through sustainable production. Indian Institute of Spices Research, Calicut, Mar 2008, pp 140–144

Sharma PV (2009) Dravyaguna-Vijnana, vol II (Vegetable Drugs), Chaukhamba Bharti Academy, Varanasi, India, pp 162–166 (Reprint 2009)

Sheeja DBA, Nair MS (2012) Phytochemical constituents of *Curcuma amada*. Biochem Systematics Ecol 44:264–266

Siddappa DR, Babu R, Varaprasad JS, Basha R, Veena V, Gawtham K (2015) Preclinical evaluation of anti-spermatogenic activity of *Curcuma amada* on cell phone radiation induced infertility in rats. Indian J Res Pharm Biotech 3(6):434–437

Siddaraju MN, Dharmesh SM (2007) Inhibition of Gastric H+, K+—ATPase and helicobacter pylori growth by phenolic antioxidsants of *Curcuma amada*. J Agric Food Chem 55:7377–7386

Silalahi M, Nisyawati WEB, Supriatna J, Mangunwardoyo W (2015) The local knowledge of medicinal plants trader and diversity of medicinal plants in the Kabanjahe traditional market, North Sumatra, Indonesia. J Ethnopharmacol 175:432–443

Singh AK (2017) Revisiting the status of cultivated plant species agrobiodiversity in India: an overview. Proc Indian Natl Sci Acad 83(1):151–174

Singh S, Kumar JK, Saikia D, Shanker K, Thakur JP, Negi AS, Banerjee S (2010) A bioactive labdane diterpenoid from *Curcuma amada* and its semisynthetic analogues as antitubercular agents. European J Med Chem 45:4379–4382

Singh S, Waman AA, Bohra P, Gautam RK, Dam Roy S (2016) Conservation and sustainable utilization of horticultural biodiversity in tropical Andaman and Nicobar Islands, India. Genet Resour Crop Evol 63:1431–1445

Sirirugosa P, Larsen K, Maknoi C (2007) The genus Curcuma L. (Zingiberaceae): distribution and classification with reference to species diversity in Thailand. Garden's Bull Singapore 59(1–2):203–220

Skornickova JL, Otakar S, Vlasta J, Sabu M, Tomas F, Pavel T, Jan S (2007) Chromosome Numbers and Genome Size Variation in Indian Species of *Curcuma* (Zingiberaceae). Ann Bot 100:505–526

Skornickova LJ, Otakar S, Karol M (2010) Back to types! Towards stability of names in Indian *Curcuma* L. (Zingiberaceae). Taxon 59(1):269–282

Srinivasan MR, Chandrashekharan N (1992) Effect of Mango ginger on lipid status in normal and hypertriglyceridemic rats. J Food Sci Technol 29:130–132

Srirod S, Tewtrakul S (2019) Anti-inflammatory and wound healing effects of cream containing *Curcuma mangga* extract. J Ethnopharmacol 238:111828. https://doi.org/10.1016/j.jep.2019.111828

Sun W, Shen W, Wenwen Z, Chuanhong W, Shuhui G, Gao H, Tao H, Jin-jian L, Yitao W, Xiu-ping C (2016) Chemical constituents and biological research on plants in the Genus *Curcuma*. Critical Rev Food Sci Nutr. https://doi.org/10.1080/10408398.2016.1176554

Swarnkar S, Katewa SS (2008) Ethnobotanical observation on tuberous plants from tribal area of Rajasthan (India). Ethnobotan Leaf 12:647–666

Syamkumar S, Sasikumar B (2007) Molecular marker based genetic diversity analysis of Curcuma species from India. Sci Hortic 112:235–241

Syiem D, Monsang ShW, Sharma R (2010) Hypoglycemic and anti-hyperglycemic activity of *Curcuma amada* Roxb. in normal and alloxan-induced diabetic mice. Pharmacol Online 3:364–372

Tewtrakul S, Subhadhirasakul S (2007) Anti-allergic activity of some selected plants in the Zingiberaceae family. J Ethnopharmacol 109(3):535–538. https://doi.org/10.1016/j.jep.2006.08.010

Varadrajan R, Mathew MCR, Souprayan S (2018) Hepatoprotective efficacy of ethanolic extracts of rhizome *Curcuma amada* Roxb. in experimental rats. Ann Plant Sci 7:1966–1972

Velayudhan KC, Muralidharan VK, Amalraj VA, Gautam PL, Mandla S, Kumar D (1999) *Curcuma* genetic resources scientific monograph No. 4. National Bureau of Plant Genetic Resources, New Delhi

Wahab IRA, Blagojevic PD, Radulovic NS, Boylan F (2011) Volatiles of *Curcuma mangga* Val. et Zijp. (Zingiberaceae) from Malaysia. Chem Biodiv 8(11):2005–2014

Waman AA (2020) Essential oil composition of clove and nutmeg from Andaman and Nicobar Islands, India. Indian J Biochem Biophy 57:95–100

Waman AA, Pooja B, Aarthi S (2018) Propagule size affects yield and quality of *Curcuma mangga* Val. et Zijp.: an important medicinal spice. Ind Crops Prod 124:36–43. https://doi.org/10.1016/j.indcrop.2018.07.011

Wong KC, Chong TC, Chee SG (1999) Essential oil of *Curcuma mangga* Val. and van Zijp rhizomes. J Essent Oil Res 11(3):349–351. https://doi.org/10.1080/10412905.1999.9701151

Yoshioka Y, Yoshimura N, Matsumura S (2019) α-Glucosidase and pancreatic lipase inhibitory activities of diterpenes from indian mango ginger (*Curcuma amada* Roxb.) and its derivatives. Molecules 24(22):4071. https://doi.org/10.3390/molecules24224071

Zaidi SF, Yamada K, Kadowaki M, Usmanghani K, Sugiyama T (2009) Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against *Helicobacter pylori*. J Ethnopharmacol 121(2):286–291.https://doi.org/10.1016/j.jep.2008.11.001

Chapter 10
Cultivation and Utilization of Red Clover (*Trifolium pratense* L.)

Grażyna Zgórka and Magdalena Maciejewska-Turska

Abstract The theoretical study provides an overview of current data on red clover (*Trifolium pratense* L.), its cultivation and breeding, other practical uses and future prospects. Particular attention is paid to this taxon as a fodder plant of global importance in traditional and modern animal husbandry systems. Evidence is presented that red clover is a leading component, influencing the pasture management on all continents due to its high biomass production, protein content and overall nutritional value associated with the ability to fix atmospheric nitrogen. Some considerations cover important agrobiological and agrotechnological issues (e.g., plant interseeding) related to increasing adaptability, sustainability and yield of red clover and other crops coexisting in the same biosystems. Interesting biotechnological approaches have been pointed out in relation to the introduction to cultivation of new varieties, which are breeding hybrids of red clover and other wild species, with greater durability and yield-forming capacity. A separate part of the review is the assessment of the phytochemical profile of red clover with particular reference to biologically active specialized metabolites. Recent data from preclinical and human studies on existing and potential applications of this taxon and individual polyphenolic components in traditional and official medicine are also discussed.

Keywords *Trifolium pratense* L. (red clover) · Geographic occurrence · Taxonomy and morphology · Specialized metabolites · Biological activity · Therapeutic uses · Agrobiological challenges · Biotechnology

10.1 Introduction

Red clover (*Trifolium pratense* L. = TP), also known as meadow, wild or purple clover, is a representative of the botanical family Fabaceae (Leguminosae), which is the third largest angiosperm family consisting of about 19,500 plant taxa worldwide (Bruneau et al. 2013). Legumes, both native and introduced, are cultivated mainly for

G. Zgórka (✉) · M. Maciejewska-Turska
Department of Pharmacognosy, Medical University of Lublin, 1 Chodźki Street, 20-093 Lublin, Poland
e-mail: grazyna.zgorka@umlub.pl; gzgorka@pharmacognosy.org

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_10

food and fodder purposes and sometimes as ornamental plants. Due to their interaction with symbiotic microorganisms (root nodul bacteria), these plants are able to fix nitrogen from the atmosphere and convert it into biomass with a significant content of well-absorbed proteins for livestock (Fauvart and Michiels 2008). In addition, these unique adaptive capacities of the leguminous plants, obtained through evolution, make it possible to enrich soils with easily assimilable nitrogen compounds of natural origin and enable the development of organic farming systems in many regions of the world (Crews and Peoples 2004). Compared to the twentieth century, when agriculture was mainly based on synthetic nitrogen fertilizers, numerous environmental risks (pollution of the seas, oceans and groundwater, eutrophication of water reservoirs, etc.) have been recognized in the last two decades, and attempts have been made to apply more ecological systems of plant cultivation, including the reintroduction of various legume taxa (Reckling et al. 2020). In this group, apart from soybean (*Glycine max* (L.) Merr.), alfalfa (*Medicago sativa* L.) and lupin (*Lupinus albus* L.), red clover plays a key role as a popular ground cover crop, which is particularly widespread in Europe and Central Asia, but is also cultivated over large areas in North Africa, Australia and America (Smýkal et al. 2015). New biotechnological and genetic approaches are currently being proposed and implemented worldwide in order to increase environmental resistance and fertility of this species (Abberton 2007; De Vega et al. 2015).

Taking into account numerous applications in modern organic agrocultures and cattle breeding, it should be remembered that TP is a rich source of both primary plant metabolites (proteins, carbohydrates, lipids, etc.) and biologically active components formed in the secondary metabolic cycle (Broderick et al. 2001; Żuk-Gołaszewska et al. 2010; Sabudak and Guler 2009). In the latter group, polyphenols are the most important constituents used by red clover in allelopathic interactions with the environment (Ohno and Doolan 2001). As germination and growth inhibitors, these compounds act like natural herbicides, limiting the growth of weeds and favoring the development of both red clover and other crops cultivated in the same area. (Conklin et al. 2002; Amossé et al. 2013; Wyngaarden et al. 2015). Polyphenols (mainly isoflavones) found in red clover, in addition to their role as allelochemicals, may have different pharmacological effects on animal organisms, which has led to an increased interest and development of scientific research on their therapeutic use. Isoflavones are particularly abundant in the family Leguminosae and belong to a class of plant constituents known as non-steroidal phytoestrogens that mimic the activity of major female hormones, namely 17β-estradiol and its derivatives (Patisaul and Jefferson 2010). Due to the above-mentioned properties, they may alleviate some undesirable physical (atherosclerosis, osteporosis), neurovegetative (sweats, hot flashes) and mental (depression) disorders occurring in peri- and postmenopausal women as a result of impairment or loss of ovarian function and estrogen deficiency (Woods and Mitchell 2005; Chen et al. 2015). The problem of aging human populations around the world will certainly promote the search for new, biologically active compounds, capable of counteracting progressive degenerative processes in tissues and vital organs. In this group, an important role is attributed to isoflavones and other polyphenolic components of red clover, which have already

been used worldwide as health-promoting ingredients in food supplements and/or medicinal products (Hidalgo et al. 2005; Dornstauder et al. 2001; Myers and Vigar 2017).

10.2 Systematic and Morphological Description of the Plant and Its Geographic Distribution

According to the taxonomic classification obtained from Germplasm Resources Information Network (GRIN), *Trifolium* L. (clover) genus is included in the clades of the Angiospermae (Magnoliophyta), comprising seed-producing taxa, and the Dicotyledons, i.e., flowering plants having two embryonic leaves within germinating seeds. Going down the systematic ladder, we encounter the order Fabales. It comprises a broad range of flowering plants with a famous family Fabaceae (Leguminosae), ranked third (after the Orchidaceae and Asteraceae) in terms of the number of species recorded on the globe (Smýkal et al. 2015; USDA 2015). Based on the generally accepted taxonomic list of the world botanical species (The Plant List 2013), all taxa of the family Leguminosae belong to 946 plant genera. One of them is the genus *Trifolium* L. At present, it comprises 244 representatives, including *T. pratense* L. (TP). In turn, apart from *T. pratense* as the main species, there are three infraspecific taxa (varieties), namely *T. pratense* var. *americanum* Harz, *T. pratense* var. *maritimum* Zabel and *T. pratense* var. *sativum* Schreb., that have been accepted in terms of the confidence level of their systematic description (The Plant List 2013).

In general, TP is an herbaceous perennial plant, with branched, rather erect, hairy stems, on average reaching from 40 to 70 cm in height. The species possesses some characteristic morphological features of the genus *Trifolium* L., which Latin name comes from compound trifoliate leaves divided into three ovate leaflet and having (at the base) a pair of ovate stipules. Depending on the location on the stem, leaves are petiolate and long hairy (those in lower part of the stem), while other, growing on the top of the shoot, have short petioles or are sessile. A distinguishing morphological feature for TP is a V-shaped pattern of white or light green color on the upper surface of each leaflet. All clovers are also easily recognizable because of the specific shape of inflorescences. These are spheroid or ovoid flowerheads growing at the ends of the stems. In TP, the inflorescences are supported on the underside by three sessile leaflets (Fig. 10.1).

Flowerheads consist of numerous sessile, tubular-shaped, five-petal flowers. Each flower has reddish-purple or pink, rarely cream or even white corolla and green, hairy calyx ended with five narrow teeth. The fruit is typical for the botanical family to which red clover belongs. It is the ovate legume with a thickened apex containing one or two heart-shaped, brown seeds (Tutin et al. 1968). As observed by plant taxonomists, TP is a highly variable species in terms of morphological characteristics both in the wild and cultivated state. The main differences concern shoot habit, total height, outer covering (indumentum), size and shape of leaflets and size and color of

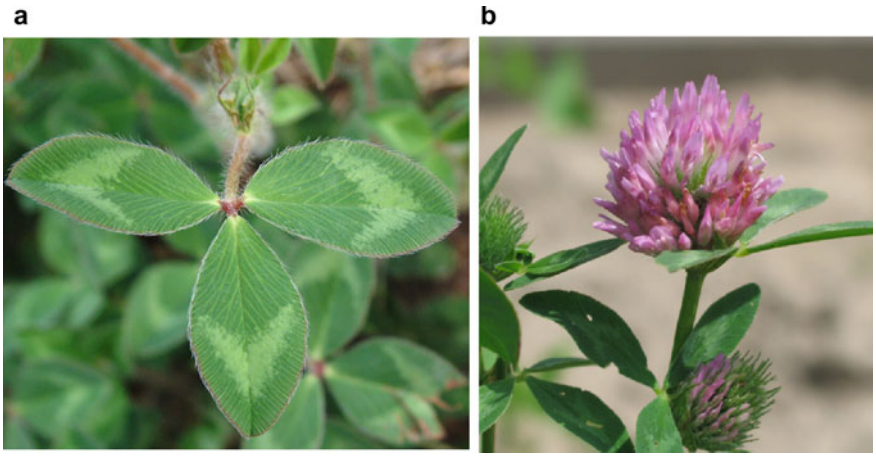


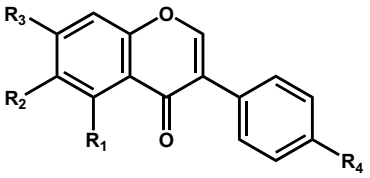
Fig. 10.1 Photographs of a leaf **a** and inflorescence, **b** of red clover

flowers. This is due to the adaptation of TP to the different environmental conditions in which it has to live. Typical habitats for this taxon are meadows, pastures and fields located on rather fertile and moist but well-drained soils that occur in lowland, upland and even mountainous regions, reaching up to an altitude of about 3000 m above sea level. In Europe, TP occurs as a native species throughout the whole continent except for parts of the extreme north (Iceland) and south (Turkey), where red clover was introduced. TP is also native to the regions of Northern and Central Asia and some South Asian (India, Iran and Iraq) and North-Western African (Morocco, Algeria, Tunisia) countries. On the other hand, red clover was introduced in vast territories of the Far East, including Siberia with Kamchatka and Sakhalin, Manchuria, Japan, Taiwan or central and eastern parts of China. TP has also been implemented into cultivation on almost the entire Australian continent and appreciated as a fodder plant in countries situated on the west and east coast of South America, except Brazil. As far as North America is concerned, red clover was acquired from Eurasia and introduced into cultivation in the United States in the eighteenth century, not only as a fodder plant but also as a cover crop, in order to improve soil quality. One of TP purple blossom cultivars (var. *americanum* Harz), obtained in breeding in this region, was re-introduced in Central Europe at the end of the nineteenth century (Roskov et al. 2019, POWO 2019).

10.3 Phytochemistry (Specialized Secondary Metabolites)

Due to biological, agricultural and economic importance of red clover, the phytochemical profile of this plant has been intensively studied especially over the last two decades. It has been documented that flavonoids are the main representatives

of polyphenolic compounds found in TP with the dominant group of isoflavone components, which are characteristic for some legumes (Křížová et al. 2019). The chemical structure of isoflavone aglycones comes from the skeleton of 3-phenyl-benzo-4-pyrone, and their diversity is due to the variability in the number and position of hydroxyl and methoxyl substitutents (Fig. 10.2). TP is known for the synthesis of large amounts of isoflavones, which occur mainly in the form of *O*-β-glucosidic conjugates, in the C-7 position of the aglycone structure (Zgórka 2011; Raju et al. 2015). Over the last 20 years, the use of sophisticated combined chromatographic (HPLC) and spectroscopic (MS and NMR) techniques has allowed to obtain more detailed information on the existence of significant quantities of new isoflavone conjugates, namely malonyl and acetyl derivatives of glycosides in various TP extracts (Lin et al. 2000; Klejdus et al. 2001; de Rijke et al. 2001; Polasek et al. 2007; Taujenis et al. 2015). Studies on the accumulation of flavonoid compounds in individual organs of red clover have shown that isoflavones occur mainly in leaves, while other types of flavonoid components dominate in inflorescences (Lin et al. 2000). TP is one of the most valuable plant sources of two isoflavone aglycones,



Isoflavone	Substituent			
	R_1	R_2	R_3	R_4
Aglycones				
Genistein	OH	H	OH	OH
Daidzein	H	H	OH	OH
Biochanin A	OH	H	OH	O-CH_3
Formononetin	H	H	OH	O-CH_3
Glycitein	H	O-CH_3	OH	OH
Prunetin	OH	H	O-CH_3	OH
Glycosides				
Genistin	OH	H	O-Glc	OH
Daidzin	H	H	O-Glc	OH
Sissotrin	OH	H	O-Glc	O-CH_3
Ononin	H	H	O-Glc	O-CH_3

Abbreviations: Glc- glucose

Fig. 10.2 Principal isoflavone aglycones and glycosides identified in red clover

namely biochanin A and formononetin. These compounds are 4′-*O*-methoxylated derivatives of genistein and daidzein found in significant quantities in soybean and, like the latter, are phytoestrogens (Wu et al. 2003; Tsao et al. 2006; Saviranta et al. 2008).

In addition, phytochemical studies on TP have shown a far lower content of remaining isoflavone aglycones, such as glycitein, pratensein, pseudobaptigenin, calycosin, prunetin, orobol, texasin, afrormosin, irilin B and irilone (Klejdus et al. 2001; Wu et al. 2003; Tsao et al. 2006; Polasek et al. 2007). Other important flavonoids identified in TP flowers include flavones (luteolin and apigenin and their derivatives) and flavonols like quercetin, kaempferol, myricetin or their glycosidic conjugates (Klejdus et al. 2001; Tundis et al. 2015). High concentration of quercetin in TP seeds was found by Oleszek and Stochmal (2002), while according to Vlaisavljević et al. (2017), hyperoside was the dominant flavonol glycoside found in red clover in the early growth phase. Another group of TP phenolics, closely related to flavonoids, are blue plant dyes (with a flavane chemical structure) known as anthocyanins. Lee et al. (2020) documented their occurrence in red clover flowers and stated that the main representative of this group was malvidin-3-*O*-galactoside. As representatives of the common shikimate metabolic pathway, several other phenolics, including cinnamic, caffeic, ferulic, *p*-coumaroylquinic, chlorogenic and *p*-hydroxybenzoic acids have been identified in different clover taxa (Klejdus et al. 2001; Kaurinovic et al. 2012; Vlaisavljević et al. 2017; Akbaribazm et al. 2020a). The presence of clovamides, which are derivatives of caffeic acid characteristic for *Trifolium* L. genus, has also been confirmed by some researchers both in the inflorescences and in extracts obtained from TP leaves. (Lin et al. 2000; Polasek et al. 2007; Tava et al. 2015). Furthermore, several saponins, namely soyasaponin I, soyasaponin II and soyasapogenol B, were found in TP seeds by Simonet et al. (1999). As far as volatile, hydrophobic components are concerned, several classes of phytoconstituents, including alcohols, aldehydes, esters, monoterpenes and hydrocarbons, have been found in essential oil and hydroalcoholic red clover extracts using combined chromatographic (GC) and spectroscopic (FID, MS) techniques (Tava et al. 2009; Akbaribazm et al. 2020a).

10.4 Pharmacological Effects and Medicinal Use

Some species of clover, including TP, have been known as medicinal products in the folk medicine of many cultures since the Middle Ages. Based on the data referring to many years of observation and experience, their use in asthma, pertussis and eye inflammation has been reported. (Wu et al. 2003; Sabudak and Guler 2009). So far, confirming the information obtained from traditional sources, it has been reported that water extracts (i.e., fresh infusions prepared from aboveground parts of red clover) effectively alleviate the symptoms accompanying skin diseases such as eczema or psoriasis (Kołodziejczyk-Czepas 2016). Aqueous rinses containing TP polyphenolic components, applied to the skin, are also known as therapeutic anti-inflammatory

preparations for acne (Widyarini et al. 2001). When consumed orally, they have a stabilizing effect on the levels of sex hormones, which are mostly responsible for acne changes (Akdoğan et al. 2018). Thanks to these properties, TP preparations can also prevent androgenic alopecia and reduce hair loss caused by hormonal disorders (Aburjai and Natsheh 2003; Loing et al. 2013).

In addition to the traditional experience-based uses, red clover is currently found in numerous dietary supplements and several herbal medicinal products recommended to alleviate menopause-related complaints resulting from ovarian failure. As far as herbal medicines are concerned, one of the first to be launched on the European pharmaceutical market in 1998 was Promensil®, which contains a standardized extract of red clover isoflavones, at a dose of 80 mg/day, intended to treat menopausal hot flushes (Myers and Vigar 2017). The estrogenic activity of some clover species was discovered in the early twentieth century by Australian scientists when reproduction disorders were observed in sheep after a high intake of *T. subterraneum* L. That isoflavone-based effect, known as "clover disease", ultimately led to infertility and has been confirmed both in sheep and other animal species (Adams 1995; Rochester et al. 2009). In turn, epidemiological data on soybean (one of the legume species containing isoflavones) indicate a direct relationship between high soybean consumption and the observed lower incidence of hormone dependent cancers, osteoporosis or menopause intensity in women from the Asian population (Villaseca 2012). These beneficial effects of soybean products have been attributed to the high content of isoflavones showing estrogen-like effects (Andres et al. 2015). In addition to soybean, the presence of isoflavones has been also documented in green, aboveground parts of numerous *Trifolium* taxa, including red clover. The similarity of their chemical structure to that of a mammalian hormone, namely 17β-estradiol (Fig. 10.3), allows them to interact with estrogen receptors (Kiyama and Wada-Kiyama 2015). However, their potency is relatively low compared to that of animal estrogens, and therefore, a high isoflavone intake is necessary to achieve the desired pharmacological activity (Moreira et al. 2014). With respect to endogenous female hormones, isoflavones have a greater affinity for the beta-type estrogen receptor (ER-β), which is mainly represented in bone tissue, prostate, skin, bladder, testicles, ovaries and brain (Jia et al. 2015; Křížová et al. 2019). The degree of affinity to ER-β and their heterogeneous distribution in human tissues explain the slightly different mode of action of isoflavones compared to synthetic estrogens used in hormone replacement therapy (Prakash and Gupta 2014). Numerous pharmacological studies (including molecular docking experiments carried out in silico) have shown that isoflavones can act as ER agonists or antagonists with similar effects to the synthetic compounds known as selective estrogen receptor modulators—SERMs (Davis 2002; Che et al. 2016; Vitale et al. 2013; Aparecida Santos et al. 2016; Powers and Setzer 2015). Through ER-agonistic effects, they can counteract estrogen deficiencies in the menopause manifested by the development of specific mental (depression), vasomotor (hot flashes, sweats) and physical (osteoporosis) disorders (Table 11.1). On the other hand, as antagonists, isoflavones can compete with estradiol for a binding ER domain, thus combating the negative effects of hyperestrogenism, e.g., ovulation disorders in young women leading to infertility. Isoflavones identified

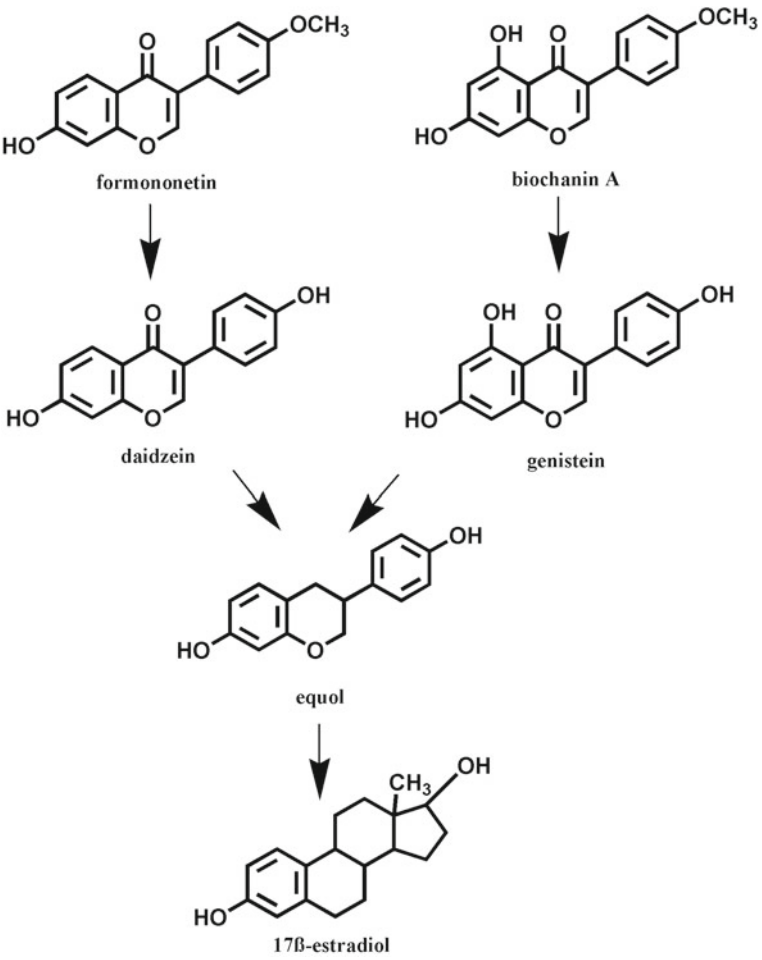


Fig. 10.3 Metabolic transformation of red clover isoflavones to active metabolites

in red clover are mainly found as glycosides. In order to become bioavailable and biologically active, they must undergo a process of hydrolysis to the corresponding aglycons. Further metabolic transformation of aglycones ultimately leads to biologically active products, mainly *S*(-)-equol (Fig. 10.3), which is formed by intestinal microbiota and shows almost 100 times higher estrogenic activity compared to genistein and daidzein (Lagari and Levis 2014; Mayo et al. 2019). Thus, equol is thought to be a key metabolite which can be responsible for most of the biological effects attributed to isoflavone compounds occurring in some legumes (soybean, red clover, etc.).

With reference to the therapeutic uses of red clover, Table 10.1 presents the most important data from current animal and human studies that have provided

Table 10.1 Biological effects confirmed for TP extracts/preparations or individual isoflavones in animal and human studies

Extract/isoflavone (dosage; administration; duration of the treatment)	Animal/human model	General biological effect	Mechanism/tissue effect observed	References
Animal studies				
Ethanolic and aqueous extracts from TP leaves (1 mL/kg; *i.p.*; 7 days)	BALB/C mice	Antioxidant	↓GSHPx, ↓GSHR, ↑Px (aqueous extract), ↑CAT, ↓XOD (aqueous extract), ↓GSH and ↓LPx/increasing serum biochemical parameters responsible for reducing cellular oxidative stress	Kaurinovic et al. (2012)
Combination of 70% ethanolic extract from TP leaves and 70% ethanolic extract from hop flowers (125, 250 and 500 mg/kg; *p.o.*; 12 weeks)	Ovariectomized female Sprague–Dawley rats	↓Symptoms associated with estrogen deficiency	↓Tail skin temperature; ↓fat weight ↓bone metabolism biomarkers (serum ALP, osteocalcin and CTX-1); ↓TC level; ↓endothelin-1 and ↑NO level	Kim et al. (2020)
TP (herbal substance) (normal standard diet supplemented with 8% dried TP; *p.o.*; 14 weeks)	Hyperlipidemic white male rabbits	↓Cardiovascular risk	↓CRP, TG, LDL and TC level ↑HDL/↓atherosclerotic plaque in the aorta and coronary arteries	Asgary et al. (2007)
50% ethanolic dried extract from TP herb (10, 20 mg/kg/day; *p.o.*; 4 weeks)	Ovariectomized female Wistar rats	Anti-osteoporotic	↑Calcium and phosphorus content in bone mineral/strengthening the femoral diaphysis and tibial metaphysis	Cegieła et al. (2012)

(continued)

Table 10.1 (continued)

Extract/isoflavone (dosage; administration; duration of the treatment)	Animal/human model	General biological effect	Mechanism/tissue effect observed	References
TP ethanolic extract standardized to a minimum 30% isoflavone aglycones (4 and 400 mg/kg/day; *p.o.*; 21 days)	Ovariectomized female Sprague–Dawley rats	↓Risk of endometrial cancer in rats with estrogenic deficiency	No estrogenic effect on uterus (lack of uterus and body weight gain)	Overk et al. (2008)
TP ethanolic extract (100–400 mg/kg in combination with doxorubicin, 5 mg/kg; *i.v.*; 5 weeks)	4T1-tumor bearing BALB/c female mice	Anticancer	↓Anti-apoptotic Bcl-2 protein expression ↑pro-apoptotic p53 protein, caspase-3 and Bax mRNA level/↓proliferation of tumor cells	Akbaribazm et al. (2020b)
Formononetin (10 mg/kg; *p.o.*; 4 weeks)	Ovariectomized female Wistar rats	Bone strengthening	↑Bone mineral content/↑mechanical strength of bones	Kaczmarczyk-Sedlak et al. (2013)
Formononetin (12.5–50 mg/kg; *i.p.*; 14 days)	Male Sprague–Dawley rats	Neuroprotective	↓Bax/Bcl-2 ratio associated with PI3K/AKT pathway/improvement of brain function in cerebral ischemia/reperfusion	Liang et al. (2014)
Formononetin (15 mg/kg; *p.o.*; 30 days)	APP/PS1 mice	Neuroprotective	↓β-amyloid formation; ↓pro-inflammatory signaling/alleviating structural changes in hippocampal vascular endothelial cells and ↑learning and memory abilities	Fei et al. (2018)

(continued)

Table 10.1 (continued)

Extract/isoflavone (dosage; administration; duration of the treatment)	Animal/human model	General biological effect	Mechanism/tissue effect observed	References
Biochanin A (100 mg/kg; *p.o.*; 14 days)	Ovariectomized F344 female rats	Vasculoprotective	↓VCAM-1-up regulation; ↓aortic neointima formation/protective effects on endothelial integrity and function	Schrepfer et al. (2006)
The ointment containing 2% of biochanin A (0.1 g/2 × daily; on skin; 3 weeks)	C57BL/6 J mice	↓Skin pigmentation	↓Cellular tyrosinase activity; ↑skin-whitening index/inhibition of melanogenesis in skin	Lin et al. (2000)
Formononetin (5, 10, 20 mg/kg; *p.o.*; 14 days)	BALB/c male nude mice	Anticancer	↓TNF-α and *NF-κB expression*/↓Tumor growth	Huang et al. (2015)
Human studies				
Menoflavon®—capsules—40 mg of standardized TP extract with average isoflavone content of 9% (40 mg; 2 × daily; *p.o.*; 90 days)	53 postmenopausal women	Alleviating menopausal symptoms	Improvement of karyopyknotic, cornification and basal cell maturation indices/positive effects on vaginal cytology ↓LDL, TG and TC/ Reducing hyperlipidemia	Hidalgo et al. (2005)
Menoflavon®—capsules—40 mg of standardized TP extract (40 mg, 2 × daily; *p.o.*; 90 days)	60 postmenopausal women with normal and increased BMI ($\geq$25 kg/m^2)	Hypolipidemic	↓LDL, lipoprotein A and TC/normalization of the serum lipid profile in patients with increased BMI	Chedraui et al. (2008)
TP standardized extract (MF11RCE) containing 40 mg isoflavone aglycones in 1 capsule (40 mg; 2 × daily; *p.o.*; 90 days)	109 postmenopausal women	Alleviating mood disorders	↓Total HADS (76,9%) and SDS (80,6%) scores/reducing symptoms of depression and anxiety accompanying the postmenopausal state	Lipovac et al. (2010)

(continued)

Table 10.1 (continued)

Extract/isoflavone (dosage; administration; duration of the treatment)	Animal/human model	General biological effect	Mechanism/tissue effect observed	References
Menoflavon®—capsules—40 mg of standardized TP extract (40 mg, 2 × daily; *p.o.*; 90 days)	109 postmenopausal women	Alleviating menopausal symptoms	Improvement of skin and hair overall condition; reduction of libido, sleep and mood complaints in treated group	Lipovac et al. (2011)
Menoflavon®—capsules—40 mg of standardized TP extract (40 mg, 2 × daily; *p.o.*; 90 days)	109 postmenopausal women	Alleviating vasomotor menopausal symptoms	↓Kupperman Index values/↓daily hot flushes and night sweat frequency	Lipovac et al. (2012)
TP dried leaves—40 mg/1 capsule (2 capsules daily; *p.o.*; 12 weeks)	72 postmenopausal women	Alleviation of climacteric symptoms	↓Total score of MRS/Significant improvement in the severity of vaginal dryness without any negative side-effects of treatment	Shakeri et al. (2015)
TP aqueous fermented extract (150 mL/day that corresponded to 37.1 mg of isoflavones; *p.o.*; 12 weeks)	60 postmenopausal women	Bone strengthening	↑spinal BMD, ↓CTX-1 in plasma/Improvement in mineral bone status and reducing bone resorption	Thorup et al. (2015)
Promensil® Forte—oral tablets containing a dry TP extract corresponding to 80 mg isoflavones in 1 tablet (80 mg daily; *p.o.*; 24 months)	42 surgically treated women receiving tamoxifen	Alleviating postoperative menopausal symptoms	↓Total score of MRS; lower reduction in BMI compared to placebo group;↑ HDL; ↓breast density/high clinical safety, no interference with tamoxifen treatment; positive effect on plasma lipid profile	Ferraris et al. (2020)

(continued)

Table 10.1 (continued)

Extract/isoflavone (dosage; administration; duration of the treatment)	Animal/human model	General biological effect	Mechanism/tissue effect observed	References
Trinovin®—oral tablets containing 40 mg of standardized TP-derived isoflavone aglycones in 1 tablet (4 tablets = 160 mg daily; *p.o.*; median, 20 days before prostatectomy)	20 men before a radical prostatectomy for prostate cancer	Anticancer	Normalizing effect on morphology of prostate gland; induction of apoptosis/positive antineoplastic effects in low to moderate-grade prostate carcinoma with minimal adverse reactions	Jarred et al. (2002)

ALP alkaline phosphatase; *Bcl-2* B cell lymphoma-2 protein; *Bax* Bcl-2-associated X protein; *BMD* bone mineral density; *BMI* body mass index; *CAT* catalase; *CTX-1* C-terminal telopeptide of type 1 collagen; *CRP* C-reactive protein; *GSH* reduced glutathione; *GSHPx* glutathione peroxidase; *GSHR* glutathione reductase; *HADS* Hospital Anxiety and Depression Scale; *HDL* high density lipoproteins; *i.p.* intraperitoneal administration; *i.v.* intravenous administration; *LDL* low density lipoproteins; *LpA* lipoprotein A; *LPx* extent of lipid peroxidation; *MRS* Menopause Rating Scale; *NF-κB* nuclear factor kappa B; *NO* nitric oxide; *p53* tumor suppressor protein; *PI3K/Akt* phosphatidylinositol 3-kinase/protein kinase B; *p.o.* oral administration; *Px* peroxidase; *SDS* Zung's self rating depression scale; *TC* total cholesterol; *TG* triglycerides; *TNF-α* tumor necrosis factor; *XOD* xanthine oxidase; *VCAM-1* vascular cell adhesion molecule 1

evidence of the efficacy of standardized TP extracts or isolated isoflavone compounds (formononetin, biochanin A) in the treatment of some biodegenerative diseases (osteoporosis or atherosclerosis) associated with estrogen deficiency. Additionally, red clover is widely used as an alternative remedy (compared to synthetic drugs) in hormone replacement therapy recommended for women with menopausal complaints. In turn, the results of preclinical tests on in vitro models indicate potentially new possibilities of therapeutic use of red clover extracts or individual isoflavones. For example, TP isoflavones are considered to affect different tissues and cell types by regulating ER-β expression, which has proved to be fundamental in the pro-apoptotic effect observed in the ER-positive breast cancer cell lines (Chen et al. 2014; Zhou et al. 2014; Zakłos-Szyda and Budryn 2020). New cytostatic effects of TP isoflavones on prostate (Bemis et al. 2004) and colorectal (Huang et al. 2015) cancer cells have also been documented. The development of more advanced scientific tools and techniques, especially in the field of genetics, biochemistry and oncopharmacology, has given an insight into the molecular and cellular mechanisms involved in the anti-tumor action of TP isoflavones. In addition to ER-mediated anticancer effects, it was found that these compounds favour apoptosis by modulating different signaling pathways and inhibit the tumor cell cycle but also enhance the immune response, antioxidant system and anti-inflammatory effects in the host animal (Beck et al. 2005; Chen et al. 2013, 2014; Zhou et al. 2014; Xiao et al. 2017; Yu et al. 2019).

According to recent preclinical studies, the concomitant administration of TP extracts (or individual isoflavones) and synthetic anticancer drugs, such as tamoxifen, temozolomide or doxorubicin, can play a pivotal role in inhibiting tumor development. Due to their synergistic effects, the possibility of dose reduction and increased cytotoxic effects on breast cancer cell lines have been demonstrated (Khazaei et al. 2018; Khazaei and Pazhouhi 2019; Akbaribazm et al. 2020b), although toxicological and other adverse effects should be carefully monitored (Van Duursen et al. 2013; Spagnuolo et al. 2014).

In developing new directions of research related to the use of TP extracts for therapeutic purposes, there is a strong need to precisely determine the phytochemical composition and content of biologically active constituents in these products. Therefore, a validated process of the phytochemical standardization should be implemented each time to guarantee the safety and clinical efficacy of plant formulations recommended for human use.

10.5 Domestication, Cultivation, Agrobiology and Environmental Aspects

The history of domesticating and cultivating red clover dates back to the tenth century and refers to the south-western part of Europe (Spain) from where the species spread to the whole continent and then successfully migrated to all parts of the world. In Europe, the cultivation of TP became particularly popular in the seventeenth

and eighteenth centuries due to increasing urbanization and the need to feed the growing urban population (Kjærgaard 2003). Nowadays, red clover is recognized as one of the most important forage legumes in temperate regions of the world. This plant is well adapted to a wide range of environmental conditions and can be grown on different types of soil, both acidic and alkaline, although it prefers areas reach in clay. TP enjoys full sunshine and does not require a high degree of substrate hydration during vegetation, but it also produces satisfactory yields in areas with higher soil moisture (FAO 2013). Due to specific symbiotic ability to absorb nitrogen from the atmosphere, it is perceived as a natural fertilizer, allowing both to increase the production of cereals and intensify animal breeding for food purposes. Nitrogen fixation by legume plants does indeed play an important role in natural ecosystems, usually characterized by a low soil nutrient content. One of the mechanisms of nitrogen transfer is realized by symbiosis of rhizobial bacteria strains with legumes, including red clover (Courty et al. 2015). The efficiency of atmospheric nitrogen allocation to herbal tissues affects the growth of their biomass which is of key importance in relation to the quantity of plant yields, i.e., the potential amount of feed for farm animals. Moreover, TP adds nitrogen to soils enriching their yield-forming quality in relation to other plant species. According to some experts in agronomy, in order to ensure proper land management in Europe and North America, in addition to new genetic technologies that sustain crop yields and resistance to adverse climatic conditions (high temperatures and lack of rainfall), diversification of northern agro-ecosystems using legumes may be an important approach (Gaudin et al. 2013). One of the plants selected for use in this system is red clover, which during the vegetation period has been successfully interseeded (introduced in crop rotation) with corn, soy or popular cereals (Schipanski and Drinkwater 2010; Wyngaarden et al. 2015). A number of benefits from the use of red clover as a cover plant in a sequential crop rotation system have already been demonstrated. A decrease in the erosion of cultivated areas and an increase in soil fertility was observed related to the improvement of their structure and increase in organic matter content (Kunelius et al. 1992; Stanger and Lauer 2008; Henry et al. 2010). Red clover also reduced the effects of biotic environmental stressors, including weeds and pests (Fisk et al. 2001; Mutch et al. 2003; den Hollander et al. 2007; Chen et al. 2006). TP cultivating also minimized the impact of abiotic factors (drought, heat and wind), interfering with the development of other plants used in crop rotation, by improving soil structure and water accumulation capacity (McKenna et al. 2018).

Regarding the environmental impact on wildlife, red clover is an important source of food for pollinating insects throughout the flowering period which starts in many regions of the temperate zone from late spring to mid-summer and lasts about 2 months (Abberton and Thomas 2011). TP flowers produce a mild honey scent, which attracts various species of insects that collect nectar and pollen. Due to the tubular structure of the individual flowers, the natural habitats and agricultural cultivars of red clover are often visited by long-tongue bumblebees, honeybees and solitary bees which represent about 60, 40 and 1% of all TP pollinating insects, respectively. The flowers of red clover are also visited by butterflies and even moths, which are active during the day. All these insects, apart from harvesting for profit, contribute

to the pollination of the flowers and thus increase the yield of clover seeds (Ishii and Kadoya 2016; Rundlöf et al. 2018; Cong et al. 2020). Other important links of TP to the wildlife are that many species of birds (e.g., geese, quails, grouses and wild turkeys) and various small mammals (wild rabbits, voles and marmots) feed on the leaves, flowers and seeds of red clover. This way, animals maintain their welfare and energy reserves necessary to survive the cold seasons (Monk 1989; Gauthier and Bedard 1991; Rochester and Millam 2009; Prieur and Swihart 2020).

10.6 Biotechnological Approaches

The ability of TP to fix atmospheric nitrogen and provide biomass with high nutritional value significantly increases the value of grasslands in temperate climate zones. However, the relatively low adaptability of this species has resulted in its partial disappearance as a component of pastures and growing areas in recent decades. Therefore, in addition to increasing yields, an important objective of red clover breeding programmes carried out around the world is to increase the longevity of individual plants and their overall ability to adapt to climate and soil conditions using techniques explored in the field of biotechnology (Taylor 2008). Despite a very old tradition of using red clover as a fodder plant, it was only in the 1960s that biotechnological methods of TP breeding began to be introduced, including free inter-layer hybridization and individual and mass selection (Taylor et al. 1963). The varieties of red clover are classified according to their degree of ploidy and maturity. Wild TP is a diploid species ($2n = 14$), while tetraploid varieties have been genetically processed by doubling the chromosomes in the cells of diploid plants using colchicine. In Europe, such efforts have been made, among others, by scientists from Lithuania, where red clover is the dominant species found in meadows and pastures. To achieve this, in vitro methods for chromosome doubling and tetraploid plant breeding have been developed. As a result, 60% of stable tetraploid plants were obtained and successfully cultured in subsequent progeny of TP (Rebāne et al. 2016). The introduction of early flowering cultivars, giving about two equal yields during the vegetation period, and late flowering varieties giving most of the biomass at the first mowing was one of the key achievements associated with the introduction of hybridized tetraploid forms of red clover (Smýkal et al. 2015). Besides, higher resistance to some fungal leaf diseases (e.g., sugar-beet powdery mildew) has also been observed in tetraploid clovers (Jakešová et al. 2011). TP is a typical outcrossing and self-incompatible crop possessing a specific genetic mechanism which prevents self-fertilization (Taylor and Smith 1979). Therefore, this taxon is difficult to propagation, because inbreeding by self-seeding usually fails to continue for more than two or three generations due to the loss of vigor and yield quality. So far, numerous attempts to improve the biological strength and adaptability of TP have been made using interspecific hybridization (Abberton 2007). For example, in the 1980s and 1990s, successful crossbreeds between zigzag (*T. medium*), owl-head (*T. alpestre*) and tetraploid red clover were developed using embryo rescue procedures in in vitro

cultures (Merker 1984; Sawai et al. 1990; Phillips et al. 1992). However, at the turn of the twentieth and twenty-first century, the research carried out by Czech plant breeding specialists (from the Research Institute for Fodder Crops in Troubsko) has proved to be an important breakthrough (Řepková et al. 2003). These studies resulted in obtaining a new stable hybrid of red and zigzag clover (variety "Pramedi", licensed in 2013) with well-developed short rhizomes, able to reproduce from seeds and giving a high yield of biomass. Owing to hybridization, TP as a non-rhizomatous perennial plant has gained new opportunities for vegetative propagation and increased environmental expansion with subsequent improved persistence (Jakešová et al. 2011 and 2014).

10.7 Perspectives

Considering the botanical origin of the red clover as an important member of a vast family of legumes entering into unique relationships with its biological environment, the future prospects of this taxon are undoubtedly linked to the development of modern agriculture and agrobiology. Deepening research on the mechanisms of various symbiotic interactions between legumes and rhizobial bacteria can contribute to the development of pro-ecological trends in global agriculture associated with increased biological fixation of atmospheric nitrogen and limited use of industrial nitrogen fertilizers. Analyzing these issues on the basis of scientific literature, we can observe a slow growth of the worldwide arable land for red clover. This shows that TP is still seen as a cultivated plant with many benefits for the quality of the soils it grows on and providing valuable high-protein feed for livestock. Therefore, further development of red clover breeding programmes based on biotechnological achievements can be expected. This may result in the introduction of new hybrids characterized by increased viability and propagation capacity, high production of green biomass, competitiveness against weeds and resistance to diseases, pests and low temperatures. Additional, important support for biotechnology may be also provided by the ongoing genetic research on red clover. These studies has already resulted in the development of a reference genome for some red clover cultivars (Dias et al. 2008; Ištvánek et al. 2014; De Vega et al. 2015; Dluhošová et al. 2018) and closely related species (*T. medium*).

A second platform, where new practical applications for red clover may arise, is scientific research on herbal medicines and health-promoting products. Preclinical and clinical studies, conducted so far, confirm the validity of the use of both extracts and isolated isoflavone components from green parts of red clover in the prevention and therapy of biodegenerative diseases that are related to disorders of sexual hormone production observed during the meno- or even andropause. This opens up the prospect of developing new food supplements and/or pharmaceutical preparations, especially for older women, which could inhibit bone degeneration (osteoporosis), dysfunction of reproductive organs or vascular endothelial damage associated with hyperlipidemia and atherosclerosis. In order to achieve appropriate

pharmacological effects in patients, new technologies and production of standardized extracts, certified by independent control agencies, should be developed. In this respect, the phytochemical standardization of plant preparations (confirming the botanical identity of the herbal substance, the content of the active ingredients and their purity) is particularly important, taking into account current international requirements in terms of the quality, safety and efficacy of herbal medicinal products (Pandey and Tripathi 2014).

References

Abberton MT (2007) Interspecific hybridization in the genus *Trifolium*. Plant Breed 126:337–342

Abberton MT, Thomas I (2011) Genetic resources in *Trifolium* and their utilization in plant breeding. Plant Genet Res Charact Util 9:38–44

Aburjai T, Natsheh FM (2003) Plants used in cosmetics. Phytother Res 17(9):987–1000

Adams NR (1995) Detection of the effects of phytoestrogens on sheep and cattle. J Animal Sci 73:1509–1515

Akbaribazm M, Khazaei MR, Khazaei M (2020b) *Trifolium pratense* L. (red clover) extract and doxorubicin synergistically inhibits proliferation of 4T1 breast cancer in tumor-bearing BALB/c mice through modulation of apoptosis and increase antioxidant and anti-inflammatory related pathways. Food Sci Nutr (in press)

Akbaribazm M, Khazaei MR, Khazaei M (2020a) Phytochemicals and antioxidant activity of alcoholic/hydroalcoholic extract of *Trifolium pratense*. Chin Herb Med (in press)

Akdoğan N, Doğan S, Atakan N, Yalçın B (2018) Association of serum hormone levels with acne vulgaris: low estradiol level can be a pathogenetic factor in female acne. Our Dermatol Online 9:249–256

Amossé C, Jeuffroy MH, Celette F, David C (2013) Relay-intercropped forage legumes help to control weeds in organic grain production. Euro J Agron 49:158–167

Andres S, Hansen U, Niemann B et al (2015) Determination of the isoflavone composition and estrogenic activity of commercial dietary supplements based on soy or red clover. Food Funct 6(6):2017–2025

Aparecida Santos M, Florencio-Silva R, Teixeira CP et al (2016) Effects of early and late treatment with soy isoflavones in the mammary gland of ovariectomized rats. Climacteric 19:77–84

Asgary S, Moshtaghian J, Naderi G et al (2007) Effects of dietary red clover on blood factors and cardiovascular fatty streak formation in hypercholesterolemic rabbits. Phytother Res 21(8):768–770

Beck V, Rohr U, Jungbauer A (2005) Phytoestrogens derived from red clover: an alternative to estrogen replacement therapy? J Steroid Biochem 94(5):499–518

Bemis DL, Capodice JL, Desai M et al (2004) A concentrated aglycone isoflavone preparation (GCP) that demonstrates potent anti-prostate cancer activity in vitro and in vivo. Clin Cancer Res 10(15):5282–5292

Broderick GA, Walgenbach RP, Maignan S (2001) Production of lactating dairy cows fed alfalfa or red clover silage at equal dry matter or crude protein contents in the diet. J Dairy Sci 84:1728–1737

Bruneau A, Doyle JJ, Herendeen P et al (2013) Legume phylogeny and classification in the 21st century: progress, prospects and lessons for other species-rich clades. Taxon 62(2):217–248

Cegieła U, Folwarczna J, Pytlik M, Zgórka G (2012) Effects of extracts from *Trifolium medium* L. and *Trifolium pratense* L. on development of estrogen deficiency-induced osteoporosis in rats. Evid-Based Compl Alt 2012 Article ID 921684:11 pages

Che CT, Wong MS, Lam CWK (2016) Natural products from Chinese medicines with potential benefits to bone health. Molecules 21:239

Chedraui P, San Miguel G, Hidalgo L et al (2008) Effect of *Trifolium pratense*-derived isoflavones on the lipid profile of postmenopausal women with increased body mass index. Gynecol Endocrinol 24(11):620–624

Chen S, Wyse DL, Johnson GA et al (2006) Effect of cover crops alfalfa, red clover, and perennial ryegrass on soybean cyst nematode population and soybean and corn yields in minnesota. Crop Sci 46:1890–1897

Chen J, Zhao X, Ye Y, Wang Y, Tian J (2013) Estrogen receptor beta-mediated proliferative inhibition and apoptosis in human breast cancer by calycosin and formononetin. Cell Physiol Biochem 32(6):1790–1797

Chen J, Hou R, Zhang X (2014) Calycosin suppresses breast cancer cell growth via ERβ-dependent regulation of IGF-1R p38 MAPK and PI3K/Akt pathways. PLoS ONE 9(3):e91245

Chen MN, Lin CC, Liu CF (2015) Efficacy of phytoestrogens for menopausal symptoms: a meta-analysis and systematic review. Climacteric 18:260–269

Cong W, Dupont YL, Søegaard K, Eriksen J (2020) Optimizing yield and flower resources for pollinators in intensively managed multi-species grasslands. Agr Ecosyst Environ 302:107062

Conklin AE, Erich MS, Liebman M (2002) Effects of red clover (*Trifolium pratense*) green manure and compost soil amendments on wild mustard (Brassica kaber) growth and incidence of disease. Plant Sci 238:245–256

Courty PE, Smith P, Koegel S et al (2015) Inorganic nitrogen uptake and transport in beneficial plant root-microbe interactions. Crit Rev Plant Sci 34(1–3):4–16

Crews TE, Peoples MB (2004) Legume versus fertilizer sources of nitrogen: ecological tradeoffs and human needs. Agr Ecosyst Environ 102(3):279–297

Davis SR (2002) Phytoestrogens in the context of SERMs. In: Manni A, Verderame MF (eds) Selective estrogen receptor modulators. Humana Press, Totowa, NJ, pp 345–363

den Hollander NG, Bastiaans L, Kropff MJ (2007) Clover as a cover crop for weed suppression in an intercropping design: II. Competitive ability of several clover species. Eur J Agron 26(2):104–112

de Rijke E, Zafra-Gómez A, Ariese F et al (2001) Determination of isoflavone glucoside malonates in *Trifolium pratense* L (red clover) extracts: quantification and stability studies. J Chromatogr A 932(1–2):55–64

De Vega J, Ayling S, Hegarty M et al (2015) Red clover (*Trifolium pratense* L.) draft genome provides a platform for trait improvement. Sci Rep 5:17394

Dias PMB, Julier B, Sampoux J et al (2008) Genetic diversity in red clover (*Trifolium pratense* L.) revealed by morphological and microsatellite (SSR) markers. Euphytica 160:189–205

Dluhošová J, Ištvánek J, Nedělník J, Řepková J (2018) Red clover (*Trifolium pratense*) and zigzag clover (*T. medium*)—a picture of genomic similarities and differences. Front Plant Sci 9:724

Dornstauder E, Jisa E, Unterrieder I et al (2001) Estrogenic activity of two standardized red clover extracts (Menoflavon) intended for large scale use in hormone replacement therapy. J Steroid Biochem Mol Biol 78:67–75

FAO (2013) Grassland index. A searchable catalogue of grass and forage legumes. Red clover (*Trifolium pratense*) FAO, Rome, Italy

Fauvart M, Michiels J (2008) Rhizobial secreted proteins as determinants of host specificity in the rhizobium-legume symbiosis. FEMS Microbiol Lett 285(1):1–9

Fei HX, Zhang YB, Liu T (2018) Neuroprotective effect of formononetin in ameliorating learning and memory impairment in mouse model of Alzheimer's disease. Biosci Biotech Biochem 82(1):57–64

Ferraris C, Ballestra B, Listorti C (2020) Red clover and lifestyle changes to contrast menopausal symptoms in premenopausal patients with hormone-sensitive breast cancer receiving tamoxifen. Breast Cancer Res Treat 180(1):157–165

Fisk JW, Hesterman OB, Shrestha A et al (2001) Weed suppression by annual legume cover crops in no-tillage corn. Agron J 93:319–325

Gaudin ACM, Westra S, Loucks CES et al (2013) Improving resilience of northern field crop systems using inter-seeded red clover: a review. Agronomy 3:148–180

Gauthier G, Bedard J (1991) Experimental tests of the palatability of forage plants in greater snow geese. J Appl Ecol 28(2):491–500
Henry DC, Mullen RW, Dygert CE et al (2010) Nitrogen contribution from red clover for corn following wheat in western Ohio. Agron J 102:210–215
Hidalgo LA, Chedraui PA, Morocho N et al (2005) The effect of red clover isoflavones on menopausal symptoms, lipids and vaginal cytology in menopausal women: a randomized, double-blind, placebo-controlled study. Gynecol Endocrinol 21(5):257–264
Huang J, Xie M, Gao P (2015) Antiproliferative effects of formononetin on human colorectal cancer via suppressing cell growth in vitro and in vivo. Process Biochem 50(6):912–917
Ishii HS, Kadoya EZ (2016) Legitimate visitors and nectar robbers on *Trifolium pratense* showed contrasting flower fidelity versus co-flowering plant species: could motor learning be a major determinant of flower constancy by bumble bees? Behav Ecol Sociobiol 70:377–386
Ištvánek J, Jaros M, Krenek A, Řepková J (2014) Genome assembly and annotation for red clover (*Trifolium pratense*; Fabaceae). Am J Bot 101:327–337
Jakešová H, Řepková J, Hampel D et al (2011) Variation of morphological and agronomic traits in hybrids of *Trifolium pratense* × *T. medium* and a comparison with the parental species. Czech J Genet Plant Breed 47:28–36
Jakešová H, Hampel D, Řepková J, Nedělník J (2014) Evaluation of feeding characteristics in variety Pramedi—interspecific hybrid *Trifolium pratense* × *Trifolium medium*. Úroda 12:183–186
Jarred RA, Keikha M, Dowling C (2002) Induction of apoptosis in low to moderate-grade human prostate carcinoma by red clover-derived dietary isoflavones. Cancer Epidem Biomar 11(12):1689–1696
Jia M, Dahlman-Wright K, Jå G (2015) Estrogen receptor alpha and beta in health and disease. Best Pract Res Cl En 29:557–568
Kaczmarczyk-Sedlak I, Wojnar W, Zych M (2013) Effect of formononetin on mechanical properties and chemical composition of bones in rats with ovariectomy-induced osteoporosis. Evid Based Compl Alt 2013, Article ID 457052:10 pages
Kaurinovic B, Popovic M, Vlaisavljevic S (2012) Antioxidant profile of *Trifolium pratense* L. Molecules 17(9):11156–11172
Khazaei M, Pazhouhi M (2019) Antiproliferative effect of *Trifolium pratens* L extract in human breast cancer cells. Nutr Cancer 71(1):128–140
Khazaei M, Mona Pazhouhi and Saber Khazaei (2018) Evaluation of hydro-alcoholic extract of *Trifolium pratense* L. for its anti-cancer potential on U87MG cell line. Cell J (Yakhteh) 20(3):412–421
Kim MR, Kim HJ, Yu SH et al (2020) Combination of red clover and hops extract improved menopause symptoms in an ovariectomized rat model. Evid Based Compl Alt 2020, Article ID 7941391:9 pages
Kiyama R, Wada-Kiyama Y (2015) Estrogenic endocrine disruptors: molecular mechanisms of action. Environ Int 83:11–40
Kjærgaard T (2003) A plant that changed the world: the rise and fall of clover 1000–2000. Landscape Res 28(1):41–49
Klejdus B, Vitamvásová-Sterbova D, Kuban V (2001) Identification of isoflavone conjugates in red clover (*Trifolium pratense*) by liquid chromatography–mass spectrometry after two-dimensional solid-phase extraction. Anal Chim Acta 450(1–2):81–97
Kołodziejczyk-Czepas J (2016) *Trifolium* species–the latest findings on chemical profile, ethnomedicinal use and pharmacological properties. J Pharm Pharmacol 68(7):845–861
Křížová L, Dadáková K, Kašparovská J, Kašparovský T (2019) Isoflavones. Molecules 24(6):1076
Kunelius HT, Johnston HW, Macleod JA (1992) Effect of undersowing barley with Italian ryegrass or red clover on yield, crop composition and root biomass. Agric Ecosyst Environ 38:127–137
Lagari VS, Levis S (2014) Phytoestrogens for menopausal bone loss and climacteric symptoms. J Steroid Biochem 139:294–301

Lee SG, Brownmiller CR, Lee SO et al (2020) Anti-inflammatory and antioxidant effects of anthocyanins of *Trifolium pratense* (Red Clover) in lipopolysaccharide-stimulated RAW-267.4 macrophages. Nutrients 12(4):1089

Liang K, Ye Y, Wang Y et al (2014) Formononetin mediates neuroprotection against cerebral ischemia/reperfusion in rats via downregulation of the Bax/Bcl-2 ratio and upregulation PI3K/Akt signaling pathway. J Neurol Sci 344(1–2):100–104

Lin LZ, He XG, Lindenmaier M et al (2000) LC-ESI-MS study of the flavonoid glycoside malonates of red clover (*Trifolium pratense*). J Agric Food Chem 48(2):354–365

Lipovac M, Chedraui P, Gruenhut C et al (2010) Improvement of postmenopausal depressive and anxiety symptoms after treatment with isoflavones derived from red clover extracts. Maturitas 65(3):258–261

Lipovac M, Chedraui P, Gruenhut Ch et al (2011) Effect of red clover isoflavones over skin appendages and mucosal status in postmenopausal women. Obst Gynecol Int 2011, Article ID 949302:6 pages

Lipovac M, Chedraui P, Gruenhut Ch et al (2012) The effect of red clover isoflavone supplementation over vasomotor and menopausal symptoms in postmenopausal women. Gynecol Endocrinol 28:203–207

Loing E, Lachance R, Ollier V, Hocquaux M (2013) A new strategy to modulate alopecia using a combination of two specific and unique ingredients. J Cosmet Sci 64(1):45–58

Mayo B, Vázquez L, Flórez AB (2019) Equol: a bacterial metabolite from the daidzein isoflavone and its presumed beneficial health effects. Nutrients 11(9):2231

McKenna P, Cannon N, Conway J, Dooley J (2018) The use of red clover (*Trifolium pratense*) in soil fertility-building: a review. Field Crop Res 221:38–49

Merker A (1984) Hybrids between *Trifolium medium* and *T. pratense*. Hereditas 101:267–268

Monk KA (1989) Effects of diet composition on intake by adult wild European rabbits. Appetite 13(3):201–209

Moreira AC, Silva AM, Santos MS, Sardão VA (2014) Phytoestrogens as alternative hormone replacement therapy in menopause: what is real, what is unknown. J Steroid Biochem 143:61–71

Mutch DR, Martin TE, Kosola KR (2003) Red clover (*Trifolium pratense*) suppression of common ragweed (Ambrosia) in winter wheat (*Triticum aestivum*). Weed Technol 17:181–185

Myers SP, Vigar V (2017) Effects of a standardised extract of *Trifolium pratense* (Promensil) at a dosage of 80 mg in the treatment of menopausal hot flushes: a systematic review and meta-analysis. Phytomedicine 24:141–147

Ohno T, Doolan KL (2001) Effects of red clover decomposition on phytotoxicity to wild mustard seedling growth. Appl Soil Ecol 16:187–192

Oleszek W, Stochmal A (2002) Triterpene saponins and flavonoids in the seeds of *Trifolium* species. Phytochemistry 61(2):165–170

Overk CR, Guo J, Chadwick LR et al (2008) In vivo estrogenic comparisons of *Trifolium pratense* (red clover) *Humulus lupulus* (hops) and the pure compounds isoxanthohumol and 8-prenylnaringenin. Chem Biol Interact 176(1):30–39

Pandey A, Tripathi S (2014) Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J Pharmacogn Phytochem 2(5):115–119

Patisaul HB, Jefferson W (2010) The pros and cons of phytoestrogens. Front Neuroendocrinol 31(4):400–419

Phillips GC, Grosser JW, Berger S et al (1992) Interspecific hybridisation between red clover and *Trifolium alpestre* using in vitro embryo culture. Crop Sci 32:1113–1115

Polasek J, Queiroz EF, Hostettmann K (2007) On-line identification of phenolic compounds of *Trifolium* species using HPLC-UV-MS and postcolumn UV-derivatisation. Phytochem Anal 18(1):13–23

Powers CN, Setzer WN (2015) A molecular docking study of phytochemical estrogen mimics from dietary herbal supplements. Silico Pharmacol 3:4

POWO (2019) Plants of the world online. Facilitated by the Royal Botanic Gardens, Kew. Published on the internet http://www.plantsoftheworldonline.org/. Retrieved on 04 Aug 2020

Prakash D, Gupta Ch (2014) Role of phytoestrogens as nutraceuticals in human health. In: Prakash D, Sharma G (eds) Phytochemicals of nutraceutical importance. CABI International, Boston, pp 148–149

Prieur AA, Swihart RK (2020) Palatability of common cover crops to voles (Microtus). Crop Prot 133:105141

Raju KSR, Kadian N, Taneja I et al (2015) Phytochemical analysis of isoflavonoids using liquid chromatography coupled with tandem mass spectrometry. Phytochem Rev 14(3):469–498

Rebāne A, Grauda D, Rancāne S et al (2016) Use of biotechnology methods in red clover (*Trifolium pratense* L.) breeding. In: Proceedings of the scientific and practical conference harmonious agriculture, Feb 25–26, 2016, Jelgava. Latvia University of Agriculture, pp 102–106

Reckling M, Bergkvist G, Watson CA et al (2020) Re-designing organic grain legume cropping systems using systems agronomy. Eur J Agron 112:125951

Řepková J, Jungmannová B, Jakešová H (2003) Interspecific hybridisation prospects in the genus *Trifolium*. Czech J Genet Plant Breed 39:306–308

Rochester JR, Millam JR (2009) Phytoestrogens and avian reproduction: exploring the evolution and function of phytoestrogens and possible role of plant compounds in the breeding ecology of wild birds. Comp Biochem Phys A 154(3):279–288

Rochester JR, Klasing KC, Stevenson L et al (2009) Dietary red clover (*Trifolium pratense*) induces oviduct growth and decreases ovary and testes growth in Japanese quail chicks. Reprod Toxicol 27(1):63–71

Roskov Y, Zarucchi J, Novoselova M, Bisby F (eds.) (2019) ILDIS world database of legumes (version 12, May 2014). In: Roskov Y, Ower G, Orrell T et al (eds) Species 2000 & ITIS catalogue of life, 2019 annual checklist. Digital resource at www.catalogueoflife.org/annual-checklist/2019. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-884X

Rundlöf M, Lundin O, Bommarco R (2018) Annual flower strips support pollinators and potentially enhance red clover seed yield. Ecol Evol 8(16):7974–7985

Sabudak T, Guler N (2009) *Trifolium* L.—a review on its phytochemical and pharmacological profile. Phytother Res 23:439–446

Saviranta NM, Anttonen MJ, Wright A et al (2008) Red clover (*Trifolium pratense* L) isoflavones: determination of concentrations by plant stage flower colour plant part and cultivar. J Sci Food Agric 88(1):125–132

Sawai A, Ueda S, Gau M, Uchiyama K (1990) Interspecific hybrids of *Trifolium medium* L. × 4x *T. pratense* obtained through embryo culture. J Japan Grassl Sci 35:267–272

Schipanski ME, Drinkwater LE (2010) Nitrogen fixation of red clover interseeded with winter cereals across a management-induced fertility gradient. Nutr Cycl Agroecos 90:105–119

Schrepfer S, Deuse T, Münze T et al (2006) The selective estrogen receptor-β agonist biochanin A shows vasculoprotective effects without uterotrophic activity. Menopause 13(3):489–499

Shakeri F, Taavoni S, Goushegir A, Haghani H (2015) Effectiveness of red clover in alleviating menopausal symptoms: a 12-week randomized, controlled trial. Climacteric 18(4):568–573

Simonet AM, Stochmal A, Oleszek W et al (1999) Saponins and polar compounds from *Trifolium resupinatum*. Phytochemistry 51(8):1065–1067

Smýkal P, Coyne CJ, Ambrose MJ et al (2015) Legume crops phylogeny and genetic diversity for science and breeding. Crit Rev Plant Sci 34(1–3):43–104

Spagnuolo P, Rasini E, Luini A et al (2014) Isoflavone content and estrogenic activity of different batches of red clover (*Trifolium pratense* L) extracts an in vitro study in MCF-7 cells. Fitoterapia 94:62–69

Stanger TF, Lauer JG (2008) Corn grain yield response to crop rotation and nitrogen over 35 years. Agron J 100:643–650

Taujenis L, Padarauskas A, Mikaliuniene J et al (2015) Identification of isoflavones and their conjugates in red clover by liquid chromatography coupled with DAD and MS detectors. Chemija 26(2):107–112

Tava A, Ramella D, Grecchi M et al (2009) Volatile constituents of *Trifolium pratense* and *T. repens* from NE Italian alpine pastures. Nat Prod Commun 4(6):1934578X0900400619

Tava A, Pecio Ł, Stochmal A, Pecetti L (2015) Clovamide and flavonoids from leaves of *Trifolium pratense* and *T. pratense* subsp. *nivale* grown in Italy. Nat Prod Commun 10(6): 1934578X1501000635

Taylor NL (2008) A century of clover breeding developments in the United States. Crop Sci 48:1–13

Taylor NL, Smith RR (1979) Red clover breeding and genetics. Adv Agron 31:125–154

Taylor NL, Stroube WH, Collins GB et al (1963) Interspecific hybridization of red clover (*Trifolium pratense* L.). Crop Sci 3:549–552

The Plant List (2013) Version 1.1. Published on the internet; http://www.theplantlist.org/. Accessed on 01 Jan

Thorup AC, Lambert MN, Kahr HS et al (2015) Intake of novel red clover supplementation for 12 weeks improves bone status in healthy menopausal women. Evid Based Compl Alt. Article ID 689138:11 pages

Tsao R, Papadopoulos Y, Yang R et al (2006) Isoflavone profiles of red clovers and their distribution in different parts harvested at different growing stages. J Agric Food Chem 54(16):5797–5805

Tundis R, Marrelli M, Conforti F et al (2015) *Trifolium pratense* and *T. repens* (Leguminosae): Edible flower extracts as functional ingredients. Foods 4(3):338–48

Tutin TG, Heywood VH, Burges NA et al (1968) Flora Europaea. In: Rosaceae to Umbelliferae, vol 2. Cambridge University Press, Cambridge, pp 157–168

USDA Agricultural Research Service (2015) Germplasm Resources Information Network (GRIN). Ag Data Commons. https://doi.org/10.15482/USDA.ADC/1212393

Van Duursen MB, Smeets EE, Rijk JC, Nijmeijer SM, van den Berg M (2013) Phytoestrogens in menopausal supplements induce ER-dependent cell proliferation and overcome breast cancer treatment in an in vitro breast cancer model. Toxicol Appl Pharmacol 269(2):132–140

Villaseca P (2012) Non-estrogen conventional and phytochemical treatments for vasomotor symptoms: what needs to be known for practice. Climacteric 15:115–124

Vitale DC, Piazza C, Melilli B et al (2013) Isoflavones: estrogenic activity, biological effect and bioavailability. Eur J Drug Metab Pharmacokinet 38:15–25

Vlaisavljević S, Kaurinović B, Popović M, Vasiljević S (2017) Profile of phenolic compounds in *Trifolium pratense* L. extracts at different growth stages and their biological activities. Int J Food Prop 20(12):3090–3101

Widyarini S, Spinks N, Husband AJ, Reeve VE (2001) Isoflavonoid compounds from red clover (*Trifolium pratense*) protect from inflammation and immune suppression induced by UV radiation. Photochem Photobiol 74(3):465–470

Woods NF, Mitchell ES (2005) Symptoms during perimenopause: prevalence, severity, trajectory, and significance. Am J Med 118(12B):14–24

Wu Q, Wang M, Simon JE (2003) Determination of isoflavones in red clover and related species by high-performance liquid chromatography combined with ultraviolet and mass spectrometric detection. J Chromatogr A 1016(2):195–209

Wyngaarden SL, Gaudin A, Deen W, Martin RC (2015) Expanding red clover (*Trifolium pratense*) usage in the corn-soy-wheat rotation. Sustainability 7:15487–15509

Xiao P, Zheng B, Sun J et al (2017) Biochanin A induces anticancer effects in SK-Mel-28 human malignant melanoma cells via induction of apoptosis inhibition of cell invasion and modulation of NF-κB and MAPK signaling pathways. Oncol Lett 14(5):5989–5993

Yu C, Zhang P, Lou L, Wang Y (2019) Perspectives on the role of biochanin A in human. Front Pharmacol 10:793

Zakłos-Szyda M, Budryn G (2020) The effects of *Trifolium pratense* L sprouts' phenolic compounds on cell growth and migration of MDA-MB-231 MCF-7 and HUVEC cells. Nutrients 12(1):257

Zgórka G (2011) Studies on phytoestrogenic and nonphytoestrogenic compounds in *Trifolium incarnatum* L. and other clover species using pressurized liquid extraction and high performance column liquid chromatography with photodiode-array and fluorescence detection. J AOAC Int 94(1):22–31

Zhou R, Xu L, Ye M et al (2014) Formononetin inhibits migration and invasion of MDA-MB-231 and 4T1 breast cancer cells by suppressing MMP-2 and MMP-9 through PI3K/AKT signaling pathways. Horm Metab Res 46(11):753–760

Żuk-Gołaszewska K, Purwin C, Pysera B et al (2010) Yields and quality of green forage from red clover di- and tetraploid forms. J Elementol 15(4):757–770

Chapter 11
Cultivation and Utilization of Diosgenin-Contained *Dioscorea* Species

Wellington Ferreira do Nascimento, Marcos Vinicius Bohrer Monteiro Siqueira, Edson Ferreira da Silva, and Elizabeth Ann Veasey

Abstract The genus *Dioscorea*, family Dioscoreaceae, has 633 species, all popularly known as yam, of which approximately 137 contain the diosgenin compound. Diosgenin, a phytosteroid sapogenin, is used as a raw material for the synthesis of steroid hormones that make up several types of drugs, such as adrenal cortical hormone, sex hormone, birth control pills, anabolic hormones, among others. Diosgenin also presents pharmacological activities such as anti-inflammatory, antimicrobial, and hypoglycemic, which allows its utilization to treat several diseases such as osteoporosis, diabetes, and obesity. Currently, the amount of diosgenin extracted from yam species have decreased due to extensive harvesting and consequently decline of *Dioscorea* spp. populations, as well as the lack of adequate technologies capable of extracting this compost on a large scale. Thus, it is necessary to identify species and/or accessions of *Dioscorea* with a higher diosgenin content to attend the demand for diosgenin extraction. However, a uniform procedure for the preparation of samples and analysis of diosgenin is highly desirable. In this review, we are providing information about the origin, domestication, geographic distribution, cultivation, utilization, and medicinal properties of diosgenin-contained *Dioscorea* species.

Keywords Domestication · Geographic distribution · Medicinal properties · Saponins · Secondary metabolites · Yams

W. F. do Nascimento
Center for Agricultural and Environmental Sciences, Federal University of Maranhão, Chapadinha, Maranhão 65500-000, Brazil

M. V. B. M. Siqueira
Campus Frutal, Minas Gerais State University, Frutal, Minas Gerais 38200-000, Brazil

E. F. da Silva
Biology Department, Federal Rural University of Pernambuco, Recife, Pernambuco 52171-900, Brazil

E. A. Veasey (✉)
Center for Agricultural and Environmental Sciences, Federal University of Maranhão, Chapadinha, Maranhão 65500-000, Brazil
e-mail: eaveasey@usp.br

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_11

11.1 Introduction

The word "yam" is derived from the languages of West Africa, Mande, "niam" or Temne, "enyame", later adopted in Portuguese as "ynhame", in Spanish as "tame", in French as "igname", and English as "yam" (Burkill 1938). Although this term is currently used loosely to refer to many species that produce edible roots, true yams are only those belonging to the family Dioscoreaceae, genus *Dioscorea* (Barton 2014).

The genus *Dioscorea*, of about 633 species (Couto et al. 2018), usually consists of climbing plants with underground tubers or aerial bulbs, with heart-shaped leaves, small green or white flowers and a winged capsule or berry fruit. Species of this genus are widely distributed in tropical, subtropical, and temperate regions of the world, and much is due to the time of great sailing, where Spanish and Portuguese sailors took the yam from Southeast Asia to the New World (Barton 2014).

Nutritionally, underground tubers and aerial yam bulbs are rich in vitamins A, C, and the B complex (thiamine, riboflavin, and niacin), in addition to being a good source of carbohydrates and presenting considerable levels of proteins and lipids (Oliveira et al. 2007). Regarding its medicinal properties, the low glycemic index of yam present when ingested in the diet allows its indication to diabetic people, with obesity problems, osteoporosis, among other hormonal and metabolic diseases (Siadjeu et al. 2015; Azeteh et al. 2019).

Among the compounds present in the yam species, diosgenin stands out. Diosgenin is a sapogenin widely used in the synthesis of steroid drugs discovered in 1936 by Fujii and Matsukawa (Martin 1969). After the Second World War, the growing need for steroid drugs and the high cost for obtaining this substance from an animal source led to the widespread search for plant sources of diosgenin, favoring the development of the steroid industry. This industry has been has been using mainly two species, *D. composita* Hemsl and *D. floribunda* Mart and Gal., originally from Mexico and Central America, respectively. However, other species of *Dioscorea* have been commercially exploited in the steroid industry, such as *D. sylvatica* Ecklon, from South Africa, and *D. deltoidea* Waal., from India (Martin 1969; Price et al. 2016).

Diosgenin, a bioactive phytochemical, is not only used as a raw material for the preparation of steroid drugs in the pharmaceutical industry but also in the treatment of various types of disorders, such as cancer, hypercholesterolemia, inflammation, and various types of infections (Jesus et al. 2016). Due to its pharmacological and industrial importance, several extractions and analysis procedures have been developed over the years in order to identify, isolate, and quantify natural sources of diosgenin (Jesus et al. 2016).

It is worth mentioning that studies related to prospecting for secondary metabolites in yam tubers have been restricted to a few species, despite the great diversity of Neotropical *Dioscorea* (Mignouna et al. 2009). Another limitation is the scarcity of new technologies capable of optimizing the extraction of diosgenin and large-scale synthesis of drugs produced from this compound.

Based on this context, the objective of this chapter was to present a review on the origin, domestication, genetic diversity and geographical distribution of *Dioscorea*

species, with emphasis on the diosgenin-contained species, as well as to present the general molecular structure, medicinal properties, and use of diosgenin by the pharmaceutical industry.

11.2 Origin, Domestication, and Genetics of *Dioscorea* Spp.

The species of the Dioscoreaceae family occur worldwide; however, their origin is still controversial (Castro et al. 2012). The *Dioscorea* genus originated in the Laurasian Palaearctic region, between the late Cretaceous (57.7–85.9 Mya) and the Mid Eocene (47.6–49.1 Mya), with subsequent radiations to the southern regions by long-distance dispersal or migration by land bridges in the Oligocene–Miocene (33.90–5.33 Mya) (Viruel et al. 2010). Although this genus has some species in the subtropical and temperate regions, its higher frequency and diversity occur in the Neotropics, where there are around 50% of the species (Couto et al. 2018). The two main Neotropical clades in a phylogenetic analysis (Couto et al. 2018) originated between the Eocene and Oligocene: the crown age for one of the clades is 31.2 Mya (23.6–39.2 Mya) and for the other clade 28.2 Mya (19.5–37.2 Mya). In the Neotropics, the species are distributed in several environments, from dry "restinga" at sea level to Andean paramos, including the edges and interior of humid forests, natural grassland ecosystems, rupicolous areas and semi-desert environments (Couto et al. 2014). As a consequence of the great variety of environmental conditions in which they occur, *Dioscorea* spp. exhibit a wide range of ecological responses, reflected in the large morphological variability found in the family. They range from large climbing vines (40 m high) to dwarf species, can be monoecious or dioecious, and present different leaf shapes, among other distinct characters (Couto et al. 2018).

Currently, of the hundreds of existing yam species, around eleven are cultivated and contribute to food security in many countries, constituting an essential source of food in Africa, Asia, Caribbean, Pacific Islands, and South America. However, many of the wild yam species have also been crucial in times of food scarcity (Shajeela et al. 2011; Dutta 2015; Padhan et al. 2020). The cultivated species of *Dioscorea* are originated from Southeast Asia and Melanesia [(*D. alata* L., *D. esculenta* (Lour.), *D. nummularia* Lam. and *D. pentaphylla* L.)], Japan and China (*D. opposita* L. and *D. japonica* Thunb), West Africa [(*D. rotundata* Poir., *D. dumetorum* (Kunth) Pax and *D. cayenensis* Lam.)], South America (*D. trifida* L.), and Africa, Asia and Melanesia (*D. bulbifera* L.) (Azeteh et al. 2019). In addition to food value, many of these species also have sociocultural and medicinal importance for local people (Azeteh et al. 2019).

Dioscorea spp. have a history linked to human beings for thousands of years by a slow and gradual process of domestication (Ayensu and Coursey 1972). Yams are considered to be domesticated at least 10.200 BP. However, the processing of yams and other plants indicates they have been domesticated and integrated into cultivation practices by at least 7.000 to 6.500 BP (Fullagar et al. 2006). Yam domestication occurred independently in distinct times in three different continents: in Asia (*D.*

alata), America (*D. trifida*), and Africa (*D. rotundata* and *D. cayenensis*) (Harlan 1992). The domestication process, implemented by yam farmers, has been described in great detail for West African species (Mignouna and Dansi 2003; Vernier et al. 2003; Scarcelli et al. 2005, 2006). The process of yam "domestication" involves the adaptation of spontaneous plants (which are plants grown without farmer´s help) to cultivation constraints without genetic changes. In this process, modifications in tuber form, size, and taste are obtained by farmers, who use only vegetative multiplication. Farmers select a spontaneous tuber for its likeness to cultivated varieties and plant it in their fields. For at least three years, farmers submit the pre-domesticated tuber to stress. If accepted by the farmers, the modified tuber is mixed with tubers of a similar variety or originates a new variety (Vernier et al. 2003; Scarcelli et al. 2005, 2006).

The genetics of yam domestication has also been well studied with morphological characters (Djedatin et al. 2017; Padhan et al. 2019) and molecular markers, such as restriction fragment length polymorphism (RFLP) (Terauchi et al. 1992), amplified fragment length polymorphism (AFLP) (Scarcelli et al. 2005, 2006), chloroplast DNA markers (Croxton et al. 2011; Barman et al. 2018), simple sequence repeat markers (SSR) (Mengesha et al. 2013; Scarcelli et al. 2017; Djedatin et al. 2017; Padhan et al. 2019), genotyping by sequence (GBS) (Girma et al. 2014), and whole-genome resequencing (Scarcelli et al. 2019).

Most of these studies have shown that yam domestication, as practiced by farmers, results in gene flow between the cultivated guinea yam species (*D. cayenensis* and *D. rotundata*) and the wild-related species, such as *D. abyssinica* Hochst. ex Kunth (originated from the savannah) and the forest species *D. praehensilis* (Benth.) A. Chev. (Terauchi et al. 1992; Scarcelli et al. 2006; Magwé-Tindo et al. 2018). However, pre-domesticated plants are not always clearly identified as belonging to either wild or cultivated species (Mignouna and Dansi 2003). Plants derived from intervarietal and interspecific hybridization also may have the same indistinction (Scarcelli et al. 2005, 2017). Girma et al. (2014) investigated the role of these two wild species plus three others concerning the origin and domestication of the two cultivated guinea yams (*D. rotundata* and *D. cayenensis*), such as *D. mangenotiana* F. Meigen, *D. togoensis* R. Knuth., and *D. burkilliana* Miege. The authors found that *D. togoensis* and *D. burkilliana* were most distant from the two cultivated species, whereas *D. abyssinica*, *D. mangenotiana*, and *D. praehensilis* were closest to cultivated yams. Using whole-genome sequencing, Scarcelli et al. (2019) found that *D. praehensilis* is the most likely progenitor of African *D. rotundata.*

In India, the relationship of several wild species with cultivated *D. alata* was also investigated using morphological traits and molecular markers (SSR). The genetic similarity analysis showed that the wild yam species such as *D. opposita*, *D. hamiltonii,* and *D. pubera* Blume had the highest genetic similarity with *D. alata* and showed their potentiality for yam improvement programs (Padhan et al. 2019). However, Sharif et al. (2020) concluded that the wild relative of *D. alata* is still unknown. Genetic analysis conducted through genotyping by sequencing of 643 greater yam (*D. alata*) accessions from four continents, using demographic inference, showed an early divergence between accessions from Mainland Southeast Asia and

Pacific, probably followed by two independent domestication events (Sharif et al. 2020). The species would then have reached the Indian Peninsula, subsequently Africa and from there the Caribbean.

Many studies on diversity and genetic structure of *Dioscorea* species have been conducted with several molecular markers in the last decade, mainly toward the cultivated species (Croxton et al. 2011; Siqueira et al. 2012, 2014; Nascimento et al. 2013; Yan et al. 2014; Ngo Ngwe et al. 2015; Silva et al. 2016, 2017; Arnau et al. 2017; Agre et al. 2019), but some studies included the wild *Dioscorea* species (Yan et al. 2013; Girma et al. 2014; Barman et al. 2018; Scarcelli et al. 2017, 2019; Padhan et al. 2019). These genetic data can contribute to the understanding with more details the plant genetic resources, especially crop wild relatives like wild yams, which are under high risks of extinction due to habitat loss, climate change, unacceptable collection practices, shifting cultivation practice and over-exploitation (Magwé-Tindo et al. (2016). For example, *D. zingiberensis* C.H. Wright, an important plant resource for diosgenin content in China (Yi et al. 2014), has its natural populations strongly declined as a result of over-exploitation (Yan et al. 2013).

11.3 Cultivation and Diosgenin-Contained *Dioscorea* Species

Yams (*Dioscorea* species) constitute the predominant starch source in sub-Saharan Africa, where food security for a growing human population is a critical issue. About 93% of total yam production of the world in 2008 was produced in five West African countries (Nigeria, Cote d'Ivoire, Ghana, Benin, and Togo) located in the traditional "Yam Zone" (Fu et al. 2011). Nigeria is the world's largest producer of yams, accounting from 70 to 76% of world production. According to the Food and Agriculture Organization report, in 2018, Nigeria produced 47.5 million tonnes of yams per year from 5.9 million hectares (FAO 2020). In other parts of the world, yam cultivation is punctual compared to African countries' production. For example, in 2018, Brazil produced 251.458 tonnes in 25.7 thousand hectares, with the highest output of yams in this country occurring mainly in the Northeast (Siqueira 2011; FAO 2020).

Cultivation and management methods for *Dioscorea* spp. have been widely discussed in the literature (Aighewi et al. 2015; Hgaza et al. 2020). However, the geographic distribution and ecological requirements of these species are still unknown, as a very limited number of studies have considered the distribution patterns of *Dioscorea* species, especially for those containing diosgenin, with very little information in the literature related to the ecological factors inherent to each species, such as climate, soil, origin, to draw an accurate map of the global cultivation region for these species (Shen et al. 2018). In general, high cost of planting materials, high labor costs, poor soil fertility, low yield potential of local varieties, pests and diseases (yam anthracnose, virus, and nematodes), and shortage of good quality yam

seeds of popular landraces and released varieties have been identified as the major constraints of yam production in Africa (Aidoo et al. 2011; Darkwa et al. 2020).

Yam planting is done with tubers, although in species that generate aerial bulbils, located at the base of petioles, as in *D. bulbifera*, the bulbils can be used for planting. Yams can also be grown from seeds, although unusual, due to the difficulty in sexually reproducing some species of yams. Cultivars of some species rarely flower and produce seeds when grown in environmental conditions different from their place of origin, mainly because they need adequate edaphoclimatic conditions, as well as specific pollinating agents for the species (Hortas 2020).

Most cultivated *Dioscorea* spp. are typical of a hot and humid climate, generally resistant to drought, grown in drained soil rich in organic matter, and require direct daily sunlight for a few hours, such as *D. alata*, *D. bulbifera* and *D. esculenta.* However, some species survive well in mild weather, such as *D. opposita* (Hortas 2020).

Regarding the distribution of diosgenin-contained *Dioscorea* species, Shen et al. (2018) observed a significant occurrence of these species in Eastern Asia, Southern North America, and Southern Africa. Also, new ecological suitability areas were found to be mainly distributed in the central region of South America, in the southern part of the European and coastal regions of Oceania. The authors concluded that annual precipitation and annual mean radiation are important climatic factors and also have decisive control on the *Dioscorea* species distribution.

Of the more than 600 yam species existent, 137 of them contain diosgenin, according to Wan et al. (1994). As mentioned above, two species, *D. composita*, originated from Mexico, and *D. floribunda*, originated from Central America, are the main diosgenin producing species for the industry, although other species have also been commercially exploited, such as *D. sylvatica*, from South Africa, and *D. deltoidea*, from India (Martin 1969; Price et al. 2016). Currently, China and Mexico are the greatest world's diosgenin producers, while China is the main international supplier of diosgenin and its derivatives (Li et al. 2010; Jin et al. 2017). Several species tested for diosgenin content and their origins are listed by Martin (1969). Among these, it is worth mentioning the higher diosgenin-content species, such as *D. composite* (from Mexico), *D. deltoidea* (from India), *D. floribunda* (from Central America), *D. prazeri* var. *glauca* (author unknown) (from India), *D. spiculiflora* Hemsl. (from Mexico), and *D. sylvatica* (from South Africa) (Table 12.1, adapted from Martin 1969), four of them commercially exploited.

Many other studies have reported diosgenin content in *Dioscorea* species, such as Edwards et al. (2002) that obtained 305.7±_45.9 and 409.3±225.6 nmol/mg of diosgenin extracted from tubers, respectively, from *D. batatas* and *D. villosa* L., both species from China. Vendl et al. (2006) extracted diosgenin from the leaves of 51 accessions of *D. alata, D. batatas*, *D. bulbifera*, *D. caucasica* Lipsky, *D. cayenensis*, *D. composita*, *D. deltoidea*, *D. discolor*, *D. japonica*, *D. mangenotiana*, *D. nipponica* Makino, *D. pentaffylla*, *D. reticulate*, *D. rotundata*, *D. sansibarensis, D. sp* and *D. vittata*, showing total diosgenin content varying from 0.036% (*D. bulbifera*) to 0.926% (*D. rotundata*). The authors also found differences between accessions of the same species, for example, varying from 0.447% to 0.926% of dry weight for

different genotypes of *D. rotundata.* Although diosgenin quantities from leaf samples range below the highest values for subterraneous organs, variation among diosgenin contents between different leaf samples is much lower than in tuber tissues, making diosgenin quantification of *Dioscorea* leaf material more comparable, according to Vendl et al. (2006).

The diosgenin content determined in a *D. polygonoides* Humb. & Bonpl. tuber collection from Colombia ranged from 0.02 to 2.64% (Niño et al. 2007). Again, intraspecific variability was detected in this study. Another study determined the diosgenin content from tubers of 54 accessions of *D. sparsiflora* and six accessions of *D. remotiflora*, from the state of Jalisco, in Mexico (Contreras-Pacheco et al. 2013). Diosgenin levels varied from 0.02 to 0.16 mg kg^{-1} in the dry base, also showing considerable intraspecific variation.

Diosgenin content was determined from four *Dioscorea* species by Yi et al. (2014), and the higher content was found for *D. zingiberensis* (varying from 8.67 to 19.52 mg g^{-1}), followed by *D. collettii* Hook f. (13.19 mg g^{-1}) and *D. septemloba* Thunb. (varying from 0.78 to 1.18 mg g^{-1}). Diosgenin was also detected in nine species, with *D. pubera* showing the highest value (7 mg g^{-1}), followed by *D. bulbifera* (6 mg g^{-1}), and the lowest value was found for the cultivated *D. alata* (4 mg g^{-1}) (Padhan et al. 2020).

As we have noticed in the above studies, different chemical and biological protocols are reported to extract diosgenin (Vendl et al. 2006; Niño et al. 2007; Zhang et al. 2007; Contreras-Pacheco et al. 2013; Yi et al. 2014; Padhan et al. 2020). Also, the analytical methods and tissue samples vary considerably between them, which makes it difficult to compare the levels of diosgenin. Therefore, the values published by these studies should be compared with caution. For these reasons, a uniform procedure for the preparation of samples and analysis of diosgenin is highly desirable, aiming to recover reliable information about the diosgenin content of various species and cultivars of of *Dioscorea* (Vendl et al. 2006).

11.4 Yam's Brief Phytochemistry

In addition to carbohydrates, essential amino acids, and vitamins, yams contain saponins and sapogenins, chemical compounds very similar to human sex hormones (Yi et al. 2014). Saponins are secondary metabolites of a glycosidic nature found naturally in edible and inedible plants that have several beneficial properties for human health and are widely used in the pharmaceutical industry (Raju and Mehta 2009). These substances are formed from a polar oligosaccharide linked to a water-insoluble nonpolar portion, generically called sapogenin. In saponins, normally the oligosaccharide is linked at the C-3 position of the sapogenin, but it can also be linked at the C-27 (in steroid saponins) or C-30 (in triterpenoid saponins) positions (Williams and Gong 2007; Raju and Mehta, 2009) (Fig. 11.1).

The nature of the oligosaccharide portion and sapogenin determine the physical, chemical, and biological properties of saponins (Rebelo 2011), which justifies the

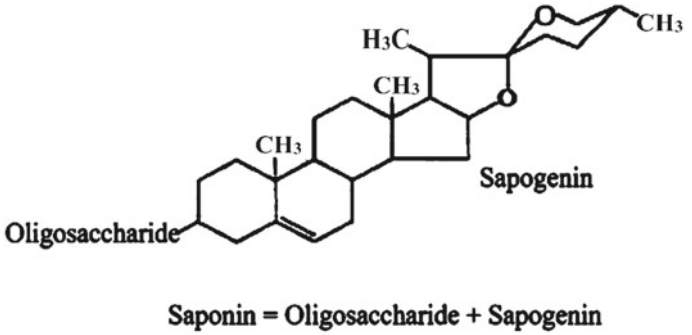


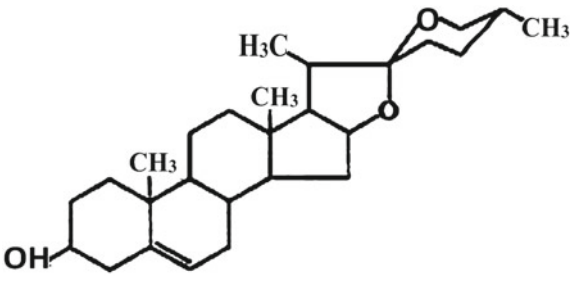
Fig. 11.1 General molecular structure of steroidal saponins

extensive variety of saponins existing in plants (Williams and Gong 2007). According to Guclu-Ustundag and Mazza (2007), saponins occur in at least 400 species of plants belonging to 60 different families. However, all the enzymes and genes involved in its biosynthesis are still unknown (Hua et al. 2017).

While diosgenin is a saponin formed by a steroidal sapogenin, with a glycosidic portion, found in several plant species, especially those of the genus *Dioscorea* (Dutta 2015), the diosgenin molecule is composed of a hydroxyl group at the C-3 position and a double bond at the C-5 position. It has six rings: A, B, C and D (cyclopentanophenanthrene system, standard on steroids), E (tetrahydrofuran), and F (tetrahydropyran). The E and F rings are fused at position C-22 to form a structure called a spiro (Fig. 11.2). These functional groups of bonds and rings are fundamental in the structural conversion of diosgenin and originate other pharmacologically active compounds (Quan et al. 2005).

As diosgenin is not metabolically synthesized by the human body, there has been an increasing demand for this substance from a plant origin in the pharmaceutical industry, which has been driven the introduction and cultivation of *Dioscorea* species in several countries. Among these, China and Mexico stand out, which together account for 67% of the world's diosgenin production (Li et al. 2010). Drugs produced from diosgenin have a turnover of more than US$ 40 billion annually in the global market, equivalent to about 10% of total medicines in the world (Long et al. 2019).

Fig. 11.2 General molecular structure of diosgenin



Currently, China is the leading international supplier of diosgenin and its derivatives, producing about 5000 tons annually (Jin et al. 2017).

On the other hand, the quantity and quality of diosgenin used in the pharmaceutical industry have not increased, mainly due to the decline in wild populations of *Dioscorea* caused by over-harvesting, the difficulty in obtaining new cultivars capable of providing satisfactory amounts of diosgenin and little exploitation of the diversity of the genetic resources (Coursey 1967; Singh et al. 2016).

11.5 Medicinal and Nutritional Properties of *Dioscorea* spp.

Several medicinal properties and pharmacological activities for diosgenin are reported in the literature, such as anti-inflammatory, antifungal, and hypoglycemic, which allows its use for cough relief, lower cholesterol, and stimulation of the liver bile secretory cell growth (Pan et al. 2013). A considerable amount of research was produced, highlighting impact/range in human health with the different *Dioscorea* spp. Ghosh et al. (2015) elaborated a complete phytochemistry review of *D. bulbifera* regarding their therapeutic importance, and Ikiriza et al. (2019) provided up-to-date information about its photochemistry, clinical benefits, conservation status, and best possible way on how this plant can be conserved for future use. These authors pointed out the medicinal uses of *D. bulbifera* such as contraceptives, sexual vigor remedy and treatment of piles, dysentery, syphilis, ulcers, tuberculosis, leprosy, cough, and diabetes. Also, Adeosun et al. (2016) described the efficacy of ethanolic extract of the peel of *D. bulbibera* as a chemotherapeutic. The authors affirmed that this species is a novel source of bioactive compounds but also ascertained its health-promoting qualities. In his pharmacological review, Subasini et al. (2013) indicated that the aerial tubers possess significant activities like purgative, deflatulent, aphrodisiac, rejuvenating and tonic, anthelmintic and is used in haematological disorders, scrofula, syphilis, haemorrhoids, flatulence, diarrhea, dysentery, worm infestations, general debility, diabetic disorders, polyuric, and skin disorders.

Many studies focus on the use of wild yam (*D. villosa*) to prevent menopause (Komesaroff et al. 2001; Hsu et al. 2008). Other problems related to women's quality of life have been the subject of studies due to the benefits of diosgenin, such as osteoporosis and premenstrual tension (Chiang et al. 2011). Das et al. (2014) listed a set of 55 species of *Dioscorea* with contraceptive and abortifacient effects, many of them present in Table 11.1.

Dutta (2015) highlighted the use of diosgenin in the treatment of syphilis and leprosy based on 16 species occurring in the state of Assam, India, which have been used as a source of food and to cure certain ailments such as cough, cold, stomach ache, leprosy, burns, fungal diseases, skin diseases, contraceptive, dysentery, arthritis, and rheumatism, and among these species, *D. alata*, *D. pentaphylla*, *D. bulbifera* and *D. villosa* showed the maximum medicinal properties. This traditional knowledge has been transmitted over generations by ethnic communities of the region. Kwon et al. (2003) highlighted the use of diosgenin in the treatment of diabetics and those

Table 11.1 List of diosgenin-contained *dioscorea* species, their origins, and respective diosgenin percentages, according to Martin (1969)

Species	Origin	Diosgenin (%)
D. abyssinica	Africa	Trace
D. alata	Philippines Islands	None or 0.25
D. althaeoides Knuth	China	Some
D. asclepiadea Prain & Burk	Japan	0.5
D. auriculata Poepp	Chile	0.2
D. balcanica Kosanin	Europe	2.0
D. bartlettii Morton	Mexico, Guatemala	0.8
D. belizensis Lundell	British Honduras	2.6
D. bulbifera	Africa, India	None or 0.5
D. capillaris Hemsl	Mexico	1.2
D. caucasica	Russia	0.6
D. cayenensis	Africa	None or 0.2
D. chiapensis Matuda	Guatemala	1.0
D. collettii	China	2.0
D. composita	Mexico	13.0
D. convolvulacea subsp. *grandifolia* (Schlecht.) Uline	Mexico	0.2
D. cyphocarpa Robinson	Mexico	0.2
D. deltoidea	India	8.0
D. dugesii Robinson	Mexico	0.2
D. escuintlensis Matuda	Guatemala	Some
D. esculenta	India	0.7
D. fastigiata Gay	Chile	Some
D. floribunda	Central America	10.0
D. floridana Bartlett	U.S.A	1.7
D. friedrichsthalii Knuth	Costa Rica	4.0
D. galeottiana Knuth	Mexico	Trace
D. glauca Muhl	North America	1.0
D. gracillima Miq	Japan	0.2
D. grandifolia (probably *D. galeottiana* Knuth)	Mexico	0.2
D. hirsuta Mart. & Gal. (probably *D convolvulacea* Chain. & Schlecht.)	Mexico	0.3
D. hirsuticaulis Rob. (probably *D jaliscana* F. Matuda)	Mexico	0.1
D. hispida Dennst	Philippine Islands	None or 0.73
D. izuensis Akahori	Japan	1.0
D. jaliscana Wats	Mexico	0.3

(continued)

Table 11.1 (continued)

Species	Origin	Diosgenin (%)
D. japonica	Japan	None or 1.0
D. laxiflora Mart	Brazil	None or some
D. lecardii De Wild	Uganda	1.0
D. lobata Uline	Mexico	0.5
D. malifolia Bak	South Africa	Trace
D. mexicana Scheidw	Mexico	0.4
D. militaris Robinson	Mexico	0.4
D. minima Rob. & Seaton	Mexico	0.3
D. minutiflora Engl	Africa, Uganda	None or trace
D. multiflora Mart. Ex Griseb	Argentina	1.0
D. multinervis Benth	Mexico	0.3
D. nelsonii Uline	Mexico	1.8
D. nervosa Phil	Chile	Some
D. nigrescens Phil	China	Some
D. nipponica	Japan	2.0
D. nummularia	India	Trace
D. orbiculata Hook	Malaya	Some
D. panthaica Prain & Burk	China	2.0
D. plumifera Rob	Mexico	0.4
D. polygonoides	Honduras	0.25
D. polystachya Turcz	Russia	0.6
D. prazeri Prain & Burk	India	2.1
D. prazeri var. *glauca*	India	4.5
D. preussii Pax	Tanzania, Uganda	None our 0.3
D. pringlei Rob	Mexico	0.4
D. pubera	India	–
D. quaternata J. F. Gmel	U.S.A	1.2
D. quinqueloba Thunb	Japan	0.4
D. remotiflora Knuth	Mexico	0.3
D. septemloba	Japan	0.1
D. sititoana Honda et Jotani	Japan	Trace
D. spiculiflora	Mexico	15.0
D. subtomentosa Miranda	Mexico	0.4
D. sylvatica	South Africa	6.0
D. tenuipes Franch & Sav	Japan	0.1

(continued)

Table 11.1 (continued)

Species	Origin	Diosgenin (%)
D. tepinapensis Uline (probably *D. composita*)	Mexico	0.7
D. testudinaria Knuth	South Africa	0.6
D. tokoro Makino	Japan	1.0
D. tomentosa	India	Trace
D. ulinei Greenm	Mexico	0.4
D. urceolata Uline	Mexico	0.5
D. villosa	U.S.A	1.3
D. wallichii Hook. F	India	Trace
D. zingiberensis	China	Some

with obesity problems from the use of *D. nipponica*. Sharma and Bastakoti (2009) identified the traditional utilization of nine species of *Dioscorea* in central Nepal and reported the use of diosgenin in the treatment of tuberculosis with *D. bulbifera* and stomach problems with *D. pentaphylla.*

Nabatanzi (2016) report on the consumption of yams (*D. cayenensis*, *D. minutiflora*, *D. odoratissima* Pax, *D. alata,* and *D. bulbifera*) in seropositive groups in Uganda. For the author, despite the already recognized medicinal properties of *Dioscorea* spp., these species need to be looked into more carefully for scientific validation of their nutrient quality and conservation measures toward their sustainable production. The South American *D. trifida* has shown reduced inflammatory parameters associated with food allergies and has the potential to prevent and treat this disease (Mollica et al. 2013).

The anticancer action of diosgenin, acting mainly by inhibiting the cell cycle and inducing apoptosis, was found among 14 *Dioscorea* species (*D. alata, D. belophylla, D. bulbifera, D. dumetorum, D. esculenta, D. hamiltonii, D. hirtiflora, D. hispida, D. kamoonensis, D. opposita, D. pentaphylla, D. pubera, D. wallichii, and D. spinosa* Wall. ex Hook. f.) (Kumar et al. 2017). An extensive review presented by Sethi et al. (2018) has compiled and analyzed the role of diosgenin in modulating various oncogenic transcription factors and intracellular molecular targets that drive tumor initiation, progression, and metastasis. The authors concluded that several challenges, such as developing novel delivery systems, pharmaceutical formulations, and semi-synthetic derivatives that are water-soluble, need to be overcome to uncover diosgenin's benefits either as a chemopreventive or therapeutic agent.

Dufie et al. (2013) underline that the low sodium but high potassium and total dietary fiber contents indicate the possible preventive role that *D. alata* could play in managing-related chronic diseases, which may be due to the action of diosgenin. Based on the nutritive evaluation studies on wild edible yams (*Dioscorea alata, D. bulbifera, D. esculenta, D. opposita, D. pentaphylla, D. spicata* Roth*, D. tomentosa* Koen ex Roxb., and *D. wallichii*) consumed by the Kanikkars tribals and Palliyars, India, it can be summarized that most of them were found to be a good

source of protein, lipid, crude fiber, starch, vitamins, and minerals (Shajeela et al. 2011). In Koraput, India, wild yams make a significant contribution to the diets and economic welfare of tribal people. A study conducted by Padhan et al. (2020) carried out to evaluate the proximate, nutritional, and anti-nutritional compositions as well as the physic-functional properties in eight wild and one cultivated *Dioscorea* species. Results showed that the wild species, such as *D. opposita*, *D. hamiltonii,* and *D. pubera,* showed better nutritional composition than the other yam species, with significantly higher amounts of nutrient and mineral content. The study also suggested that these wild yam species are a safe food source for local consumption and domestication, leading to potential improvement of food security.

Childhood malnutrition is a current and perpetual public health concern in many African countries. Challenges remain for the difficulty in formulating nutritionally adequate diets. Leng et al. (2019) investigated the effect of *D. schimperiana* Kunth pulp color on nutritional composition and antioxidant activity of formulated yam-based complementary food. The authors found a positive correlation between the yam color and the contents in carbohydrates (0.64), total phenols (0.82), ß-criptoxanthine (0.6), zeaxanthin (0.86), and a significant correlation for antioxidant activity, such as alpha (1.00) and beta carotene (0.91) and total carotenoid provitamin A levels (0.94). In Brazil, Teixeira et al. (2013) highlighted the feasibility of purple yam (*D. trifida*) bread as a health-promoting food-based also in the nutritive evaluation profile. Beyerlein and Pereira (2018), in a morphological characterization of 20 *D. trifida* landraces from the Amazon in Brazil, found that pulp color was the main character dividing the accessions into two groups, one with white pulp tubers (seven accessions, but one of them had purple pulp color) and another with purple pulp tubers (13 accessions). Nascimento et al. (2015) also found high variability for pulp color among *D. trifida* accessions from the South, Southeast, Central-West, and North regions in Brazil, with the purple (13 accessions) and white-purple (18 accessions) tuber colors predominating in the Central-West and North regions. Therefore, further studies on the nutritional properties of purple pulp color of *D. trifida* tubers seem to be promising, since there is plenty of availability of this tuber color. Increasing provitamin A carotenoid intake through biofortification using some of the underutilized root tuber staples can reduce the prevalence of vitamin A deficiency among the vast consumers of yams (Ukom et al. 2014).

11.6 Perspectives

As the production and quality of diosgenin have decreased due to the lack of high-quality germplasm, the scarcity of information related to the region where species with a high content of diosgenin are found, as well as the decline of *Dioscorea* populations due to extensive harvesting, the identification of the new potential ecological distribution of diosgenin-contained *Dioscorea* species is required.

Little attention to research, minimal commercialization, and deficient political structures are the main obstacles to harnessing the real potential of *Dioscorea* spp. The most important compound of *Dioscorea* is diosgenin, currently used in the

synthesis of steroid drugs; however, other potential uses of these compounds and related compounds need to be studied extensively to validate their quality and adapt their biological potentials.

An attempt should also be made to determine the mechanism of action, bioavailability, and physiological pathways of diosgenin and its derivatives for its possible applications in drug discovery and the cure of various diseases. Studies should also be carried out in order to use diosgenin to formulate new drugs to combat pathogens and microorganisms. Research on these species of *Dioscorea* will open new perspectives in the study of biodiversity management for sustainability, development, germplasm conservation, pharmacology, and many other new fields of research in plant and pharmaceutical science.

Acknowledgements The authors would like to thank São Paulo Research Foundation (FAPESP), the Coordination for the Improvement of Higher Education Personnel (CAPES), and the Brazilian National Research Council (CNPq) for their support in the form of financial assistance and scholarships granted for studies on *Dioscorea* spp. in Brazil.

References

Adeosun OM, Arotupin DJ, Toba AO, Adebayo AA (2016) Antibacterial activities and phytochemical properties of extracts of *Dioscorea bulbifera* Linn (Air Potatoes) tubers and peels against some pathogenic bacteria. J Phytopharmacol 5(1):20–26

Agre P, Asibe F, Darkwa K et al (2019) Phenotypic and molecular assessment of genetic structure and diversity in a panel of winged yam (*Dioscorea alata*) clones and cultivars. Sci Rep 9:18221

Aidoo R, Nimoh F, Bakang JEA (2011) Economics of small-scale seed yam production in Ghana: Implications for commercialization. Afr J Sustain Dev 13:65–78

Aighewi BA, Asiedu R, Maroya N, Balogun M (2015) Improved propagation methods to raise the productivity of yam (*Dioscorea rotundata* Poir.). Food Sec 7:823–834

Arnau G, Bhattacharjee R, Mn S et al (2017) Understanding the genetic diversity and population structure of yam (*Dioscorea alata* L.) using microsatellite markers. PLoS ONE 12(3):e0174150

Ayensu ES, Coursey DG (1972) Guinea yams the botany, ethnobotany, use and possible future of yams in West Africa. Econ Bot 26:301–318

Azeteh IN, Hanna R, Sakwe PN, Njukeng AP, Kumar PL (2019) Yam (*Dioscorea* spp.) production trends in Cameroon: A review. Afr J Agric Res 14:1097–1110

Barman P, Bhat KV, Geeta R (2018) Phylogenetic analysis of Indian *Dioscorea* and comparison of secondary metabolite content with sampling across the tree. Genet Resour Crop Evol 65:1003–1012

Barton H (2014) Yams: origins and development. In: Smith C (ed) Encyclopedia of global archaeology. Springer, Heidelberg, pp 7943–7947

Beyerlein P, Pereira HS (2018) Morphological diversity and identification key for landraces of the Amerindian yam in central Amazon. Pesq Agropec Bras 53(4):405–418

Burkill IH (1938) The contact of the Portuguese with African food plants which gave words such as “yam” to European languages. Proc Linn Soc Lond 150:84–95

Castro AP, Fraxe TJP, Pereira HS, Kinupp VF (2012) Etnobotânica das variedades locais do cará (*Dioscorea* spp.) cultivados em comunidades no município de Caapiranga, estado do Amazonas. Acta Bot Bras 26(3):658–667

Chiang SS, Chang SP, Pan TM (2011) Osteoprotective effect of monascus-fermented *Dioscorea* in ovariectomized rat model of postmenopausal osteoporosis. J Agr Food Chen 59(17):9150–9157

Contreras-Pacheco ML, Santacruz-Ruvalcaba F, Garcia-Fajardo JA et al (2013) Diosgenin quantification, characterisation and chemical composition in a tuber collection of *Dioscorea* spp. in the state of Jalisco, Mexico. Int J Food Sci Tech 48:2111–2118
Coursey DG (1967) Yams: an account of the nature, origins, cultivation and utilisation of the useful members of the Dioscoreaceae. Longmans, London
Couto RS, Martins AC, Bolson M, Lopes RC, Smidt EC, Braga JMA (2018) Time calibrated tree of *Dioscorea* (Dioscoreaceae) indicates four origins of yams in the Neotropics since the Eocene. Bot J Linn Soc 20:1–17
Couto RS, Tenorio V, Alzer FC et al (2014) Taxonomic revision of the *Dioscorea campestris* species assemblage (Dioscoreaceae). Syst Bot 39:1056–1069
Croxton MD, Andreu MA, Williams DA, Overholt WA, Smith JA (2011) Geographic origins and genetic diversity of air-potato (*Dioscorea bulbifera*) in Florida. Invas Plant Sci Manage 4:22–30
Darkwa K, Olasanmi B, Asiedu R, Asfaw A (2020) Review of empirical and emerging breeding methods and tools for yam (*Dioscorea* spp.) improvement: Status and prospects. Plant Breed 139:474–497
Das B, Talukdar ADAS, Dutta CM, Talukdar ADAS (2014) A few traditional medicinal plants used as antifertility agents by ethnic people of Tripura, India. Int J Pharm Pharm Sci 6(3):47–53
Djedatin G, Adeoti K, Ewedje E, Agbangla C (2017) Does the domestication of yam wild type *Dioscorea praehensilis* and *Dioscorea abyssinica* has a genetic basis? Int J Sci 6:18–28
Dufie WMF, Oduro I, Ellis WO, Asiedu R, Maziya-Dixon B (2013) Potential health benefits of water yam (*Dioscorea alata*). Food Funct 4:1496–1501
Dutta B (2015) Food and medicinal values of certain species of *Dioscorea* with special reference to Assam. J Pharmacogn Phytochem 3:15–18
Edwards AL, Jenkins RL, Davenport LJ, Duke JA (2002) Presence of diosgenin in *Dioscorea batatas* (Dioscoreaceae). Econ Bot 56(2):204–206
FAO (2020) FAOSTAT database. Rome, Italy. Available online at http://faostat3.fao.org/home/E
Fu RHY, Kikuno H, Maruyama M (2011) Research on yam production, marketing and consumption of Nupe farmers of Niger State, central Nigeria. Afr J Agric Res 6(23):5301–5313
Fullagar RA, Field J, Denham T, Lentfer C (2006) Early and mid-Holocene tool use and processing of taro (*Colocasia esculenta*), yam (*Dioscorea* spp.) and other plants at Kuk Swamp in the highlands of Papua New Guinea. J Archeol Sci 33(1):595–614
Girma G, Hyma KE, Asiedu R, Mitchell SE, Gedil M, Spillane C (2014) Next-generation sequencing based genotyping, cytometry and phenotyping for understanding diversity and evolution of guinea yams. Theor Appl Genet 127:1783–1794
Ghosh S, Parihar VS, More P, Dhavale DD, Chopade BA (2015) Phytochemistry and therapeutic potential of medicinal plant: *Dioscorea bulbifera*. Curr Med Chem 5(4):160–172
Guclu-Ustundag O, Mazza G (2007) Saponins: properties, applications and processing. Crit Rev Food Sci Nutr 47(3):231–258
Harlan JR (1992) Crops and man, 2nd edn. American Society of Agronomy, Madison
Hgaza KV, Oberson A, Kiba ID, Diby LN'G, Ake S, Frossard E (2020) The nitrogen nutrition of yam (*Dioscorea* spp) J Plant Nutr 43(1):64–78
Hortas (2020) Como plantar inhame. Available online at https://hortas.info/como-plantar-inhame
Hsu K, Chang C, Tsai H, Tsai F, Hsieh YY (2008) Effects of yam and diosgenina on calpain systems in skeletal muscle of ovariectomized rats. Taiwan J Obstet Gynecol 47(2):180–185
Hua W, Kong W, Cao X et al (2017) Transcriptome analysis of *Dioscorea zingiberensis* identifies genes involved in diosgenin biosynthesis. Genes Genom 39:509–520
Ikiriza H, Ogwang PE, Peter EL et al (2019) *Dioscorea bulbifera*, a highly threatened African medicinal plant, a review. Cogent Biol 5:1–6
Jesus M, Martins APJ, Gallardo E, Silvestre S (2016) Diosgenin: recent highlights on pharmacology and analytical methodology. J Anal Methods Chem 1:1–16
Jin M, Xian L, Sun L (2017) Isolation and structure identification of steriod saponuns from *Dioscorea zingiberensis* C. H. Wright. Northwest Pharm J 32:395–400

Komesaroff PA, Black CVS, Cable V, Sudhir K (2001) Effects of wild yam extract on menopausal symptoms, lipids and sex hormones in healthy menopausal women. Climacteric 4:144–150
Kumar S, Das G, Shin HS, Patra JK (2017) *Dioscorea* spp. (a wild edible tuber): a study on its ethnopharmacological potential and traditional use by the local people of similipal biosphere reserve, India. Front Pharmacol 8:1–17
Kwon CS, Sohn HY, Kim SH et al (2003) Anti-obesity effect of *Dioscorea nipponica* Makino with lipase-inhibitory activity in rodents. Biosci Biotechnol Biochem 67(7):1451–1456
Leng MS, Tobit P, Demasse AM et al (2019) Nutritional and anti-oxidant properties of yam (*Dioscorea schimperiana*) based complementary food formulation. Sci Afr 5:1–9
Li X, Ma JZ, Shi YD (2010) Research progress and prospects of *Dioscorea* and diosgenin. Chem Ind Forest Prod 30:107–112
Long X, Liang Q, Gao H, Politov A, Li Y, Cheng M (2019) Physical and chemical analysis of diosgenins prepared by water separation and high temperature and pressure hydrolysis from different kinds of *Dioscorea* rhizomes. J Med Plants 7:1–8
Magwé-Tindo J, Wieringa JJ, Sonké B et al (2018) Guinea yam (*Dioscorea* spp., Dioscoreaceae) wild relatives identified using whole plastome phylogenetic analyses. Taxon 67:905–915
Magwé-Tindo J, Zapfack L, Sonké B (2016) Diversity of wild yams (*Dioscorea* spp., Dioscoreaceae) collected in continental Africa. Biodivers Conserv 25:77–91
Martin FW (1969) The species of *Dioscorea* containing sapogenin. Econ Bot 23(4):373–379
Mengesha WA, Demissew S, Fay MF, Smith RJ, Nordal I, Wilkin P (2013) Genetic diversity and population structure of Guinea yams and their wild relatives in South and South West Ethiopia as revealed by microsatellite markers. Genet Resour Crop Evol 60:529–541
Mignouna HD, Dansi A (2003) Yam (*Dioscorea* sp.) domestication by the Nago and Fon ethnic groups in Benin. Genet Resour Crop Evol 50:519–528
Mignouna HD, Abang MM, Geeta R (2009) True yams (*Dioscorea*): a biological and evolutionary link between eudicots and grasses. Cold Spring Harb Protoc 4:1–7
Mollica JQ, Cara DC, Auriol MD, Oliveira VB, Cesar IC, Branca MGL (2013) Anti-inflammatory activity of American yam *Dioscorea trifida* L. in food allergy induced by ovalbumin in mice. J Funct Foods 5:1975–1984
Nabatanzi A (2016) Wild food plants used by people living with HIV/AIDS in Nakisunga sub-county, Uganda. Afr J Food Agric Nutr Dev 16:11310–11330
Nascimento WF, Siqueira MVBM, Ferreira AB, Ming LC, Peroni N, Veasey EA (2015) Distribution, management and diversity of the endangered Amerindian yam (*Dioscorea trifida* L.). Braz J Biol 75:104–113
Nascimento WF, Rodrigues JF, Koehler S, Gepts P, Veasey EA (2013) Spatially structured genetic diversity of the Amerindian yam (*Dioscorea trifida* L.) assessed by SSR and ISSR markers in Southern Brazil. Genet Resour Crop Evol 60:2405–2420
Ngo Ngwe MFS, Omokolo DN, Joly S (2015) Evolution and phylogenetic diversity of yam species (*Dioscorea* spp.): implication for conservation and agricultural practices. PLoS ONE 10(12):e0145364
Niño J, Jiménez DA, Mosquera OM, Correa YM (2007) Diosgenin quantification by HPLC in a *Dioscorea polygonoides* tuber collection from Colombian flora. J Braz Chem Soc 18(5):1073–1076
Oliveira AP, Barbosa LJN, Pereira WE, Silva JEL, Oliveira ANP (2007) Produção de túberas comerciais de inhame em função de doses de nitrogênio. Hortic Bras 25(1):73–76
Padhan B, Biswas M, Panda D (2020) Potential of neglected and underutilized yams (*Dioscorea* spp.) for improving nutritional security and health benefits. Front Pharmacol 11:1–13
Padhan B, Mukherjee AK, Mohanty SK, Lenka SK, Panda D (2019) Genetic variability and inter species relationship between wild and cultivated yams (*Dioscorea* spp.) from Koraput, India based on molecular and morphological markers. Physiol Mol Biol Plants 25(5):1225–1233
Pan CH, Tsai CH, Liu FC (2013) Influence of different particle processing on hypocholesterolemic and antiatherogenic activities of yam (*Dioscorea psecudojaponica*) in cholesterol-fedrabbitmode. J Sci Food 93(6):1278–1283

Price EJ, Wilkin P, Sarasan V, Fraser PD (2016) Metabolite profiling of *Dioscorea* (yam) species reveals underutilized biodiversity and renewable sources for high-value compounds. Sci Rep 6:1–10

Quan HJ, Koyanagi J, Fusao K, Saito S (2005) Preparations of vitamin D analogs, spirostanols and furostanols from diosgenin and their cytotoxic activities. Eur J Med Chem 40:662–673

Raju J, Mehta R (2009) Cancer chemopreventive and therapeutic effects of diosgenin, a food saponin. Nutr Cancer 61(1):27–35

Rebelo AIMA (2011) Diosgenina e derivados oxidados: potenciais agentes antitumorais e antifúngicos. Thesis (Dissertation), Universidade da Beira Interior, Covilhã, Portugal

Scarcelli N, Daïnou O, Agbangla C, Tostain S, Pham JL (2005) Segregation patterns of isozyme loci and microsatellite markers show the diploidy of African yam *Dioscorea rotundata* (2n= 40). Theor Appl Genet 111:226–232

Scarcelli N, Tostain S, Vigouroux Y, Agbangla C, Daïnou O, Pham JL (2006) Farmers' use of wild relative and sexual reproduction in a vegetatively propagated crop. The case of yam in Benin. Mol Ecol 15:2421–2431

Scarcelli N, Chaïr H, Causse S, Vesta R, Couvreur TLP, Vigouroux I (2017) Crop wild relative conservation: wild yams are not that wild. Biol Conserv 210:325–333

Scarcelli N, Cubry P, Akakpo R et al (2019) Yam genomics supports West Africa as a major cradle of crop domestication. Sci Adv 5:1–7

Sethi G, Shanmugam MK, Warrier S et al (2018) Pro-apoptotic and anti-cancer properties of diosgenin: a comprehensive and critical review. Nutrients 10:2–12

Shajeela OS, Mohan VR, Jesudas LL, Soris PT (2011) Nutritional and antinutritional evaluation of wild yam (*Dioscorea* spp.). Trop Subtrop Agroecosyst 14:723–730

Sharif BM, Burgarella C, Cormier F et al (2020) Genome-wide genotyping elucidates the geographical diversification and dispersal of the polyploid and clonally propagated yam (*Dioscorea alata* L.). Ann Bot 126:1029–1031

Sharma LN, Bastakoti R (2009) Ethnobotany of *Dioscorea* L. with emphasis on food value in Chepang communities in Dhading district, central Nepal. J Plant Sci 6:12–17

Shen L, Xu J, Luo L et al (2018) Predicting the potential global distribution of diosgenin-contained *Dioscorea* species. Chin Med 13:58

Siadjeu C, Toukam GM, Bell JM, Nkwate S (2015) Genetic diversity of sweet yam "*Dioscorea dumetorum*" (Kunth) Pax revealed by morphological traits in two agro-ecological zones of Cameroon. Afr J Biotechnol 14(9):781–793

Silva D, Siqueira MVBM, Carrasco N, Mantello C, Nascimento WF, Veasey EA (2016) Genetic diversity among air yam (*Dioscorea bulbifera*) varieties based on single sequence repeat markers. Genet Mol Res 15(2):gmr7929

Silva LRG, Mezette TF, Nascimento WE, Silva EF, Veasey EA (2017) Spatially structured morphological and molecular diversity among *Dioscorea cayenensis* and *D. rotundata* yam accessions. Plant Genet Resour 15(4):296–309

Singh R, Kharat SN, Mendhulkar VD (2016) Chromatographic analysis for "diosgenin" content in Abutilon indicum (L) sweet. Int J Pharm Pharm Sci 8(2):178–182

Siqueira MVBM, Bonatelli ML, Günther T et al (2014) Water yam (*Dioscorea alata* L.) diversity pattern in Brazil: an analysis with SSR and morphological markers. Genet Resour Crop Evol 61:611–624

Siqueira MVBM, Dequigiovanni G, Guivin M, Feltran JC, Veasey EA (2012) DNA fingerprinting of water yam cultivars in Brazil based on microsatellite markers. Hortic Bras 30:653–659

Siqueira MVBM (2011) Yam: a neglected and underutilized crop in Brazil. Hortic Bras 29(1):16–20

Subasini U, Thenmozhi S, Sathyamurthy D, Vetriselvan S, Rajamanickam GV, Dubey GP (2013) Pharmacognostic and phytochemical investigations of *Dioscorea bulbifera* L. Int J Pharm Life Sci 4(5):2693–2700

Teixeira AP, Oliveira IMA, Lima ES, Matsuura T (2013) The use of purple yam (*Dioscorea trifida*) as a health-promoting ingredient in bread making. J Res Biol 3(1):747–758

Terauchi R, Chikaleke VA, Thottappilly G, Hahn SK (1992) Origin and phylogeny of Guinea yams as revealed by RFLP analysis of chloroplast DNA and nuclear ribosomal DNA. Theor Appl Genet 83:743–751

Ukom NA, Ojimelukwe PC, Ezeama CF, Ortiz DO, Aragon II (2014) Proximate composition and carotenoid profile of yams (*Dioscorea spp.)* and cocoyam (*Xanthosoma maffa (Scoth*)) root tubers from Nigeria. Am J Food Nutr 4(1):1–10

Vendl O, Wawrosch C, Noe C, Molina C, Kahl G, Kopp B (2006) Diosgenin contents and DNA fingerprint screening of various yam (*Dioscorea sp.*) Genotypes. Z Naturforsch 61:847–855

Vernier P, Orkwor GC, Dossou AR (2003) Studies on yam domestication and farmers' practices in Benin and Nigeria. Outlook Agric 32:35–41

Viruel J, Segarra-Moragues JG, Pérez-Collazos E, Villar L, Catalán P (2010) Systematic revision of the Epipetrum group of *Dioscorea* (Dioscoreaceae) endemic to Chile. Syst Bot 35:40–63

Wan JR, Ding ZZ, Qin HZ (1994) A phytogeographical study on the Family Dioscoreaceae. Acta Bot Boreal-Occident Sin 14(2):128–135

Williams JR, Gong H (2007) Biological activities and syntheses of steroidal saponins: the shark repelling pavoninins. Lipids 42(1):77–86

Yan QQ, Li Y, Sun XQ et al (2014) Isolation and characterization of polymorphic microsatellite loci from aerial yam (*Dioscorea bulbifera* L.). Genet Mol Res 13:1514–1517

Yan QQ, Sun XQ, Guo JL, Hang JL, Li MM (2013) Development of polymorphic microsatellite markers for *Dioscorea zingiberensis* and cross-amplification in other *Dioscorea* species. Genet Mol Res 12(3):3788–3792

Yi T, Fan LL, Chen HL et al (2014) Comparative analysis of diosgenin in *Dioscorea* species and related medicinal plants by UPLC-DAD-MS. BMC Biochem 15:1–19

Zhang YQ, Liang JH, Fu EH, Li BX (2007) Effect of modified enzymatic catalysis on the extraction of diosgenin from *Dioscorea zingiberensis* C. H Wright Chem Eng Technol 30:1488–1494

Chapter 12
Cultivation, Chemical Constituents and Utilization of *Lonicera caerulea* L. (Blue Honeysuckle) in Poland

Katarzyna Sobkowicz, Agnieszka Szewczyk, Beata Ornat, and Małgorzata Bedra-Tokarz

Abstract *Lonicera caerulea* was commonly named among others as a haskap, blue honeysuckle and honeyberry. It is a culinary plant which was known for many years in the region of Northeast Asia, where the fruits from wild-grown plants have been collected. Russia was considered to be the oldest breeding center of the blue honeysuckle. Today, the plantations of blue honeysuckle are located in many countries, e.g., Canada, USA, China, Great Britain and Poland, and the area of cultivation is constantly increasing. In many countries, there are research centers trying to create new cultivars of blue honeysuckle. This article reviews basic information on the botanical characteristic, chemical composition, utilization and the requirements for cultivation of blue honeysuckle. Much attention has been paid to the chemical composition of the fruits and their therapeutic, health-promoting properties and culinary use. The presence of flavonoids, anthocyanins and iridoids makes blue honeysuckle fruits a high health potential. Fruits are used mainly for fresh eating, freezing, drying or the production of many articles such as juices and concentrates. Polish producers use the harvested fruits for processing purposes. These culinary food products which were produced on their farms might promote the region and supplement the household budget. The knowledge about the utilization and cultivation of this plant is broadening in Poland.

Keywords Lonicera caerulea var. kamtschatica · Haskap · Honeyberry · Botanical characteristic · Chemical composition · Biological activities

K. Sobkowicz (✉) · B. Ornat · M. Bedra-Tokarz
Garden of Medicinal Plants, Medical College, Jagiellonian University, Medyczna 9 str, 30-688, Cracow, Poland
e-mail: katarzyna.kaluzny@uj.edu.pl

A. Szewczyk (✉)
Department of Pharmaceutical Botany, Medical College, Jagiellonian University, Medyczna 9 str, 30-688, Cracow, Poland
e-mail: agnieszka.szewczyk@uj.edu.pl

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_12

Abbreviations

DW Dry weight
FW Fresh weight
HFD High-fat diet

12.1 Introduction

The fruits of blue honeysuckle had been collected from the plants growing in the wild in northeastern part of Asia which are their natural sites (Kaniewska et al. 2013; Bell and Williams 2019; Plekhanova 2000). The therapeutic, strengthening and flavor properties of these fruits were appreciated by the local populations. Over time, researchers became interested in blue honeysuckle and began breeding work in order to select new varieties of this species (Sabitov 1986; Skupień et al. 2007; Kaczmarska et al. 2015). Blue honeysuckle fruits were used in the form of infusions and juices, to improve the functioning of the digestive, urinary systems. It was also used to treat hypertension, as well as to fight against malaria, anemia and osteoporosis (Anikina et al. 1998; Plekhanova 2000; Thompson and Barney 2007).

Blue honeysuckle is a novel, niche market fruit with a valuable, natural source of phytochemicals, vitamins and bioelements essential for human health (Grobelna et al. 2019; Gerbrandt et al. 2020). According to Rupasinghe, the vitamin C content in blue honeysuckle fruits ranges from 29 to 187 mg/100 g and it is higher than in oranges or strawberries (Rupasinghe et al. 2018). Blue honeyberry fruits are a good source of bioactive molecules. Fruits are dark purple to blue and oval, and have a savory flavor and bitter–sweet to sweet taste. Therefore, blue honeysuckle berries are willingly consumed by people all over the world (Chmiel et al. 2014; Anton et al. 2019). There are many possibilities of using blue honeysuckle, e.g., fresh eating, freezing, drying, processing into fruit juices, processing into freeze-dried products (Litwin 2019).

Due to the affordable cultivation, blue honeysuckle shrubs are suitable for commercial cultivation and amateur gardening at home (Lauritzen et al. 2015). They are also suitable for organic farming (Sołtysiak 2019). Research carried out at the experimental station in Cologne (Germany) showed that it is possible to cultivate Haskap in foil tunnels also using the ecological method (Stremer 2019). For several years, blue honeysuckle has been available for domestic sale, in the form of nursery material, as well as fresh fruit or ready-made food products. Although blue honeysuckle fruits are still insufficiently appreciated by domestic consumers, they have a wide range of applications. Blue honeysuckle berries are mainly cultivated for harvesting fresh fruits.

12.2 Taxonomy and Botanical Characteristics

In plant taxonomy, *Lonicera caerulea* belongs to the Caprifoliaceae family (Wu and Hou 2021). The Caprifoliaceae family comprises more than 200 species (Poyarkova 2000). One of popular varieties of this plant species is *Lonicera caerulea var. kamtschatica.* In botanical terminology, variety is a rank below that of species and subspecies but above that of form. Cultivar is a rank below that of variety and nomenclatures as a basic classification category of cultivated plants (mostly used in horticulture), and it is a plant with special utility or aesthetic features (Kulpa 1964). The genus *Lonicera* is commonly known as an ornamental plant (Hummer 2006). There are two subspecies of *Lonicera caerulea*: *Lonicera caerulea ssp. caerulea* and *Lonicera caerulea ssp. pallasii.* (Batoczenko 2019). *Lonicera caerulea* originated from the regions of Northeast Asia and grows and yields well in North America and parts of Europe characterized by a cooler climate. It can be found in the literature as blue honeysuckle, fly honeysuckle, honeyberry and sweetberry honeysuckle, while it is customary to refer to the fruits of this plant as 'haskap' (Thompson and Chaovanalikit 2003; Hummer 2006; Thompson 2006; Miyashita et al. 2011; Bors et al. 2012; Hayes and Peterson 2020) (Fig. 12.1).

Depending on the variety, this deciduous, cold hardy plant grows up to 0.8 m and can reach a height of over 2 m. The plant can be of various shapes, more or

Fig. 12.1 Frozen fruits of blue honeysuckle (photo Kiełkowski R.)

Fig. 12.2 Blue honeysuckle shrub growing in Garden of Medicinal Plants, Jagiellonian University, Medical College, Cracow. (photo Sobkowicz K.)

less compact and spherical, with raised shoots and peeling bark (Figs. 12.2 and 12.4). Its leaves are placed opposite to the shoots and are simple and numerous, with various sizes ranging from 2 to 7 cm long and over 1 cm wide and shapes such as oval, lanceolate or elongated (Fig. 12.3) (Svarcova et al. 2007; Hummer et al. 2011; Lauritzen et al. 2015; Wu and Hou 2021).

The flowers of blue honeysuckle are tubular and small, with a yellow, cream or white color, and generally do not exceed 2 cm in diameter. Some cultivars bloom very early (in March), when the thermal conditions do not allow bees to fly around. Therefore, their main pollinators are bumblebees (Hummer et al. 2011; Bieniasz et al. 2017). The shrubs of this plant require cross-pollination with pollen from another plant that blossoms at the same time (Lauritzen et al. 2015). Depending on the variety, the fruits have an elongated shape, appear dark purple or navy blue, with a coating, and are juicy in nature (Figs. 12.1 and 12.5). They have different flavors such as tart, sweet–tart, sweet, and sour or sweet, which may be a characteristic of the variety. The fruits mature from the end of May (Hummer 2006; Hummer et al. 2011; Szot et al. 2014; Lauritzen et al. 2015). Their weight ranges from 0.3 to 2.0 g (Auzanneau et al. 2018).

Fig. 12.3 Blue honeysuckle leaves (photo Sobkowicz K.)

12.3 Cultivation

Russia was considered to be the oldest breeding center of blue honeysuckle (Wawiłow Institute in St. Petersburg and South Ural Institute of Horticulture and Potatoes in Chelyabinsk) (Plekhanova 1996). At the end of the twentieth century, some varieties of blue honeysuckle from that location were brought to Poland. Currently, Russian varieties from the Bakcharskoe Center of Northern Pomology, such as 'Vostorg,' 'Jugana,' 'Docz Velikana' and 'Bakczarskij Velikan,' are cultivated in Poland. Another center that conducts research on blue honeysuckle is the University of Saskatchewan in Canada, where breeding works on varieties such as 'Boreal Beauty,' 'Boreal Beast,' 'Boreal Blizzard,' 'Aurora,' 'Indigo Gem,' 'Honey Bee' and 'Tundra' have been carried out. These varieties are characterized by later flowering and fruit ripening, as well as high taste and suitability for mechanical harvesting.

Fig. 12.4 Blue honeysuckle shrubs growing on the farm 'Gospodarstwo Szkółkarskie Kiełkowscy', Pszów, Poland (photo Kiełkowski R.)

Blue honeysuckle breeding was also performed in the USA, which resulted in the production of varieties with high taste. Zofia and Hieronim Łukaszewski were the first Polish breeders to create domestic varieties of blue honeysuckle. Their breeding works resulted in varieties such as 'Wojtek,', 'Rebeka,' 'Ruben,' 'Zojka,' 'Iga' and 'Klon 44.' Other Polish varieties of blue honeysuckle are 'Atut,' 'Duet,' 'Kasia' and 'Krystyna' (Król-Dyrek 2017). There are about 50 named blue honeysuckle varieties in trade (Bors 2019).

Determining the exact world acreage of blue honeysuckle is difficult due to insufficient data from Asian countries. Experts estimate that the world cultivation area of this plant is about 5500 hectares, of which about 1000 hectares is grown in Canada and about 20 hectares is grown in the USA. Important producers from Europe are Great Britain—about 40 ha, Slovenia—about 40 ha and Poland, which is considered a leader among European blue honeysuckle producers (Cassells 2017). According to Czernienko, the area of blue honeysuckle commercial crops exceeds 735 ha in Russia, and the largest plantations are located in Western Siberia. Fresh and frozen fruit as well as the blue honeysuckle fruit preserves are available in the Russian market. There are companies that deal with fruit processing mainly into juices and jams, but also sweets, lyophilisates, sauces, concentrates and batches for the dairy and confectionery industries (Czernienko 2019).

The cultivars and selected clones of blue honeysuckle plant differ in terms of flowering, fruit ripening and morphological features, such as the shape, size, taste

Fig. 12.5 Blue honeysuckle shrubs with fruits (photo Kiełkowski R.)

and chemical composition of the fruits. In Poland, foreign and domestic varieties are available for sale. Currently, the cultivation area of blue honeysuckle in Poland exceeds 2000 ha and is increasing every year (Podymniak 2017). The cultivation of this plant is not limited to one region, and the national climate and soil conditions allow for its cultivation in various parts of Poland. Domestic and foreign varieties are grown on Polish plantations. Table 12.1 presents the details of randomly selected domestic farms cultivating haskap (over 1 ha), with the area of cultivation and cultivars. Data used for its cultivation were obtained from the Internet resources (Internet resources 1.) and by contacting the farms.

The cultivation of blue honeysuckle is relatively easy. These shrubs are resistant to cold, pests and various soil acidities. They tolerate pleasant temperatures well and do not require protection during the winter period. According to Pierzga, blue honeysuckle shrub can stand −40 °C, and open flowers −8 °C (Pierzga 2001). In addition, their generative organs are not damaged under low and negative temperatures. Depending on the weather and variety, the flower buds may open in March, although a large proportion of the cultivated shrubs show full flowering in April. The plant is characterized by average soil requirements; for good growth, it requires fertile, well-drained and moderately moist soil, with a pH of 5.5–6.5 (even higher pH values are tolerated). The bushes grow well in sunny, bright or slightly shaded places (Lauritzen et al. 2015).

Table 12.1 Selected farms cultivating blue honeysuckle in Poland

Town	Province/voivodeship	Blue honeysuckle cultivation area [ha]	Cultivated varieties of blue honeysuckle
Bożatki	Wielkopolskie	1	'Aurora' 'Honey Bee' 'Indigo Gem'
Chrzanowo	Pomorskie	1.75	'Vostorg' 'Jugana' 'Nimfa' 'Silginka' 'Docz Velikana' 'Bakczarskij Velikan'
Dąbrowa Białostocka	Podlaskie	2	'Wojtek' 'Klon 44'
Dąbrowica	Lubelskie	1	'Aurora' 'Honey Bee' 'Indigo Gem' 'Wojtek' 'Zojka' 'Ruben' 'Rebeka' 'Atut' 'Duet' 'Karina' 'Jolanta' 'Tola'
Izabelmont	Lubelskie	2.5	'Wojtek' 'Zojka' 'Aurora' 'Indigo Gem' 'Honey Bee'
Katarzynów	Mazowieckie	3	'Wojtek' 'Indigo Gem'
Krasnopol	Podlaskie	1 <	'Vostorg' 'Honey Bee'
Mokra Prawa	Łódzkie	1 <	'Aurora' 'Jugana' 'Vostorg'

(continued)

Table 12.1 (continued)

Town	Province/voivodeship	Blue honeysuckle cultivation area [ha]	Cultivated varieties of blue honeysuckle
Muniakowice	Małopolskie	3	'Aurora' 'Jugana' 'Vostorg' 'Indigo Gem' 'Sinij Utes' 'Lawina' 'Honey Bee' 'Uslada' 'Ussulga' 'Boreal Blizzard' 'Boreal Beast' 'Boreal Beauty' 'Blue Banana' 'Blue Treasure' 'Giant's Heart' 'Strawberry Sensation'
Palczew	Mazowieckie	1 <	'Aurora' 'Honey Bee' 'Indigo Gem' 'Wojtek' 'Jolanta'
Romanówka	Świętokrzyskie	3.5	'Wojtek' 'Julia' 'Zojka'
Szarek	Warmińsko-Mazurskie	10	'Vostorg' 'Docz Velikana' 'Honey Bee' 'Tundra' 'Indigo Gem' 'Czułymskaja' 'Bakczarskij Velikan'
Szewna	Świętokrzyskie	7	'Boreal Beast' 'Boreal Beauty' 'Boreal Blizzard' 'Vostorg' 'Aurora' 'Indigo Gem'

The quality of the fruit, its appearance and taste are particularly important in commercial crops. Therefore, plants which grow in plantations should be provided with adequate hydration (especially in periods with low rainfall), protection against pests and fertilization (Cassells 2017). Plant nutrients are essential to increase productivity (Iheshiulo et al. 2019), and it might be helpful to find blue honeysuckle growing guidelines that have been developed by specialists (Cassells 2017). The occurrence of pests on the blue honeysuckle plantation can be minimized by using healthy nursery material, selection of appropriate sites for blue honeysuckle cultivation, analysis of the plantation surroundings, selection of appropriate varieties that are less sensitive to pests, systematic inspection of plantations and elimination of infected plants (Kałużna 2019).

12.4 Chemical Composition

Blue honeysuckle fruits have been consumed as food for centuries due to their high nutritional value. These berries are rich in fiber, saccharides, lipids, proteins, organic acids, polyphenols, vitamins and bioelements (iron, silicon, magnesium, manganese, copper, phosphorus) (Palikova et al. 2009). The fresh blue honeysuckle fruits contain 82.9 g/100 g FW of water. Depending on the cultivar, their energy value varies from 70 to 330 kcal/100 g FW (Molina et al. 2019).

Saccharides

Carbohydrates are major macronutrients in blue honeysuckle berries, which are found at a concentration of 14.3–15.87 g/100 g FW. The fruits of *Lonicera caerulea var. kamtschatica* contain free sugars such as fructose and glucose (at high concentration-4.02 and 3.86 g/100 g FW, respectively) and sucrose (in small amounts) (Molina et al. 2019). The content of individual sugars in the fruits largely differs depending on the cultivar. For instance, the content of fructose and glucose ranges from 14 to 33.7 and from 8 to 32.7 g/100 g DW, respectively (Auzanneau et al. 2018).

Lipids

The fruits *Lonicera caerulea var. kamtschatica* contain 0.38 g/100 g FW of lipids. About 20 fatty acids are found in the blue honeysuckle berries, of which the main unsaturated fatty acids are: linoleic acid (71.79%), α-linolenic acid (4.24%) and γ-linolenic acid (1.4%). The main saturated fatty acid is palmitic acid (5.39%). Table 12.2 shows the composition of fatty acids in blue honeysuckle fruits (Molina et al. 2019).

Proteins

The protein content of blue honeysuckle berries varies from 0.87 to 1.6 g/100 g FW depending on the cultivar and soil and cultivation conditions (Molina et al. 2019).

Table 12.2 Fatty acid composition in blue honeysuckle fruits (Molina et al. 2019)

Fatty acid	Relative %
Caproic acid (C6:0)	0.078 ± 0.001
Caprolic acid (C8:0)	0.067 ± 0.001
Capric acid (C10:0)	0.012 ± 0.001
Lauric acid (C12:0)	0.366 ± 0.003
Myristic acid (C14:0)	0.304 ± 0.008
Myristoleic acid (C14:1)	0.067 ± 0.001
Pentadecylic acid (C15:0)	0.092 ± 0.002
Palmitic acid (C16:0)	5.39 ± 0.01
Palmitoleic acid (C16:1)	0.098 ± 0.001
Margaric acid (C17:0)	0.082 ± 0.001
Stearic acid (C18:0)	0.986 ± 0.001
Oleic acid (C18:1n9)	14.153 ± 0.006
Linoleic acid (C18:2n6)	71.79 ± 0.08
γ-Linolenic acid (C18:3n6)	1.4 ± 0.1
α-Linolenic acid (C18:3n3)	4.24 ± 0.01
Arachidic acid (C20:0)	0.158 ± 0.001
Eicosenoic acid (C20:1)	0.086 ± 0.001
Eicosadienoic acid (C20:2)	0.227 ± 0.002
Behenic acid (C22:0)	0.308 ± 0.001
Lignoceric acid (C24:0)	0.069 ± 0.004
Saturated fatty acids	7.91 ± 0.02
Monounsaturated fatty acids	14.403 ± 0.006
Polyunsaturated fatty acids	77.69 ± 0.03

Organic acids

The studies by Molina et al. (2019) showed that the total organic acid content of blue honeysuckle berries was 3.93 g/100 g FW. Table 12.3 summarizes the content of organic acids. Wojdyło et al. (2013) additionally determined the content of phytic and shikimic acids (0.047 and 0.0039 g/100 g FW, respectively) (Molina et al. 2019; Wojdyło et al. 2013).

Table 12.3 Organic acid composition of blue honeysuckle fruits (Molina et al. 2019)

Organic acids	Content [g/100 g FW]
Oxalic acid	0.041
Quinic acid	0.37
Malic acid	0.77
Citric acid	2.76

Vitamins

The content of ascorbic acid (vitamin C) is three to ten times higher in blue honeysuckle berries than in blueberries (Molina et al. 2019). Auzanneau et al. (2018) found that, depending on the cultivar, the ascorbic acid content varies from 1.78 to 4.21 mg/g DW, while Zlabur et al. (2019) reported that the vitamin C content was 5.348 g/100 g DW (Auzanneau et al. 2018; Zlabur et al. 2019).

The dominant isoform of tocopherols in blue honeysuckle fruits is α-tocopherol (0.77 mg/100 g FW), while β-, γ- and δ-tocopherol isoforms are found in small amounts (Molina et al. 2019).

Phenolic compounds

Jurikova et al. (2012) showed that the phenolic fraction in blue honeysuckle berries (4% of FW) comprised 3.5% phenolics (anthocyanins, flavonoids, phenolic acids) (Jurikova et al. 2012). The phenolic fraction of blue honeysuckle berries has a high nutraceutical value. The major polyphenolic compounds found in these berries are mono-, di- and tri-polymers of flavan-3-ols (61% of total phenolic compounds). The other phenolic compounds found are anthocyanins (29%), phenolic acids (7%) and flavonols + flavones (3%) (Oszmiański et al. 2016).

Flavan-3-ols

Oszmiański et al. (2016) determined that flavan-3-ols are the major group of polyphenols in blue honeysuckle berries, with the total content amounting to 690.77 mg/100 g FW. The dominant compounds (87% of total flavan-3-ols) were found to be polymeric procyanidins. The concentrations of (+)-catechin and (-)-epicatechin were estimated at 80.42 and 3.19 mg/100 g FW, respectively (Oszmiański et al. 2016).

Anthocyanins

The total content of monomeric anthocyanins in blue honeysuckle berries varies between 8.4 and 65 mg/g DW, depending on the cultivar and cultivation conditions (Auzanneau et al. 2018).

Chaovanalikit et al. (2004) determined the quantitative composition of the anthocyanin fraction as follows: cyanidin-3-O-glucoside (79–92% of anthocyanin total content), cyanidin-3,5-O-diglucoside (4.27%), cyanidin-3-O-rutinoside (2.07%), peonidin-3-O-glucoside (3.44%) and pelargonidin-3-O-glucoside (0.83%) (Chaovanalikit et al. 2004).

The studies by Molina et al. confirmed that cyanidin-3-O-glucoside (Fig. 12.6) is the dominant among the anthocyanins, but the qualitative and quantitative composition of anthocyanins was different. Table 12.4 presents the individual contents of anthocyanins in blue honeysuckle berries (according to Molina et al. 2019).

In the studies by Oszmiański et al. (2016), the total content of anthocyanins in blue honeysuckle berries was determined at 335.24 mg/100 g FW. The content of the dominant compound cyanidin-3-O-glucoside was 295.3 mg/100 g FW. The remaining

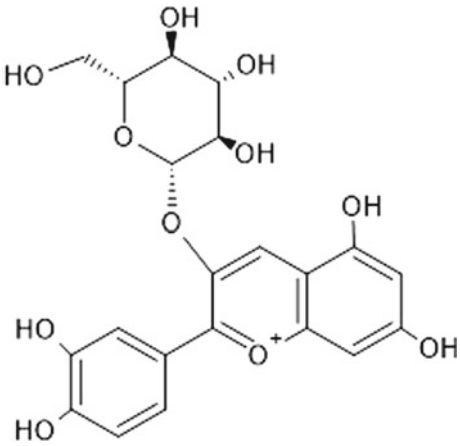


Fig. 12.6 Chemical structure of cyanidin-3-O-glucoside

Table 12.4 Anthocyanin composition of blue honeysuckle fruits (Molina et al. 2019)

Anthocyanin	Content [mg/g dry extract]
Cyanidin-O-hexoside-O-hexoside	6.75
Cyanidin-3-O-glucoside	61.7
Cyanidin-O-rhamnoside-O-hexoside	10.1
Pelargonidin-3-O-glucoside	10.3
Peonidin-3-O-glucoside	4.872
Peonidin-O-rhamnoside-O-hexoside	4.142
Total	97.9

seven anthocyanins (cyanidin-3,5-diglucoside, peonidin-3,5-dihexoside, cyanidin-3-O-rutinoside, pelargonidin-3-O-glucoside, peonidin-3-O-glucoside, peonidin-3-O-rutinoside and cyanidin-3-hexoside-ethyl-catechin) were found in smaller amounts (<30 mg/100 g FW) (Oszmiański et al. 2016). The total anthocyanin content varied from 0.009 to 0.253 g % FW, depending on the method used for extraction (Myjavcova et al. 2010).

Flavonoids

The total content of flavonoids in blue honeysuckle berries was estimated at about 1.9 g/100 g DW. Among the flavonoids, quercetin glycosides were found to be dominant (Jurikova et al. 2012). The content of rutoside (Fig. 12.7) varies from 1.02 to 3.67 mg/g DW, depending on the cultivar (Auzanneau et al. 2018).

Phenolic acids

Jurikova et al. (2012) reported that the berries of *Lonicera caerulea* L. *var. kamtschatica* contain chlorogenic, caffeic, ferulic, protocatechuic, gentisic, rosmarinic and vanillic acids (Fig. 12.8, Jurikova et al. 2012).

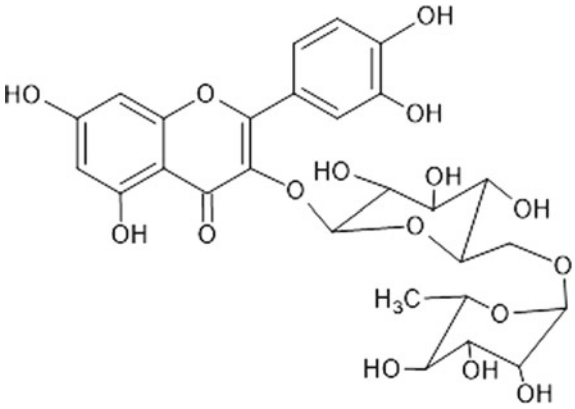
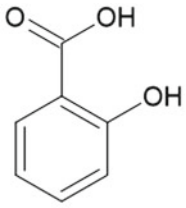
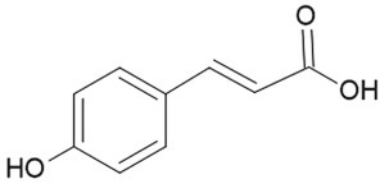


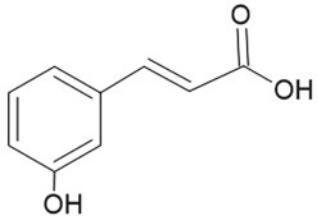
Fig. 12.7 Chemical structure of rutoside



Salicylic acid



p-coumaric acid



m-coumaric acid

Fig. 12.8 Chemical structures of the main phenolic acids in blue honeysuckle berries

Table 12.5 Phenolic acid composition of blue honeysuckle fruits (Zadernowski et al. 2005)

Phenolic acids	Content [mg/kg DW]
Gentisic acid	153.5
Gallic acid	44.3
p-Pyrocatechuic acid	28.6
Protocatechuic acid	144.4
Salicylic acid	1234.9
Vanillic acid	21.1
Caffeic acid	598.2
m-Coumaric	2014.5
p-Coumaric	987.1
3,4-Dimetoxycinnamic	44.2
Ferulic	36.9
Hydroxycaffeic	51.9

In the study of Zadernowski and co-workers, the total content of 12 phenolic acids was determined at 5418.2 mg/kg DW. Table 12.5 presents the individual contents of phenolic acids in blue honeysuckle berries (Zadernowski et al. 2005).

Iridoids

Iridoids are rarely found in fruits. However, studies on blue honeysuckle berries focusing on the content of iridoids have proven the presence of these compounds (Kucharska and Fecka 2016; Kucharska et al. 2017). Oszmiański and Kucharska (2018) identified five iridoids (loganic acid, 7-*epi*-loganic acid 7-O-pentoside, loganin, sweroside and secologanin) in blue honeysuckle berries with the total content of these compounds estimated at 46.32 mg/100 g FW. The major iridoids in these berries were determined as loganic acid and loganin (44 and 41%, respectively) (Oszmiański and Kucharska 2018).

Monoterpenoids

The blue honeysuckle berries contain over 40 different terpenoid compounds. These include monoterpenoid hydrocarbons and monoterpenoid oxygen-containing compounds (oxides, alcohols, aldehydes and ketones). Eucalyptol was identified as the major component of the volatile fraction (Kupska et al. 2014).

Triterpenoids

Among the triterpenoids, the tetracyclic triterpenoids were the major compounds. Becker et al. (2017) found that the total content of free tetracyclic triterpenoids was 46.61 μg/mg of blue honeysuckle fruit wax extract, pentacyclic triterpenoid was 12.21 μg/mg and ester was 3.69 μg/mg (Becker et al. 2017).

12.5 Application in Treatment and Prevention of Various Diseases

Blue honeysuckle berry has high health and healing properties. In the natural medicine of Russia, China and Japan, it is commonly used for treating diseases of the digestive and cardiovascular systems. In Japan, blue honeysuckle berry is considered a valuable source of medicinal substances and hence called the 'elixir of life' in the folk tradition. As mentioned above, blue honeysuckle berry is rich in phenolic compounds, such as anthocyanins, flavonoids, proanthocyanidins, catechins and phenolic acids, which reduce the harmful effects of free oxygen radicals and exhibit high antioxidant properties. Phenolic compounds also prevent the emergence of many civilization diseases such as diabetes, heart failure, stroke or cancer. Thus, including blue honeysuckle berry in the diet can help in significantly reducing the risk of heart attack and atherosclerosis (Molina et al. 2019).

Antimicrobial properties

Studies have shown that blue honeysuckle berry extract is active against bacteria and other microorganisms. It was reported that 20% ethanol extract of blue honeysuckle berries showed moderate activity against Gram-positive and Gram-negative bacteria (Raudsepp et al. 2019).

Blue honeysuckle extract was found to inhibit the growth of all the studied strains of bacteria at concentrations of 3.41 mg/mL (*Listeria monocytogenes*, *Escherichia coli*, *Enterobacter cloacae*, *Salmonella typhimurium*) and 6.81 mg/mL (*Bacillus cereus* and *Staphylococcus aureus*). The extract also has antifungal properties and was shown to exhibit a stronger inhibitory activity against *Trichoderma viride* (2.13 mg/mL) but the lower against *Aspergillus versicolor* (6.81 mg/mL) (Molina et al. 2019).

Antioxidant and radical oxygen species scavenging activities

Free radicals cause the oxidation of molecules including proteins, lipids and DNA. Their deteriorative effects can be diminished by natural antioxidants such as polyphenolic compounds. The individual cultivars of blue honeysuckle berries differ in the content of these compounds and thus exhibit different levels of antioxidant activity (DPPH test) ranging from 6.59 to 10.17 g of ascorbic acid equivalent/kg FW (Rop et al. 2011).

The acidified water extract of blue honeysuckle fruits was found to show DPPH radical scavenging activity (68.24 ± 1.13%) and ABTS radical scavenging activity (70.05 ± 0.84%) (Li et al. 2019).

Depending on the cultivar, large differences are observed in the antioxidant activity. For example, the FCR-reducing capacity of the extract varies between 12.6 and 42.3 mg gallic acid equivalent/g DW, while antioxidant capacity in DPPH assay ranges between 60 and 228 μmol Trolox equivalent/g DW (Auzanneau et al. 2018).

Protective effect against UV-induced damage

The phenolic fraction of blue honeysuckle fruits is capable of protecting skin against the deleterious effects of sunlight. Its UVA protective activity was assessed in human keratinocytes HaCaT after pre- or posttreatment with different concentrations of the phenolic fraction (1–250 mg/L). The results showed that the phenolic fraction of blue honeysuckle fruits significantly suppressed UVA-induced production of reactive oxygen species. The protective effect was found to be concentration-dependent with the maximum value observed at 50 mg/L (Svobodowa et al. 2008).

The phenolic fraction of blue honeysuckle fruits can also moderate UVB-induced damage. A study analyzed its effect of reducing the extent of DNA breakage together with caspase-3 and caspase-9 activity and DNA laddering induced by UVB. The extract of blue honeysuckle fruits significantly decreased the generation of reactive oxygen and nitrogen species, partially diminished the expression of interleukin (IL)-6 and prevented the proliferation of keratinocytes (Svobodowa et al. 2009).

Erythrocyte protection

The extracts of blue honeysuckle fruits and leaves can protect the physical properties and lipid membranes of erythrocytes. Both extracts were shown to protect the red blood cell membrane against oxidation induced by UVC irradiation and AAPH (2,2'-azobis(2-methylpropionamidine) dihydrochloride). The extract components (polyphenols) were incorporated in the external part of the erythrocyte membrane. Furthermore, studies of electric parameters of membranes modified by the extracts showed that the extract slightly stabilized the lipid membranes and did not reduce their specific resistance or capacity. The polyphenolic compounds located in the hydrophilic part of the membrane protected the cells against the reactive forms of oxygen and other substances (Bonarska-Kujawa et al. 2014).

Effect of blood pressure

In a study, a 400 mg dose of blue honeysuckle fruit extract elicited (1.5 h post-prandially) significantly lower diastolic blood pressure and heart rate in older adults, compared to placebo. The blood pressure outcome was consistent with the vasodilatory mechanism of action (Bell and Williams 2019).

Effect of cognition

The anthocyanin-rich extract of blue honeysuckle fruits was proven to improve the episodic memory of older adults. In a double-blind trial, the researchers observed higher word recall and improvement in word recognition scores (Bell and Williams 2019).

Antifibrosis effect of lung

The polyphenol-rich blue honeysuckle extract was shown to alleviate silica-induced pulmonary fibrosis in mice. This involved alteration in the activity of regulatory T cells and mitigation of T helper (Th)1/Th17 responses, which resulted in reduced levels of Th2 cytokine and transcription factor mRNA. Cell-signaling detection

revealed that p38 and c-Jun N-terminal kinase phosphorylation was effectively blocked by the blue honeysuckle extract, which also downregulated the expression of inducible nitric oxide (NO) synthase and upregulated two antioxidant mediators in the lung nuclear factor (erythroid-derived 2)-like 2 and heme oxygenase-1. Thus, the extract of blue honeysuckle berries may modulate immune responses and alleviate lung fibrosis (Zhao et al. 2019).

Application in metabolic diseases

A study examined the antidiabetic potential of blue honeysuckle berry extract in high-fat-diet (HFD)-induced mild diabetic mice. After 12 weeks of oral administration of the extract, a noticeable increase was observed in the level of blood glucose, insulin, glycated hemoglobin (HbA1c), blood urea nitrogen (BUN) and creatinine in the studied mice. This confirmed that blue honeysuckle berry extract could act as a potential herbal agent to cure diabetes (type II) (Sharma et al. 2019).

The use of blue honeysuckle berry extract (75% aqueous ethanol extract, freeze-dried into powder) in the diet of mice led to the improvement of glucose metabolism, by increasing insulin sensitivity. Supplementation of blue honeysuckle berry extract suppressed HFD-induced obesity and hepatic fat deposition (Liu et al. 2018).

Furthermore, continuous administration of blue honeysuckle berry extract (for 84 days) was shown to significantly prevent obesity and nonalcoholic fatty liver disease in high-fat-diet-fed mice (Kim et al. 2018).

In a study analyzing the use of anthocyanin-rich (327 mg/g) blue honeysuckle berry extract in high-fructose diet in rats, it was found that the addition of the extract to the diet normalized the plasma concentration of triglyceride and insulin (Jurgoński et al. 2013).

Hepatoprotective effects

The oral administration of blue honeysuckle berry extract in mice with CCl_4-induced acute liver damage resulted in a hepatoprotective effect, and liver damage was significantly inhibited compared to the control group (Lee et al. 2019).

The phenolic fraction (18.5% anthocyanins) of blue honeysuckle berry extract was found to inhibit rat liver microsome peroxidation, induced by *tert*-butyl hydroperoxide with IC_{50} values of 160 ± 20 μg/mL (Palikova et al. 2009).

Anti-inflammatory effect

The anti-inflammatory and antioxidant effects of blue honeysuckle berry extract were investigated in a study on adjuvant-induced arthritis rat model and macrophage-like (RAW264.7) cell model. The serum levels of proinflammatory factors including tumor necrosis factor alpha (TNT-α), interleukin IL-1β, IL-6, and nitric oxide (NO) were significantly reduced in rats fed with blue honeysuckle berry extract (Wu et al. 2015).

Toxicity

In a study on female and male specific pathogen-free ICR mice (OrientBio, Korea) was concluded that concentrated and lyophilized powder of blue honeysuckle (BHcL)

is a practically nontoxic. BHcL contained 0.93 g/g of carbohydrates, 0.02 g/g of proteins, 0.2 mg/g of sodium, 210.63 mg/g of total phenolics, 159.3 mg/g of total flavonoids and 133.57 mg/g of total anthocyanins. No treatment-related mortalities, changes in body or organ weight, clinical signs, necropsy or histopathological findings up to 2 mg/kg body weight were observed in the studied animal model (Kim et al. 2015).

12.6 Utilization

Blue honeysuckle fruits are used in folk medicine, mainly in Russia, China and Japan, in the form of infusions and juices, to improve the functioning of the digestive and urinary systems. Fruits were also used to treat hypertension, as well as to fight against malaria, anemia and osteoporosis (Anikina et al. 1998; Thompson and Barney 2007).

Blue honeysuckle fruits can be eaten fresh, and according to one of the Polish growers, they are tasty for children (Poseł 2019). They are used mainly for the production of juices and concentrates, as well as jams, marmalades, syrups, fruit mousses, jellies, meat sauces and alcoholic beverages such as flavored vodka liqueurs or wine (deep or dark in color and a rich bouquet), and also consumed in dried form. They are also used as an additive to dishes such as muffins and dumplings (Kozłowska and Troszyńska 1999; Liu et al. 2009, 2010; Krasnowska and Sikora 2011; Lauritzen et al. 2015; Cassells 2017; Kowalczyk 2019).

Blue honeysuckle juice can also be used to produce multifruit juices. By combining apple juice, which is rich in natural antioxidants, with blue honeysuckle juice with prohealth properties, a completely new product with increased content of vitamin C and anthocyanins was formulated (Grobelna et al. 2019). Some producers also add freeze-dried fruits to confection or use them to make local tea blends or combine them with other products such as honey, chocolates and sweets. Furthermore, blue honeysuckle fruits and their extracts are used in the phytocosmetics industry as an ingredient of soaps, creams and body lotions (Kowalczyk 2019; Litwin 2019). In the Polish company 'Blue Haskap,' blue honeysuckle fruits are used to produce juice and jams at first. As the company grows, they expanded its range with new products such as freeze-dried fruits and honey with freeze-dried blue honeysuckle and continue to develop (Litwin 2019). One of the Polish producers of blue honeysuckle—the Plantin Company donated the harvest to the dessert fruit market, processing into fruit juices and processing into freeze-dried products and sale of frozen fruit (Kusibab and Kusibab-Mruk 2019).

Assessment of juice composition has revealed that the content of sugars, organic acids, ascorbic acid, antioxidants and polyphenols, and the pH potential of fruits are important aspects of the processing industry. The level of sugars (°Brix) is particularly important in the production of juices and wine. Research on sugar content showed that even fruit samples of the same variety may have different sugar contents depending on the place of cultivation (tunnel or field cultivation). It was found that the samples of 'Indigo Gem' and 'Ruben' varieties grown in the field had higher sugar content than

the samples of the same varieties grown in tunnels and thus were more suitable for the production of juices and wine. Due to the fact that individual varieties cultivated in different ways may contain different amounts of ingredients, they may be suitable for different applications (Jarret 2017).

After harvesting, blue honeysuckle fruits have a fairly short shelf life. Therefore, research works are underway to extend their shelf life, for example, in a controlled atmosphere (Błaszczyk et al. 2017). A good way to extend the shelf life of fruits is to dry them. Studies have shown that exposure of fresh fruits to different temperatures for different durations can clearly affect the content of metabolites such as sugars and organic acids. A particular change is observed in the content of ascorbic acid, which can be degraded in dried fruits under the influence of heat treatment (Senica et al. 2019).

12.7 Conclusions

Currently, the blue honeysuckle is receiving increasing attention. This is manifested, inter alia, by the constant emergence of research centers dealing with breeding works on new varieties, as well as an increase in the number of scientific publications about this plant species. The frequent appearance of trade stands with food products made using the blue honeysuckle baize at agrarian fairs throughout the world. In Poland, blue honeysuckle is grown widely, and new plantations of this plant are established every year. Both domestic and foreign varieties of blue honeysuckle are grown in the country. Although the popularity of blue honeysuckle fruits continues to increase, the producers find it challenging to promote blue honeysuckle and the products derived from them, and to win new markets.

A growing number of local producers on the Polish market offer blue honeysuckle fruit products or their combination with other products. The producers commonly process the fruits grown by them at home, and the finished products are sold as regional products or directed to specialized grocery stores that focus on the sale of prohealth products, organic food stores or the so-called health food stores. These products often have quality certificates. There is also growing awareness of the health aspects of consuming blue honeysuckle and their preserves. Due to the high-quality composition, blue honeysuckle berries are considered among the fruits of the future, and hence, there are prospects for the improvement of their production.

The Polish producers use the harvested fruits for processing purposes and produce in their farms food products such as juices, syrups, jams and alcoholic beverages to improve the regional income as well as supplement their household income. They are aware of the necessity to popularize the blue honeysuckle and therefore organize meetings, as well as local and national cultural events aimed at promoting this fruit, including the campaigns ‘Time for Polish Super-fruit’ and ‘June in the Haskap Berry Plantations.’ In such campaigns, which are conducted in individual farms cultivating

blue honeysuckle throughout the country, open days combined with workshops are organized on a given date. Since 2017, the Kamchatka Berry Growers Association has been operating, and their task is to represent and protect the rights of the Kamchatka berry growers associated with them.

References

Anikina EV, Syrchina AI, Vereshchagin AL, Larin MF, Semenov AA (1998) Bitter iridoid glucoside from the fruit of Lonicera caerulea L. Chem Nat Compd 24(4):512–513

Anton D, Koskar J, Raudsepp P, Meremae K et al (2019) Antimicrobial and antioxidative effects of plant powders in raw and cooked minced pork. Foods 8:661–679

Auzanneau N, Weber P, Kosińska-Cagnazzo A, Andlauer W (2018) Bioactive compounds and antioxidant capacity of *Lonicera caerulea* berries: comparison of seven cultivars over three harvesting years. J Food Compos Anal 66:81–89. https://doi.org/10.1016/j.jfca.2017.12.006

Batoczenko W (2019) Jadalny wiciokrzew z ukraińskich karpat, In: Konferencja Kamczacka Hortus Media Krakow, Poland, pp 135–138

Becker R, Pączkowski C, Szakiel A (2017) Triterpenoid profile of fruit and leaf cuticular waxes of edible honeysuckle *Lonicera caerulea var. kamtschatica.* Acta Soc Bot Pol 86(1):35–39. https://doi.org/10.5586/asbp.3539

Bell L, Williams CM (2019) A pilot dose-response study of the acute effects of haskap berry extract (*Lonicera caerulea* L.) on cognition, mood, and blood pressure in older adults. Eur J Nutr 58:3325–3334. https://doi.org/10.1007/s00394-018-1877-9

Bieniasz M, Dziedzic E, Błaszczyk J (2017) Możliwe sposoby zwiększenia atrakcyjności kwiatów jagody kamczackiej dla owadów zapylających, In: Konferencja Kamczacka, Hortus Media, Krakow, Poland, pp 49–54

Błaszczyk J, Dziedzic E, Bieniasz M (2017) Wpływ zróżnicowanych warunków przechowywania na jakośc i trwałość owoców jagody kamczackiej, In: Konferencja Kamczacka, Hortus Media, Krakow, Poland, pp 113–118

Bonarska-Kujawa D, Pruchnik H, Cyboran S, Żyłka R, Oszmiański J, Kleszczyńska H (2014) Biophysical mechanism of the protective effect of Blue Honeysuckle (*Lonicera caerulea* L. *var. kamtschatica* Sevast) polyphenols extracts against lipid peroxidation of erythrocyte and lipid membranes. J Membrane Biol 247:611–625. https://doi.org/10.1007/s00232-014-9677-5

Bors B (2019) Planowane zapylanie haskapu. In: Konferencja Kamczacka, Hortus Media, Krakow, Poland, pp 47–58

Bors B, Thomson J, Sawchuk E, Reimer P et al (2012) Haskap breeding and production; final report. Saskatchewan Agriculture, Saskatoon, SK, Canada, pp 1–142

Cassells LJ (2017) Wnioski z ostatnich siedmiu lat uprawy jagody kamczackiej w Ameryce Północnej, In: Konferencja Kamczacka, Hortus Media, Krakow, Poland, pp 78–88

Chaovanalikit A, Thompson MM, Wrolstad RE (2004) Characterization and Quantification of Anthocyanins and Polyphenolics in Blue Honeysuckle (*Lonicera caerulea* L.). J Agric Food Chem 52:848–852. https://doi.org/10.1021/jf0305090

Chmiel T, Abogado D, Wardencki W (2014) Optimization of capillary isotachophoretic method for determination of major macroelements in blue honeysuckle berries (Lonicera caerulea L.) and related products. Anal Bioanal Chem 406:4965–4986. https://doi.org/10.1007/s00216-014-7879-4

Czernienko A (2019) Trendy w rozwoju ogrodnictwa przemysłowego w Rosji oraz ocena odmian pod kątem potrzeb rynkowych. In: Konferencja Kamczacka, Hortus Media, Krakow, Poland, pp 107–114

Dziedzic E, Błaszczyk J, Bieniasz M, Dziadek K, Kopeć A (2020) Effect of modified (MAP) and controlled atmosphere (CA) storage on the quality and bioactive compounds of blue honeysuckle fruits (*Lonicera caerulea* L.). Sci Hort 265. https://doi.org/10.1016/j.scienta.2020.109226

Gerbrandt EM, Bors RH, Meyer D, Wilen R, Chibbar RN (2020) Fruit quality of Japanese, Kuril and Russian blue honeysuckle (Lonicera caerulea L.) germplasm compared to blueberry, raspberry and strawberry. Euphytica 216:59. https://doi.org/10.1007/s10681-020-02587-w

Grobelna A, Kalisz S, Kieliszek M (2019) The effect of the addition of Blue Honeysuckle berry juice to apple juice on the selected quality characteristics, anthocyanin stability, and antioxidant properties. Biomolecules 9(11):744. https://doi.org/10.3390/biom9110744

Hayes DJ, Peterson BJ (2020) Growth of *Lonicera caerulea* across fertility and moisture conditions: Comparisons with *Lonicera villosa* and invasive congeners. Hort Sci 55(2):149–155. https://doi.org/10.21273/HORTSCI14318-19

Holubec V, Smekalova T, Leisova-Svobodova L (2019) Morphological and molecular evaluation of the Far East fruit genetic resources of Lonicera caerulea L.—vegetation, ethnobotany, use and conservation. Genet Resour Crop Evol 66:121–141. https://doi.org/10.1007/s10722-018-0701-y

Hummer KE (2006) Honeysuckle: A new Berry Crop for North America. J Amer Pomol Soc 60(1):3–8

Hummer KE, Pomper KW, Postman J, Graham CJ et al (2011) Emerging fruit crops. Fruit Breeding 97–147. https://doi.org/10.1007/978-1-4419-0763-9_4

Iheshiulo EM-A, Abbey L, Hammermeister A (2019) Nutrient sufficiency levels for haskap (*Lonicera caerulea* L.) using the boundary-line approach. Can J Plant Sci 99(2). https://doi.org/10.1139/cjps-2018-0151

Jarret D (2017) Rozwój uprawy i badania jakościowe jagody kamczackiej w Szkocji, In: Konferencja Kamczacka, Hortus Media, Krakow, Poland, pp 7–18

Jurgoński A, Juśkiewicz J, Zduńczyk Z (2013) An anthocyanin-rich extract from Kamchatka honeysuckle increases enzymatic activity within the gut and ameliorates abnormal lipid and glucose metabolism in rats. Nutrition 29:898–902. https://doi.org/10.1016/j.nut.2012.11.006

Jurikova T, Rop O, Mlcek J, Sochor J et al (2012) Phenolic profile of edible Honeysuckle Berries (Genus *Lonicera*) and their biological effects. Molecules 17:61–79. https://doi.org/10.3390/molecules17010061

Kaczmarska E, Gawroński J, Dyduch-Sieminska M et al (2015) Genetic diversity and chemical characterization of selected Polish and Russian cultivars and clones of blue honeysuckle (Lonicera caerulea). Turkish J Agric Forestry 39:394–402. https://doi.org/10.3906/tar-1404-149

Kałużna M (2019) Zagrożenia chorobami w uprawie jagody kamczackiej. In: Konferencja Kamczacka, Hortus Media. Krakow, Poland, 7–14.

Kaniewska J, Gozdecka G, Domoradzki M, Szambowska A (2013) Przydatność przetwórcza i charakterystyka owoców jagody kamczackiej I jej przetworów. Eng Sci Technol 4(11):58–66

Kim HS, Park SI, Choi SH et al (2015) Single oral dose toxicity test of blue honeysuckle concentrate in mice. Toxicol Res 31(1):61–68. https://doi.org/10.5487/TR.2015.31.1.061

Kim JW, Lee YS, Seol DJ et al (2018) Anti-obesity and fatty liver-preventing activities of *Lonicera caerulaea* in high-fat diet-fed mice. Int J Molecular Med 42:3047–3064. https://doi.org/10.3892/ijmm.2018.3879

Kowalczyk B (2019) Wina i nalewki z jagody kamczackiej, In: Konferencja Kamczacka, Hortus Media, Krakow, Poland, pp 129–134

Kozłowska H, Troszyńska A (1999) Rola naturalnych substancji nieodżywczych pochodzenia roślinnego jako składników żywności funkcjonalnej. Żywność. Nauka. Technologia Jakość 4(21):63–73

Krasnowska G, Sikora T (2011) Suplementy diety a bezpieczeństwo konsumenta. Żywność. Nauka. Technologia. Jakość 4 (77):5–23

Król-Dyrek K (2017) Przydatność odmian suchodrzewu jadalnego do uprawy w Polsce w zależności od ich pochodzenia. Konferencja Kamczacka. Hortus Media Krakow, Poland, pp 19–28

Kucharska AZ, Fecka I (2016) Identification of iridoids in edible Honeysuckle Berries (*Lonicera caerulea var. kamtschatica* Sevast.) by UPLC-ESI-q TOF-MS/MS. Molecules 21:1157. https://doi.org/10.3390/molecules21091157

Kucharska AZ, Sokół-Łętowska A, Oszmiański J, Piórecki N, Fecka I (2017) Iridoids, phenolic compounds and antioxidant activity of edible Honeysuckle Berries (*Lonicera caerulea var. kamtschatica* Sevast.). Molecules 22:405. https://doi.org/10.3390/molecules22030405

Kulpa W (1964) Zasady nomenklatury roślin uprawnych. Acta Agrobotanica 16:12–13

Kupska M, Chmiel T, Jędrkiewicz R, Wardencki W, Namieśnik J (2014) Comprehensive two-dimensional gas chromatography for determination of the terpenes profile of blue honeysuckle berries. Food Chem 52:88–93. https://doi.org/10.1016/j.foodchem.2013.11.129

Kusibab T, Kusibab-Mruk J (2019) Obserwacje I wnioski po 4 latach uprawy jagody kamczackiej na plantacji. Konferencja Kamczacka. Hortus Media Krakow, Poland, pp 59–70

Lauritzen E, Maughan T, Black BL (2015) Haskap (Blue Honeysuckle) in Garden. Horticulture/Fruit/ Extension Utah State University 04. https://doi.org/10.13140/RG.2.1.2978.6645

Lee YS, Cho IJ, Kim JW et al (2019) Hepatoprotective effects of blue honeysuckle on CCl_4-induced acute liver damaged mice. Food Sci Nutr 7:322–338. https://doi.org/10.1002/fsn3.893

Li F, Zhao H, Xu R et al (2019) Simultaneous optimization of the acidified water extraction for total anthocyanin content, total phenolic content, and antioxidant activity of blue honeysuckle berries (*Lonicera caerulea* L.) using response surface methodology. Food Sci Nutr 7:2968–2976. https://doi.org/10.1002/fsn3.1152

Litwin A (2019) Możliwości zagospodarowania owoców, na podstawie obserwacji bieżących trendów krajowych i zagranicznych. Konferencja Kamczacka. Hortus Media Krakow, Poland, pp 123–128

Liu C, Zheng X, Jia S, Ding N, Gao X (2009) Comparative experiment on hot-air and microwave-vacuum drying and puffing of blue honeysuckle snack. Inter J Food Eng 5:1–9. https://doi.org/10.2202/1556-3758.1683

Liu C, Zheng X, Shi J, Xue J, Lan Y (2010) Optimising microwave vacuum puffing for blue honeysuckle snacks. Inter J Food Eng 133:108–115. https://doi.org/10.1111/j.1365-2621.2009.02156

Liu M, Tan J, He Z et al (2018) Inhibitory effect of blue honeysuckle extract on high-fat-diet-induced fatty liver in mice. Animal Nutrition 4:288–293. https://doi.org/10.1016/j.aninu.2018.06.001

Miyashita T, Araki H, Hoshino Y (2011) Ploidy distribution and DNA content variations of *Lonicera caerulea* (Caprifoliaceae) in Japan. J Plant Res 124:1–9

Molina AK, Vega EN, Pereira C et al (2019) Promising Antioxidant and antimicrobial food colourants from *Lonicera caerulea* L. *var*. kamtschatica. Antioxidants 8:394. https://doi.org/10.3390/antiox8090394

Myjavcova R, Marhol P, Kren V et al (2010) Analysis of anthocyanin pigments in *Lonicera* (*Caerulea*) extracts using chromatographic fractionation followed by microcolumn liquid chromatography-mass spectrometry. J Chromato A 1217:932–7941. https://doi.org/10.1016/j.chroma.2010.05.058

Oszmiański J, Kucharska AZ (2018) Effect of pre-treatment of blue honeysuckle berries on bioactive iridoid content. Food Chem 240:1087–1091. https://doi.org/10.1016/j.foodchem.2017.08.049

Oszmiański J, Wojdyło A, Lachowicz S (2016) Effect of dried powder preparation process on polyphenolic content and antioxidant activity of blue honeysuckle berries (*Lonicera caerulea* L. *var. kamtschatica*). LWT-Food Sci Technol 67:214–222. https://doi.org/10.1016/j.lwt.2015.11.051

Palikova I, Valentova K, Oborna I, Ulrichova J (2009) Protectivity of Blue Honeysuckle extract against oxidative human endothelial cells and rat hepatocyte damage. J Agric Food Chem 57:6584–6589. https://doi.org/10.1021/jf9003994

Pierzga K (2001) Suchodrzew jadalny. In- Szkolkarstwo 5:14–15

Plekhanova MN (1996) Blue Honeysuckle: a new berry from Russia. Pomona 29(1):46–48

Plekhanova MN (2000) Blue honeysuckle (*Lonicera caerulea* L.)—a new commercial berry crop for temperate climate: Genetic resources and breeding. Acta Hort 538:159–164

Podymniak M (2017) Introduction, In: Konferencja Kamczacka. Hortus Media Krakow, Poland, p 5

Poseł P (2019) Pięć lat z jagoda kamczacką. Konferencja Kamczacka. Hortus Media Krakow, Poland, pp 141–151

Poyarkova AI (2000) *Lonicera* L. In- Schischkin B ed, Flora of the USSR, 23 Science Publishers Moscow, Russia, pp 446–549

Raudsepp P, Koskar J, Anton D, Meremae K et al (2019) Antibacterial and antioxidative properties of different parts of garden rhubarb, blackcurrant, chokeberry and blue honeysuckle. J Sci Food Agric 99:2311–2320. https://doi.org/10.1002/jsfa.9429

Rop O, Reznicek V, Mlcek J et al (2011) Antioxidant and radical oxygen species scavenging activities of 12 cultivars of blue honeysuckle fruit. Hort Sci 38(2):63–70

Rupasinghe HPV, Arumuggam N, Amararathna M. De Silva ABKH (2018) The potential health benefits of haskap (*Lonicera caerulea* L.): Role of cyanidin -3-*O*-glucoside. J Funct Foods 44:24–39

Sabitov A (1986) Species of blue fruited honeysuckle, prospects for breeding. All union Order of Lenin and Order of Friends of Nature Science Research Institute of Plant Growing, Leningrad, Russia

Senica M, Stampar F, Mikulic-Petkovsek M (2019) Different extraction processes affect the metabolites in blue honeysuckle (*Lonicera caerulea* L. subsp. *edulis*) food products. Turkish J Agric for 43:576–585

Sharma A, Kim JW, Ku SK, Choi JS, Lee HJ (2019) Anti-diabetic effects of blue honeyberry on high-fed-diet-induced type II diabetic mouse. Nutr Res Pract 13(5):367–376. https://doi.org/10.4162/nrp.2019.13.5.367

Skupień K, Oszmiański J, Ochmian I, Grajkowski J (2007) Characterization of selected physicochemical features of blue honeysuckle fruit cultivar Zielona. Polish J Nat Sci 4:101–107

Sołtysiak U (2019) Uprawy jagodowe w systemie produkcji ekologicznej. Konferencja Kamczacka. Hortus Media Krakow, Poland, pp 71–78

Stremer P (2019) Sukcesy i niepowodzenia w uprawie jagody kamczackiej w tunelach. Konferencja Kamczacka. Hortus Media Krakow, Poland, pp 15–22

Svarcova I, Heinrich J, Valentova K (2007) Berry fruits as a source of biologically active compounds: the case of Lonicera cearulea. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 151(2):163–174

Svobodova A, Rambouskova J, Walterova D, Vostalova J (2008) Protective effects of phenolic fraction of blue honeysuckle fruits against UVA-induced damage to human keratinocytes. Arch Dermatol Res 300:225–233. https://doi.org/10.1007/s00403-008-0850-5

Svobodova A, Zdarilova A, Vostalova J (2009) *Lonicera caerulea* and *Vaccinium myrtillus* fruit polyphenols protect HaCaT keratinocytes against UVB-induced phototoxic stress and DNA damage. J Dermatol Sci 56:196–204. https://doi.org/10.1016/j.jdermsci.2009.08.004

Szot I, Lipa T, Sosnowska B (2014) Jagoda kamczacka—właściwości prozdrowotne owoców i możliwości ich zastosowania. Żywność. Nauka. Technologia Jakość 4(95):18-29. https://doi.org/10.15193/ZNTJ/2014/95/018-029

Thompson MM (2006) Introducing haskap, Japanese blue honeysuckle. J Amer Pomol Soc 60(4):164–168

Thompson MM, Barney DL (2007) Evaluation and breeding of haskap in North America. J Amer Pomol Soc 61(1):25–33

Thompson M, Chaovanalikit A (2003) Preliminary observations on adaptation and nutraceutical values of blue honeysuckle (Lonicera caerulea) in Oregon, USA. Acta Hortic 626:65–72

Wojdyło A, Jauregui PNN, Carbonell-Barrachina AA, Oszmiański J, Golis T (2013) Variability of phytochemical properties and content of bioactive compounds in *Lonicera caerulea* L. *var. kamtschatica* Berries. J Agric Food Chem 61:12072–12084. https://doi.org/10.1021/jf404109t

Wu S, Hou DX (2021) Haskap (*Lonicera caerulea*) Berries. In: Asian berries health benefits, vol 16. Functional Foods and nutraceuticals series CRC Press FL USA, pp 327–328

Wu S, He X, Wu X et al (2015) Inhibitory effects of blue honeysuckle (*Lonicera caerulea* L.) on adjuvant-induced arthritis in rats: Crosstalk of anti-inflammatory and antioxidant effects. J Funct Foods 17:514–523. https://doi.org/10.1016/j.jff.2015.06.007

Zadernowski R, Naczk M, Nesterowicz J (2005) Phenolic acid profiles in some small berries. J Agric Food Chem 3:2118–2124. https://doi.org/10.1021/jf040411p

Zhao J, Zang J, Lin Y, Wang Y, Li D, Meng X (2019) Polyphenol-rich blue honeysuckle extract alleviates silica-induced lung fibrosis by modulating Th immune response and NRF2/HO-1 MAPK signaling. J Funct Foods 53:176–186. https://doi.org/10.1016/j.jff.2018.12.030

Zlabur JS, Colnar D, Voca S, Lorenzo JM et al (2019) Effect of ultrasound pre-treatment and drying method on specialized metabolites of honeyberry fruits (*Lonicera caerulea* var. *kamtschatica*). Ultrason Sonochem 56:372–377. https://doi.org/10.1016/j.ultsonch.2019.04.034. http://polskiesuperowoce.pl – Poznaj jagodę kamczacką na jednej z 27 polskich plantacji - 8.06.2020

Chapter 13
Cultivation and Utilization of Shiitake Mushroom

F. Atila

Abstract Shiitake (*Lentinula edodes*) is the third most commonly cultivated edible mushroom species in the world. It has attracted people's attention with its medical properties as well as taste and nutritional value. Shiitake which has been known and used in Chinese medicine for more than 2000 years is now considered a great resource for modern clinical and pharmacological research. This mushroom contains many biologically active compounds (polysaccharides, lentinan, LEM and KS–2, ergosterol, nucleic acid derivatives, water-soluble lignins, eritadenine, etc.) which possess different medicinal effects such as antitumor, immunomodulatory, hypocholesterolemic, antibacterial, antifungal, anti–inflammatory and antioxidant. The chapter presents an overview of the research on the shiitake mushroom including its taxonomy, cultivation techniques, biotechnological approach, functional compounds and medicinal properties.

Keywords *Lentinula edodes* · Medicinal mushroom · Lentinan · Eritadenine · Anticancer

13.1 Introduction

Shiitakae (*Lentinula edodes)* is the third most commonly cultivated edible mushroomin the world, ranking just behind *Agaricus bisporus* and *Pleurotus* spp. representing about 17% of worldwide production (Zervakis and Koutrotsios 2017).

Although it is also known by different names such as the oakwood mushroom, the golden oak mushroom and the black forest mushroom (USA), xiang–gu and dong–gugo (China), lectin (France) in different parts of the world, today the name shiitake is the most widely used name for this mushroom. "Shiitake" name was derived from two words, "shii" (shii tree (*Castanopsis cuspidata* (Thunb.) Schottky)) and "take" (mushroom in Japanese), so shiitake means "mushroom of the shii or oak tree" in Japanese.

F. Atila (✉)
Faculty of Agriculture, Department of Horticulture, Kirsehir Ahi Evran University, 40200 Kırşehir, Turkey
e-mail: funda.atila@ahievran.edu.tr

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_13

The shiitake mushroom has been a symbol of healthy and youth for thousands of years in Far Eastern culture. Although shiitake production started in China and Japan, today there is also a significant increase in the production and consumption of shiitake in other parts of the world.

Until the mid-1980s, Japan that grown shiitake on natural logs was the main producer of shiitake in the world, while China became the major producer of shiitake in a short time with the development of sawdust-based techniques. According to the data of China Edible Mushrooms Association, in 2015, shiitake mushroom production accounted for 20% of the total edible mushroom production in China (Li et al. 2018), and this amount is also estimated to be approximately 98% of worldwide shiitake production (Yamanaka 2017).

Shiitake is a mushroom species that take people's attention with its high nutritional value and taste. Besides nutritional value, shiitake is one of the most widely known medical mushrooms in the world. The shiitake mushroom was known and used in classical Chinese medicine since more than 2000 years ago (Mizuno 1995). Shiitake contains several bioactive compounds, including polysaccharides, lentinan, LEM and KS–2, ergosterol, nucleic acid derivatives, water-soluble lignins and eritadenine. Shiitake has immune modulating, anticarcinogenic and antitumor, antioxidative, antilipidemic, hepatoprotective, antiviral, antibacterial and antiparasitic effects in relation to these bioactive compounds contained in fruitbody and mycelium.

Shiitake can be consumed directly as fresh and dried food as well as health supplements. Nowadays, different types of supplements obtained from shiitake are sold as capsules, tonics or tablet in many of countries in the world (Bisen et al. 2010).

The chapter focuses on cultivation and medicinal properties of shiitake which is an edible and medicinal mushroom. The aim of this article is to gather and summarize available information on the shiitake mushroom, including its taxonomy, enzyme production, cultivation techniques, functional compounds and medicinal properties.

13.2 Geographic Distribution

Lentinula genus includes five morphologically defined species that were identified on the basis of morphology characteristics and geographical distribution by Pegler (1983) (Table 13.1). However, through subsequent research, it was determined that the phylogenetic relationships of *Lentinula* genus were more complex than the Pegler's would suggest, and apart from these five species, new *Lentinula* species were also identified (Mata and Petersen 2000; Hibbett 2001; Mata et al. 2001).

Shiitake (*L. edodes*) grows naturally throughout Southeast Asia, but the exact limits are uncertain. Samgina (1981) reported that *L. edodes* was found in Kazakhstan. This report notes that the mushroom was found on conifer wood. But *L. edodes* is usually grow on *Quercus, Castanopsis and Lithocarpus* (Pegler 1983). Therefore, the species idetification may have been incorrect.

L. edodes was first identified as *Agaricus edodes* by Miles Joseph Berkeley in 1877. Then, Singer placed shiitake in *Lentinus* genus in 1936. David Pegler suggested

Table 13.1 Geographical distribution of *Lentinula* genus (Pegler 1983)

Species	Geographic distribution
Lentinula boryana (Berk. & Mont.) Pegler	Central America, northern South America, and the Gulf Coast states of North America
Lentinula guarapiensis (Speg.) Pegler	Paraguai
Lentinula edodes (Berk.) Pegler	North-east Asia
Lentinula lateritia (Berk.) Pegler	Southeast Asia and Australasia (except New Zealand
Lentinula novaezelandieae (Stev.) Pegler	New Zealand

transferring this species to genus *Lentinula* based on microscopic observations in 1975. Molecular phylogenetic studies also support relocation of shiitake from the genus *Lentinus* to genus *Lentinula* (Molina et al. 1992; Hibbett and Vilgalys 1993; Hibbett and Donoghue 1996). Today, shiitake is classified in the genus *Lentinula*, the family *Tricholomataceae*, the order *Agaricales* and the subphylum of *Basidioimycotina.* But even now, the shiitake is often still being misspelt as *Lentinus edodes* (Berk.).

Shiitake is a fleshy gilled mushroom. The mushroom produces white-colored spores and white mycelia. Shiitake pileus that are light tan to dark brown is convex to applanate, and size of pileus ranges from 5 to 25 cm. The stipe is usually attached to the pileus centrally. Deep cracks can occur that reveal the underlying white tissue on pileus when shiitake grown on hardwood logs.

Shiitake is a saprophytic white-rot fungi that has the ability to enzymatically degrade cellulose, lignin and other macromolecules (Asgher et al. 2008). In the nature, this mushroom grows in cutting or dead logs particularly of the oak family (*Quercus* spp.) and various deciduous or broad-leaved trees in warm and humids regions (Royse 1997).

13.3 Nutritional Properties of Shiitake

Shiitake has high nutritional content as well as excellent flavor. This high-quality mushroom has important nutrients including dietary fiber (Mattila et al. 2002), minerals (George et al. 2014), vitamin B_{12} (Bito et al. 2014) and vitamin D (Jasinghe and Perera 2006), while it does not have vitamins A and C.

Also, the shiitake mushroom represents an excellent protein supplement. Dried shiitake contains a level of protein comparable to that of several different types of meat. In addition, shitake has 18 different amino acids and almost ideal ratios of eight essential amino acids (Turło et al. 2008). The essential amino acid content of shiitake is better than soybeans, meat, milk or eggs (Vetter 1995), especially they are rich in arginine and lysine (Liu and Bau 1980).

Dietary fiber plays an *important* role in preventing type 2 diabetes, insüline resistance, obesity, hypertension and some type of cancers (Galisteo et al. 2008). The dietary fiber content of shiitake is significantly higher than meats, whereas its fat content is much lower. Moreover, 77.7% of fatty acid content of shiitake consist of unsaturated fatty acids (Bisen et al. 2010). High dietary fiber and unsaturated fatty acid content of shiitake may help protect against cardiovascular diseases by lowering cholesterol values.

13.4 Major Active Compounds Isolated from Shiitake

13.4.1 Lentinan

Lentinan (β–(1→3)–D–glucan) is a polysaccaride isolated from the fruiting bodies or mycelium of shiitake. It is situated in cell wall and has a high molecular weight. The estimated molecular weight of lentinan was reported as 400– 800 $\times$ 10^3 Da by Ooi and Liu (2000). The chemical structure of lentinan is consisting of five β–(1 → 3)–D–glucopyranoside in a linear linkages and two β–(1 → 6)–D– glucopyranoside branches in side chains. This structure results in a right-handed triple-helical form (Wang et al. 2020). The biological activity is assossiated with the position of the glucose molecules in the helix structure (Surenjav et al. 2006). The molecular weight of polysaccades has also an influence on the immune stimulating effect as well as the degree of branching and chain conformation of them (Kulicke et al. 1997).

The lentinan displays numerous bioactivities such as immunomodulator and antitumor (Chihara et al. 1970; Zheng et al. 2005), antivirus (Guo et al. 2009), stimulating the expression of cytokines (Kupfahl et al. 2006) and hypocholesterolemic (Gu and Belury 2005). It does not have direct cytotoxic effects on cancer cells, instead it displays antitumor activity by strengthening the host immune system (Chihara et al. 1970) Moreover, lentinan has been recognized as a promising compound for the formulation of new functional foods and nutraceuticals due to its minimal side effects in addition to medicinal properties mentioned above. The sulfated derivatives of lentinan also exhibit bioactivities similar to lentinan such as strengthening the immune system (Guo et al. 2009).

13.4.2 Lem

Lentinula edodes mycelium (LEM) is a bioactive substance derived from powdered mycelia of shiitake harvested before fructification. The major active compound od LEM is a heteroglycan protein conjugate, being a protein-bound polysaccaride and largely composed of sugar (44%) and protein (24.6%) (Sugano et al. 1982). In addition to heteroglycan protein complex in the structure of LEM, various nucleic acid

derivatives, thiamine (vitamin B1), riboflavin (vitamin B2), ergosterol and eritadenine (Breene 1990), water-soluble lignins (Suzuki et al. 1990) and KS–2 (Fujii et al. 1978) are also presented.

LEM extract has thus been used as a medicinal food for at least 30 years in Japan (Yoshioka et al. 2012). LEM has been determined to have some bioactivity such as antioxidant (Akamatsu et al. 2004), hepatoprotective (Watanabe et al. 2006; Yoshioka et al. 2012), immunoregulatory activity and anticancer (Sugano et al. 1982; Kojima et al. 2010) activity. The antitumor activity of LEM is also thought to be associated with strengthening the host immune system rather than direct cytotoxicity, similar to lentinan. Macrophages are vital components of immune system, and LEM may play a significant role in macrophage stimulation. This macrophage activation is thought to be related to the antitumor and immunoregulatory activity of LEM (Morinaga et al. 1992).

13.4.3 Ks–2

KS–2 is a peptide–polysaccharide complex isolated from shiitake. KS–2 is consist of a-linked mannose and a small amount of peptide which is composed of serine, threonine and alanine with residual amounts of the other amino acids (Bisen et al. 2010). KS–2 polysaccharides possess antitumor and antiviral properties, and the molecular weight of KS–2 was reported as between 6.0×10^4 and $9.5 \times l0^4$ (Fujii et al. 1978).

13.4.4 Eritadenine

Eritadenine (2(R),3(R)–dihydroxy–4–(9– adenyl)–butyric acid), also known as lentinacin or lentysine, is a nucleic acid derivative produced mainly by shiitake. It was isolated from shiitake first time by Chibata et al. (1969) and Rokujo et al. (1970). Eritadenine is considered to be one of the major active substances accountable for hypocholesterolemic activity of shiitake (Sugiyama et al. 1995; Shimada et al. 2003; Enman et al. 2008; Bisen et al. 2010). To date, a number of studies (Sugiyama et al. 1995) have reported on the mechanism by which eritadenine exerts its cholesterol and triglyceride lowering properties, but the detailed mechanism is not yet completely explained.

13.4.5 Lectins

Lectins are carbohydrate-binding proteins (glycoproteins) of non-immunoglobulin origin, with an ability to produce cell agglutination (Dixion 1981). Various biological activities of mushroom lectins such as immunomodulatory and anti-tumor (Wang et al. 1996; Zhang et al. 2010), antivirus (Li et al. 2008) antifungal (Chandrasekaran et al. 2016), antibacterial (Chandrasekaran et al. 2016) and hypotensive (Wang et al. 1996) have been reported. However, there are few studies on the lectins of shiitake mushrooms (Jeune et al. 1990; Wang et al. 1999; Vetchinkina et al. 2008). Extracellular lectin activity of shiitake grown in submerged cultures was reported in some studies (Tsivileva et al. 2005; Wang et al. 1999; Vetchinkina et al. 2008).

The saline extract of the fruiting bodies of shiitake contains an amount of lectin that represents about 10% of the protein content of the fruitbody (Li et al. 2018). Lectin activity of fruiting bodies of shiitake is usually higher that that of lectins of mycelia. Mitogenic effects of lectin isolated from shiitake in human and murine were determined by Jeune et al. (1990) and Moon et al. (1995). A lectin isolated from shiitake has ability to agglutinate L1210 cell lines and HeLa cells (Moon et al. 1995).

13.4.6 Lentin

The lentin, a antifungal protein, isolated from the fruiting bodies of shiitake, exhibiting strong antifungal activity. It also showed an ability of inhibit proliferation of HIV–1 reverse transcriptase and leukemia cells (Ngai and Ng 2003).

13.4.7 Ergesterol

Ergosterol, (ergosta–5,7,22–trien–3β–ol) is a sterol found in cell membranes of mushrooms. It is abundant in shiitake as most of edible mushrooms. Ergosterol is provitamin form of D_2, and ultraviolet irradiation can convert these bioactive sterols to vitamin D_2 (Jasinghe and Perera, 2006; Morales et al. 2017). Shiitake mushrooms containing about 0.5% ergosterol (dry weight) were able to produce 400 IU of vitamin D per gram after being exposed to a fluorescent sunlamp (Breene 1990). Although mushrooms are traditionally dried under the sun, today this process is mostly performed in mechanical dryers.

Vitamin D_2 content of shiitake mushroom can be increased up to 5 times by exposure to direct sunlight for 3 h/day. Exposure to sunlight also increases the free amino acid content of the fruitbodies, making them sweeter and less bitter (Kiribuchi 1991).

13.5 Shiitake Cultivation

Shiitake is a saprotrophic mushroom, which means that they obtain the nutrients they need by decomposing various lignocellulosic waste. The ability of shiitake in converting complex lignocellulosics into simple organic compounds has been allowed many agricultural waste to be used in the cultivation of shiitake. In the commercial production of shiitake mushroom, two different techniques are applied, namely natural log cultivation and bag cultivation techniques in the world.

13.5.1 Natural Log Cultivation Technique

Cultivation of shiitake on natural logs began in far east almost a thousand years ago (Chang and Miles 2004). The first traditional cultivation method used natural logs, usually from the oak family such as shii tree (*Castanopsis cuspidata*) under outdoor conditions. This method has been the most common cultivation method until the mid-1980s.

Shiitake can be grown on many kind of hardwood and softwood trees, but oak (*Quercus*) are the most widely used species in natural log cultivation. Although logs from hardwood trees have longer fruiting period, harvest starts later than those of softwood logs. One of the most important points in the selection of logs is that shiitake mycelia colonize easily on the sapwood. Sapwood is living portion of log and contains polysaccharides needed for mycelial development, whereas colonization is difficult in the heartwood, a dead portion. For this reason, logs with a wide sapwood portion should be preferred while selecting for the production of shiitake.

Spawn running period in the log cultivation of shiitake may take 6–18 months, whereas cultivation cycle takes approximately 6 years depending on spawn, tree species, log size, moisture content of logs and climate factors, etc. Maximum biological efficiency is around 33%. Approximately 75% of the total yield is obtained the 2nd and 3rd years (Royse 2001).

Production of shiitake on log has been steadily declining with the development of sawdust-based techniques that has the shorter crop cycle and quick return of the money invested. However, the natural log technique has also some advantages such as requiring less care and labor, less susceptibility to microorganisms, rich in flavor and bioactive content. High molecular weight polysaccharide contents of shiitake mushrooms grown on logs are higher than those of fruitbodies grown on bags (Brauer et al. 2002).

13.5.2 Bag Cultivation Technique

After the middle of the 80s, a new method that uses plastic bags which are filled with lignocellulosic substrates has gradually replaced the traditional system on tree logs (Chang and Miles 2004). The time between the start and end of the crop cycle of the bag cultivation system is approximately 3 months, which corresponds to approximately 6% of the that of the log system. Moreover, in this technique, the biological efficiency is on average 75 to 125%, depending on the substrates used (3 times higher than the log system). The bag cultivation technique has advantages such as a shorter cultivation cycle, higher yields and year-round mushroom production, even if a bag cultivation technology needs relatively high initial investment cost of installation.

13.5.2.1 Growing Substrates

Although hardwood sawdust is the most commonly substrate for shiitake cultivation, various studies have shown that different agricultural or agro-industrial by-products that are locally abundant and cheaper such as cotton straw (Levanon et al. 1993), wheat straw and corn cobs (Philippoussis et al. 2003), sunflower hulls (Curvetto et al. 2002), hazelnut husk (Özçelik and Pekşen 2007), chickpea straw, sunflower head residue, alfalfa hay, corn stalk (Atila 2019a) may be alternative substrates for shiitake cultivation.

Supplementation of sawdust with millet, rice bran, sugarcane molasses, maize powder, rye, soy flour, grape pomace has improved mushrooms yields considerably (Royse 1996; Rossi et al. 2003; Royse and Sanchez 2007; Moonmoon et al. 2011; Atila 2019b), and but the use of alternative substrates rich in phenolic content such as olive press cake (Gregory and Pohleven 2014 and green walnut husk (Atila 2019b) seem to affect negatively mycelia growth and yield of shiitake. The situation could be associated with the presence of phenolic compound inhibiting mycelium growth and fructification.

13.5.2.2 Substrate Preparation

Optimal environmental conditions and choosing the suitable substrates are essential for success of shiitake cultivation. The first step of shiitake production is the preparation of the growing medium. Substrate preparation is based on process involving shredding, wetting, mixing and sterilization.

If sawdust is used as basal substrate in the preparation of the growing medium, no shredding is required. However, straw and other agricultural wastes such as corn cobs, cotton stalk and corn stalk need to be shredded into pieces 3–5 cm in length to facilitate disinfection and bagging process. Then, the substrates are soaked in water 6–12 h at room temperature and drained. Shiitake mycelium needs nitrogen sources as well as carbon. Therefore, several supplements rich in nitrogen such as bran, soybean

flour and maize powder are added to basal substrate. Overuse of supplements may cause the growth of some competitive organisms such as green mold *(Trichoderma* sp). After the mixing process is completed, the moisture of the growing medium is adjusted to 60–65% and filled in bags. Substrate disinfection is an important stage for maximum yield and quality in shiitake production. Autoclave sterilization method is generally used for substrate disinfection. However, it is difficult to use this method by some farmers due to the high cost of production and the need for expensive equipment. For this reason, steam sterilization has been recommended by some researchers (Mata and Savoie 1998; Savoie et al. 2000). However, this method is not commercially available.

13.5.2.3 Spawning Substrate and Incubation

After cooling, sterilized bags are inoculated with spawn at 1–5% (w/w) ratio in sterile environment. Incubation period must be carried out at 25 ºC ± 2 with a 12-hour light and 12-hour dark cycle. Unlike the other commercial mushroom species, in the production of shiitake, vegetative phase takes place in two stages, spawn run and browning. At the end of the spawn running period, the entire surface of the substrate turns from white to brown, and a hard hipha crust forms on the surface of the substrate, indicating that mycelium is ready for fructification. Although it is not a general rule, after the incubation period, generally the plastic bags are removed, the substrate blocks are soaked or sprinkled with cold water for fruiting induction, and then, bags are transferred to the production room at 17 to 19 ºC with a humidity of 90%. Moreover, lighting should be provided 12 h daily. Mushrooms should be harvested when they are turgid and before the pileus extends fully. Harvest may be done by hand, grasping the mushrooms at the base and turning them slightly so that they can be removed without physical damage. After obtaining the first harvest, blocks can be rehydrated to induce a second flush by soaking them in water for 12 h (Gaitán-Hernández and Mata 2004).

13.5.2.4 Yield

Shiitake mushrooms yield better in substrates containing moderate amounts of N, hemicellulose and lignin and with a low cellulose: lignin ratio (Atila 2019a). Biological efficiency differs considerably by ranging from 2.8 to 124.1% in sawdust-based substrates (Diehle and Royse 1996; Pire et al. 2001; Atila 2019b), up to 99.3% in straw-based substrates (Gaitan-Hernandez and Mata 2004; Philippoussis et al. 2007; Elisashvili et al. 2015) or from 102 to 112% in sunflowers hulls (Curvetto et al. 2002) (Table 13.2).

Table 13.2 Biological efficiency of shiitake grown on different substrates

Substrate	Biological efficiency (%)	References
Sawdust (maple and Birch) Rice bran, millet	6.11–124.1	Diehle and Royse (1986)
Cotton straw (CS) Cotton straw + wheat straw (CWS)	CS–46 CWS–82	Levanon et al. (1993)
Sawdust Wheat bran, white millet, rye, CO_3	59.1–99.6	Royse (1996)
Sugarcane baggase, Sugarcane leaves Pineapple crown	36.3–133.0	Salmones et al. (1999)
Various formulations of rice straw, chestnut sawdust, pinus sawdust, soyflour, rice, barley, maize flour, some chemical fertilizer	42.3–59.5	Morais et al. (2000)
Different types of sawdust	2.8–52.3	Pire et al. (2001)
Sugarcane bagasse + rice bran (25–30%)	98.42–99.84	Rossi et al. (2003)
Oak sawdust, wheat straw, corn cobs	19.44–54.17	Philippoussis et al. (2003)
Pasteurized Wheat Straw	24.8–55.6	Gaitan-Hernandez and Mata (2004)
Sunflower hulls + wheat bran	102–112	Curvetto et al. (2002)
Different formulations of corn cob, Euclyptus sawdust and rice bran	18.88–43.87	Eira et al. (2005)
Vineyard pruning, barley straw, wheat straw	37.02–93.25	Gaitan-Hernandez et al. (2006)
Different mixtures of wheat straw (WS), corn-cobs (CC), and oak wood sawdust (OS) and millet, wheat bran, soybean flour	41.07–80.64	Philippoussis et al. (2007)
HH alone and its mixtures with wheat straw (WS), beech wood-chip (BWC) and wheat bran (WB) in different ratios	43.73–87.73	Özçelik and Pekşen (2007)
Wheat straw Oak sawdust Rye, wheat bran, white millet	80.4–98.9	Royse and Sanchez (2007)
Sawdust with 10%–40% of rice bran wheat bran and maize powder	53.5–153.3 g/500 g substrate	Moonmoon et al. (2011)

(continued)

Table 13.2 (continued)

Substrate	Biological efficiency (%)	References
Various formulations of oak sawdust, corn cobs, wheat straw, maize stubble, chopped cardboard, cotton waste, peanut husk, wheat kernels, wheat bran, rice meal, $MgSO_4$, $CaCO_3$, thiamine, urea	4.9–61	Martínez-Guerrero et al. (2012)
Sawdust (S) and straw (St) with different ratio of wheat bran (Wb)	S + Wb = 7.20 − 81.0 St + Wb = 11.8 – 66.8	Sharma et al. (2013)
Pasteurized wheat straw	66–320	Gaitan-Hernandez et al. (2014)
Chickpea straw, sunflower head residue, alfalfa hay; corn stalk	20.1–51.0	Atila (2019a)
Oak sawdust, grape pomace, green walnut hulls, tea wastes, olive press cake	38.2–70.7	Atila (2019b)

13.6 Medicinal Properties and Usage

Shiitake has been considered as a medicament for the inhibition and treatment of many diseases in the Orient for thousands of years. Today, medicinal properties of the shiitake have been also confirmed by a large number of high-quality scientific researches involving in vitro, in vivo and clinical studies. Extracts from shiitake, entire or part of fruit body, have been stated as having anti-cancer and antitumor, antihypercholesterolemic, hypoglycemic, hepatoprotective, antioxidant and antimicrobial (Table 13.3). In the following sections, major published laboratory and clinical studies on the some medicinal properties of shiitake were summarized.

13.6.1 Antitumor and Immunostimulating Activity

Cancer is the second leading cause of death globally. Although treatment methods such as chemotherapy and radiotherapy are widely used in cancer treatment, they are not always effective and often cause a number of side effects. Therefore, alternative methods of treatment, such as medicinal mushrooms, have attracted great attention of people all over the world.

Shiitake is a mushroom species known for its antitumor properties. Antiproliferative activities of this mushroom against prostate cancer, gastric cancer, breast cancer, colon carcinoma, lung cancer, skin cancer lines have been explored, and promising results have been obtained from many clinical and experimental trials carried out in shiitake (Vere White et al. 2002; Ng et al. 2002; Gu and Belury 2005; Fang et al.

Table 13.3 Biologically active constituents isolated from shiitake and their medicinal properties

Major Bioactive Compounds	Source	Extract	Pharmacological effects	Applications		References
				In vitro	In vivo	
Lentinan	Fruitbody	Ethanol	Immunomodulator		Mice	Chihara et al. 1970
	Fruitbody	5% NaOH–0.05% NaBH4	Immunomodulator	A colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method	Rats	Zheng et al. (2005)
	Mushroom fruiting bodies, mushroom spores and mushroom cultured broth	Ethanol	Hypocholesterolemic	mouse skin carcinoma cell line, CH72, and the non-tumor cell line, C50 cultured in modified Eagle's Minimal Essential Medium		Gu and Belury (2005)
	Fruitbody	Boiling water and ethanol	Antiviral		Chickens	Guo et al. (2009)
	Fruitbody		Antitumor		Mice	Ng and Yap (2002)
LEM	Culturated mycelia of shiitake	Ethanol	Antiviral	Cultured medium		Tochikura et al. (1988)
	Culturated mycelia of shiitake	Hot water and ethanol	Antioxidant Hepatoprotective	İnvitro	Dimethyl nitrosamine-injured mice	Akamatsu et al. (2004)
	Mycelia	Hot water	Hepatoprotective	primary cultured rat hepatocytes		Watanabe et al. (2006)
	LEM extract powder	Hot water and ethanol (50%)	Hepatoprotective	primary cultures of rat hepatocytes exposed to CCl4		Yoshioka et al. (2012)
	Mycelia	Water	Anticancer		Rats	Sugano et al. (1982;)

(continued)

Table 13.3 (continued)

Major Bioactive Compounds	Source	Extract	Pharmacological effects	Applications		References
				In vitro	In vivo	
	Cultured mycelia	Hot water	Anticancer, immunoregulatory activity	In vitro		Kojima et al. (2010)
KS-2	Cultured mycelia	Hot water	Antitumor		Mice	Fujii et al. (1978)
Eritadenine	Dry mushroom	Ethanol	Hypocholesterolemic		Rats	Chibata et al. (1969)
	Fruitbody		Hypocholesterolemic		Rats	Rokujo et al. (1970;)
	Fruitbody		Hypocholesterolemic		Rats	Sugiyama et al. (1995;)
	Pure eritadenine		Hypocholesterolemic		Rats	Shimada et al. (2003)
	Mycelia	Methanol	Hypocholesterolemic	Submerged culture		Enman et al. (2008)
Lectin	Fruitbody	Physiological saline	Antitumor		Animals and human	Moon et al. (1995)
	Mycelium, brown mycelial film, primordium and fruitbody	Water	Hemagglutinating activity	Several agar media		Tsivileva et al. (2001)

(continued)

Table 13.3 (continued)

Major Bioactive Compounds	Source	Extract	Pharmacological effects	Applications		References
				In vitro	In vivo	
O-sulfonated α-D-glucan	Fruitbody	5% NaOH/0.05% NaBH4	Antitumor		Mice	Unursaikhan et al. (2006)
	Fruitbody	Ethanol	Antiviral		Tobacco plants infected tobacco mosaic viruses	Wang et al. (2015)
Mycelial extracts	Mycelia	Water	Antiviral	Culture media		Sasaki et al. (2001)
LEP	Fruitbody	Aqueous and ethanol extracts	Antiviral	Poliovirus, Bovine herpesvirus cells grown in Dulbecco's Modified Eagle Medium (DMEM)		Rincão et al. (2012)
Chitosan	Stripe of fruitbody	Aqueous NaOH	Antioxidant	The conjugated diene method		Yen et al. (2007)

2006; Ina et al. 2013; Zhang et al. 2018). Biologically active substances isolated from shiitake exhibit numerous mechanisms of anticancer activity such as the inhibition of angiogenesis (Deocaris et al. 2005), stimulation of the cancer cells for apoptosis (Fang et al. 2006) or retarding the development of tumors (Ng et al. 2002).

Lentinan, known as immunomodulatory and anticancer agents, is the most broadly studied compound in the shiitake mushroom. The antitumour effect of lentinan is attributed to stimulation of the immune response in various investigations (Chihara et al. 1970; Fujii et al. 1978; Ina et al. 2013). Moreover, lentinan is reported to trigger hematopoietic stem cells, macrophages and natural killer cells (Akramiene et al. 2007). Similarly, macrophage achieved from the KS–2 treated mice. KS–2 strongly inhibited tumor growth in mices who administered orally in both doses between 1 and 100 mg/kg (Fuji et al. 1978).

Antitumor properties of shiitake are not only due to polysaccharides, and a lectin isolated from shiitake was an agglutinin of tumor cell lines tested by L1210 and HeLa cells (Moon et al. 1995). Arginine, a substance used in the supplement of cancer patients, is also abundant in shiitake (Eghianruwa et al. 2011). IA–a (a glycogen-like structure) and IA–b (arabinoxylan-like polysaccharide) isolated from LEM stimulate cytokine production and phagocytosis in RAW264.7 cells (Kojima et al. 2010). The *O*–sulfonated α–D–glucan of shiitake has higher antitumor activity than those of the native glucan (1 → 3)–α–D –glucans and the antitumor activity of the native glukans against S–180 can be enhanced by *O*–sulfonation of these glukans (Unursaikhan et al. 2006).

Several researcher reported that use of the combination of lentinan and chemotherapy drugs has inhibited proliferation and induced apoptosis than use of chemoterapy drugs alone (Zhao et al. 2013; Liu et al. 2015; Sun et al. 2015). Clinical researches have revealed that lentinan is effective in prolonging survival in patients with stomach, ovarian or colorectal cancer (Borchers et al. 1999; Fujimoto et al. 2006) and prevents side effects such as nausea and asthenia, which are common in chemotherapeutic treatment of D. Moreover, administration of lentinan in combination with Bacillus Calmette–Guerin (BCG) vaccine which is used against tuberculosis induces activation of immune cells in the lung tissue (Drandarska et al. 2005).

13.6.2 Hypocholesterolemic Activity

Coronary artery disease (CAD) is the first cause of death worldwide. The most important risk factors of the disease are hypercholesterolemia, obesity, diabetes, high triglycerides and low density lipoprotein cholesterol (LDLc) levels, hypertension and cigarette smoking as well as genetic factors (Abdel-aziz and Mohamed 2013).

The ability of shiitake to lower cholesterol was described for the first time by Kamiya et al. (1969). The major active hypocholesterolemic component in the shiitake mushroom is a adenosine derivative eritadenine. (Takashima et al. 1973). In addition to eritadenine, nucleic acid compounds extracted from shiitake were found to be inhibitors of platelet agglutination (Sugiyama et al. 1995).

Eritadenine can reduce cholesterol level in plasma and expedite lipid accumulation in the liver by removing it from the circulations. LDL is converted into high-density lipoprotein (HDL) cholesterol which is beneficial for the human system in the liver (Kabir and Kimura 1989). Another suggestion regarding the activity of eritadenine is that high dosages of eritadenine may break down the secretion of very low density lipoprotein (VLDL) and reduce cholesterol by lowering the ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE) in liver microsomes (Sugiyama et al. 1995). However, the mechanism that reveals the cholesterol-lowering effect of eritadenine has not been fully elucidated. Eritadenine signafically reduced serum cholesterol, phospholipids and triglycerides, both in intact rats and in animals fed a high-fat diet (Rokujo et al. 1970). The eritadenine content in the shiitake mushrooms was in the range 3.2–6.3 mg/g dried mushrooms (Enman et al. 2007). A diet containing eritadenine (0.005%) caused a 25% reduction in total cholesterol within 1 week (Chibata et al. 1969). The use of eritadenine 10–21 mg/kg/day in male and female rats decreased the atherogenic index (TC/HDL) in rat sera (Morales et al. 2018). Although the hypocholesterolemic effect of eritadenine has been investigated in several studies on rats (Sugiyama et al. 1995; Shimada et al. 2003), there are few human studies in the literature (Suzuki and Ohshima 1976).

The experimental and clinical data show that shiitake has beneficial effects on lowering low-density lipoproteins, total cholesterol and triglycerides, as well as in preventing the diseases such as arterial hypertension and high blood sugar levels which are effective in the development of cardiovascular diseases. (Kabir and Kamura 1989; Yang et al. 2002).

13.6.3 Antioxidant Activity

Oxidative stress occurs as a result of an imbalance caused by increased reactive oxygen species (ROS) and/or decreased antioxidant defense systems of the body. Numerous studies have demonstrated that oxidative stress plays a role in the emergence and development of some diseases such as atherosclerosis (Kattoor et al. 2017), muscle wasting (Moylan and Reid 2006), hypertension (Higashi et al. 2002), neurodegeneration (Brown 2005) and stroke (Cherubini et al. 2005).

Methanolic extract of shiitake is a promising alternative for use as an antioxidant (Sasidharan et al. 2010). LEP (polysaccharides isolated from shiitake), which can act as an antioxidant, may play a role in healing oral ulceration. LEP administration, in rats with oral ulceration, significantly was increased activities of serum antioxidant enzymes, whereas it was decreased levels of serum, mucosal interleukin–2 (IL–2) and tumor necrosis factor alpha (TNF–a) (Yu et al. 2009). Moreover, the administration of LEP can stimulate the expression of genes encoding antioxidant enzymes, reducing the increased oxidation stress-induced feeding by high-fat diet in rats. In addition, this treatment would decrease expression of VCAM–1mRNA of thoracic aorta endothelial cell in rats (Xu et al. 2008). Another substance that

has strong antioxidant and anti-inflammatory properties in the shiitake mushroom is ergothioneine, and it is an amino acid analog (Jang et al. 2016).

Heat treatment significantly increases the antioxidant activities of shiitake mushrooms. The antioxidant activity of raw shiitake mushroom is increased about 2.0–fold by heat treated at 121 °C for 30 min (Choi et al. 2006). Although freeze drying is suggested for the protection of eritadenine and protein content of fruitbody, hot air drying at 50 °C has been proposed for the stability or formation of total phenolics in shiitake (Zhang et al. 2013).

13.6.4 Hepatoprotective Activity

Shiitake has direct protective effects on hepatocytes. Methanolic extract of shiitake fruitbody can protect liver cells from paracetamol-induced liver damage, with its antioxidative effect on hepatocytes, thus reducing or eliminating the harmful effects of toxic metabolites of paracetamol. Administration of shiitake extract at a dose of 200 mg/kg for seven days to paracetamol-induced hepatatoxic mice reduces the activity of serum enzymes and bilirubin, resulting in significant hepatoprotective effects (Sasidharan et al. 2010). A significant reduction in liver injury was also noted when mice with severe liver damage were fed vitamin D–enriched shiitake mushroom extracts (Drori et al. 2016).

Not only the fruit bodies of shiitake, but also its mycelia has hepatoprotective activity. Polyphenolic compounds contained in the L.E.M. seemed to be responsible for the protective effect (Watanabe et al. 2006). Oral administration of the extracts of LEM has the protective effect against CCl_4 (Chen 2012) and D–galactosamine (Watanabe et al. 2006) induced hepatic injury in rats. Hot water and ethanol extracts of L.E.M. repress the development of liver fibrosis induced by dimethylnitrosamine (DMN) and inhibit proliferation and morphological change of isolated rat hepatic stellate cells (HSCs) (Akamatsu et al. 2004). LEM containing anti-oxidation and anti-inflammation activities might be used for alleviating side effects of chemotherapy and preventing the progression of liver cancer for patients with chronic hepatitis (Yagi 2012).

13.6.5 Antimicrobial Activity

Antibiotics are widely used as therapeutic agents in the treatment of many diseases. But, with increasing bacterial resistance to antibiotics, plants and mushrooms with antibacterial activity have attract attention. The superior abilities of fungi in improving host immunity can be very useful in fighting infection. Shiitake contains several compounds such as lentinan, which have the ability to stimulate humoral immunity to help prevent bacterial infections that are resistant to antibiotics (Markova et al. 2003; Hatvani 2001).

Table 13.4 Antimicrobial activity of shiitake in vitro

Sources	Target organisms	References
Aqueous extracts of dry and fresh mushroom	*Bacillus subtilis, Esherichia coli*	Casaril et al. (2011)
Chloroform, ethylavetate and water extracts of dried mushroom	*Streptococcus* spp., *Actinomyces* spp., *Lactobacillus* spp., *Provotella* spp., *Porphyromonas* spp.	Hirasawa et al, (1999)
The culture filtrate after 18–25 days of cultivation of shiitake	*Bacillus subtilis*	Ishikawa et al. (2001)
Mushroom extract	*Aspergillus ochraceus and Penicillium verrucosum*	Ricelli et al. (2002)
Lentin isolated from fruitbody of shiitake	*Physalospora piricola, Botrytis cinereal, Mycosphaerella arachidicola*	Ngai and Ng, (2003)
Mycelial extracts of shiitake	*Helminthosporium euphorbiae, Helminthosporium sp, Fusarium solani and Phomopsis sojae*	Sasaki et al. (2001)
Mushroom extract	*Aspergillus parasiticus, Aspergillus flavus*	Reverberi et al. (2011)

Extracts and pure compounds of shiitake exhibit high levels of antimicrobial activity, including the antibacterial and antifungal action. The effect of shiitake on the growth of various bacteria and fungi was revealed by several authors (Hirasawa et al. 1999; Ishikawa et al. 2001; Sasaki et al. 2001; Ricelli et al. 2002; Ngai and Ng 2003; Reverberi et al. 2011; Casaril et al. 2011) in vitro studies (Table 13.4).

13.6.6 Antiviral Activity

Various extracts of shiitake mushroom and some polysaccharides isolated from shiitake have been suggested as sources of potential antiviral agents. Aqueous and ethanol extracts and polysaccharide (LeP) from shiitake are effective in the replication of poliovirus type 1 (PV–1) and bovine herpes virus type 1 (BoHV–1) (Rincão et al. 2012). Improvement was observed in liver function tests in patients with cornic hepatitis B and seropositive for hepatitis B (Hbe) antigenemia who consumed 6 g of LEM orally daily for 4 months, while some patients undergo a change from HBeAg-positive to anti-HBe positive (Amagase 1987). A laccase isolated from fresh fruitbody of shiitake mushroom exhibited inhibitory activity to HIV–1 (Sun et al. 2011). LEM and ethanol extract of LEM blocked the HIV virus at the initial stage of its development (Tochikura et al. 1988). Clinical and in vitro studies shown that LEM has ability of inhibition of HIV infection of cultured T-cells. LEM increased the T-cell count in

HIV patients with AIDS symptoms from 1250/mm^3 to 2550/mm^3, and the symptoms were much improved after 60 days (Izuka 1990). Administration of aqueous extracts from shiitake in dose of 0.4–2 mg/mice protected mice against lethality induced by the herpes simplex type 2 virus (Razumov et al. 2013). The feeding of influenza virus-infected mice for 2 weeks with a mixture of glucans obtained mycelial mushroom powders of Shiitake significantly reduced the clinical symptoms of infection. (Vetvicka and Vetvickova 2015). The possibility of using shiitake polysaccarides and other compounds in the control and prevention of viral infections that affect plants and animals was also reported (Sasaki et al. 2001; Wang et al. 2015).

13.6.7 Dosage and Toxicity

It is important to know the efficacious and safety doses of dietary supplements and nutraceuticals in order to benefit effectively from them. The recommended dose is 6–16 g for dried shiitake and about 90 g for fresh fruitbody (Liu and Bau 1980). One study on mice showed that the daily intake of 100 mg/kg of shiitake mushroom could have potential health-improving effects (Grotto et al. 2016). Since an aqueous extract of shiitake fruitbody reduces the activity of blood platelets in the process of coagulation, especially people who are taking blood thinners should be careful when using shiitake or water-soluble fractions (Yang and Jong 1989).

The doses of compounds isolated from shiitake such as lentinan are lower than that of mushroom consumption. Lentinan has been found safe to be administered to humans by IV injection in a dose range of 1–5 mg/day once or twice a week, and greater doses can cause immune suppression (Taguchi et al. 1982; Aoki 1984). In the early stages of AIDS or chronic hepatitis, the best dose of LEM was recommended between 2–6 g per day in 2 or 3 divided doses orally, while the dose may be reduced to 1/2–1 g per day once the disease becomes more stable (Sharon 1988). Lentinan and LEM have no known serious side effects (Aoki 1984). Some people may experience minor side effects or allergic reactions, known as shiitake dermatitis, caused by glucan lentinan (Nguyen et al. 2017).

13.7 Biotechnological Approach

13.7.1 Submerged Liquid Fermentation with Shiitake

Submerged liquid fermentation (SLF) techniques are used in different areas such as liquid spawn production, enzyme production and biomass production for pharmaceutical and nutraceutical applications.

Several researchers reported that liquid has a shortened spawn running period and a higher yield in comparison with the grain spawn (Kawai et al. 1996; Leatham

and Griffin 1984; Lee et al. 2019). On the other hand, thanks to the liquid spawn technology, mycelia can be stored for a long time (Zilly et al. 2011). But there may be some problems in use of liquid spawn such as degeneration and mutation (Itävaara 1993). Submerged liquid cultivation can also be a promising alternative method for bioactive molecules to be obtained in a shorter time, with a higher amount and with less risk of contamination (Harvey et al. 2001; Tepwong et al. 2012). Moreover, various enzyme activities of shiitake have been determined in submerged liquid cultures (Buswell et al. 1996; Nagai et al. 2002). It is possible to eliminate the toxicity of wastewaters by using this feature of shiitake mycelia (D'Annibale et al. 2004). The medium composition and environmental parameters are crucial for optimal biomass, enzyme or metabolite production (Enman et al. 2008; Lee et al. 2019).

13.7.2 Utility of Spent Shiitake Mushroom Substrate

The total production of cultivated mushrooms in the world was approximately 34 million tons in 2013, and shiitake accounted for 7.48 million tons (Royse et al. 2017). Every kg of mushroom produced 5 kg of wet mass SMS (Medina et al. 2012). When 35–40% of the waste compost is calculated as dry matter, the resulting spent shiitake mushroom substrate (SSMS) in 2013 can be estimated about 13 million tons.

Shiitake mycelia secrete various enzymes capable of breaking down polyphenols, including lignin peroxidase, Mn-dependent peroxidase and laccase (Asgher et al. 2008). SSMS contains plenty of shiitake mycelia and can biodegrade organic xenobiotic compounds found in soil and water and adsorb some pollutants (Ahlawat and Sing 2009). This allows the use of SSMS in some biotechnological applications easily and cheaply. The biotechnological areas, in which SSMS can be used, are reviewed in the following section.

13.7.2.1 Bioremediation

The extracellular enzyme system of shiitake developed unique non-specific enzyme systems with the ability to attack not only lignin but also a broad spectrum aromatic compounds as well as some non-aromatic organopollutants such as pentachlorophenol (Okeke et al. 1993), 17α–ethinylestradiol (Eldridgea et al. 2017) and 2,4–dichlorophenol (Tsujiyama et al. 2013). The use of shiitake cultures for these remediation of contaminated soils could be less expensive and beneficial for human health and environment. Moreover, the biological degradation of pollutants using shiitake spent substrates would reduce the cost of disposal.

13.7.2.2 Dye Wastewater Degradation

A large amount of dye wastewaters, which are harmful to the environment and human health, are released into the environment from paint factories and other dye-using industries. Although some methods are applied to dispose of these wastewaters, they are expensive applications (Moreira et al. 2000). Biodegradation of colored wastewater by ligninolytic enzymatic system of white rot fungus appears to be an attractive alternative. Use of shiitake in the degredation processes has a important potential. Several strains of shiitake also have been reported as the dye- or colored material-degrading organisms (Hatvani and Mecs 2002; Boer et al. 2004). On the other hand, olive oil mill water (OMWW) has inhibitor effects of on plant growth, microbial activity and soil properties (Rusan et al. 2016; Mekki et al. 2013). The main cause of the harmful effects of OMWW is considered to be the presence of high concentrations of phenolic compounds (Azam et al. 2002). Some of strain of shiitake show high performance for treatment of OMW, resulting a significant decrease in the color and phenolics concentration of OMWW (Lakhtar et al. 2010).

13.7.2.3 Ethanol Production

In the traditional production of bioethanol, which attracts attention as an alternative fuel today, lignocellulosic materials with high sugar and starch content such as straw, corn, sugar cane, potatoes are used (Watanabe et al. 2010; Belal 2013; Patni et al. 2013). However, uses of most of these products as food cause significant cost increases.

Shiitake growing medium is prepared from various agricultural and forest wastes. SSMS remaining after mushroom production contains high amount of lignin. The use of this waste material with high lignocellulosic content as a raw material in ethanol production reduces the cost (Asada et al. 2011). The producing ethanol from SSMS has been demonstrated in the several studies (Asada et al. 2011; Hiyama et al. 2016; Xiong et al. 2019). However, further studies are needed to develop technologies that will integrate mushroom and biofuel production.

13.8 Future and Perspective

Shiitake is a type of mushroom that is appreciated not only for its unique flavor and nutritional value, but also for its health benefits. Despite these superior properties, some problems with the production and medicinal use of this mushroom need to be addressed in order for shiitake to reach its deserved place.

Although shiitake is widely consumed in some countries, the amount of consumption in some countries is very low or absent. The reasons for low consumption are high prices, lack of familiarity with the mushroom species and low production quantities. Increasing local production and developing new technologies to get higher

yield of shiitake can lead to a decrease in the retail price. Moreover, shiitake production can be a good source of income for farmers, especially living in rural areas of less developed countries, and limited resources can be used beneficially.

In the rural areas, the biggest problem for the farmers could be the supply of growing substrate for shiitake cultivation. In countries where the shiitake production sector does not improve, it is not possible for farmers to obtain the growing substrates from a supplier. On the other hand, it is difficult to prepare the growing medium by himself, because the disinfection of the growing medium in the cultivation of shiitake is carried out by autoclave sterilization method and the establishment and implementation costs of this method are high. Therefore, it is important to develop alternative disinfection methods in order to expand cultivation of shiitake in rural areas. Selection of strains adapted to different substrates and disinfection methods is critically important for prolonged crop cycle and high mushroom yield. Moreover, researches for the selection and breeding of genotypes with high production capacity at high temperatures will be of great benefit in reducing energy consumption in tropical regions and summer production.

Consumers are increasingly interested in functional foods that are proven to help improve human health. Some bioactive substance of shiitake are especially effective at strengthening the immune system and lowering cholesterol and used as active compounds in the development of various functional foods. Although in vitro and in vivo studies conducted with identified bioactive compounds isolated from shiitake offer exciting results for this compounds to be qualified as a functional food and source of potential drugs, more information is required about the therapeutic effects of bioactive ingredients. Further studies, including clinical trials, to determine dose ranges that are both safe and useful in the treatment or prevention of diseases are required to obtain maximum benefit from bioactive compounds without negative consequences. Moreover, detailed studies on the chemical structures and mechanisms of action of some less researched bioactive substances isolated from shiitake may contribute to the determination of the new medicinal properties of the fungus.

Although the content of bioactive substance of shiitake may be affected by the strain, growing substrates and culture conditions, there are no protocols yet to ensure standardization of the quality and quantity of bioactives. For this reason, it is one of the conditions that should be taken into consideration that approved standard production protocols may be required to guarantee the quality and effectiveness of fungal products to be used for pharmaceutical applications. The production of high-quality products of standard quality and the provision of sustainable production under controlled conditions should be determined as the most important targets. The submerged liquid fermentation method appears to be much more useful in achieving these targets.

On the other hand, factors such as temperature and humidity applied in the cultivation of mushrooms provide optimum conditions for the development of harmful fungi species, bacteria and pests. For this reason, it is common to use pesticides to solve the problems related to diseases and pests that are frequently encountered in mushroom production. Considering the negative effects of these pesticides on human health, it should be preferred to apply controlled and certified production systems

such as organic agriculture or good agricultural practices in the production of mushrooms that will be used for medical purposes. Another solution proposal in this regard is to use a submerged culture instead of fruitbody to produce bioactive metabolites for pharmaceutical applications. Mushroom mycelium is known to be very rich in bioactive content. While it is possible to produce mycelium without using chemicals in the production of submerged mycelia culture, it will be much easier to set this type of production to standards.

References

Abdel-aziz TA, Mohamed RH (2013) Association of endothelial nitric oxide synthase gene polymorphisms with classical risk factors in development of premature coronary artery disease. Mol Biol Rep 40:3065–3071

Ahlawat OP, Sing R (2009) Influence of pH, temperature and cultural media on decolorization of synthetic dyes through spent substrate of different mushrooms. J Sci End Res 68:1068–1074

Akamatsu S, Watanabe A, Tamesada M, Nakamura R, Hayashi S, Kodama D, Kawase M, Yagi K (2004) Hepatoprotective effect of extracts from *Lentinus edodes* mycelia on dimethylnitrosamine—induced liver injury. Biol Pharm Bull 27(12):1957–1960

Akramiene D, Kondrotas A, Didziapetriene J, Kevelaitis E (2007) Effects of beta-glucans on the immune system. Medicina (kaunas) 43(8):597–606

Amagase H (1987) Treatment of hepatitis B patients with *Lentinus edodes* mycelium. In: Proceedings of the XII international congress of gastroenterology. Lisbon, p 197

Aoki T (1984) Lentinan. In: Fenichel RL, Chirgis MA (eds) Immune, modulation agents and their mechanisms, vol 25. Immunol Studies, pp 62–77

Asada C, Asakawa A, Sasaki C, Nakamura Y (2011) Characterization of the steam-exploded spent Shiitake mushroom medium and its efficient conversion to ethanol. Bioresour Tech 102:10052–10056

Asgher M, Bhatti HN, Ashraf M, Legge RL (2008) Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. Biodegradation 19:771–783

Atila F (2019a) Compositional changes in lignocellulosic content of some agro-wastes during the production cycle of shiitake mushroom. Sci Hort 245:263–268

Atila F (2019b) The use of phenolic-rich agricultural wastes for *Hericium erinaceus* and *Lentinula edodes* cultivation. Ege Üniv Ziraat Fak Derg 56(4):417–425

Azam F, Muller C, Weiske A, Benckiser G, Ottow J (2002) Nitrification and denitrification as sources of atmospheric nitrous oxide—role of oxidizable carbon and applied nitrogen. Biol Fert Soil 35(1):54–61

Belal EB (2013) Bioethanol production from rice straw residues. Braz J Microbiol 44(1):225–234

Berkeley MJ (1857) Introduction to cryptogamic botany. H.Bailliere, London

Bisen PS, Baghel RK, Sanodiya BS, Thakur GS, Prasad GBKS (2010) *Lentinus edodes*: a macrofungus with pharmacological activities. Curr Med Chem 17:2419–2430

Bito T, Teng F, Ohishi N, Takenaka S, Miyamoto E, Sakuno E, Terashima K, Yabuta Y, Watanabe F (2014) Characterization of vitamin B_{12} compounds in the fruiting bodies of shiitake mushroom (*Lentinula edodes*) and bed logs after fruiting of the mushroom. Mycoscience 55:462–468

Boer CG, Obici L, Souza CGM, Peralta RM (2004) Decolorization of synthetic dyes by solid state cultures of *Lentinula* (*Lentinus*) *edodes* producing manganese peroxidase as the main ligninolytic enzyme. Bioresour Technol 56:1384–1387

Borchers AT, Stern JS, Hackman RM, Keen CL, Gershwin ME (1999) Mushrooms, tumors, and immunity. Proc Soc Exp Biol Med 221:281–293

Brauer D, Kimmons T, Phillips M (2002) Effects of management on the yield and highmolecular-weight polysaccharide content of shiitake (*Lentinula edodes*) mushrooms. J Agri Food Chem 50:5333–5237

Breene W (1990) Nutritional and medicinal value of specialty mushrooms. J Food Prot 53:883–994

Brown DR (2005) Neurodegeneration and oxidative stress: prion disease results from loss of antioxidant defence. Folia Neuropathol 43(4):229–243

Buswell JA, Cai YJ, Chang ST, Peberdy JF, Fu SY, Yu H-S (1996) Lignocellulolytic enzyme profiles of edible mushroom fungi. World J Microbiol Biotechnol 12:537–542

Casaril KBPB, Kasuya MCM, Vanetti MCD (2011) Antimicrobial activity and mineral composition of shiitake mushrooms cultivated on agricultural waste. Braz Arch Biol Technol 54(5):991–1002

Chang S, Miles P (2004) Cultivation, nutritional value, medicinal effect, and environment impact. CRC Press, Florida

Chandrasekaran G, Lee YC, Park H, Wu Y, Shin HJ (2016) Antibacterial and antifungal activities of lectin extracted from fruiting bodies of the Korean cauliflower medicinal mushroom, *Sparassis latifolia* (Agaricomycetes). Int J Med Mushroom 18(4):291–299

Chen J (2012) Recent advance in the studies of β-Glucans for cancer therapy. AntiCancer Agents Med Chem 13:679–680

Cherubini A, Ruggiero C, Polidori MC, Mecocci P (2005) Potential markers of oxidative stress in stroke. Free Radic Biol Med 39(7):841–852

Chibata I, Okumura K, Takeyama S, Kotera K (1969) Lentinacin: a new hypocholesterolemic substance in *Lentinus edodes*. Experientia 25:1237–1238

Chihara G, Hamuro J, Maeda Y, Arai Y, Fukuoka F (1970) Fractionation and purification of the polysaccharides with marked antitumour activity especially lentinan from *Lentinus edodes*. Cancer Res 30:2776–2781

Choi Y, Lee SM, Chun J, Lee HB, Lee J (2006) Influence of heat treatment on the antioxidant activities and polyphenolic compounds of shiitake (*Lentinus edodes*) mushroom. Food Chem 99:381–387

Curvetto N, Figlas D, Delmastro S (2002) Sunflower seed hulls as substrate for the cultivationof shiitake mushrooms. Hort Technol 12:652–655

D'Annibale A, Casa R, Pieruccetti F, Ricci M, Marabottini R (2004) Lentinula edodes removes phenols from olive-mill wastewater: impact on durum wheat (Triticum durum Desf.) germinability. Chemosphere 54:887–894

Deocaris CC, de Castro MC, Oabel AT, Co EL, Mojica ER (2005) Screening for anti–angiogenic activity in Shiitake mushroom (*Lentinus edodes* Berk) extracts. J Med Sci 5(1):43–46

deVere White RW, Hackman RM, Soares SE, Beckett LA, Sun B (2002) Effects of a mushroom mycelium extract on the treatment of prostate cancer. Urology 60(4):640–644

Diehle DA, Royse DJ (1986) Shiitake cultivation on sawdust: evaluation of selected genotypes for biological efficiency and mushroom size. Mycologia 78(6):929–933

Dixion HBF (1981) Defining a lectin. Nature 292:192

Drandarska I, Kussovski V, Nikolaeva S, Markova N (2005) Combined immunomodulating effects of BCG and *Lentinan* after intranasal application in guinea pigs. Int Immunopharm 5(4):795–803

Drori A, Shabat Y, Ya'acov AB, Danay O, Levanon D, Zolotarov L, Ilan Y (2016) Extracts from *Lentinula edodes* (Shiitake) edible mushrooms enriched with vitamin D exert an anti-inflammatory hepatoprotective effect. J Med Food 19(4):383–389

Eghianruwa Q, Odekanyin O, Kuku A (2011) Physicochemical properties and acute toxicity studies of a lectin from the saline extract of the fruiting bodies of the shiitake mushroom, *Lentinula edodes* (Berk). Int J Biochem Mol Biol 2(4):309–317

Eira FCD, Meirelles WF, Paccola-Meirelles LD (2005) Shiitake production in corncob substrates. Rev Bras Milho Sorgo 4(2):141–148

Eldridgea HC, Milliken A, Farmerd C, Hamptone A, Wendlanda N, Cowardf L, Gregory DJ, Johnson CM (2017) Efficient remediation of 17α–ethinylestradiol by Lentinula edodes (shiitake) laccase. Biocatal Agric Biotechnol 10:64–68

Elisashvili V, Kachlishvili E, Asatiani M (2015) Shiitake medicinal mushroom, *Lentinus edodes* (higher Basidiomycetes) productivity and lignocellulolytic enzyme profiles during wheat straw and tree leaf bioconversion. Int J Med Mushrooms 17:77–86

Enman J, Rova U, Berglund KA (2007) Quantification of the bioactive compound eritadenine in selected strains of shiitake mushroom (*Lentinus edodes*). J Agric Food Chem 55:1177–1180

Enman J, Hodge D, Berglund KA, Rova U (2008) Production of the bioactive compound eritadenine by submerged cultivation of Shiitake (*Lentinus edodes*) mycelia. J Agric Food Chem 56:2609–2612

Fang N, Li Q, Yu S, Zhang J, He L, Ronis MJJ, Badger TM (2006) Inhibition of growth and induction of apoptosis in human cancer cell lines by an ethyl acetate fraction from shiitake mushrooms. J Altern Complement Med 12(2):125–132

Fujii T, Aeda H, Suzuki F, Shida N (1978) Isolation and characterization of a new antitumor polysaccharide, KS-2 extract from culture mycelia of *Lentinus edodes*. J Antibiot 11:1079–1090

Fujimoto K, Tomonaga M, Goto S (2006) A case of recurrent ovarian cancer successfully treated with adoptive immunotherapy and lentinan. Anticancer Res 26:4015–4018

Gaitan-Hernandez R, Mata G (2004) Cultivation of the edible mushroom *Lentinula edodes* (shiitake) in pasteurized wheat straw-alternative use of geothermal energy in Mexico. Eng Life Sci 4(4):363–367

Gaitán-Hernández R, Esqueda M, Gutiérrez A, Sánchez A, Beltrán-García M, Mata G (2006) Bioconversion of agrowastes by *Lentinula edodes*: the high potential of viticulture residues. Appl Microbiol Biot 71:432–439

Gaitan-Hernandez R, Cortes N, Mata G (2014) Improvement of yield of the edible and medicinal mushroom *Lentinula edodes* on wheat straw by use of supplemented spawn. Braz J Microbiol 45(2):467–474

Galisteo M, Duarte J, Zarzuelo A (2008) Effects of dietary fibers on disturbances clustered in the metabolic syndrome. J Nutr Biochem 19:71–84

George PL, Ranatunga TD, Reddy SS, Sharma GC (2014) A comparative analysis of mineral elements in the mycelia and the fruitbodies of shiitake mushrooms. Am J Food Tech 9(7):360–369

Gregory A, Pohleven F (2014) Cultivation of three medicinal mushroom species (G*anoderma lucidum*, L*entinula edodes* and *Grifola frondosa*) on olive oil press cakes containing substrates and their bioactivity assessment. Ann Agrar Sci 12(3):35–38

Grotto D, Bueno DCR, Ramos GK, da Costa ASR, Spim SRV, Gerenutti M (2016) Asse ssment of the safety of the shiitake culinary-medicinal mushroom, *Lentinus edodes* (Agaricomycetes), in rats: Biochemical, hematological, and antioxidative parameters. Int J Med Mushroom 18(10):861–870

Gu Y, Belury MA (2005) Selective induction of apoptosis in murine skin carcinoma cells (CH72) by an ethanol extract of *Lentinula edodes*. Cancer Lett 220:21–28

Guo Z, Hu Y, Wang D, Ma X, Zhao X, Zhao B, Wang J, Liu P (2009) Sulfated modification can enhance the adjuvanticity of lentinan and improve the immune effect of ND vaccine. Vaccine 27:660–665

Harvey L, McNeil B, Kristiansen B, Smith JE, Kheng Tan K (2001) Production of lentinan by submerged cultivation of *Lentinus edodes* (Berk.) Sing. Int J Med Mush 3:161

Hatvani N (2001) Antibacterial effect of the culture fluid of *Lentinula edodes* mycelium grown in submerged liquid culture. J Antibact Antifungal Ag 17(1):71–74

Hatvani N, Mécs I (2002) Effect of the nutrient composition on dye decolorisation and extracellular enzyme production by *Lentinus edodes* on solid medium. Enzyme Microb Tech 30:381–386

Hibbett DS (2001) Shiitake mushrooms and molecular clocks: historical biogeography of *Lentinula*. J Biogeog 28:231–241

Hibbett DS, Donoghue MJ (1996) Implications of phylogenetic studies for conservation of genetic diversity in shiitake mushrooms. Conserv Biol 10:1321–1327

Hibbett DS, Vilgalys R (1993) Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. Syst Bot 18:409–433

Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Oshima T, Chayama K (2002) Endothelial function and oxidative stress in renovascular hypertension. N Engl J Med 346:1954–1962

Hirasawa M, Shouji N, Neta T, Fukushima K, Takada K (1999) Three kinds of antibacterial substances from *Lentinus edodes* (Berk.) Sing. (shiitake, an edible mushroom). Int J Antimicrob Agents 11(2):151–157

Hiyama R, Harada A, Seiki G, Orihashi K (2016) Ethanol production from unpretreated waste medium of Shiitake mushroom (*Lentinula edodes*) by semi-simultaneous saccharification and fermentation under high substrate concentration conditions. Cellulose Chem Technol 59(7–8):771–780

Ina K, Kataoka T, Ando T (2013) The use of lentinan for treating gastric cancer. Anti-Cancer Agents in Med Chem 13:681–688

Ishikawa NK, Kasuya MCM, Vanetti MCD (2001) Antibacterial activity of *Lentinula edodes*. Braz J Microbiol 32(3):206–210

Itävaara M (1993) Problems associated with the liquid cultivation of shiitake, *Lentinula edodes* (Berk.) Pegler, vol 150. VTT Publications (Technical Research Centre of Finland), pp 11–62

Izuka C (1990) Antiviral composition extracts from basidiomycetes. European Patent Application EP 464,311. In: CA, 116, 76351z

Jang Y, Park J, Ryoo R, Park Y, Hyeon Ka K (2016) Ergothioneine contents of shiitake (*Lentinula edodes*) fruiting bodies on sawdust media with different nitrogen sources. Kor J Mycol 44(2):100–102

Jasinghe VJ, Perera CO (2006) Ultraviolet irradiation: the generator of vitamin D_2 in edible mushrooms. Food Chem 95(4):638–643

Jeune KH, Moon IJ, Kim MK, Chung SR (1990) Studies on lectins from Korean higher fungi; IV. A mitogenic lectin from the mushroom *Lentinus edodes*. Planta Med 56:592

Kabir Y, Kimura S (1989) Dietary mushrooms reduce blood pressure in spontaneously hypertensive rats (SHR). J Nutr Sci Vitaminol 35:91–94

Kamiya T, Saito Y, Hashimoto M, Seki H (1969) Structure and synthesis of lentysine, a new hypocholesterolemic substance. Tetrahedron Lett 10:4729–4732

Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL (2017) Oxidative stress in atherosclerosis. Curr Atheroscler Rep 19:42

Kawai G, Kobayashi H, Fukushima Y, Ohsaki K (1996) Effect of liquid mycelial culture used as a spawn on sawdust cultivation of shiitake (*Lentinula edodes*). Mycoscience 37:201–207

Kiribuchi T (1991) Effective uses of fungi by UV irradiation. 3. Changes of free amino acid composition in fungi by sun or ultraviolet light irradiation. Nippon Kasei Gakk 42(5):415–21

Kojima H, Akaki J, Nakajima S (2010) Structural analysis of glycogen–like polysaccharides having macrophage—activating activity in extracts of *Lentinula edodes* mycelia. J Nat Med 64:16–23

Kulicke WM, Lettau AI, Thielking H (1997) Correlation between immunological activity, molar mass, and molecular structure of different (1→3) β–D–glucans. Carbohydr Res 297:135–143

Kupfahl C, Hof GH (2006) Lentinan has a stimulatory effect on innate and adaptive immunity against murine Listeria monocytogenes infection. Int Immunopharmacol 6:686–696

Lakhtar H, Ismaili-Alaoui M, Philippoussis A, Perraud-Gaime I, Roussos S (2010) Screening of strains of *Lentinula edodes* grown on model olive mill wastewater in solid and liquid state culture for polyphenol biodegradation. Int Biodeterior Biodegradation 64:167–172

Leatham GF, Griffin TJ (1984) Adapting liquid spawn *Lentinus edodes* to oak wood. Appl Microbiol Biotechnol 20:360–363

Lee SJ, KimHH KSH, Kim SH, Sung NJ (2019) The effect of different culture conditions of liquid spawn on the quality characteristics of shiitake mushroom (*Lentinula edodes*). J Mushrooms 17(3):99–106

Levanon D, Rothschild N, Danai O, Masaphy S (1993) Bulk treatment of substrate for the cultivation of shiitake mushrooms (*Lentinus edodes*) on straw. Bioresource Technol 45(1):63–64

Li YR, Liu QH, Wang HX, Ng TB (2008) A novel lectin with potent antitumor, mitogenic and HIV–1 reverse transcriptase inhibitory activities from the edible mushroom Pleurotus citrinopileatus. Biochim Biophys Acta 1780:51–57

Li S, Wang A, Liu L, Tian G, Wei S, Xu F (2018) Evaluation of nutritional values of shiitake mushroom (*Lentinus edodes*) stipes. J Food Meas Charact 12:2012–2019

Liu B, Bau YS (1980) Fungi pharmacopoeia. Kinoko, Oakland, CA. A translation of Chinese medicinal fungi, 2nd edn. Shansi People's Publishing House. With added material, pp 302
Liu W, Gu J, Qi J, Zeng XN, Ji J, Chen ZZ, Sun XL (2015) Lentinan exerts synergistic apoptotic effects with paclitaxel in A549 cells via activating ROS–TXNIP–NLRP3 inflammasome. J Cell Mol Med 19(8):1949–1955
Markova N, Kussovskia V, Drandarska I, Nikolaeva S, Georgieva N, Radoucheva T (2003) Protective activity of *Lentinan* in experimental tuberculosis. Int Immunopharm 3:1557–1562
Martínez-Guerrero MA, Sihuanca D, Macías-López A, Pérez-López RI, Martínez-Madrigal JD, López-Olguín JF (2012) Characterization and production of shiitake (*Lentinula edodes*) in Mexico using supplemented sawdust. Afr J Biotechnol 11(46):10582–10588
Mata JL, Petersen RH (2000) A new species of *Lentinula* (Agaricales) from Central America. Mycoscience 41:351–355
Mata G, Savoie J-M (1998) Extracellular enzyme activities in six *Lentinula edodes* strains during cultivation in wheat straw. World J Microbiol Biotechnol 14(4):513–519
Mata JL, Petersen RH, Hughes KW (2001) The genus *Lentinula* in the Americas. Mycologia 93(6):1102–1112
Mattila P, Salo-Vaananen P, Konko K, Aro H, Jalava T (2002) Basic composition and amino acid contents of mushrooms cultivated in Finland. J Agric Food Chem 50:6419–6422
Medina E, Paredes C, Pérez-Murcia MD, Bustamante MA, Moral R (2009) Spent mushroom substrates as component of growing media for germination and growth of horticultural plants. Bioresour Technol 100(18):4227–4232
Medina E, Paredes C, Bustamante MA, Moral R, Moreno-Caselles J (2012) Relationships between soil physic-chemical, chemical and biological properties in a soil amended with spent mushroom substrate. Geoderma 173–174:152–161
Mekki A, Dhouib A, Sayadi S (2013) Review: effects of olive mill wastewater application on soil properties and plants growth. Int J Recycl Org Waste Agricult 2:15
Mizoguchi Y, Yamamoto S (1985) Immunotherapy in liver diseases. Rinsyo Menneki 17:452–460
Mizuno T (1995) Shiitake, *Lentinus edodes*: functional properties for medicinal and food purposes. Food Rev Int 11:7–21
Molina FI, Shen P, Jong SC, Orikono K (1992) Molecular evidence supports the separation of *Lentinula edodes* from *Lentinus* and related genera. Can J Bot 70:2446–2452
Moon IJ, Chung SR, Jeune KH (1995) Mitotic Stimulation and cancer cell agglutination of the lectin from *Lentinus edodes.* Yakhak Hoeji 39(3):260–267
Moonmoon M, Shelly NJ, Md Khan A, Md Uddin N, Hossain K, Tania M, Ahmed S (2011) Effects of different levels of wheat bran, rice bran and maize powder supplementation with sawdust on the production of shiitake mushroom (*Lentinus edodes* (Berk.) Singer). Saudi J Biol Sci 18(4):323–328
Morais MH, Ramos AC, Matos N, Santos-Olivera EJ (2000) Production of shiitake mushroom (*Lentinula edodes*) on lignocellulosicresidues. Food Sci Tech Int 6:123–128
Morales D, Gil-Ramirez A, Smiderle FR, Piris AJ, Ruiz-Rodriguez A, Soler-Rivas C (2017) Vitamin D—enriched extracts obtained from shiitake mushrooms (*Lentinula edodes*) by supercritical fluid extraction and UV–irradiation. Innov Food Sci Emerg Technol 41:330–336
Morales D, Smiderle FR, Piris AJ, Soler-Rivas C, Prodanov M (2018) Production of a β–d–glucan–rich extract from Shiitake mushrooms (*Lentinula edodes*) by an ex-traction/microfiltration/reverse osmosis (nanofiltration) process. Innov Food Sci Emerg Technol 41:330–336
Moreira MT, Mielgo I, Feijoo G, Lema JM (2000) Evaluation of different fungal strains in the decolourisation of synthetic dyes. Biotechnol Lett 22:1499–1503
Morinaga H, Tazawa K, Sakamoto K, Katsuyama S (1992) Inhibitive effect of LEM by oral administration of AH–60C and RL1 for liver metastasis. Biotherapy 6:763–765
Moylan JS, Reid M (2006) Oxidative stress, chronic disease, and muscle wasting. Muscle Nerve 35(4):411–429
Ng TB, Yap AT (2002) Inhibition of human colon carcinoma development by lentinan from shiitake mushrooms (*Lentinus edodes*). J Altern Complement Med 8(5):581–589

Nagai M, Sato T, Watanabe H, Saito K, Kawata M, Enei H (2002) Purification and characterization of an extracellular laccase from the edible mushroom *Lentinula edodes*, and decolorization of chemically different dyes. Appl Microbiol Biot 60:327–335

Ngai PHK, Ng TB (2003) Lentin, a novel and potent antifungal protein from shiitake mushroom with inhibitory effects on activity of human immunodeficiency virus–1 reverse transcriptase and proliferation of leukemia cells. Life Sci 73:3363–3374

Nguyen AH, Gonzaga MI, Lim VM, Adler MJ, Mitkov MV, Cappel MA (2017) Clinical features of shiitake dermatitis: A systematic review. Int J Dermatol 56:610–616

Okeke BC, Smith JE, Paterson A, Watson-Craik IA (1993) Aerobic metabolism of pentachlorophenol by spent Sawdust culture of "shiitake" mushroom (*Lentinus edodes)* in soil. Biotechnol Lett 15(10):1077–1080

Ooi VE, Liu F (2000) Immunomodulation and anti-cancer activity of polysaccharide–protein complexes. Curr Med Chem 7:715–729

Özçelik E, Pekşen A (2007) Hazelnut husk as a substrate for the cultivation of shiitake mushroom (*Lentinula edodes*). Bioresource Technol 98:2652–2658

Patni N, Pillai SG, Dwivedi AH (2013) Wheat as a promising substitute of corn for bioethanol production. Procedia Eng 51:355–362

Pegler DN (1983) The genus *Lentinula* (Tricholomataceae tribe Collybieae). Sydowia 36:227–239

Philippoussis AN, Diamantopoulou PA, Zervakis GI (2003) Correlation of the properties of several lignocellulosic substrates to the crop performance of the shiitake mushroom *Lentinula edodes*. World J Microbiol Biotechnol 19:551–557

Philippoussis A, Diamantopoulou P, Israilides C (2007) Productivity of agricultural residues used for the cultivation of the medicinal fungus *Lentinula edodes*. Int Biodeterior Biodegradation 59:216–219

Pire DG, Wright JE, Albertó E (2001) Cultivation of shiitake using sawdust from widely available local woods in Argentina. Micol Apl Int 13:87–91

Razumov IA, Kazachinskaia EI, Puchkova LI, Kosogorova TA, Gorbunova IA, Loktev VB, Tepliakova TV (2013) Protective activity of aqueous extracts from higher mushrooms against Herpes simplex virus type–2 on albino mice model. Antibiot Chemotherapy 58:8–12

Reverberi M, Zjalic S, Ricelli A, Di Meo C, Scarpari M, Fanelli C, Fabbri AA (2011) Mushroom versus fungi: natural compounds from *Lentinula edodes* inhibit aflatoxin biosynthesis by *Aspergillus parasiticus*

Ricelli A, Fabbri AA, Trionfetti-Nisini P, Reverberi M, Zjalic S, Fanelli C (2002) Inhibiting effect of different edible and medicinal mushrooms on the growth of two Ochratoxigenic microfungi. Int J Med Mushrooms 4(2):173–179

Rincão VP, Yamamoto KA, Ricardo NMPS, Soares SA, Meirelles LDP, Nozawa C, Linhares REC (2012) Polysaccharide and extracts from *Lentinula edodes*: structural features and antiviral activity. Virol J 9(37):1–6

Rokujo T, Kikuchi H, Tensho A, Tsukitani Y, Takenawa T, Yoshida K, Kamiya T (1970) Lentysine: a new hypolipidemic agent from a mushroom. Life Sci 9:379–385

Rossi IH, Monteiro AC, Machado JO, Andrioli JL, Barbosa JC (2003) Shiitake (*Lentinula edodes*) production on a sterilized bagasse substrate enriched with rice bran and sugarcane molasses. Braz J Microbiol 34(1):66–71

Royse DJ (1996) Yield stimulation of shiitake by millet supplementation of wood chip substrate. In: Royse DJ (ed) Mushroom biology and mushroom products. University Park, PA, Penn State University Press, pp 277–283

Royse DJ (1997) Speciality mushrooms and their cultivation. Hortic Rev 19:59–97

Royse DJ (2001) Cultivation of shiitake on synthetic and natural logs. Cooperative Extension, Pennsylvania State University, University Park, PA, USA, College of Agricultural Sciences, p 12p

Royse DJ, Sanchez JE (2007) Ground wheat straw as a substitute for portions of oak wood chips used in shiitake (*Lentinula edodes*) substrate formulae. Bioresource Technol 98:2137–2141

Royse DJ, Baars J, Tan Q (2017) Current overview of mushroom production in the World. In: Diego CZ, Pardo-Gimenez A (eds). Edible and medicinal mushrooms: technology and applications pp 5–13

Rusan MJM, Albalasmeh AA, Malkawi HI (2016) Treated olive mill wastewater effects on soil properties and plant growth. Water Air Soil Pollut 227:135

Salmones D, Mata G, Ramos LM, Waliszewski KN (1999) Cultivation of shiitake mushroom, *Lentinula edodes*, in several lignocellulosic materials originating from subtropics. Agronomie 19(1):13–19

Samgina DI (1981) Agailkovie gribi I. Agaricales. Fl Spor rast Kazakhst 18:1–268

Sasaki SH, Linhares REC, Nozawa CM, Moltalván R, Paccola-Meireles LD (2001) Strains of *Lentinula edodes* suppress growth phytopathogenic fungi and inhibit Alagoas serotype of vesicular stomatitis virus. Braz J Microbiol 32:52–55

Sasidharan S, Aravindran S, Latha LY, Vijenthi R, Saravanan D, Amutha S (2010) In vitro antioxidant activity and hepatoprotective effects of *Lentinula edodes* against paracetamol–induced hepatotoxicity. Molecules 15:4478–4489

Savoie JM, Delpech P, Billette C, Mata G (2000) Inoculum adaptation changes the outcome of the competition between *Lentinula edodes* and Trichoderma spp. During shiitake cultivation on pasteurized wheat straw. Mush Sci 15:667–674

Sharma S, Khanna PK, Kapoor S (2013) Effect of supplementation of wheat bran on the production of shiitake (*Lentinus edodes* (Berk.) Pegler) using wheat straw and saw dust substrates. Bioscan 8(3):817–820

Sharon TM (1988) Personal observations: *Lentinus edodes* (shiitake) mycelial extract. Typescript

Shimada Y, Morita T, Sugiyama K (2003) Eritadenine–induced alterations of plasma lipoprotein lipid concentrations and phosphatidylcholine molecular species profile in rats fed cholesterol–free and cholesterol–enriched diets. Biosci Biotech Bioch 67:996–1006b

Sugano N, Hibino Y, Choji Y, Maeda H (1982) Anticarcinogenic actions of watersoluble and alcohol-insoluble fractions from culture medium of *Lentinus edodes* mycelia. Cancer Lett 17:109–114

Sugiyama K, Akachi T, Yamakawa A (1995) Hypocholesterolemic action of eritadenine is mediated by a modification of hepatic phospholipid metabolism in rats. J Nutr 125:2134–2144

Sugiyama K, Yamakawa A, Kawagishi H, Saeki S (1997) J Nutr 127:593–599

Sun J, Wang H, Ng TB (2011) Antitumor activities of *O*-sulfonated derivatives of (1→3)–α–D–Glucan from different *Lentinus edodes*. Ind J Biochem Biophys 48:88–94

Sun M, Zhao W, Xie Q, Zhan Y, Wu B (2015) Lentinan reduces tumor progression by enhancing gemcitabine chemotherapy in urothelial bladder cancer. Surg Oncol 24:28–34

Surenjav U, Zhang L, Xu X, Zhang X, Zeng F (2006) Effects of molecular structure on antitumor activities of β–(1→3)–D–glucans from different *Lentinan edodes*. Carbohydr Polym 63:97–104

Suzuki S, Ohshima S (1976) Influence of *L. edodes (Lentinus edodes)* on human serum cholesterol. Mushroom Sci 9(1):463–467

Suzuki H, Iiyama K, Yoshida O, Yamazaki S, Yamamoto N, Toda S (1990) Structural characterization of the immunoactive and antiviral water–solubilized lignin in an extract of the culture medium of *Lentinus edodes* mycelia (LEM). Agric Biol Chem 54(2):479–487

Taguchi T et al (1982) Clinical trials on lentinan (polysaccharide). In: Yamamura Y et al (eds) Immunomodulation by microbial productsand related synthetic compounds. Elsevier, New York, pp 467–475

Takashima K, Izumi K, Iwai H, Takeyame S (1973) The hypocholesterolemic action of eritadenine in the rat. Atherosclerosis 17(3):491–502

Tepwong P, Giri A, Ohshima T (2012) Effect of mycelial morphology on ergothioneine production during liquid fermentation of *Lentinula edodes*. Mycoscience 53:102–112

Tochikura TS, Nakashima H, Ohashi Y, Yamamoto N (1988) Inhibition (in vitro) of replication and of the cytopathic effect of human immunodeficiency virus by an extract of the culture medium of *Lentinus edodes* mycelia. Med Microbial Immunol 177:235–244

Tsivileva OM, Nikitina VE, Garibova LV (2005) Effect of culture medium composition on the activity of extracellular lectins of *Lentinus edodes*. Appl Biochem Microbiol 41:174–176

Tsujiyama S, Muraoka T, Takada N (2013) Biodegradation of 2,4–dichlorophenol by shiitake mushroom (*Lentinula edodes*) using vanillin as an activator. Biotechnol Lett 35:1079–1083

Turło J, Gutkowska B, Herold F, Krzyczkowski W, Błazewicz A, Kocjan R (2008) Optimizing vitamin B12 biosynthesis by mycelial cultures of Lentinula edodes (Berk.) Pegl. Enzyme Microb Technol 43(4–5):369–374

Unursaikhan S, Xu X, Zeng F, Zhang L (2006) Antitumor activities of *O*-sulfonated derivatives of (1→3)–α–D–glucan from different *Lentinus edodes*. Biosci Biotechnol Biochem 70(1):38–46

Vetchinkina EP, Nikitina VE, Tsivileva OM, Garibova LV (2008) Activity of *Lentinus edodes* intracellular lectins at various developmental stages of the fungus. Appl Biochem Microbiol 44(1):66–72

Vetter J (1995) Mineralstoff and aminosauregehalte des esbaren, kultivilten Pilzes Shii–take (*Lentinus edodes*). Z Lebensm Unters Forsch 201:17–19

Vetvicka V, Vetvickova J (2015) Glucan supplementation enhances the immune response against an influenza challenge in mice. Ann Transl Med 3(2):22

Wang HX, Liu WK, Ng TB, Ooi VEC, Chang ST (1996) The immunomodulatory and antitumor activities of lectins from the mushroom *Tricholoma mongolicum*. Immunopharmacol 31:205–211

Wang HX, Ng TB, Ooi VEC (1999) Studies on purification of a lectin from fruiting bodies of the edible shiitake mushroom *Lentinus edodes*. Int J Biochem Cell Biol 31:595–599

Wang J, Yu G, Li Y, Shen L, Qian Y, Yang J, Wang F (2015) Inhibitory effects of sulfated lentinan with different degree of sulfation against tobacco mosaic virus (TMV) in tobacco seedlings. Pestic Biochem Physiol 122:38–43

Wang Y, Jin H, Yu J, Qu C, Wang Q, Yang S, Ma S, Ni J (2020) Quality control and immunological activity of lentinan samples produced in China. Int J Biol Macromol 159:129–136

Watanabe A, Kobayashi M, Hayashi S, Kodama D, Isoda K, Kondoh M, Kawase M, Tamesada M, Yagi K (2006) Protection against D–galactosamine-induced acute liver injury by oral administration of extracts from *Lentinus edodes* mycelia. Biol Pharm Bull 29:1651–1654

Watanabe T, Srichuwong S, Arakane M, Tamiya S, Yoshinaga M, Watanabe I, Yamamoto M, Ando A, Tokuyasu K, Nakamura T (2010) Selection of stress–tolerant yeasts for simultaneous saccharification and fermentation (SSF) of very high gravity (VHG) potato mash to ethanol. Bioresour Tech 101:9710–9714

Xiong S, Martín C, Eilertsen L, Wei M, Myronycheva O, Larsson S, Lestander T, Atterhem L, Jönsson L (2019) Energy-efficient substrate pasteurisation forcombined production of shiitake mushroom (*Lentinula edodes*) and bioethanol. Bioresour Technol 274:65–72

Xu C, HaiYan Z, Jian Hong Z, Jing G (2008) The pharmacological effect of polysaccharides from *Lentinus edodes* on the oxidative status and expression of VCAM–1mRNA of thoracic aorta endothelial cell in high–fat–diet rats. Carbohydr Polym 74:445–450

Yagi K (2012) Liver protective effect of *Lentinula edodes* mycelia (LEM). Gan to Kagaku Ryoho 39:1099–1102

Yamanaka K (2017) Cultivation of mushrooms in plastic bottles and small bags. In: Zied DC, Pardo-Gimenez A (eds) Edible and medicinal mushrooms technology and applications

Yang QY, Jong SC (1989) Medicinal mushrooms in China. Mushr Sci 12:631–642

Yang BK, Kim DH, Jeong SC, Das S, Choi YS, Shin JS, Lee SC, Song CH (2002) Hypoglycemic effect of a *Lentinus edodes* exopolymer produced from a submerged mycelial culture. Biosci Biotechnol Biochem 66:937–942

Yen MT, Tseng YH, Li RC, Mau JL (2007) Antioxidant properties of fungal chitosan from shiitake stipes. LWT Food Sci Technol 40(2):255–261

Yoshioka Y, Kojima H, Tamura A, Tsuji K, Tamesada M, Yagi K, Murakami N (2012) Low–molecular–weight lignin–rich fraction in the extract of cultured *Lentinula edodes* mycelia attenuates carbon tetrachloride-induced toxicity in primary cultures of rat hepatocytes. J Nat Med 66:185–191

Yu Z, LiHua Y, Qian Y, Yan L (2009) Effect of Lentinus edodes polysaccharide on oxidative stress, immunity activity and oral ulceration of rats stimulated by phenol. Carbohydr Polym 75:115–118

Zervakis GI, Koutrotsios G (2017) Solid–State Fermentation of plant residues and Agro-industrial wastes for the production of medicinal mushrooms. In: Agrawal et al (eds) Medicinal plants and fungi: recent advances in research and Development, Medicinal and Aromatic Plants of the World, , pp 365–396

Zhang Y, Ming G, Kaiping W, Zhixiang C, Liquan D, Jingyu L, Fang Z (2010) Structure, chain conformation and antitumor activity of a novel polysaccharide from *Lentinus edodes*. Fitoterapia 81(8):1163–1170

Zhang N, Chen H, Zhang Yu, Ma L, Xu X (2013) Comparative studies on chemical parameters and antioxidant properties of stipes and caps of shiitake mushroom as affected by different drying methods. J Sci Food Agric 93:3107–3113

Zhang Y, Liu W, Xu C, Huang W, He P (2017) Characterization and antiproliferative effect of novel acid polysaccharides from the spent substrate of shiitake culinary-medicinal mushroom *Lentinus edodes* (Agaricomycetes) cultivation. Int J Med Mushrooms 19(5):395–403

Zhang Y, Zhang M, Jiang Y, Li X, He Y, Zeng P, Guo Z, Chang Y, Luo H, Liu Y, Hao C, Wang H, Zhang G, Zhang L (2018) Lentinan as an immunotherapeutic for treating lung cancer: a review of 12 years clinical studies in China. J Cancer Res Clin 144:2177–2186

Zhao L, Xiao Y, Xiao N (2013) Effect of lentinan combined with docetaxel and cisplatin on the proliferation and apoptosis of BGC823 cells. Tumor Biol 34:1531–1536

Zheng R, Jie S, Hanchuan D, Moucheng W (2005) Characterization and immunomodulating activities of polysaccharide from Lentinus edodes. Int Immunopharmacol 5:811–820

Zilly A, Bracht A, Coelho-Moreira JS, Maciel GM, Soares AA, Bazanella GCS, Peralta RM (2011) Enzymes from Ganoderma lucidum: production and applications. Curr Top Biochem Res 13:1–11

Chapter 14
Cultivation and Breeding of Commercial Perfumery Grass Vetiver

Sunita Singh Dhawan, Pankhuri Gupta, and Raj Kishori Lal

Abstract *Chrysopogon zizanioides* (Roberty) or Vetiver is a member of the grass family Poaceae. In the Indian subcontinent, the Vetiver, a tropical grass has been known since long for its several characteristics, the grass promises for commercial benefits for farmers for its high-cost essential oil and for several industries along with sustainable management of soil erosion in tropical and semi-arid zones. Since long Vetiver was known to be used and recognized for hedging in the field on the contour to stop soil erosion, Vetiver can improve crop production through retaining moisture and nutrient conservation. Vetiver and its products were used in India for household needs, in perfumery, and many other traditional usages along with Indian Ayurvedic system medicines. In addition to its specific aromatic values, it has numerous applications in traditional medicines as well. It is rich in Khusimol, Khusinol, Vetivone, and Khusimone. Genus Vetiver shows variation, and DNA fingerprinting was used extensively to analyze the variability found in the Vetiver genus. This is of utmost benefit in creating a well-organized way of maintaining the genetic diversity in Vetiver. In this chapter, we have summarized different morphological characteristics, chemical composition, and its cultivation strategies along with molecular fingerprints developed in this perfumery grass Vetiver. Because of increasing population and less income, indigenous system of farming in the tropical and subtropical areas are changing drastically. Therefore, farmers have increased the proportion of land under cultivation for enhancing crops per capita per year and cultivating aromatic crops in the marginal land for increasing per capita produce thus also for increasing income. *C. zizanoides* is very much suitable for fulfilling all these needs in this challenging scenario; therefore, we have discussed significance of this Poaceae family member for preventing soil erosion and increase in essential oil yields by developing superior elite genotypes, cultivars along with its various improved methods of cultivation and breeding.

S. S. Dhawan (✉) · P. Gupta
Biotechnology Division, CSIR-Central Institute of Medicinal and Aromatic Plants, PO CIMAP, Lucknow, U.P 226015, India
e-mail: sunita.dhawan@cimap.res.in

R. K. Lal
Division of Genetics and Plant Breeding, CSIR-Central Institute of Medicinal and Aromatic Plants, PO CIMAP, Lucknow, U.P 226015, India

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_14

Keywords *Chrysopogon zizanioides* · Sesquiterpene · Essential oil yield · Khus · Cultivation · Industrial applications

14.1 Introduction

Chrysopogon zizanioides (Roberty) or Vetiver belongs to grass family Poaceae which is an important perennial and aromatic C4 plant. In India, it is popularly known as Khus, found in plains and hills of India and distributed worldwide nearly all riverside and marshy land. Vetiver essential oil is used in perfumery, for fragrances, toiletries, and cosmetics industries. It has extensive use in aromatherapy. Its roots are used as a carminative, stimulant, diaphoretic, and in many traditional medicines. Vetiver oil also holds sedative properties. Vetiver is planted in June–July and harvested in the month of September to October of next year. Growth of Vetiver roots is affected by different climatic parameters like rainfall intensity, temperature, and soil. Essential oil of Vetiver is composed of many complex chemicals containing sesquiterpenes and its derivatives. In this, Vetivone and khusimone are main and important constituents. Other than this Vetiverols, ester and carbonyl group is the main compound, which is responsible for essential oil quality. Globally, Vetiver essential oil production is 250 tons per annum, globally approximately 50 million USD (250 tons/annum) http://www.synbiowatch.org/commodities/Vetiver. This volume of production comprises various varieties of Vetiver oil: Haiti (100 tons), Indonesia (80 tons), China (20 tons), India (20 tons), Brazil (15 tons), Dominican Republic (12 tons), Vietnam (3 tons), Madagascar (2 tons), Nepal (0.5 tons), Reunion (0.5 tons), and Ghana (0.4 tons) (Thwaites 2010). Unique and most diverse varieties with high essential oil yield of Vetiver are developed and released by CSIR—Central Institute of Medicinal and Aromatic Plants, Lucknow, India. (Lal et al. 2013). There are two forms of Vetiver grass: one originated from North India and other from South India. DNA-based molecular marker, miRNA, DNA barcoding, microarrays, and next-generation sequencing (NGS) technology are useful in molecular tagging. Molecular marker technologies are useful in management of germplasm collections, classification, and phylogenetic studies not only by avoiding redundancy but also in authentication of the genus (Chakrabarty et al. 2015). The present chapter describes that Vetiver is a crop of choice for farmers by providing additional income with very low maintenance that will improve their livelihood.

14.2 Geographical Distribution of Vetiver

In India, *Vetiveria zizanioides* grow usually in wild throughout tropical and subtropical plains, near the river side and in marshy areas. It has extensive range of natural distribution that ranges from sandy costal area to hills and plains in the Kumaun hills of Uttar Pradesh. In India, two most diverse morphological variations in Vetiver

are present in diverse geographical regions: First is found in the Indogangetic plains of north in the states of Rajasthan, Madhya Pradesh, Uttar Pradesh, and Bihar and second in the southern region of east and west coast of Indian peninsula in the states of Andhra Pradesh, Karnataka, Tamil Nadu, and Kerala. These two types of Vetiver are different from each other. The north type is known as "Bharatpur type," and its characteristics features are like the leaves are narrow, with high seed and flowering and produce high quality of root oil (Khus oil), whereas the southern type is known as "cultivated type." In this, no seed formation usually occurs with late flowering with broad leaves and produces low root oil (Guenther 1972; Gupta and Pareek 1995). These two morphologically distinct Vetiver complexes were differentiated in Table 14.1 (Chahal et al. 2015).

In India, the diverse type of Vetiver is found due to different environmental and geographical changes. It has variation at phenotypic, at molecular level as well as physiological behavior (Lal et al. 1997, Lal 2000, Lal and Sharma 2000). However, other than India, Vetiver is also distributed in major parts of the world like Asia, South Africa, and USA, etc.

Table 14.1 Comparative analysis of northern and southern types of Vetiver

S. No.	Parameters	North Indian or Bharat type	South Indian or cultivated type
1	Geographical regions	Indo-Gangetic Plains adjoining areas mainly in the states of Rajasthan, Madhya Pradesh, Uttar Pradesh, and Bihar	The east and west coast of Indian peninsula in the states of Andhra Pradesh, Karnataka, Tamil Nadu, and Kerala
2	Morphological characters	Profuse flowering, high seed setting having narrow leaves with vigorous roots	Late and low flowering with high pollen sterility and non-seed setting with wider leaves
3	Essential oil type	Produce low concentration superior quality laevorotatory root oil (Khus oil)	Producing low-quality dextrorotatory root oil (Vetiver oil) known as Java Vetiver
4	Essential oil quality	Higher specific gravity, free alcohol, and ester value after acetylation	Higher refractive index, acid value, ester value, combined alcohols, ester content, and carbonyl values
5	Yield (%)	0.28	2.37
6	General appearance	Brown, clear liquid	Yellow brown, clear liquid
7	Odor	Heavy woody, earthy, sweet, persistent	Harsh woody, spicy

14.3 Phytochemistry of Vetiver Root Essential Oil Composition

Vetiver is a perennial grass with stiff erect leaves and aromatic roots. Vetiverroot oil is very complex in composition and therefore very complicated with more than hundred sesquiterpene constituents and their derivatives. Main chemical constituents in Vetiver essential oil are **sesquiterpene hydrocarbons, sesquiterpene alcohol derivatives,** (Vetiverol, Khusimol), **sesquiterpene carbonyl derivatives** (Vetivone, Khusimone), and **sesquiterpene ester derivatives** (Khusinolacetate). The major constituents that are known to influence the aroma are **α-Vetivone, β-Vetivone**, and **Khusinol** (Ramanujam et al. 1964; Smith et al. 2012)**.**The major compound in Vetiver essential oil includes firstly, (sesquiterpene hydrocarbons) clovene, cadenene, amorphine, aromadendrine, junipene, secondly, (sesquiterpene alcohol derivatives) Vetiverols–khusimol, epiglobulol, spathulenol, khusinol, thirdly, (sesquiterpene carbonyl derivatives) vetivones–vetivone, khusimone, and lastly (sesquiterpene ester derivatives) khusinol acetate. The most important components used in Vetiver oil have the highest boiling points (Lavania 2003) (Lavania et al. 2000, 2009).

In another report, *V. zizanioides* Nash roots from Thai-type plant, total 36 volatiles, were detected in the oil. Khusimone (20.91%), (Z)-9, 10-dehydro–2–norzizaene (14.71%), khusimol (12.21%), and (E)–opposita-4(15), 7(11)–dien–12–al (10.55%) were present as the major odors were extracted by using GC-MSand SPME method (Pripdeevech et al. 2006). Matsuo et al. (2016) reported three unique sesquiterpenoids: Vetiverianines A, B and C and a known eudesmane sesquiterpenoid in Vetiver root, and the structures were determined by NMR spectroscopic, X-ray crystallography, and vibrational circular dichroism data analysis. GC-FID and GC–MS have studied the essential oils extracted from Vetiver (*V. zizanioides* (L.) Nash.) roots obtained from four different sites of South India. Eighty constituents were identified, accounting for 94.5–97.8% of the oils. Bangalore, Hyderabad, Kundapur, and Mettupalayam oils were rich in sesquiterpenes and oxygenated sesquiterpenes with skeletons of cedrane, bisabolane, eudesman, eremophilane, and zizaane. Major constituents found in different varieties of Vetiver developed by CSIR-CIMAP are discussed in Table 14.2.

Interestingly, the Vetiver oil is one of the most complex of the essential oils. Its chemistry is complicated, and steam distillation is slow, but as the Vetiver essential oil is least volatile compared to other essential oils, it retains its place in perfumery for fixing of more volatile, other costly essential oils for preventing them from volatilizing fast. For extraction of the high-quality essential oils through steam distillation, specially designed separators were used for further value-added processing into important constituents such as Vetiverol and Vetiveryl acetate. The Vetiver essential oil trade is highly specialized and operated in a specific chain of farmers, processors, exporters, distillers, chemists, and industries being a commodity crop. Demand for unprocessed Vetiver root is increased immensely being a premier commodity cash crop also added benefits of hedging, for conservation of soil nutrient and water.

Table 14.2 Chemical diversity among different varieties of Vetiver released by CSIR-CIMAP

S.No	Varieties	Major constituents
1	KS-1	> 30% Khusimol
2	KS-2	> 20% Khusimol
3	Sugandha	> 21% Khusimol
4	Dharini	> 8.9% Khusol
5	Gulabi	> 23.98% Khusimol
6	Kesari	> 24.21% Khusimol
7	CIM Viriddhi	> 25% Khusimol
8	CIMAP Khus-40	> 45% Khusinol
9	G-15	> 18% Khusimol
10	G-22	> 20% Khusimol
11	Khusnalika	> 45–50% Khusinol
12	CIM-Samriddhi	> 30% Khusilaland > 19% Khusol

14.4 Medicinal Properties and Various Usage of Vetiver

Vetiver essential oil contains many benefits. It is traditionally used in aromatherapy to release stress, anxiety, tension, and depression in brain. This is also useful for stretch marks, fat cracks, rashes, and burning, etc. Further, it helps get rid of nervous system disorders, afflictions, epileptic and hysteric attacks, and nervous and neurotic disorders such as Parkinson's disease and deficiency in control over limbs. (Upadhyay et al. 2007). It regulates sebaceous oil gland function, has deodorizing properties, and helps to normalize oily skin and clear acne. It helps in treatment of cut, wound, and inflammation in skin (Lavania 2003). Additional, advantages of Vetiver are that its essential oil helps in strengthening of bones, muscle aches, rheumatism, arthritis, gout, cramps, and dry skin. Extract of Vetiver helps in enhancing and boosting up the metabolism as well as in digestive system. Antiseptic properties of this plant are recognized to help in the healing of wounds and protecting from fungus and bacterial infections growth (Kumar 2008).

In the recent study, Lavanya et al. (2016) investigated the antiviral medicinal properties present in the *V. zizanioides* against dengue virus. Analysis of active substance was examined for antiviral properties using docking method along with reference ligand. As a result, it showed that Ethyl 4–(4–methylphenyl)–4–pentenoate is a good candidate for the development of an effective anti-dengue compound from Vetiver plant. Root extracts and fractions of *V. zizanioides* were evaluated by Saikia et al. (2012) for antimycobacterial activity against *Mycobacterium tuberculosis* H(37)Rv and H(37)Ra strains using radiometric BACTEC 460 TB that showed the promising candidature of *V. zizanioides* root extract and hexane fraction act as antituberculosis agent.

Vetiver is also known for its medicinal properties in Ayurvedic literature. It is mentioned that plant is used to cure problem related to digestive system, antigout

carminative stomachic, antispasmodic, hematinic, antimicrobial, anti-asthmatic, diuretic, and anthelmintic. Vetiver roots are also used in the treatment of anemia, boils, fever, epilepsy, rheumatism, weakness, mouth and stomach ulcer, etc. (Jain 1991) It is also used in malaria treatment (Rao and Suseela 2000; Jain 1991; Singh and Maheshwari 1983). Vetiver roots are also used in making of the roofs, hats, and other household products in India. Local villagers are making beautifully hand-crafted items from Vetiver leaves, stems, and roots by weaving. The unprocessed cut leaves could be utilized for mulching. Mulch is much in demand in agriculture and horticulture, Vetiver yields higher volume of leaves for such purposes, because its high carbon-to-nitrogen ratio ensures its long life within heat and humidity of the tropical regions Therefore, Vetiver is a unique incredible grass with many beneficial usages as for conservation of soil and moisture, restoration of industrial wasteland, purifying polluted water bodies, providing shades and shelter for cattles, stabilizing dam river banks, and many others.

14.5 Cultivation, Breeding, and Domestication of Different Varieties of Vetiver

There are several specific characteristics of Vetiver grass which makes it as a special essential oil commodity for farmers, because of roots and many physiological, ecological characteristics and ability to grow without attracting pests. Vetiver has a unique fine root system. The native strength of Vetiver root enables it to grow well through difficult soils due to their tensile strength, and this deep root system also makes the plant drought tolerant. Vetiver can tolerate prolonged drought, fire, flood, submergence, and extreme temperatures. And in many of the cases, it may be the only plant to survive. Its ability to regrow quickly after being affected by drought, fire, frost, salt, soil salinity, soil sodicity and varied range of pH, and other unfavorable soil conditions is reasonably incomparable.

Therefore, CSIR-CIMAP, India, has developed twelve superior elite varieties of *C. zizanioides* through mutation, selection, and breeding approaches (Bahl et al. 2018). Specific characteristics of these high yielding genotypes are discussed below in Table 14.3, and the field images of the varieties are depicted in Figs. 14.1, 14.2, 14.3, 14.4, 14.5, 14.6, 14.7, 14.8, 14.9, 14.10, 14.11 and 14.12. US patents of variety CIMAP Khus-40 (https://patents.google.com/patent/US20120278945P1/en) and Variety Khusnalika (https://patents.google.com/patent/USPP28388P3/en) are also with CSIR-CIMAP. Now the varieties developed were immensely popular among farmers as being high yielder and superior to others with diverse chemical note as described in Table 14.3.

Table 14.3 High yielding varieties of Vetiver released by CSIR-CIMAP

S.No.	Varieties	Dry root yield (ql/ha)	Oil yield (kg/h)	Note/odor	Characters	Remarks
1	KS-1	25.00	26.00	Khus note	Tall, medium, light green leaves, inflorescence long, white color	For drought/marginal land
2	KS-2	24.00	25.00	Khus note	Medium tall, yellowish green leaves, inflorescence long, purple color	For drought/marginal land
3	Sugandha	24.00	22.00	Khus note	Tall, medium, light green leaves, inflorescence long, white colure	Induced tetraploid variety developed from KS-1
4	Dharini	31.00	32.00	Khus note	Very tall, very fast growth, long and broad dark green leaves, early flowering, inflorescence long, dance light brown color	Suitable for flood areas having dense and long network of roots a suitable soil binder–cum high oil yielder
5	Gulabi	28.00	34.00	Rose note	Medium dwarf, light green leaves, late flowering, inflorescence dark in purple color	Suitable for drought/marginal, water logging areas, high pH, alkaline soils
6	Kesari	29.00	30.00	Saffron note	Dwarf, very light green thin leaves, earliest flowering, inflorescence greenish white color	Suitable for irrigated and non-irrigated both areas and specific purpose

(continued)

Table 14.3 (continued)

S.No.	Varieties	Dry root yield (ql/ha)	Oil yield (kg/h)	Note/odor	Characters	Remarks
7	CIM Viriddhi	27.00	33.00	Khus note	Medium tall, light green leaves, very late flowering inflorescence very dark purple color	Suitable for irrigated and non-irrigated both areas
8	CIMAP Khus-40 Patent:USPP28388P3	25–30	25–30	Khus note	Lax inflorescence with enlarged floret size	A novel seed infertile autotetraploid (4x = 40)clone of Vetiver
9	G-15	9–22	35–40	Khus note	Plant has robust erect growth habit	Suitable for wide climatic conditions
10	G-22	18–20	28–30	Khus note	Plant has robust erect growth habit	Suitable for wide climatic conditions
11	Khusnalika Patent:USPP28388P3	18–20	18–20	Khus note	Spreading canopy and inflorescence bearing flowers with white feathery stigma	Suitable for wide climatic conditions northern climates
12	CIM-Samriddhi	30–35	35	Khus/fruity note	Plant has yellow–green inflorescence and broad dark green leaves are the two unique distinctive features of this variety	Suitable for wide climatic conditions northern climates

14.5.1 Planting of the Material

Vegetative propagation through stem and root cuttings is a cheaper source of cultivation of Vetiver. Nurseries should be fertilized (150 kg/ha of nitrogen) and irrigated timely mostly in dry areas. Loamy to sandy–clay soils are best for Vetiver nurseries. Vetiver can grow in drought areas for long period of time. Vetiver should be planted

Fig. 14.1 Var. KS-1 of Vetiver released by CSIR-CIMAP (A) field view of plant (B) root

Fig. 14.2 Var. KS-2 of Vetiver released by CSIR-CIMAP (C) field view of plant (D) root

Fig. 14.3 Var. Sugandha of Vetiver released by CSIR-CIMAP (E) field Plant (F) Root

on the edges of the field on very small farms and fields where land is limited and where farmers are unable to plant through their fields. Gap filling is necessary and should be done at the start of the wet season.

Fig. 14.4 Var. Dharani of Vetiver released by CSIR-CIMAP (G) field plant (H) root

Fig. 14.5 Var. Gulabi of Vetiver released by CSIR-CIMAP (I) field view of plant (J) root

Fig. 14.6 Var. Kesariof Vetiver released by CSIR-CIMAP (K) field view of plant (L) root

Fig. 14.7 Var. Vriddhi of Vetiver released by CSIR-CIMAP (M) field view of plant (N) Root

Fig. 14.8 Var. CIMAP Khus-40 of Vetiver released by CSIR-CIMAP (M) field view of plant (N) root

Fig. 14.9 Var. G-15 of Vetiver released by CSIR-CIMAP (Q) field view of plant (R) root

Fig. 14.10 Var. G-22 of Vetiver released by CSIR-CIMAP (S) field view of plant (T) root

Fig. 14.11 Var. Khusnalika of Vetiver released by CSIR-CIMAP (U) field view of plant (V) root

Fig. 14.12 Var. CIM-Samriddhi of Vetiver released by CSIR-CIMAP (W) field view of plant (X) root

14.5.2 Propagation Methods

Vetiver was propagated mainly by root division or slips. The slip was separated from the main clump and planted in soil as seedlings, and the plants develop quickly once roots are established. The plant responds well to fertilizer and irrigation. Ratooning-like sugarcane, the Vetiver plant, can be cut and sown in the light soil to sprout again. Lateral buds could be utilized successfully for developing Vetiver "eyes" which are intercalary buds on the surface of the crown developed. Through culms, young stems easily form new roots, therefore culms can be utilized effectively to propagate the new plants. Through cuttings,Vetiver could be grown from stem cuttings with two nodes with treatment with a rooting hormone like indole acetic acid (IAA). Multiplication by using culm–cuttings, back clumps of Vetiver of about 30 to 50 cm could be utilized for tillering.

14.5.3 Planting Time

In general, planting of Vetiver is done in monsoon (mid-June–September), but the ideal time of planting of Vetiver as an annual crop is winter season. The other most suitable time of planting is January and February in North India. In South Indian conditions, where temporal and diurnal variation in temperature are not significant and monsoon sets early, the ideal planting time is February to April. But for the rainfed area, monsoon planting only is considered to be the most ideal. On undulating and problematic soils also, it should be planted in rainy season only.

14.5.4 Cultivation Calendar

Major activity	Month	Activity details
Land preparation	May–June	2–3 deep plowing and removal of perennial weeds
Manure and fertilizer	May–June	Application of basal dose of recommended dose of FYM/compost and fertilizers
Plantation	June–July	Slips from healthy, disease-free clumps with rhizome portion intact having 15–20 cm of aerial portion are planted at a spacing of 60 × 30 cm/60 × 45 cm/60 × 60 cm
Irrigation	June–July	Irrigation should be given immediately after transplanting and up to establishment. Later on 8–10 irrigations are required throughout the cropping period

(continued)

(continued)

Major activity	Month	Activity details
Fertilizer	July–August	Application of first top dressing of nitrogen 25 kg/ha at one month after planting
Harvesting	December–February	Digging the clumps along with its roots at eighteen months after planting either by manual or mechanical means

The input cost for various intercultural operations is clearly defined in Table 14.4, in which cost is in INR based on recent rates approximately.

Cultivation costs are based on the prices as prevailing in March 2019, and distillation rates are based on the rates charged by the private distillers.

14.6 Biotechnological Interventions for Analyzing Diversity in Vetiver

Vetiver grass (*Veteveria zizanioides*), because of its outstanding qualities, has now become important to classify for using the correct species and types for producing inferior-quality Vetiver oil. DNA-based markers have been utilized extensively for studying genetic relationship in different species of *V. zizanioides*, as summarized in Table 14.5. Whole genomic and transcriptomic sequences have become available in

Table 14.4 Economics of the cultivation of Vetiver

Operations	Cost of cultivation (Rs/ha)
Field preparation	3000
Layout preparation	1000
Planting material	35,000
Planting	3000
Water management	3000
Weed management	4000
Nutrient management	4000
Plant protection and contingency	1000
Digging	36,000
Distillation	5000
Land rent	15,000
Total	1,10,000
Oil yield (Kg/ha)	>25
Gross return @Rs 8000/Kg oil	2,00,000
Net return (Rs./ha)	90,000

Source Farm bulletin CSIR-CIMAP

Table 14.5 Molecular markers used for analyses of polymorphism in *Vetiveria zizanioides*

S. No.	Name of species/cultivars	Place of collection	Type of Marker used	% polymorphism or % similarity	Purpose of the study	References
1	21 accessions	USA, Louisiana, Colombo, Lilongwe, Northern India, Kassel, Germany, Guang Dong, China, Florida, Nepal, and Portugal	RAPD	100% polymorphism	Genetic diversity	(Adams et al. 2003)
2	131 accessions	Northern and Southern parts of India	RAPD ISSR	89.02% polymorphism 85.18% polymorphism	Genetic diversity	(Singh et al. 2014)
3	10 accessions	Brazil	AFLP	61.73% polymorphism	Molecular characterization	(Celestino et al. 2015)
4	18 accessions	CSIR-CIMAP	RAPD	73% polymorphism	Genetic diversity	(Dhawan et al. 2018)
5	25 accessions	India	ISSR	79.4% polymorphism	Genetic diversity	(Raja et al. 2019)

many model organisms over the last several years, which have significantly enhanced knowledge of the nature of physiological processes in higher plants like Vetiver. Chakrabarty et al. (2015) reported transcriptome analysis of North and South Indian type Vetiver to know the role of genetic makeup on oil quality and root morphology. North Indian type showed higher activity of flavonoid and terpenoid biosynthesis-related genes, i.e., ERF, MYB, bHLH, bZIP, and WRKY and were upregulated in development of root and regulation in hormones.

In another study, numerous genes reported by George et al. (2017) during drought and salt stress to be upregulated in *C. zizanioides*. This includes genes encoding dehydration responsive proteins, peroxidases, late embryogenesis abundant (LEA) proteins, enzymes scavenging reactive oxygen species (ROS), transporters, enzymes in the flavonoid biosynthetic pathways, protein kinases, ethylene receptors, etc. Responsive genes expressed toward both salt and drought stress were found total 108 in both tissues.

In a recent study, the complete and annotated chloroplast genome sequences of *C. zizanioides* reported by Sigmon et al. (2017) include three Sunshine, Capitol, and Huffman non-fertile cultivars and two fertile accessions from Punjab and Allahabad from northern India sites. Non-fertile accessions of Vetiver grass have been used for environmental remediation and erosion control in many parts of the world but fertile plants can turn into harmful weeds. Unique polymorphisms are important to differentiate between non-fertile and fertile plants; therefore, cp genomes of both were sequenced. Total 28 polymorphisms, which include 14 SNPs, 11 microsatellites, 2 small indels, and one micro inversion, were reported in the sterile Sunshine from fertile accessions of Vetiver. This study will help in conservation of germplasm. Micro-RNAs are small, non-coding RNAs which regulate posttranscription gene expression. They typically bind to their target mRNAs' 3'-UTR (untranslated region) and repress protein production by destabilizing mRNA and translational silencing. Recently, total 80 miRNA were identified with 25 miRNA families in leaf and 31 in root. miR169 and miR5021 were reported to regulate most of the target in leaf and root. Some miRNA like miR2102, miR854, and miR5658 regulate terpenoid metabolism as well as primary metabolism like photosynthesis (miR5021 and miR854), etc., in Vetiver. The sesquiterpene (+)-zizaene is the direct precursor of Khusimol, the main fragrance constituent of the Vetiver essential oil. Improved production and in situ recovery of sesquiterpene (+)-Zizaene were done by using metabolically engineered *E. coli* (Aguilar et al. 2019). This research provides additional information for the incorporation of terpene bioprocesses by in situ product recovery, which could be extended to industrializing fragrant molecules in other terpene studies.

V. zizanoides L. Nash is considered to be effective for the heavy metal phytoremediation. In *V. zizanoides* plantlets, an arsenic (As) accumulation, translocation, and tolerance investigation were performed by (Singh et al. 2017) upon exposure to specific arsenic concentrations (10–200 μM). The upregulation of the antioxidant enzyme activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT), and glutathione s-transferase (GST) showed increased tolerance to plants against arsenic-induced oxidative stress.

14.7 Future Perspectives

Traditionally, Vetiver is used in several countries as aromatic and medicinal plants. Vetiver is the most important, highly diverse herb with vast potential. It is known to produce aroma from its roots essential oil and is used in fragrance/perfume, cosmetic, and pharma industries. Different communities use the various sections of Vetiver such as mouth ulcer, boiling, epilepsy, fire, snakebite, nausea, rheumatism, and headache. There is increasing demand and interest in this plant as it is useful for human health and wellness that is why Vetiver can be a natural defense against in the treatment for several diseases. Therefore, C4 plant that is Vetiver would become a profitable crop for providing specific molecules as well as a hub for basic understanding of biosynthesis of various chemical compounds and the gene regulation involved in this complex cross-linking, thus, providing support to farmers to improve their livelihood. Future research should focus on Vetiver for the evaluation of its pharmacological properties and for the control of various diseases for human welfare. The genome analysis of Vetiver provides a basic model for the understanding of biosynthesis and gene regulation of other aromatic plant species. Therefore, this plant is providing a model plant system for medicinal as well as for aromatic plant species.

14.8 Conclusion

The immediate advantages of Vetiver plants are for retaining the moisture in the soil, thus conserving the soil moisture. Wherever Vetiver grass were planted by the farmers, their fields retained sufficient moisture to sustain the seedlings and, even in drought conditions, the farmers were able to harvest a good crop, not only the large-scale farmers, but because of its low cost, it can benefit the small marginal farmer as well. *V. zizanioides* are used to stabilize mine dumps, landfills, road cuttings, eroded slopes, etc., and Vetiver helps agricultural production by maintaining moisture and nutrients in the soil in rainfed cultivation, depending on the weather to get a harvest. The economic rate of Vetiver is good in market. Improved elite cultivars with superior features for disease and drought resistance, animal fodder, formation of hedge, fodder, and insect have been established in India. For environmental conservation purposes, the Vetiver could be efficiently used. Once successfully developed, the Vetiver will provide a low-cost, natural method of environmental protection, particularly for the restoration of polluted lands, as well as for arid and desert areas.

Due to its unique features by maintaining soil moisture and preventing soil erosion, Vetiver can significantly improve in harsh environments. In holistic view, the Vetiver is a nature-gifted plant material, which could be utilized for improving income by utilizing superior, high yielding low-cost-consuming varieties with suitability to grow in almost all diverse climates and soil types. It will provide good alternate for marginal farmers and waste lands with additional income to the farmer and boon for industry

based on its high-valued essential oil. The superior varieties developed at CSIR-CIMAP, India, are developed with unique qualities like with diversified chemical note and immensely popular among farmers because of the increasing demand at industrial level providing additional income by using marginal and wastelands, and this is again beneficial with greater impact on to soil conservation.

References

Adams RP, Pandey RN, Dafforn MR, James SA (2003) Vetiver DNA-fingerprinted cultivars: effects of environment on growth, oil yields and composition. J Essent Oil Res 15:363–371

Aguilar F, Scheper T, Beutel S (2019) Improved production and in situ recovery of sesquiterpene (+)-zizaene from metabolically-engineered E. coli. Molecules 24(18):3356–3375

Bahl JR, Singh AK, Lal RK, Gupta AK (2018) High-yielding improved varieties of medicinal and aromatic crops for enhanced income. In: Singh B, Peter KV (eds) New age herbals: resource, quality and pharmacognosy. Springer, Singapore, pp 247–265

Celestino RS, Zucchi MI, Pinheiro JB, Campos JB, Pereira AA, Bianchini FG, Lima RN, Arrigoni-Blank MF, Alves PB, Blank AF (2015) Molecular and chemical characterization of Vetiver, *Chrysopogon zizanioides* (L.) roberty, germplasm. Genet Mol Res 14:9452–9468

Chahal KK, Bhardwaj U, Kaushal S, Sandhu AK (2015) Chemical composition and biological properties of *Chrysopogon zizanioides* (L) roberty syn. Vetiveria zizanioides (L) nash-a review. Indian J Nat Prod Resour 6(4):251–260

Chakrabarty D, Chauhan PS, Chauhan AS, Indoliya Y, Lavania UC, Nautiyal CS (2015) De novo assembly and characterization of root transcriptome in two distinct morphotypes of Vetiver, *Chrysopogon zizaniodes* (L.) roberty. Sci Rep 5:18630

Dhawan SS, Gupta P, Mishra A, Singh SK, Chauhan HS (2018) Genetic differentiation within *Vetiveria zizanioides*: selection and breeding of commercial perfumery grass Vetiver. J Herbs Spices Med Plants 0:1–9

George S, Manoharan D, Li J, Britton M, Parida A (2017) Drought and salt stress in *Chrysopogon zizanioides* leads to common and specific transcriptomic responses and may affect essential oil composition and benzylisoquinoline alkaloids metabolism. Curr Plant Biol 11(12):12–22

Guenther E (1972) The essential oils, vol 4. Rober E Krieger Publishing Co., Inc., Huntington, New York, pp 156–180

Gupta RK and Pareek SK (1995) Vetiver. In: Chadha KL, Gupta R (eds) Advances in Horticulture, vol 11. Malhotra publishing House, New Delhi, India, pp 773–787

Jain SK (1991) Dictionary of Indian folk medicine and ethno-botany. Deep Publ, New Delhi, India

Kumar A (2008) Ayurvedic medicines: some potential plants for medicine from India. In: Kumar A, Spory S (eds) Recent advances in plant biotechnology. I.K. International, New Delhi, pp 680–694

Lal RK, Sharma JR, Mishra HO (1997) Genetic diversity in germplasm of Vetiver grass (*Vetiveria zizanioides*, L. Nash). J Herb Spice Med Plant 5 (1):1–12

Lal RK (2000) Genetic variability and association analysis for yield and components in indigenous and exotic collections of vetiver (*Vetiveria zizanioides* (L.) Nash). J Spices Aromat Crops 9(2):133–136

Lal RK, Sharma JR (2000) Ascendancy of clonal selection on genetic variability and associations in vetiver (*Vetiveria zizanioides*). J Med Aromat Plant Sci 22(1B):572–578

Lal RK, Gupta P, Gupta V, Sarkar S, Singh S (2013) Genetic variability and character associations in Vetiver (*Vetiveria zizanioides* L. Nash). Ind Crops Prod 49:273–277

Lavania UC, Lavania S (2000) Vetiver grass technology for environmental protection and sustainable development. Curr Sci 78:944–946

Lavania UC (2003) Vetiver root – oil and its utilization. PRVN Tech. Bull. No. 2003/1, ORDPB, Bangkok

Lavania UC, Lavania S (2009) Sequestration of atmospheric carbon into subsoil horizons through deep-rooted grasses–Vetiver grass model. Curr Sci 97:618–619

Lavanya P, Ramaiah S, Anbarasu A (2016) Ethyl 4-(4-methylphenyl)-4-pentenoate from *Vetiveria zizanioides* Inhibits Dengue NS2B–NS3 protease and prevents viral assembly: a computational molecular dynamics and docking study. Cell Biochem Biophys 74:337–351

Matsuo Y, Maeda S, Ohba C, Fukaya H, Mimaki Y (2016) Vetiverianines A, B, and C: sesquiterpenoids from *Vetiveriazizanioides* roots. J Nat Prod 79:2175–2180

Mishra A, Gupta S, Gupta P, Dhawan SS, Lal RK (2020) In: Silico identification of miRNA and targets from *Chrysopogon zizanioides* (L.) roberty with functional validation from leaf and root tissues. Appl Biochem Biotechnol. https://doi.org/10.1007/s12010-020-03381-z

Pripdeevech P, Wongpornchai S, Promsiri A (2006) Highly volatile constituents of *Vetiveria zizanioides* roots grown under different cultivation conditions. Molecules 11:817–826

Raja MB, Rajamani K, Suresh J, Joel AJ, Uma D (2019) Molecular characterization of Vetiver [*Chrysopogon zizanioides* Roberty] genotypes using ISSR markers. Med Plants Int J Phytomedicines Relat Ind 11:246

Ramanujam S, Kumar S (1964) Metroglyph analysis of geographical complexes in Indian Vetiver. Indian J Genetics 24:144–150

Rao RR, Suseela MR (2000) *Vetiveria zizanioides* (Linn.) Nash–a multipurpose ecofriendly grass of India. Paper presented at ICV-2 held in Cha-am, Phetchaburi, Thailand. In: Preceedings of ICV-2. pp 444–8

Saikia D, Parveen S, Gupta VK, Luqman S (2012) Anti-tuberculosis activity of Indian grass Khus (*Vetiveria zizanioides* L. Nash). Complement Ther Med 20(6):434–436

Sigmon BA, Adams RP, Mower JP (2017) Complete chloroplast genome sequencing of Vetiver grass (*Chrysopogon zizanioides*) identifies markers that distinguish the non-fertile 'Sunshine' cultivar from other accessions. Ind Crops Prod 108:629–635

Smith S, Smet ID (2012) Root system architecture: insights from *Arabidopsis* and cereal crops. PhilTrans R Soc B 367:1441–1452

Singh KK, Maheshwari JK (1983) Traditional phytotherapy amongst the tribals of Varanasi district U.P. J Econ Tax Bot 4:829–838

Singh R, Narzary D, Bhardwaj J, Singh AK, Kumar S, Kumar A (2014) Molecular diversity and SSR transferability studies in Vetiver grass (*Vetiveria zizanioides* L. Nash). Ind Crops Prod 53:187–198

Singh S, Sounderajan S, Kumar K, Fulzele DP (2017) Investigation of arsenic accumulation and biochemical response of in vitro developed *Vetiveria zizanoides* plants. Ecotoxicol Environ Saf 145:50–56

Thwaites CH (2010) Vetiver: uprooted? Perf Flav 35(5):22–23

Upadhyay PB, Roy S, Kumar A (2007) Traditional use of medicinal plants among the rural communities of churu district in the thar desert, India. J Ethnopharma 113:387–399

Chapter 15
Cultivation and Utilization of *Pandanus odorifer* for Industrial Application

Noohi Nasim, I. Sriram Sandeep, Sanghamitra Nayak, and Sujata Mohanty

Abstract *Pandanus odorifer* (Forssk.) Kuntze (Kewda) is an industrially important aromatic plant currently having huge demand for the unique fragrance possessed by its essential oil. Phenyl ethyl methyl ether is the major component which imparts this exquisite odour to the kewda male flower essential oil. This distinctive aroma instigates its extensive usage in the cosmetic, pharmaceuticals and flavour and fragrance industries. Almost every part of the plant (flower, stem, root, leaves) possess numerous pharmacological and ethnic utilities. The plant propagation using elite genetic material is therefore imperative to produce improved quality kewda plants to meet the global need. The accelerated demand of kewda perfumes has resulted in a hike in the price of kewda oil. Hence, the farmers require its large-scale cultivation mainly in the coastal and sub-coastal regions. The present chapter focuses on the botanical, phytochemical, pharmacological, agronomical and biotechnological aspects of *Pandanus odorifer*. This comprehensive information will conclusively allow better utilization of this industrially important plant for various industrial uses and improve the socio-economic growth of low-income coastal villagers.

Keywords *Pandanus odorifer* · Essential oil · Perfume · Chemotype · Genotype · Pharmacology · Aromatic plant · Flavour and fragrance industry

15.1 Introduction

Since ancient times plants have been an exorbitant source of natural products and have been widely used to treat various health-related disorders; natural products include numerous pharmaceutical compounds, colouring agents, dyes, and aromatic essential oils. The essential oils are the secondary metabolites stored in the glandular trichomes or cavities and provide a defence system to the plant against herbivores (Glas et al. 2012). Several aromatic plants have been identified and explored for their therapeutic essential oils which are used extensively in the flavor and fragrance

N. Nasim · I. S. Sandeep · S. Nayak · S. Mohanty (✉)
Department of Biotechnology, Rama Devi Women's University, Vidya Vihar, Bhubaneswar, Odisha 751022, India
e-mail: sujatamohantyils@gmail.com

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_15

industry as well as the pharmaceutical industries (Mohanty et al. 2017; Stringaro et al. 2018; Hanif et al. 2019; Manilal et al. 2020). About 90% of the essential oil is consumed by the flavour and fragrance industry in the production of perfumes, cosmetics, food flavouring agents and other healthcare products (Swamy and Sinniah 2016).

Pandanus odorifer (Forssk.) Kuntze (kewda) is a perennial evergreen dioecious monocotyledonous aromatic plant native to South Asia (Nadaf and Zanan 2012; Solomon Raju and Lakshminarayana 2020). The plant sees flowering during the rainy season and is highly valued for its fragrant male flowers. The essential oil from the male flowers is isolated by the hydro-distillation method, which possesses a unique fragrance (Nasim et al. 2018). The phytochemical analysis of kewda essential oil has revealed phenyl ethyl methyl ether (PEME) as the major component that imparts the characteristic smell to the oil (Naqvi and Mandal 1996). Very limited reports are available for the GC-MS analysis to identify the phytochemical composition of kewda flower essential oil (Naqvi and Mandal 1996; Misra et al. 2000; Raina et al. 2004; Nasim et al. 2017a, 2018). Because of its distinct aroma, of kewda oil has a massive demand in pharmaceutical, cosmetic and flavour and fragrance industry.

15.1.1 Botanical Description

15.1.1.1 Taxonomic Position

Pandanus odorifer (Forssk.) Kuntze (kewda) belongs to an ancient family Pandanaceae (Gallaher et al. 2015). The taxonomic classification of *Pandanus odoriferis* as follows:

Kingdom	Plantae
Division	Angiospermae
Class	Monocotyledons
Order	Pandanales
Family	Pandanaceae
Genus	Pandanus
Species	*Pandanus odorifer*

The name Pandanus was derived from a Malayan vernacular name of the trees, "pandan" in 1743 by Rumphius (John 1960). In India, the genus is represented by 30–40 species, which are not well defined (Kirtikar et al. 1991). Among these, *P. odorifer* is a dominant species found mainly in Odisha's coastal regions (Nasim et al. 2017a). Commonly known as kewda, the plant has various synonyms (Table 15.1).

15.1.1.2 Morphological Description

The plant has a palm-like appearance, usually growing to a height of 3–5 m (Fig. 15.1A, B). The kewda leaves are deep green, glaucous, oblong, ensiform with coriaceous margins and tapering with spiny midribs, arranged spirally on the branches and measure 1–3 m long and 5–6 cm broad (Fig. 15.1C). The plant is dioecious with unisexual flowers. Male flowers appear as clusters with androecia having a unique fragrance and are surrounded by tender white bracts (Fig. 15.1 D). The male spadix is 25–50 cm in length and has numerous subsessile cylindrical spikes, which are 5–10 cm long (Padhy et al. 2016). The female flowers lack any fragrance and look like a pineapple. The female inflorescence consists of a single spadix (5 cm in diameter) and gynoecia without perianth and is made up of lots of carpels (Padhy et al. 2016). The male flowers last only a single day. The fruits of this plant are odourless with 15–25 cm width and are ellipsoid, ovoid, globose or subglobose. Unripen fruits are green, whereas mature fruits are yellow/red (Jose et al. 2016; Padhy et al. 2016). It has greyish- or reddish-brown smooth barks. Braches and stems are ringed with distinct leaf scars (Lim 2012). It has a spinous trunk (12–25 cm across) with the thick and strong prop or adventitious stilt roots that arise from the stems (Fig. 15.1E) (Panda et al. 2009). Ecologically, the plant has been reported to bear immense potential in its intricate root system, and thereby controlling soil erosion, fixing sand dunes and protecting from damage caused by tsunami (Tanaka et al. 2011; Thuy et al. 2018).

Table 15.1 Synonyms of *Pandanus odorifer*

Botanical	*Pandanus fascicularis Lam*
	Pandanus odoratissimus L.f
	Pandanus tectorius
English	Screwpine
	Umbrella tree,
Hindi	*Kewda*
	Kewra
Sanskrit	*Ketaka*
Urdu	*Keora*
Odiya	*Kiya*
	Ketki
Malayalam	Kaitha
Tamil	Kaida
Thai	Ka-Ra-Ket

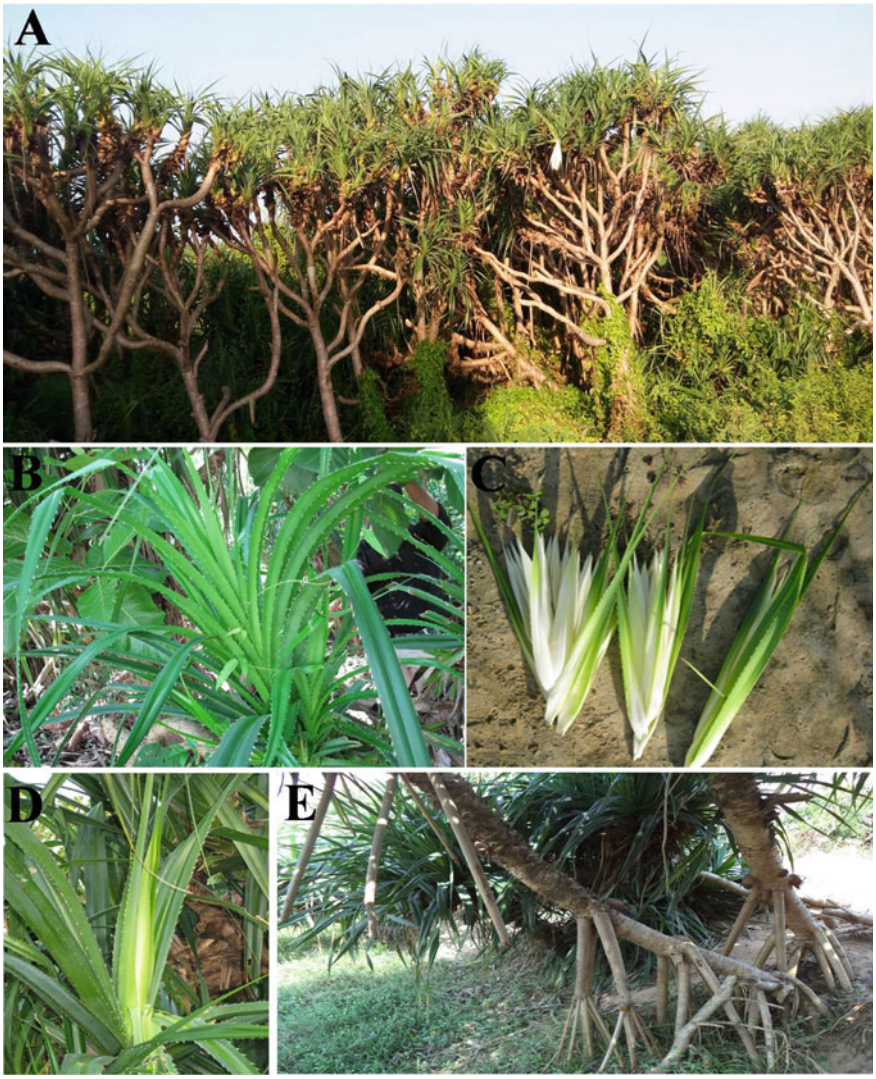


Fig. 15.1 Dense kewda plantation in Ganjam district of Odisha, (B) kewda plant, (C) kewda male flowers, (D) kewda spiny leaves, (E) stilt/prop roots of kewda

15.1.2 Industrial Importance of Pandanus Odorifer (Kewda)

Pandanus odorifer (kewda) is an industrially important essential oil-bearing plant with high priced flower essential oil. Economically, it is an important natural bio-resource for the perfumery industry due to its unique fragrance (Panda et al. 2009).

The characteristic smell of the male flowers owes their application to the manufacturing of various perfumery products through hydro-distillation. Three types of end products are produced from the hydro-distilled kewda flowers, i.e. kewda oil, kewda attar and kewda water. Kewda oil has substantial demand in the perfumery industry, whereas kewda attar and kewda water are predominantly used for flavouring purposes in the food and cosmetic industry (Panda et al. 2012).Ganjam district of Odisha has been reported to have a superior quality of kewda essence (Raina et al. 2004). Hence, it provides 85–90% of kewda essence in India and about 50% of the world with an approximate turnover of Rs. Fifty crores (Padhy et al. 2016). The kewda products have seen an upsurge in their price due to the prompt demand of kewda perfume in the national and international markets, especially in the Arab countries (Sahu and Misra 2007). Kewda essential oil is approximately priced to be 2.5 to 4 lakh per litre, kewda attar is 0.2 lakh per litre and kewda water is Rs 300 per litre (Padhy et al. 2016).

Pandanus odorifer plant parts have multipurpose industrial applications and a broad ethnic value. Though the male flowers are extensively used for perfumery production, they also have broad pharmaceutical properties. Other plant parts such as leaves, roots and fruits are also employed in the food, fibre, handcraft and pharmaceutical industries. Kewda leaves are rigid and spiny and often used as fences across the crop fields to protect them from livestock. The low-income coastal villagers also use them in making various handicraft products such as ropes, mats, baskets, table lamps, files and wall hangings. (Abral et al. 2012; Teli and Jadhav 2017). The leaf extracts are also used for food colouring purposes. In Sri Lanka, the leaves are used for cooking (Takeda et al. 2008). The pulp and polyester composites of the leaves are used in the paper and fibre industry. The fibre obtained from *P. odorifer* leaves also has excellent potential for being used as a textile and composite material. The thick and strong prop roots are used as supports and fabrication of houses. The spinous trunks of mature plants are used in building thatched roof. It is also used for preparing glue and making string. The branches are used to make compost and wood fuel. In India and Sri Lanka, the flowers are used for decoration and are offered to God. Fruits are used as firewood and foodstuff (Nadaf and Zanan 2012; Baba et al. 2016).

Hence, cultivation practice and the marketing of kewda products have become an additional source of income for the deprived coastal villagers, resulting in their socio-economic growth. Thus, the plant has become an essential bio-resource with a positive impact on the local economy of Ganjam district, Odisha, India (Panda et al. 2007; Panda et al. 2009; Panda et al. 2010b; Jose et al. 2016).

15.2 Geographic Distribution

15.2.1 Origin

Pandanus is a pleiotropic genus, belonging to an ancient family Pandanaceae, representing dioecious monocotyledons having Gondwanan origin (Gallaher et al. 2015). Among all the genera of the Pandanaceae family, Pandanus is the largest genus and has the broadest geographical distribution with immense economic and medicinal importance (Buerki et al. 2012). The diversity of habitats included by the genus occupies the tropical and sub-tropical zones, riversides, rocky or sandy coasts, swamp forests, mangrove forests, savannas, lowland dipterocarp forest and mountain forest (Susanti et al. 2012).

15.2.2 Distribution

In India, Pandanaceae family represents about 30–40 species under three genera *Pandanus, Benstonea* and *Freycinetia* (Table 15.2). *Pandanus odorifer* is an important member of the genus *Pandanus* with a higher concentration in Andaman and Nicobar Islands and Northern and Southern India. It is an aromatic monocot species, native to Australia, Indonesia, South Asia and Philippines. It is widely distributed in South America, Micronesia, Papua New Guinea, Melanesia, Polynesia, India and Pacific Islands (Nadaf and Zanan 2012; Adkar et al. 2014; Nasim et al. 2020). In India, it is massively distributed in the Western Ghats zone and the coastal zone of Odisha, Kerala, Tamil Nadu, West Bengal, Andhra Pradesh, Gujarat and Uttar Pradesh (Padhy et al. 2016; Nasim et al. 2017a). In Odisha, the plant is found in Ganjam, Cuttack, Khorda, Jagatsinghpur, Bhadark, Puri and Balasore. Although the plant covers the entire coast of Odisha, the Ganjam district is the only growth centre where the plant is cultivated for commercial purposes (Nasim et al. 2017a).

15.3 Brief Phytochemistry

Aromatic plants have a wide range of chemical components that seeks the attention of pharmaceutical industries. A large number of phytoconstituents have been identified in the flower essential oil of *Pandanus odorifer*. The available reports showed a diverse chemical profile with a broad range of chemical constituents with a wide range of volatility, including ethers, esters, aldehydes, alcohol, ketones, acids, sulphur and nitrogen-containing compounds. The major constituent identified in the kewda oil was 2-phenylethyl methyl ether (75.0%) which is the key component responsible for the distinct aroma of kewda (Naqvi and Mandal 1996). Terpinen-4-ol (15.2%)

Table 15.2 Distribution of some Indian *Pandanus* species

	Species	Distribution	References
1	*Pandanus odorifer*	Odisha, Andhra Pradesh, Kerala, Tamil Nadu, West Bengal, Uttar Pradesh, Gujarat	Nasim et al. (2020)
2	*Pandanus kaida*	Goa, Karnataka, Kerala, Tamil Nadu, Odisha,	Nadaf and Zanan (2012)
3	*Pandanus furcatus*	Maharashtra, Goa, Karnataka and Kerala	Zanan and Nadaf (2013)
4	*Pandanus thwaitesii*	Goa, Karnataka, Kerala, Maharashtra	Zanan and Nadaf (2011)
5	*Pandanus canaranus*	Goa, Karnataka, Kerala, Maharashtra	Zanan and Nadaf (2011)
6	*Pandanus dubius*	Kerala and Karnataka	Zanan and Nadaf (2013)
7	*Pandanus amaryllifolius*	Gujarat, Maharashtra, Goa, Karnataka, Kerala, Tamil Nadu, Orissa, and West Bengal	Wakte et al. (2012)
8	*Pandanus emarginatus*	Arunachal Pradesh	Nadaf and Zanan (2012a)
9	*Pandanus leram*	Andaman and Nicobar Islands	Nadaf and Zanan (2013)
10	*Pandanus nepalensis*	WB, Sikkim	Nadaf and Zanan (2013)
11	*Pandanus unguifer*	WB, Sikkim	Nadaf and Zanan (2013)
12	*Pandanus palakkadensis*	Palakkad, Kerala state	Nadaf et al. (2011)
13	*Pandanus mangalorensis*	Mangalore district, Karnataka state	Zanan and Nadaf (2012a)
14	*Pandanus martinianus*	Arunachal Pradesh, Assam	Zanan and Nadaf (2012b)
15	*Pandanus foetidus*	Kerala, Karnataka, Goa	Zanan and Nadaf (2011)
16	*Pandanus diversus*	Assam	Nadaf and Zanan (2012)
17	*Pandanus unipapillatus*	Maharashtra, Goa, Karnataka, Kerala	Nadaf and Zanan (2013)

was reported as the second major constituent for the first time by Naqvi and Mandal (1996). Raina et al. (2004) reported 2-phenyl ethyl methyl ether (37.7%), terpinen-4-ol (18.6%), α-terpineol (8.3%) and 2-phenyl ethyl alcohol (7.5%) as the dominating phyto-compounds in the hydro-distilled kewda flower oil obtained from Ganjam district of Odisha. The study also reported a comparative analysis of hydro-distilled kewda oil with oil purchased from the local market. Market kewda oil was reported to have 2-phenyl ethyl alcohol (33.2%) as the major compound followed by 2-phenyl ethyl methyl ether (16.1%), benzyl benzoate (11.0%), viridine (8.8%) and germacrene B (8.3%) (Raina et al. 2004). GC and GC-MS analysis of the kewda flower extract was reported by Rout et al. (2005). Rout et al. (2011) reported the chemical composition of the extract obtained by liquid CO_2 extraction of the kewda flowers. The above studies show the better quality of kewda essential oil from Ganjam district, but there was unpredictability for the oil quality from different zones of Ganjam.

In this context, a study was done by collecting kewda oil from twelve different zones of Ganjam district (Rushikulya River Bank, Kalipalli, Keluapalli, Markandi, Indrakhi, Mantridi, Kaliabali, Basanaputty, Chamakhandi, Podapadar, Chilika and Tampara) and GC-MS analysis was done. The study reported the PEME to be the major constituent highest in Rushikulya River Bank (81.86%) and lowest in Mantridi (58.03%). The second major compound reported was terpinen-4-ol (7.81–21.46%) (Nasim et al. 2018). The study also reported the role of soil factors for secondary metabolites of kewda. Among the five analysed parameters, i.e., soil pH, soil nitrogen (N), organic carbon (OC), potassium (K) and phosphorous (P), N was found to be the most influential factor for kewda oil yield and PEME content followed by OC, pH, P and K (Nasim et al. 2018). For a more detailed characterization of the volatile constituents present in kewda oil, GCxGC-TOFMS analysis was also done. The study identified 159 chemical compounds in kewda oil out of which kewda ether, *ortho*-cymene and terpinen-4-ol were the predominant constituents (Nasim et al. 2017a). The structures of some of the major chemical components found in kewda essential oil are shown in Fig. 15.2.

Other than *Pandanus odorifer*, very few reports are available on other *Pandanus* species (Table 15.3). MacLeod and Pieris (1982) analysed the essential oil of *Pandanus latifolius* leaves and reported the presence of mainly sesquiterpene hydrocarbons, and the only monoterpene that was reported was linalool. The chemical profile of the red fruit oil from *Pandanus conoideus* showed the presence of 1, 3-dimethylbenzene (27.46%), N-glycyl- L-alanine (17.36%), trichloromethane (15.22%) and ethane (11.43%) (Rohman et al. 2012). *Pandanus amaryllifolius* leaf oil

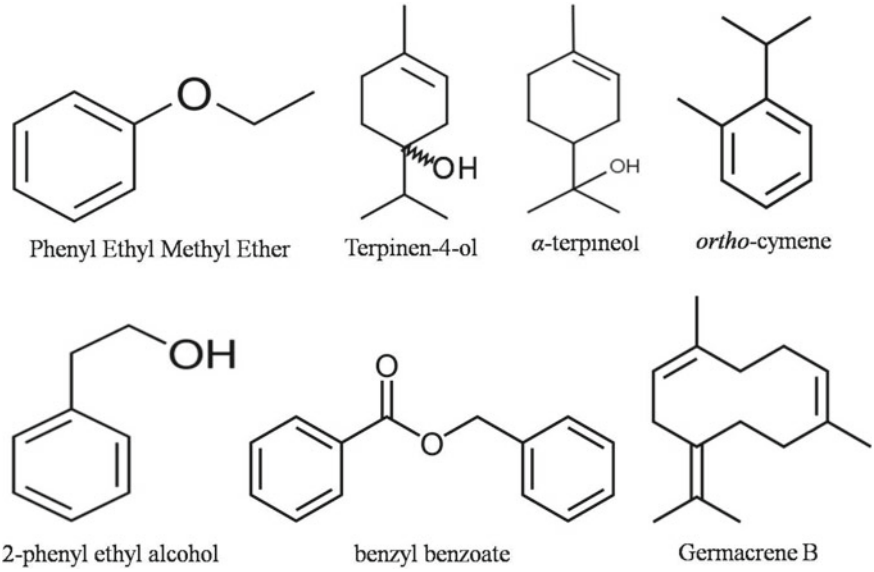


Fig. 15.2 Structures of chemical compounds found in *Pandanus odorifer* male flowers essential oil

Table 15.3 Analysis of essential oil constituents in different *Pandanus* species

S.No.	Pandanus species	Origin	Major constituents	Plant part	Analysis	References
1	*Pandanus latifolius*	Sri Lanka	Linalool (5.7%), beta-selinene (24.3%), beta-caryophyllene (10.8%), styrene (12%), formylthiophen (14.9%), 1,2 dimethoxybenzene (2.9%), beta-farnesene (3.6%)	Leaves	GC–MS	MacLeod and Pieris (1982)
2	*Pandanus conoideus*	Indonesia	1,3-dimethylbenzene (27.46%), N-glycyl- L-alanine (17.36%), trichloromethane (15.2%), and ethane (11.43%)	Fruit	GC–MS	Rohman et al. (2012)
3	*Pandanus amaryllifolius*	Singapore	3-Methyl-2-(5H)-furanone(73.07%),3-hexanol(7.09%), 4-methylpentanol(6.13%), 3-Hexanone (2.97%), 2-hexanone (2.65%)	Leaves	GC–MS	Jiang (1999)
4	*Pandanus tectorius*	Polynesia	Geranyl acetate(27.5%), 3-Methyl-3-buten- 1-yl cinnamate(17.1%), Ethyl cinnamate(10.2%), 3-Methyl-3-buten- 1-yl acetate(10.1%), Cinnamyl acetate(5.2%), 3-Methyl-2-buten- 1-yl acetate(4.7%), 3-Methyl-2-buten- 1-yl cinnamate(4.5%), 2-Phenylethyl acetate(2.9%), Linalool(1.9%), C15H24(1.8%),	Fruit	GC and GC–MS	Vahirua-Lechat et al. (1996)
5	*Pandanus Tectorius*	China	Asarone(26.7%),longipinocarvone(15.2%) and 2-methyl-6-(4-methylphenyl)hept-2-en-4-one(14.8%)	Root	GC and GC–MS	Liu et al. (2012)
6	*Pandanus fasciculuris*	India	2-phenylethyl methyl ether(75%), terpinen-4-ol (15.2%), p-cymene(1.8%), a-pinene(1.6%), sabinene(1.2%), linalool(1.1%), b-pinene(1%)	Flower	GC and GC–MS	Naqvi and Mandal (1996)
7	*Pandanus odoratissimus*	India	2-phenyl ethyl methyl ether (37.7%), terpinen-4-ol (18.6%), α-terpineol (8.3%) and 2-phenyl ethyl alcohol (7.5%),	Flowers	GC and GC–MS	Raina et al. (2004)

(continued)

Table 15.3 (continued)

S.No.	Pandanus species	Origin	Major constituents	Plant part	Analysis	References
8	*Pandanus fascicularis*	India	2-phenyl ethyl methyl ether, terpinen-4-ol and a-terpineol	Flower	GC and GC–MS	Rout et al. (2011)
9	*Pandanus fascicularis*	India	kewda ether, ortho-cymene and terpinen-4-ol	Flower	GCxGC-TOFMS	Nasim et al. (2017a)
10	*Pandanus odorifer*	India	Phenylethyl methyl ether (58.03–81.86%), terpinen-4-ol (7.81–21.46%)	Flower	GC and GC–MS	Nasim et al. (2018)
11	*Pandanus amaryllifolius*	Myanmar	Phytol (21.35%), α-thujaplicin (18.64%), dodecanol (12.55%), n-tetradecanol (8.93%), benzyl acetate (8.08%)	Leaves	GC–MS	Mar et al. (2019)

chemical composition was reported by Jiang (1999). About twenty-two compounds were identified and the major components were 3-methyl-2-(5H)-furanone, 3-hexanol, 4-methylpentanol, 3-exanone and 2-hexanone. Isopentenyl and dimethylallyl acetates and cinnamates were reported as the dominant phytoconstituents in the essential oil of ripe fruit of *Pandanus tectorius* (Vahirua-Lechat et al. 1996). *Pandanus tectorius* root oil was analysed by Liu et al. (2012) and major compounds reported were asarone, longipinocarvone and 2-methyl-6-(4-methylphenyl) hept-2-en-4-one. The leaf extract of *Pandanus odorus* was analysed with GC–MS and α-tocopherol, β-sitosterol, hexadecanoic acid, campesterol, squalene, stigmasterol and 9,12,15-octadecatrien-1-ol were identified as major components by Rahman et al. (1999).

15.4 Medicinal Properties and Usage

Pandanus odorifer has a broad range of pharmacological properties. Different parts of the plant have been used as one of the ingredients in several Ayurvedic formulations (Nasim et al. 2020). In Ayurveda, kewda has been used for treated many human health problems such as indigestion, headache, anorexia, constipation, leprosy and rheumatism. (Udupa et al. 2011). Since ancient times kewda oil has been used for healing skin diseases, small-pox, earache, rheumatoid arthritis, headache, spasms and leprosy and as a laxative for colic infections (Adkar et al. 2014). The fruit is an excellent source of carotenoids and has been used to treat vitamin deficiencies and certain heart-related infections (Lim 2012). As a traditional practice, tablets made up of kewda leaf extracts are used for getting relief from pain and inflammation (Panda et al. 2009). The leaves are also used for curing syphilis, leprosy, leucoderma, scabies and small-pox (Padhy et al. 2016). The plant extract also possesses diuretic and anti-spasmodic properties (Rajeswari et al. 2012).

The biological potencies of *Pandanus odorifer* were reported as anti-inflammatory (Del mundo et al. 2020), anti-oxidant (Londonkar and Kamble 2011), anti-diabetic (Kumari et al. 2012), anti-cancer (Gowtham et al. 2014), anti-bacterial and thrombolytic (Penu et al. 2020), anti-fungal (Rahayu et al. 2013), cardio-protective (Sobhana et al. 2014), cytotoxic activity (Jitu et al. 2017), hepato-protective (El-Shaibany et al. 2016), protective effect on UV-B-induced DNA damage (Kaewklom and Vejaratpimol 2011), anti-stress (Adkar et al. 2014), anti-ulcer (Abirami et al. 2015), neuro-pharmacological activities (Kuber and Santhrani 2010), etc. Comprehensive information regarding pharmaceutical properties present in extracts of different parts of kewda is summarized in Table 15.4.

Table 15.4 Biological activities reported in *Pandanus odorifer*

SI. No.	Activity	Extract	Active constituents	References
1	Anti-ulcer	Aqueous extract of leaves	Steroids, saponin, sterol,alkaloids, quinone, phenol, coumarin, glycosides	Abirami et al. (2015)
2	Anti-convulsant	Ethanol extract of leaves	Glycosides, flavonoids, alkaloids	Adkar et al. (2014)
3	Nocturnal enuresis	Methanolic peduncle extract	Flavonoids and phenolic compounds	El-shaibany (2014)
4	Anti-oxidant, anti-bacterial	Fruits extract	Phenolics, flavonoids, terpenoid, steroids, saponins and glycosides	Londonkar and Kamble (2011); Andriani et al. (2019)
5	Anti-inflammatory	Methanolic and aqueous extracts	Ethyl caffeate and dihydroconiferyl alcohol	Del mundo et al. (2020)
6	Neurobehavioral activity	Ethyl acetate fraction of leaves	Flavonoids	Bhatt and bhatt (2015)
7	Cytotoxic activity	Chloroform extract of fruits	Alkaloids, steroids, terpenoids, flavonoids, tannins, saponins	Jitu et al. (2017)
8	Anti-fertility	Hydroalcoholic leaves extract	Alkaloids, carbohydrates, flavonoids, saponins	Kumar et al. (2017)
9	Anti-microbial	Hydroalcoholic leaf extract	Alkaloids and flavonoids	Kumar et al. (2010)
10	Anti-diabetic	Methanolic extract of aerial roots	Flavonoids and phenolic compounds	Rajeswari et al. (2012)
11	Hepato-protective	Ethanolic extract of roots	Alkaloids, flavonoids, glycosides	Mishra et al. (2015)
12	Analgesis	Aqueous extract of prop roots	Carbohydrates, proteins, aminoacids, saponins, tannins, phenolic compounds, alkalodies and flavonoids	Rajeswari et al. (2011)
13	Anti-cancer	Aqueous extract of roots and leaves	Alkaloids, flavonoids, glycosides and phenolic content	Gowtham et al. (2014)

(continued)

Table 15.4 (continued)

SI. No.	Activity	Extract	Active constituents	References
14	Anti-hyperglycemic	Alcohol and aqueous root extracts	Phenolic compounds, tannins and flavonoids	Madhavan et al. (2008)
15	Anti-nociceptive	Chloroform extract of leaves	Steroids, terpenoids, flavonoids, saponins, tannins	Panda et al. (2008)
16	Wound-healing agent	Chloroform extract of leaves	Steroids, terpenoids, flavonoids, saponins, tannins	Panda et al. (2010b)
17	Anti-fungal activity	Ethanol extract of leaves	Alkaloids and tannins	Rahayu et al. (2013)
18	Anti-diarrheal activity	Methanol extracts of leaf and fruit	Flavonoids, saponins, alkaloids, steroids, terpenes	Rahman et al. (2014)
19	CNS depressant	Methanolic leaf extract	Steroids, saponins, terpenoids, glycosides, tannins, flavonoids and phenolics	Raju et al. (2011)
20	Cardio-protective activity	Hydroalcoholic leaves extracts	Flavonoids, tannins, saponins, proteins, aminoacids, alkaloidsglycosides, phenols, carbohydrates	Sobhana et al. (2014)

15.5 Agro-Technology/Cultivation/Domestication

15.5.1 Vegetative Propagation

Pandanus odorifer propagate by vegetative methods. The adventitious aerial roots and the branch cuttings are used widely for its propagation. About 60–80 cm long and 8–10 cm thick branch cuttings are implanted during the rainy season. The spacing between each plantlet is maintained at 3–7 m apart. It is highly salt-tolerant and can withstand strong winds (Rashmi and Nadaf 2017). The plant is adapted well to light and heavy well-drained soil. It is well acclimatized to saline, sandy and marshy wastelands. Among the different edaphic factors influencing the oil yield and PEME content of the kewda flower essential oil, nitrogen has been reported to be the most influential factor followed up by organic carbon, pH, phosphorous and potassium (Nasim et al. 2018).

15.5.2 Climate

The plant grows abundantly in high rainfall areas and is found profusely in a 45 × 15-km stretch in Ganjam district along the coast of the Bay of Bengal. The flowering starts after 3–4 years of the plantation and the rainy season (July to October) sees the maximum flowers. A mature kewda tress produces about 30–40 flowers spikes yearly. Branching, rainfall and nearness to the water body affect the flowering of the plant. The plant has a life span of 50–80 years which might last up to 100–150 years also. However, the fruiting stage is only for 20–25 years (Adkar et al. 2014).

15.5.3 Crop Nutrition

Nutritionally, the fruit of the plant is rich in provitamin A, vitamin C and total carotenoids. Foods rich in carotenoids are consumed to protect against anaemia, vitamin A deficiency and chronic diseases such as diabetes, cancer and heart diseases (Lim 2012). It is consumed in the form of a paste and contains protein, calcium, iron, thiamine and beta-carotene. The juice is also used in the form of a beverage (Englberger et al. 2009). The fruit seed oil has also been reported to be a promising source for non-edible biodiesel production (Mahlinda et al. 2017).

15.5.4 Diseases and Pests

The senescence of the plant is mainly due to the infection of insect pests such as bagworm, beetles and thrips causing economic losses. Diseases like foot rot of central shoot, leaf blight and fruit rot have also been reported in this plant (Jagadev et al. 2001).Arbuscular mycorrhizal fungi (AMF) association has also been reported in this plant which helps in strengthening the ecological efficacy in coastal regions (Kamble et al. 2013).

15.5.5 Harvesting

Kewda male flowers are the prime source of the essential oil and the harvesting time and extraction procedures of the essential oil play a significant role in maintaining its quality and yield. Depending on the flowering season which occurs thrice a year, the inflorescences are harvested. The male flowers are plucked manually early morning between 7–9 a.m. with the help of a long bamboo stick fitted with a hook and immediately subjected to essential oil extraction. Large quantities of flowers are collected and hydro-distilled by the traditional method of oil extraction employing

copper vessel, copper lead and bamboo pipes. It has been reported that delay in the transportation of kewda flowers deteriorates the quality and aroma of kewda oil (Nasim et al. 2017a). This leads to decreased price of the oil and in turn causes a huge loss to the farmers. Hence, harvesting time and the extraction of essential oil have to be a rapid process to maintain the oil quality and quantity, thereby minimizing the loss to the kewda growers (Nasim et al. 2017a).

15.6 Biotechnological Approaches

15.6.1 Overview of Molecular Markers Technologies Employed in Kewda

Plant genetic resources are valuable and irreplaceable resources for current and future crop improvement strategies. Genetic diversity accounts for heritable genetic variability within and among populations of a species and forms the basis for survival, selection, adaptation and plant improvement (Rao and Hodgkin 2002; Laurentin 2009). Molecular markers have been extensively used to study genetic diversity (Agarwal et al. 2008; Omondi et al. 2016). Different types of molecular markers like RAPD, ISSR, AFLP, SSR and SNP have been developed and are being used for crop improvement (Nadeem et al. 2018). These molecular marker techniques can be employed to study genetic diversity within and among the species (Cervera et al. 2000; Zou et al. 2011). In comparison with other marker types, these DNA-based markers techniques can unmask the genetic diversity of almost all species at various developmental stages, remaining unaffected by environmental conditions (Shah et al. 2018). Molecular markers also provide information about the diversity at the nucleotide level (SNPs), population structure, frequencies of gene and allele (genotype information), distribution and range of genetic diversity. Besides, molecular markers can also be used for resolving taxonomic problems and help in providing exact taxonomic hierarchies important for phylogenetic studies (Sarwat et al. 2012). Characterization based on molecular markers is highly reliable and effective in studying variation in different genotypes (Kaur et al. 2015).

To date, there are only a few reports of the utilization of RAPD, ISSR and SSR for assessing genetic diversity in kewda. RAPD profile of three morphotypes (spinous, ketaki and spineless.) of *Pandanus fascicularis* was developed by Panda et al. (2007). Cluster analysis by the UPGMA method from their study resulted in a phylogenetic dendrogram which produced two groups, one separating ketaki morphotype while the second group was separating spinous and spineless morphotype suggesting ketaki as a distinct variety in the Pandanus genus. Panda et al. (2010a) utilized 30 RAPD primers to detect sex differences in *Pandanus tectorius* Parkinson (*P. fascicularis, P. odorifer)* from seven populations of Odisha. Dendrogram from their study divided the seven populations into two distinct groups of male and female plants. AMOVA show 3% molecular variance among populations and ~95% within populations. Molecular

markers were also used for differentiating male and female genotypes in *Pandanus fascicularis.* Vinod et al. (2007) developed a male-specific SCAR marker for differentiating the sexes in Pandanus. Another report revealed a considerable amount of polymorphism at the interspecific level and a lower degree of polymorphism at the intraspecific level in the genus Pandanus when analysed by RAPD (Sarile and Menguito 2010). In a more recent study, genetic diversity analysis of *Pandanus odorifer* (Forssk.) Kuntze was carried out by utilizing 13 ISSRs and 30 SSRs (Nasim et al. 2020). A total of 84 accessions from different regions of Ganjam were utilized in the study which revealed SSRs being more effective than ISSR for genetic diversity evaluation in kewda accessions. Further studies in this direction will help in proper identification and characterization of kewda species and the data from genetic diversity analysis can be utilized for kewda improvement to meet its increasing demand in the perfumery industry.

15.6.2 Genomics Aspect of P. Odorifer

With the rapid advancement of genomics and transcriptomics, the identification of key genes involved in the synthesis of specialized metabolites in medicinal and aromatic plants has become much faster (Yamazaki et al. 2018). The next-generation sequencing strategies have enabled the researchers to sequence huge and complex genomes of medicinal plants effortlessly. Despite its medicinal and economic importance, there are only limited reports on the exploration of genes associated with *Pandanus odorifer*'s unique fragrance. Vinod et al. (2010) for the first time constructed a male-specific cDNA library in *P. odorifer* and identified specific transcripts involved in PEME biosynthesis. A total of 977 ESTs were generated from their study. Rashmi et al. (2019) utilized integrative transcriptomics and metabolomics approach for the understanding of salinity tolerance in *P. odorifer*. Results from their study revealed the up-regulation of Asparagine (Asn) biosynthesis genes. Higher transcript accumulation of genes, *viz. glutamine synthetase, glutamine synthase, aspartate kinase, pyruvate kinase, aspartate aminotransferase, phosphoenolpyruvate, carboxylase and asparagine synthetase* (AS) was observed under salt stress. Subsequently, the genomic resources generated from these studies will largely benefit our understanding of primary and secondary metabolite pathways and pave the way for metabolite engineering to increase essential oil yield and quality in *P. odorifer*.

15.7 Perspectives

In recent years, medicinal and aromatic plants have gained huge attention due to the stimulated demand from the pharmaceutical, cosmetic and aromatic industries in the local, national and international markets. *Pandanus odorifer* is an economically and

industrially important aromatic plant. The exquisite fragrance owned by the kewda male inflorescence has captivated the perfumery industry. Different parts of the plant possess numerous pharmaceutical properties. It is an important natural bio-resource of Ganjam district, Odisha, and provides livelihood to the huge population there. The flower essential oil of kewda is in high demand for kewda perfumes, especially in the national and international markets, especially in the Arab countries.

In spite of so much economic value and numerous pharmaceutical properties in the oil and extracts, the plant still lacks proper exploration in the global market due to restricted plantation and high cost of kewda oil.

Use of elite chemotypes and elite genotypes of kewda is the need of the hour to improve the quality and quantity of kewda, thereby meeting the increased demand of this plant in the perfumery industry. Furthermore, future research is anticipated in developing efficient protocols for in vitro studies for maintaining the bioactive chemical entities naturally present in the essential oil. The plant is vegetatively propagated; hence, the development of different propagation strategies is needed for crop management with improved cultivation practices. Large-scale kewda cultivation can be achieved through the proper application of advanced biotechnological applications to study the genetic variation by various types of molecular markers to improve the quality of kewda cultivars and meet the global market requirement. Also, kewda growers should use high yielding kewda chemotypes as mass planting material and follow the rapid extraction of essential oil immediately after harvesting to improve the quality of different kewda products like kewda attar and kewda water. Different edaphic factors should also be taken care of with balanced nutrient application, weed control, timely planting, micronutrient use and timely harvesting to meet the future market demand.

References

Ab Rahman N, Hassan M, Omar A, Ab Kadir M (1999) Major chemical constituents of supercritical carbon dioxide extract of *Pandanus odorus* leaves. Nat Prod Sci 5(2):75–79

Abirami J, Sathya P, Joshi Eminal J, Brindha P (2015) Anti ulcer activity of *Pandanus fascicularis* (lam.) on ethanol induced ulcer in wistar albino rats. World J Pharm Pharm Sci 4(10):1698–1705

Abral H, Andriyanto H, Samera R, Sapuan S, Ishak M (2012) Mechanical properties of screw pine (*Pandanus odoratissimus*) fibers—unsaturated polyester composites. Polym-Plast Technol Eng 51(5):500–506

Adkar PP, Jadhav PP, Ambavade SD, Shelke TT, Bhaskar VH (2014) Protective effect of leaf extract of Pandanus odoratissimus Linn on experimental model of epilepsy. Int J Nutr, Pharmacol, Neurol Dis 4(2):81

Agarwal M, Shrivastava N, Padh H (2008) Advances in molecular marker techniques and their applications in plant sciences. Plant Cell Rep 27(4):617–631

Andriani Y, Ramli NM, Syamsumir DF, Kassim MNI, Jaafar J, Aziz NA, Marlina L, Musa NS, Mohamad H (2019) Phytochemical analysis, antioxidant, antibacterial and cytotoxicity properties of keys and cores part of *Pandanus tectorius* fruits. Arab J Chem 12(8):3555–3564

Armayni UA, Ahmad MS, Yusof NM, Mohsin HF, Wahab IA (2015) The identification of biphenyl from Pandanus Pygmaeus. Procedia Environ Sci 30:68–72

Baba S, Chan HT, Kezuka M, Inoue T, Chan EW (2016) Artocarpus altilis and Pandanus tectorius: Two important fruits of Oceania with medicinal values. Emir J Food Agric 28(8): 531–539
Bhatt PR, Bhatt KP (2015) Neurobehavioral activity of *Pandanus tectorius* Parkinson (Pandanaceae) leaf extract in various experimental models. J Pharma Sci Tech 5(1):16–18
Buerki S, Callmander MW, Devey DS, Chappell L, Gallaher T, Munzinger J, Haevermans T, Forest F (2012) Straightening out the screwpines: A first step in understanding phylogenetic relationships within Pandanaceae. Taxon. 61(5):1010–20
Butaud J-F, Jacq F, Callmander MW (2019) *Pandanus papateaensis* (Pandanaceae): une nouvelle espècemenacée de l'atollsoulevé de Makatea (Tuamotu, Polynésiefrançaise). Candollea 74(2):137–144
Callmander MW, Laivao MO, Randrianaivo R (2010) A new species of Pandanaceae from northern Madagascar, *Pandanus ankaranensis.* Novon: J Bot Nomenclature/Missouri Bot Gard 20(3):243
Cervera MT, Remington D, Frigerio J-M, Storme V, Ivens B, Boerjan W, Plomion C (2000) Improved AFLP analysis of tree species. Can J for Res 30(10):1608–1616
Del Mundo CR, Castillo AL, An SS, Tan MA (2020). In vivo COX-2 modulation and metabolite profiling of Pandanus tectorius leaves extracts. 3 Biotech 10(3):1–10
Dutta P, Saxena H, Brahmam M (1987) Kewda Perfume Industry in India1. Econ Bot 41(3):403–410
El-Shaibany A (2014) Nocturnal enuresis, antioxidant and antimicrobial activities of *Pandanus odoratissimus* L. peduncle. Int J Pharmaceut Sci Res 5(3):811–818
El-Shaibany A, Molham AH, Al-Massarani S, El-Gamal A, Al-Ajami A, Al-Adhl A (2016) Hepato-protective effect of Pandanus odoratissimus l inflorescence extracts in acetaminophen-treated guinea pigs. Trop J Pharm Res 15(2):259–267
Englberger L, Schierle J, Hofmann P, Lorens A, Albert K, Levendusky A, Paul Y, Lickaneth E, Elymore A, Maddison M (2009) Carotenoid and vitamin content of Micronesian atoll foods: Pandanus (*Pandanus tectorius*) and garlic pear (Crataevaspeciosa) fruit. J Food Compos Anal 22(1):1–8
Gallaher T, Callmander MW, Buerki S, Keeley SC (2015) A long distance dispersal hypothesis for the Pandanaceae and the origins of the Pandanus tectorius complex. Molecular phylogenetics and evolution. 83:20–32
Glas JJ, Schimmel BC, Alba JM, Escobar-Bravo R, Schuurink RC, Kant MR (2012) Plant glandular trichomes as targets for breeding or engineering of resistance to herbivores. Int J Mol Sci 13(12):17077–17103
Gowtham Raj G, Varghese HS, Kotagiri S, Swamy BM V (2014) Evaluation of anti-cancer potential of aqueous extract of *Pandanus odoratissimus* (Y. Kimura) Hatus. forma ferreus, by in vivo ascitic tumor model in swiss albino mice. Pharmacognosy J 6(1)
Hanif MA, Nisar S, Khan GS, Mushtaq Z, Zubair M (2019) Essential oils. In: Essential oil research. Springer, Cham, pp 3–17
Jagadev PN, Beura S, Maharana T (2001) Insect pests and diseases of Kewda (Pandanus fascicularis Lam.). Indian Perfumer 45(4):265–268
Jiang J (1999) Volatile composition of pandan leaves (*Pandanus amaryllifolius*). In: Flavor chemistry of ethnic foods. Springer, pp 105–109
Jitu KRM, Debnath D, Asad S, Das RC, Sultana A (2017) Phytochemical screening and evaluation of cytotoxic activity of *Pandanus fascicularis* L. (Fruits). Discov Phytomedicine 4(3):31
Jose B, Harikumar K, Krishnan PN, Satheeshkumar K (2016) In vitro mass multiplication of screw pines (Pandanus spp.)-an important costal bio-resource. J Coast Conserv 20(6):443–53
Kaewklom S, Vejaratpimol R (2011) In vitro protective effect of Pandanus ordoratissimus extract on ultraviolet B (UVB)-induced DNA damage. Afr J Biotechnol 10(45):9165–9169
Kamble VR, Kanoujiya LR, Rahate HL, Agre DG (2013) AM Fungal Status in Ketaka: Pandanus fascicularis From Coastal Region of Konkan, Maharahshtra. IOSR J Pharm Biol Sci 5(6):01–06
Kaur S, Panesar PS, Bera MB, Kaur V (2015) Simple sequence repeat markers in genetic divergence and marker-assisted selection of rice cultivars: a review. Crit Rev Food Sci Nutr 55(1):41–49
Kirtikar KR, Basu BD, Blatter E (1991) Indian Medicinal Plants, 2nd ed., vol 4. Indian Book Center, New Delhi, pp 2591–2593

Kuber BR, Santhrani T (2010) Assessment of Neuropharmacological Activities Of Pandanus Odoratissimus Root In Mice. J Pharm Chem 4: 114–117

Kumar D, Kumar S, Kumar S, Singh J, Sharma C, Aneja K (2010) Antimicrobial and preliminary phytochemical screening of crude leaf extract of *Pandanus odoratissimus* L. Pharmacol Online 2:600–610

Kumar S, Dagar S, Kumar P, Singh J, Kumar S, Kumar D (2017) Antifertility effect of hydroalcoholic extract of *Pandanus odoratissimus* L. leaves. Porto Biomed J 2(5):167–169

Kumari S, Wanjari M, Kumar P, Palani S (2012) Antidiabetic activity of *Pandanus fascicularis* Lamk-aerial roots in alloxan-induced hyperglycemic rats. Int J Nutr, Pharmacol, Neurol Dis 2(2):105

Laurentin H (2009) Data analysis for molecular characterization of plant genetic resources. Genet Resour Crop Evol 56(2):277–292

Lim TK (2012) Edible medicinal and non-medicinal plants, vol 1. Springer

Liu J, Peng L, Xian M, Cheng J-L, Chen W-W (2012) Supercritical carbon dioxide extraction of volatile oil from *Pandanus tectorius* Soland and analysis by GC-MS'J. Mod Chin Med 4:4–6

Londonkar R, Kamble A (2011) Hepatotoxic and invivo antioxidant potential of *Pandanus odoratissimus* against carbon tetrachloride induced liver injury in rats. Orient Pharm Exp Med 11(4):229–234

MacLeod AJ, Pieris NM (1982) Analysis of the volatile essential oils of Murrayakoenigii and *Pandanus latifolius*. Phytochemistry 21(7):1653–1657

Madhavan V, Nagar JC, Murali A, Mythreyi R, Yoganarasimhan S (2008) Antihyperglycemic activity of alcohol and aqueous extracts of *Pandanus fascicularis* lam. roots in alloxan induced diabetic rats. Pharmacologyonline 3:529–536

Mahlinda S, Husin H, Riza M, Muslim A (2017) A comparative study of biodiesel production from screw pine fruit seed: using ultrasound and microwave assistance in in-situ transesterification. J Eng Sci Technol 12(12):3412–3425

Manilal A, Sabu KR, Shewangizaw M, Aklilu A, Seid M, Merdekios B, Tsegaye B (2020) In vitro antibacterial activity of medicinal plants against biofilm-forming methicillin-resistant Staphylococcus aureus: efficacy of *Moringa stenopetala* and *Rosmarinus officinalis* extracts. Heliyon 6(1):e03303

Mar A, Mar AA, Thin PP, Zin MM (2019). Study on the phytochemical constituents in essential oil of pandanus amaryllifolious Roxb. Leaves and their Anti-bacterial Efficacy. Yadanabon Univ Res J 10:1

Mishra G, Khosa R, Singh P, Jha K (2015) Hepatoprotective potential of ethanolic extract of *Pandanus odoratissimus* root against paracetamol-induced hepatotoxicity in rats. J Pharm Bioallied Sci 7(1):45

Misra R, Dash P, Rao Y (2000) Chemical composition of the essential oils of Kewda and Ketaki. J Essent Oil Res 12(2):175–178

Mohanty SK, Swamy MK, Sinniah UR, Anuradha M (2017) Leptadenia reticulata (Retz.) Wight & Arn. (Jivanti): botanical, agronomical, phytochemical, pharmacological, and biotechnological aspects. Molecules 22(6):1019

Murtini E, Yuwono S, Setyawan H, Nadzifah N Pandan(2020) Leaf powder: characteristics and its application in Pandan sponge cake making. IOP Conf Ser: Earth Environ Sci 475:012041. https://doi.org/10.1088/1755-1315/475/1/012041

Nadaf A, Zanan R (2012) Economical importance of Indian Pandanus species. In: Indian Pandanaceae-an overview. Springer, pp 127–137

Nadaf A, Zanan R, Wakte K (2011) A new endemic species of Pandanaceae from India: *Pandanus palakkadensis*. Kew Bull 66(1):183–186

Nadeem MA, Nawaz MA, Shahid MQ, Doğan Y, Comertpay G, Yıldız M, Hatipoğlu R, Ahmad F, Alsaleh A, Labhane N (2018) DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. Biotechnol Biotechnol Equip 32(2):261–285

Naqvi AA, Mandal S (1996) Investigation of the essential oil of Pandanus fascicularis Lam. by GC/MS. J Essent Oil Res 8(5):571–572

Nasim N, Behera JK, Sandeep IS, Kar B, Ramarao V, Srivastava R, Nayak S, Mohanty S (2017a) Effect of harvesting time and storage on essential oil and PEME content of *Pandanus fascicularis*. J Appl Pharm Sci 7(10):185–189

Nasim N, Behera JK, Sandeep IS, RamaRao V, Kar B, Mishra A, Nayak S, Mohanty S (2018) Phytochemical analysis of flower from *Pandanus odorifer* (Forssk.) Kuntze for industrial application. Nat Prod Res 32(20):2494–2497

Nasim N, Ray A, Singh S, Jena S, Sahoo A, Kar B, Sandeep IS, Mohanty S, Nayak S (2017b) Characterization of Kewda volatile components by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry. Nat Prod Res 31(7):853–856

Nasim N, Sandeep IS, Sahoo A, Das S, Panda MK, Acharya L, RamaRao V, Nayak S, Mohanty S (2020) Population genetic structure and diversity analysis in economically important *Pandanus odorifer* (Forssk.) Kuntze accessions employing ISSR and SSR markers. Ind Crops Prod 143:111894

Omondi EO, Debener T, Linde M, Abukutsa-Onyango M, Dinssa FF, Winkelmann T (2016) Molecular markers for genetic diversity studies in African leafy vegetables. Advan Biosci Biotechnol 7(3):188–197

Padhy S, Dash SK, Panda BB, Misra MK, Padhi NP, Sahu D, Das M, Nayak SP, Dash B, Rao VR (2016) *Pandanus fasicularis* Lamk. (Kewda): the prime vegetation in the hinterland biodiversity of coastal Odisha, with unique ethnic utility, genetic variation and economics-a review. J Biodivers 7(1):33–49

Panda KK, Das AB, Panda BB (2009) Use and variation of *Pandanus tectorius* Parkinson (P. fascicularis Lam.) along the coastline of Orissa, India. Genet Resour Crop Evol 56(5):629–637

Panda KK, Panigrahy RK, Das AB (2007) Analyses of chromosome number, nuclear DNA content and RAPD profile in three morphotypes. Plant Genet Resour Newsl 149–152:15212

Panda KK, Sahoo B, Das AB, Panda BB (2010a) Use of RAPD markers to detect sex differences in *Pandanus tectorius* Parkinson, an important bioresource plant in Orissa, India. Int J Biodivers Sci, Ecosyst Serv Manage 6(1–2):28–34

Panda P, Nayak S, Panda DP (2010b) Formulation and evaluation of cream prepared form *Pandanus fascicularis* Lamk. and their wound healing activity. Drug Invention Today 2(9)

Panda P, Panda D, Panda P, Nayak S (2008) Antinociceptive and anti-inflammatory activities of *Pandanus fascicularis* Lamk. leaves in animal models. Orient Pharm Exp Med 7(5):485–493

Panda K, Das AB, Panda BB (2012). Genomics of Pandanus: A useful plant resource of coastal Odisha, India. Lambert Academic Publishing

Penu F, Ivy S, Ahmed F, Uddin J, Hossain M, Labu Z (2020) In vitro assessment of antioxidant, thrombolytic, antimicrobial activities of medicinal plant *Pandanus odoratissimus* L. leaves extract. J Sci Res 12(3):379–390

Qu J, Hao Y, Zhu Y, Wu C, Wang W, Leng P (2011) Studies on germplasm of Juglans by EST-SSR markers. Acta Hortic Sinica 38(3):441–448

Rahayu SE, Handayani S, Noverita ISJ (2013) Antifungal and preliminary phytochemical screening of leaf extract of *Pandanus odoratissimus* L.f. Proceeding international conference pp 100–105

Rahman MK, Islam MF, Barua S, Rahman MM, Sayeed MA (2014) Comparative study of antidiarrheal activity of methanol extracts from leaf and fruit of *Pandanus odoratissimus* Linn. Orient Pharm Exp Med 14(4):363–367

Raina V, Kumar A, Srivastava S, Syamsundar K, Kahol A (2004) Essential oil composition of 'kewda' (*Pandanus odoratissimus*) from India. Flavour Fragr J 19(5):434–436

Rajeswari J, Kesavan K, Jayakar B (2011) Phytochemical and pharmacological evaluation of prop roots of *Pandanus fascicularis* Lam. Asian Pac J Trop Med 4(8):649–653

Rajeswari J, Kesavan K, Jayakar B (2012) Antidiabetic activity and chemical characterization of aqueous/ethanol prop roots extracts of *Pandanus fascicularis* Lam. in streptozotocin-induced diabetic rats. Asian Pac J Trop Biomed 2(1):S170–S174

Raju S, Subbaiah NV, Reddy KS, Das A, Murugan KB (2011) Potential of *Pandanus odoratissimus* as a CNS depressant in Swiss albino mice. Braz J Pharm Sci 47(3):629–634

Rao VR, Hodgkin T (2002) Genetic diversity and conservation and utilization of plant genetic resources. Plant Cell, Tissue Organ Cult 68(1):1–19

Rashmi D, Barvkar VT, Nadaf A, Mundhe S, Kadoo NY (2019) Integrative omics analysis in *Pandanus odorifer* (Forssk.) Kuntze reveals the role of Asparagine synthetase in salinity tolerance. Sci Rep 9(1):1–16

Rashmi D, Nadaf A (2017) Understanding the mechanism of salt tolerance in pandanus odorifer L. P Natl A Sci India B. 1–7

Rohman A, Sugeng R, Man YC (2012) Characterizaton of red fruit (*Pandanus conoideus* Lam) oil. Int Food Res J 19(2):563

Rout P, Misra R, Sahoo S, Sree A, Rao Y (2005) Extraction of kewda (*Pandanus fascicularis* Lam.) flowers with hexane: composition of concrete, absolute and wax. Flavour Fragrance J 20 (4):442–444

Rout PK, Naik S, Rao YR (2011) Liquid co_2 extraction of flowers of *Pandanus fascicularis* Lam. and fractionation of floral concrete and comparative composition of the extracts. J Food Biochem 35(2):500–512

Rout PK, Rao YR, Prakash O, Khare P (2015) Adsorptive recovery of high value essential oil from kewda (*Pandanus fascicularis* Lam.) distillation condensate. Asia-Pac J Chem Eng 10(5):659–669

Sarile AS, Menguito CA (2010) Molecular differentiation of selected Pandanus spp. by Random amplified polymorphic DNA (RAPD) analysis. Acta Manilana 55:59–68

Sarwat M, Nabi G, Das S, Srivastava PS (2012) Molecular markers in medicinal plant biotechnology: past and present. Crit Rev Biotechnol 32(1):74–92

Shah UN, Mir J, Ahmed N, Fazili KM (2018) Assessment of germplasm diversity and genetic relationships among walnut (*Juglans regia* L.) genotypes through microsatellite markers. J Saudi Soc Agric Sci 17(4):339–350

Sobhana K, Nirosha K, Ravikumar A, Sravanthi R, Chandrkala P (2014) Evaluation of cardio-protective activity of *Pandanus odoratissimus* leaves against isoproterenol induced myocardial infarction in albino rats. Int J Novel Trends Pharm Sci 4(5):151–158

Solomon Raju A, Lakshminarayana G (2020) In: Prasada Rao C., Dileepu Kumar B, Santhi Kumari M, Prasad KBJ, Divyasree M, Suneetha Rani T. Pollination and fruit dispersal in the Fragrant Screw Pine, *Pandanus odorifer* (Forssk.) Kuntze (Pandanaceae), vol 21. Species

Son NT (2019) Secondary metabolites of genus *Pandanus*: an aspect of phytochemistry. Mini-Rev Org Chem 16(7):689–710

St John H (1960) Revision of the genus *Pandanus* stickman: part 1. Key to the sections

Stringaro A, Colone M, Angiolella L (2018) Antioxidant, antifungal, antibiofilm, and cytotoxic activities of Mentha spp. essential oils. Medicines 5(4):112

Susanti R, Suzuki E, Miyamoto J, Abe M, Uchiumi T (2012) Differences in the growth of beach Pandan, Pandanus odoratissimus, between tropical (Java) and subtropical (southern Japan) zones. Tropics 21(3):81–90

Swamy MK, Sinniah UR (2016) Patchouli (PogostemoncablinBenth.): botany, agrotechnology and biotechnological aspects. Ind Crops Prod 87:161–176

Takeda J, De Silva S, Muthuraman P, Rahman SM, Kawet L (2008) Spices in Sri Lanka, India and Bangladesh with special reference to the usages and consumptions. Bull Facul Agric (Saga Univ.) 93:1–25

Tanaka N, Jinadasa KB, Mowjood MI, Fasly MS (2011) Coastal vegetation planting projects for tsunami disaster mitigation: effectiveness evaluation of new establishments. Landscape Ecol Eng 7(1):127–135

Teli M, Jadhav A (2015) Mechanical extraction and physical characterization of *Pandanus Odorifer* Lignocellulosic fibre. Int J Sci Res (IJSR) ISSN 6

Teli M, Jadhav A (2017) Effect of mercerization on the properties of *Pandanus odorifer* lignocellulosic fibre. IOSR J Polym Text Eng (IOSR-JPTE) 4:7–15

Thuy NB, Nandasena NA, Dang VH, Tanaka N (2018) Simplified formulae for designing coastal forest against tsunami run-up: one-dimensional approach. Nat hazards 92(1):327–46

Tibpromma S, Daranagama DA, Boonmee S, Promputtha I, Nontachaiyapoom S, Hyde KD (2017) Anthostomelloideskrabiensis gen. et sp. nov.(Xylariaceae) from *Pandanus odorifer* (Pandanaceae). Turkish J Bot 41(1):107–116

Udupa AL, Ojeh N, Gupta S, Rathnakar UP, Rajput R, Shubha HV, Benegal D, Benegal A, Rao S, Rao S (2011) Analgesic activity of Pandanus fascicularis Lam. J Pharm Res 4(4):1234–1236

Vahirua-Lechat I, Menut C, Roig B, Bessiere J, Lamaty G (1996) Isoprene related esters, significant components of *Pandanus tectorius*. Phytochemistry 43(6):1277–1279

Vinod M, Raghavan PS, George S, Parida A (2007) Identification of a sex-specific SCAR marker in dioecious *Pandanus fascicularis* L. (Pandanaceae). Genome 50(9):834–839

Vinod M, Sankararamasubramanian H, Priyanka R, Ganesan G, Parida A (2010) Gene expression analysis of volatile-rich male flowers of dioecious *Pandanus fascicularis* using expressed sequence tags. J Plant Physiol 167(11):914–919

Wakte KV, Nadaf AB, Thengane RJ, Jawali N (2009) *Pandanus amaryllifolius* Roxb. cultivated as a spice in coastal regions of India. Genet Resour Crop Evol 56(5):735–740

Wakte KV, Zanan RL, Saini A, Jawali N, Thengane RJ, Nadaf AB (2012) Genetic diversity assessment in *Pandanus amaryllifolius* Roxb. populations of India. Genet Resour Crop Evol 59(7):1583–1595

Yamazaki M, Rai A, Yoshimoto N, Saito K (2018) Perspective: functional genomics towards new biotechnology in medicinal plants. Plant Biotechnol Rep 12(2):69–75

Zanan RL, Nadaf AB (2011) Collection, characterization and olfactory evaluation of Pandanus species in Southern India. Plant Genet Resour 1–3

Zanan RL, Nadaf AB (2012a) *Pandanus mangalorensis*: a new species of Pandanaceae from Southern India. Kew Bull 67(3):555–559

Zanan RL, Nadaf AB (2012b) *Pandanus martinianus* (Pandanaceae), a new endemic species from northeastern India. Phytotaxa 73(1):1–7

Zanan RL, Nadaf AB (2013) Conservation status of Indian pandanaceae. Am J Plant Sci 4(6A):51–56. https://doi.org/10.4236/ajps.2013.46A008

Zou Z, Gui Z, Yu L, Chen F, Xia B (2011) Genetic diversity within and among populations of Acarus siro L. revealed by inter simple sequence repeats (ISSR). Procedia Environ Sci 8:707–714

Part II
Biotechnology in Medicinal Plants

Chapter 16
Medicinal Plant Research at Crossroads: Biotechnological Approaches for Conservation, Production and Stability in Tissue Cultures and Regenerated Plants

Mihir Halder, Anrini Majumder, Smita Ray, and Sumita Jha

Abstract Medicinal plants are treasures of nature with almost never-ending resource of unlimited, diverse, complex and valuable natural compounds with a variety of pharmacological properties. They are used worldwide by all human beings to maintain and restore good health since ancient times. The cumulative effect of heterogeneous distribution and availability throughout the world, anthropogenic interferences like habitat destruction, overexploitation due to increasing demand, unsustainable harvesting practices from the wild, introduction of exotic species, climatic changes, lack of knowledge about medicinal properties and domestication, less agronomical preference in comparison with food crops, weakening of biodiversity protection laws and/or tendency to undermine these laws by modern socio-economic forces are responsible for the loss of medicinal plant biodiversity. Classical plant tissue culture (PTC)-based biotechnological approaches have been efficiently utilized as sustainable platforms for the conservation of endangered, disease-prone and recalcitrant medicinal plant species via in vitro micropropagation and in vitro production of pharmaceutically important bio-active phytochemicals in different transformed and/or non-transformed cells, organs or regenerated plants that effectively decrease harvesting pressure from wild populations of medicinal plants. Advancement in genomics, transcriptomics, proteomics, nanoscience and synthetic biology in recent times have started to revolutionize medicinal plant research in many dimensions where PTC techniques play very important role for the implementation of these

M. Halder
Department of Botany, Barasat Government College, 10 K.N.C. Road, Kolkata, Barasat, West Bengal 700124, India

A. Majumder
Department of Botany, Harimohan Ghose College, J-206 & 208A, Paharpur Road, Kolkata, West Bengal 700024, India

S. Ray
Department of Botany, Bethune College, 181, Bidhan Sarani, Kolkata, West Bengal 700006, India

S. Jha (✉)
Centre of Advanced Study, Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata, West Bengal 700019, India

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_16

modern fields of science by providing constant aseptic control culture condition and better scope to utilize totipotency in comparison with wild cultivation. Hairy root culture, ploidy engineering, metabolic pathway engineering, transgenic plant development, large-scale production in bioreactors, application of Zinc-finger nucleases, TALENs and CRIPR/Cas9 are promising new biotechnological approaches. In spite of various applications and advantages of the PTC techniques, the broader commercial utilization of in vitro techniques as an important tool for the germplasm conservation of threatened commercially important medicinal plant species via mass proliferation and industrial production of SMs can be challenged by genetic, phenotypic and phytochemical instability often developed by somaclonal variations in long-term in vitro culture. The present paper reviews the recent (from 2010 upto June 2020) achievements in PTC research for conservation of medicinal plants, production of important SMs, and the stability of in vitro cultures along with case studies of two endangered species, *Podophyllum hexandrum* and *Rauvolfia serpentina*. There are immense opportunities to produce pharmaceutical important SMs with minimal threat to the biodiversity by the application of suitable conventional and upcoming PTC techniques along with screening for stability.

Keywords Genetic stability · Micropropagation · Podophyllotoxin · *Rauvolfia serpentina* · Secondary metabolite

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
ABA	Abscisic acid
AFLP	Amplified fragment length polymorphism
AgNP	Ag-SiO_2 core–shell nanoparticle
ARC	Adventitious root culture
BAP	6-Benzylaminopurine
BCR	Bubble column reactor
BTAB	Balloon-type airlift bioreactor
BTBB	Balloon-type bubble (air-lift) bioreactor
CAD	Cinnamyl alcohol dehydrogenase
CAOMT	Caffeic acid-O-methyltransferase
CCOMT	Caffeoyl CoA-O-methyltransferase
cDNA	Complementary DNA
CRISPR	Clustered regularly interspaced short palindromic repeats
Crtdc	Tryptophan decarboxylase gene isolated from *Catharanthus roseus*
CSC	Cell suspension culture
CuONP	Copper oxide nanoparticle
DMSO	Dimethylsulfoxide
DTAB	Drum type airlift bioreactor
DW	Dry weight

EC	Enzyme Commission
EST-SSRs	Expressed sequence tag-derived simple sequence repeat markers
FeNP	Iron oxide nanoparticle
g	Gram
GA_3	Gibberellic acid
GC-MS	Gas chromatography mass spectrometry
HPLC	High performance liquid chromatography
HRC	Hairy root culture
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
ISSR	Inter simple sequence repeat
Kn	Kinetin
l	Liter
MeJA	Methyl jasmonic acid
Mg	Miligram
MIA	Monoterpene indole alkaloid
MP	Medicinal plant
MS medium	Murashige and Skoog medium
NAA	1-Naphthaleneacetic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
NSB	Nutrient sprinkle bioreactor
PGRs	Plant growth regulators
PSC	Protocorm suspension culture
PTC	Plant tissue culture
PVP	Polyvinylpyrrolidone
RAPD	Random amplified polymorphic DNA
RNA	Ribonucleic acid
RNAi	RNA interference
ROS	Reactive oxygen species
SA	Salicylic acid
SC	Shoot culture
SCoT	Start codon targeted
SE	Somatic embryo
SEC	Somatic embryo culture
SM	Secondary metabolite
SOD	Superoxide dismutases
SPAR	Single primer amplification reaction
SSR	Simple sequence repeat
STB	Stirred tank bioreactor
TA	Tropane alkaloid
TALENs	Transcription activator-like effector nucleases
TDC	Tryptophan decarboxylase
T-DNA	Transfer DNA
TIA	Terpenoid indole alkaloid

WPM	Woody plant medium
ZFNs	Zinc-finger nucleases
ZnONP	Zinc oxide nanoparticle

16.1 Introduction

Plants are treasures of nature that act as perpetual sources of diverse groups of compounds. Plants with health-promoting constituents are commonly termed as medicinal plants (MPs), which have been used worldwide by all human beings for their medicinal power to maintain and restore good health since ancient times. The identification of MPs, their bioactive constituents and the source organs/parts were undoubtedly more difficult due to the limited knowledge about the bioactive principles and lack of sophisticated instruments. The gradual progression in science and technology, integrated approach of appropriate analytical techniques, improvement in in vitro and in vivo bioassay models, the study of the molecular targets and networking of the bioactive molecules have led to the isolation and identification of several new MPs and their active constituents with improved healthcare efficacy for the treatment of different health problems.

The overwhelming phyto-diversity is used as a valuable, almost never-ending resource of unlimited novel, diverse and complex natural compounds with a variety of pharmacological properties that offer compound scaffolds in the pharmaceutical industry. It is estimated that 70–80% of the world population relies mainly on traditional and herbal medicine for their primary healthcare needs. Recently, World Health Organization (WHO) estimated that 80% of people in developing countries such as India depend on plant-derived medicines, while more than 25% of the total prescribed drugs used in developed countries such as the USA are also plant-derived medicines (Chen et al. 2016). According to the recent document published from Kew Royal Botanic Garden, out of 28,187 plant species with the potential for medicinal use, only 4478 species are cited in a medicinal regulatory publication (Willis 2017). This clearly indicates the huge opportunity in MP research as many more currently unanalyzed medicinally valuable plant-derived natural products are awaiting to be discovered.

The pharmacological properties of MPs are governed by their bioactive principles which are a wide variety of low molecular weight secondary metabolites (SMs), synthesized from divergent metabolic pathways as a by-product of primary metabolites, mostly in tissue or organ or at developmental stage-specific manner in response to various forms of biotic and abiotic stress in a limited plant species (Halder and Jha 2020; Halder et al. 2019). Plant SMs are a fascinating class of phytochemicals with diverse and complex structures that include alkaloids, terpenoids, flavonoids, quinones, coumarins, glycosides, polyketides, volatile oils, resins, tannins, glucosinolates, cyanogenic glycosides, etc., which provide survival, adaptive and competitive benefits to the plants against different biotic and abiotic factors encountered by

them (Halder and Jha 2020). They also represent highly economically valuable plant-based natural products used as drugs, cosmetics, dietary supplements, fragrances, flavours, dye, etc. (Chandran et al. 2020).

The demand for herbal medicine is gaining more attention globally year by year for the treatment of a wide variety of human ailments mainly due to human population explosion, considered very safe as there is no or minimal side effect compared to several synthetic drugs, their broad spectrum of activity, age and sex independent effect, frequent inadequate availability of synthetic drugs in developing countries, the excessive cost of treatments and development of resistance to currently used drugs for infectious diseases. Additionally, public interest in the preventive medicine and integrated medicine in recent times significantly increased the demand of MPs and natural drug therapy. Moreover, the herbal medicine industry is one of the quickest emerging industries worldwide and it is anticipated that the world trade in MPs and their metabolites will touch US$ 5 trillion in 2050 from US$ 60 billion in the year 2000 with an average yearly growth rate of 7% (Government of India 2000).

The conventional method of commercial production of bioactive, plant-derived SMs relies on the extraction of the metabolite from naturally grown MPs after harvesting from wild habitat. But commercial production of these bioactive SMs from natural population is limited due to regional or global constraint of plant material availability, slow growth and multiplication rates, tissue- or organ-specific metabolite production, low productivity, geographical and seasonal variation in content, purification difficulties, application of chemicals to combat with biotic stress, comparatively large production cycles, economic cost involved in implementation of appropriate high-throughput screening bioassays and continuous reduction in land availability. Additionally, overexploitation, insufficient attempts for replenishment after procurement and limited cultivation resulted in gradual depletion of the wild population of many MPs.

There is an enormous discrepancy between demands of consumers for plant-derived medicines and phytochemicals, and the availability of MPs from natural habitat due to gradual increase in demand against the available synthetic medicine and different problems associated with conventional natural supply. De novo chemical synthesis of these complex SMs is often both arduous and commercially expensive due to stereo-specificity (Nandagopal et al. 2018). There has been an impetus to develop viable, biotechnological methods of production of SMs (Nandagopal et al. 2018). In this context, plant tissue culture (PTC) techniques serve as eco-friendly, potential alternatives that minimize these limitations and offer a viable platform for the rapid mass multiplication and medicinally important SM production. Culture of plant protoplasts, cells, tissues, organs or complete plantlets in in vitro aseptic condition on or in certain artificial culture media under defined, controlled physical conditions like light, temperature and humidity is broadly called PTC. In vitro PTC provides an aseptic, stable and controlled culture condition that is always preferred to determine the relationship among different experimental conditions and plant materials with SMs productivity. Additionally, it is used as an alternative approach for rapid proliferation of rare, endangered and threatened MP species.

It is believed that the journey of PTC started through the introduction of the totipotency concept by Gottlieb Haberlandt in 1902, followed by organogenesis in tobacco pith, morphogenesis in carrot phloem cells, micropropagation of *Cymbidium* using meristem culture, development of germplasm conservation method, development of regeneration and multiplication protocols of various plants, conservation of phytodiversity and production of high-value bioactive metabolites from MPs. Nowadays, transfer of the gene of interest, ploidy and metabolic engineering and production of transgenic culture or plant have been made possible through the integration of PTC techniques and recombinant DNA technology.

In recent times, the trend of PTC-based research on MPs has been shifted from micropropagation and ex-situ conservation by organogenesis, morphogenesis and regeneration to commercial production of various pharmaceutically demanded phytochemicals by the implementation of different strategies like optimization of culture medium and conditions, hairy root culture (HRC), metabolite engineering, polyploidy induction and large-scale production in bioreactors. Publication data shown in Fig. 16.1 clearly shows the gradual increase in PTC-based research in last few years due to immense application potentiality of modern PTC for plant improvement and conservation.

Whole genome projects in plants have significantly risen due to plummeting of sequencing cost and the data of assembled draft whole genome sequence of 225 plants available online in early 2017 (Willis 2017). Nowadays, modern PTC approaches are not only confined to the conservation of MPs and enhancement of the productivity of their active SMs, but also exploit the genome of MPs and synthetic biology strategies for the production of engineered molecules and/or custom-designed MPs (Espinosa-Leal et al. 2018).

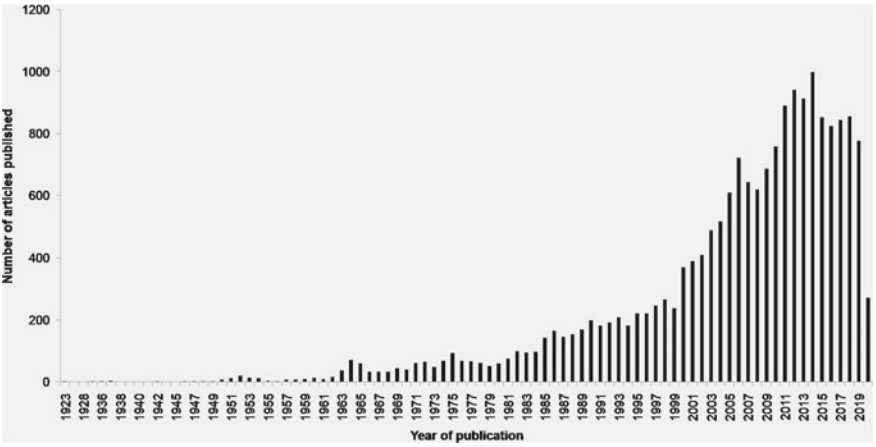


Fig. 16.1 Graphical representation of year-wise research publication data from 1923–June 2020 based on data search result in PubMed database with keyword "Plant tissue culture"

Although the different domains of PTC-based MP research and their potential applications are very vast, PTC has been mostly exploited as a major platform for in vitro conservation of MPs and to find strategies to enhance the production of SMs within a short span of time, without disturbing their natural populations to fulfil the increasing demand for such compounds. In this context, biochemical and genetic stability of in vitro cultures (transformed or non-transformed cells, tissues, organs, whole plants) are also very important in both conservation and SM production in long-term cultures. Thus, the present review paper intends to assess the recent (2010–June 2020) achievements in PTC research for conservation of MPs and production of medicinally important SMs as well as their class, distribution in plant families and important medicinal uses with special emphasis on their stability. It also includes case studies in two species, *Podophyllum hexandrum* and *Rauvolfia serpentine,* reviewing important achievements over several decades of research.

16.2 Problems Associated with Improvement of Medicinal Plants

16.2.1 Availability of Resources

Climatic variation throughout the earth and habitat-specific growth of MPs has led to heterogeneous distribution and availability in all the parts of the world. Many plant species are naturally and exclusively restricted to a specific small geographic area, a province, a nation or a continent and known as endemic plants. These region specific, unique MPs are usually more vulnerable to anthropogenic threats and/or natural changes due to their restricted distribution, one or few populations, small population size, declining population size, excessive collection by humans, short reproduction capacity, specific habitat conditions and necessity of stable and constant environments (Coelho et al. 2020; Fig. 16.2). Hence, endemic species should be carefully monitored and managed, and their conservation is considered a global priority (Coelho et al. 2020).

16.2.2 Loss of Biodiversity of Medicinal Plants

In the recent past, biodiversity of MPs at the level of genes, species and ecosystems encountered enormous pressure throughout this planet due to habitat destruction, rapid increase in human population, rapid urbanization and overexploitation caused by increasing demand coupled with expanding international trade and unsustainable harvesting practices from the wild (Fig. 16.2). Additionally, climate changes, plant

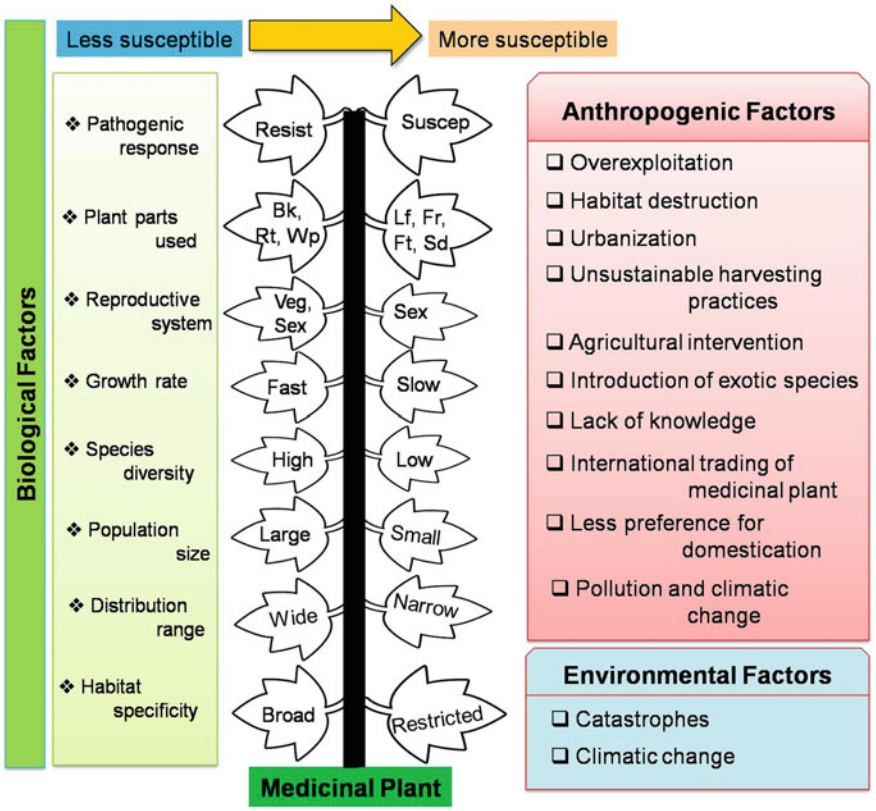


Fig. 16.2 Major factors that are responsible for loss of biodiversity in medicinal plants (Bk = Bark, Fr = Flower, Ft = Fruit, Lf = Leaf, Resist = Resistance, Rt = Root, Sd = Seed, Sex = Sexual, Suscep = Susceptible Veg = Vegetative, Wp = Whole plant)

diseases, the introduction of exotic species, lack of knowledge of medicinal properties and domestication and agronomical preference of other food crops often create serious threats to many wild populations of economically valuable MPs (Fig. 16.2).

It has been roughly estimated that 20% of wild resources of MPs have already been nearly exhausted with the increase in human population and plant consumption. A highly conservative estimate states that the present loss of plant species is between 100 and 1000 times higher than the expected natural extinction rate that leads to loss of at least one potential major drug every 2 years (Chen et al. 2016). Unfortunately, no reliable estimation of globally threatened MPs is available, only some of the MPs that suffer from genetic erosion and resource destruction have been listed as threatened (Chen et al. 2016). Thus, conservation of biodiversity is an urgent necessity to ensure the sustainability and availability of the variety of MP germplasm that provide raw materials for industries and explore the possibility of future development.

16.2.3 Decline in Local Knowledge and Cultural Survival

Since ancient times, MPs are an integral component of the life of tribal communities as these plants support their need for medicine, livelihood security and financial income. They also use these naturally occurring medicinal resources as ethnoveterinary medicine for the maintenance and conservation of the health care of livestock. Apart from the economic importance of conserving biodiversity, several ethnobotanical, cultural, moral and ethical aspects that are associated with the sanctity of all forms of life, also promote conservation. These local communities continually played a pivotal role in the conservation of nature and genetic resources of MPs either through in-situ conservation and/or domestication over several generations. In India, a large number of sacred groves around ancient sacred sites and temples have been preserved by tribal people in several states and these sites act as gene bank of wild plants.

Interestingly, important indigenous knowledge of MPs and their potential applications related to improving health and life privilege associated with these folklore communities have been conserved and transmitted from generation to generation in an undocumented form. These regional cultures and communities are also facing different challenges due to civilization, privatization or nationalization of land, and many of them may have already become extinct or are in the verge of extinction in the near future. Documentation of this knowledge about the MPs, their bioactivity and conservation of those tribal groups are also required.

16.2.4 Public Awareness and Need for Further Research Medicinal Plant

A mass extinction of species is occurring currently worldwide, and a significant number of the plants is diminishing at an alarming rate, where anthropogenic reasons being the most dominant weapon that destroying the wild population of MPs. Public awareness and participation are necessary to address the issue, and the conservation and sustainable use of these natural resources is critical to save the diversity of MPs in nature. Involvement of local communities is an important strategy to make conservation sustainable. Effective conservation strategies for MPs should thus take place within four main areas: in-situ conservation, ex-situ conservation, education and research (Sharma and Pandey 2013).

Mother earth has an enormous diversity of plants, and plant cells are the reservoirs of diverse phytochemicals. Only a few diamonds have been collected from the huge pool of bioactive compounds in the plants and a major portion is still not exploited by humans. Recent public awareness towards natural products and advancement in extraction method, purification procedure, detection technique, genomics, transcriptomics, proteomics and metabolomics significantly accelerate research of MPs that can be predicted by the published articles (Fig. 16.1). In the present scenario, the

major concerning areas related to the MP research are the plant identification, their bioactive components, development of sustainable production system through in-situ or ex-situ conservation or through other biotechnological approaches and new product discovery. Availability of the plants, impurity, seasonal variation, tissue or organ-specific production, production restricted to selected taxa, low amount, structural diversity and complexity and lack of universal extraction methods are the major problems in MP research.

16.3 Conservation of Medicinal Plants

Nowadays, despite the economical and pharmaceutical demand of MP-derived SMs, indiscriminate use, excessive harvesting, continuous increase of human populations and per capita consumption, deforestation, industrialization, fires, development of land for agriculture, environmental damage and environmental changes create tremendous pressure on the wild population of MPs leading to the extinction and rapid reduction in their indigenous lands, which have put natural populations at risk in future (Fig. 16.2). Besides these anthropogenic factors, the knowledge about multiple biological characters that are correlated with extinction risk of a specific MP is also essential for their sustainable growth in natural habitats. Environmental changes and natural calamities such as floods, fires have also caused loss of biodiversity. The cumulative effect of the above-mentioned factors along with the weakening of customary laws and/or tendency to undermine these laws by modern socio-economic forces is the main causative reasons for the loss of biodiversity throughout the world. According to the IUCN (International Union for Conservation of Nature and Natural Resources) Red List of threatened species, the number of threatened species increases dramatically every year and recent report showed 15,774 threatened plant species out of 38,630 evaluated species, whereas the total number of plant species described is 422,683 (IUCN 2019).

The status of biodiversity of earth greatly relies on the balance between loss of biodiversity and conservation (Fig. 16.3). Both in-situ and ex-situ conservation methods are very effective in biodiversity conservation of plants. These scientific conservation approaches have been implemented or should be implemented globally to prevent the loss of MP biodiversity at the present alarming situation. Protection, preservation, maintenance, conservation and sustainable utilization through scientific conservation could be applied to prevent loss of biodiversity. The in-situ conservation of MPs may be done in naturally occurring forests and non-forest areas such as traditional farming sites and wild nurseries, by clearly demarcating them as “Medicinal Plant Conservation Reserves”, while ex-situ conservation may be done outside natural habitats by cultivating and maintaining plants in botanical gardens, nurseries, other types of gardens (garden of schools, museums and other institutions and personal gardens) or through long-term preservation of seeds in seed banks and long-term preservation of plant propagules in tissue culture repositories. Besides these, identification of new MPs from existing biodiversity or more effective compounds

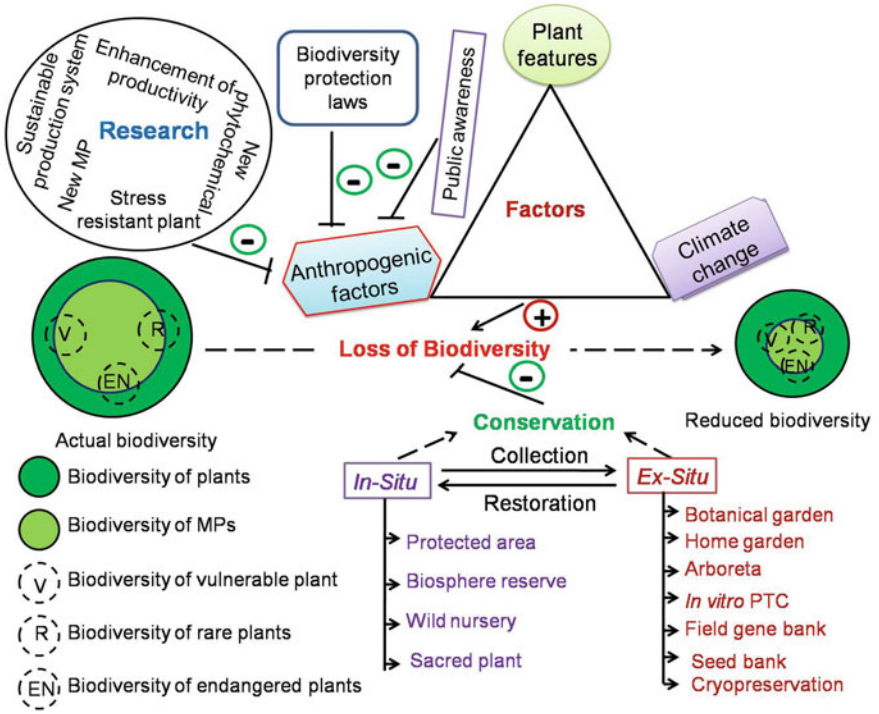


Fig. 16.3 Schematic representation of the relationship between different positive and negative factors that control medicinal plants biodiversity along with types of conservation

from well-known MPs can stabilize biodiversity by reducing exploitation pressure (Fig. 16.3).

In-situ conservation of MPs is recommended for the sustainable conservation and management of MP as this approach is cost-effective, feasible and do not require special skills or technology as species survive and perpetuate in their own niche or microclimate available in the natural habitat. It may be often challenged by the requirement of robust management systems and gradual increase pressures on land cause disappearance of large wild areas. Additionally, safeguarding via in-situ conservation is not always sufficient to protect valuable MPs in their natural ecological niche. Ex-situ conservation techniques are very important for complementation of in-situ approaches that protect MP populations from the danger of destruction, replacement or deterioration at outside the native habitat, but preferably within the natural areas of distribution by acting as a backup of plant germplasm which might be lost from their wild habitats. In some instances, ex-situ conservation may be the only option for many species which survive and perpetuate optimally only in their own niche or the microclimate.

Although cultivation and maintenance of selected MPs in botanical gardens, arboreta, nurseries, etc., directly strengthen in-situ conservation, generally such sites

have limited numbers of specimens of each species and they are poorly represented in comparison with wild populations. In spite of these, botanic gardens can play major roles in MP conservation through developing propagation and cultivation protocols and undertaking programmes of domestication and variety breeding by the acquisition, implementation and validation of traditional knowledge. Seed banks are quite popular and attractive ex-situ conservation methods of storing the genetic diversity of many plants far distant from their natural habitats under the supervision of the specialist scientists. However, representation of seeds of MPs in seed banks is not always satisfactory.

In this alarming situation, the development and advancement of different biotechnological approaches are promising alternatives for conservation of commercially important, rare, endangered and threatened MP species via rapid mass propagation and germplasm conservation as well as for large-scale production of desirable bioactive phytochemicals and genetic improvement of the MPs. These include different in vitro PTC methods such as micropropagation, somatic embryogenesis, culture of regenerated plants and genetic transformation. During tissue culture and the domestication process of MPs, medicinal efficacy should not be compromised. Conventional, common PTC methods for germplasm conservation and production of bioactive phytochemicals along with route of discovery of new bioactive compound have been shown in Fig. 16.4.

16.4 Plant Tissue Culture for Conservation of Medicinal Plants

PTC is a quick and efficient in vitro technique for rapid growth and proliferation of cells, tissues and organs of plants on artificial solid or liquid media under the aseptic condition that has opened extensive areas of research for the conservation of elite germplasms for virtually unlimited time span in limited space by exploring totipotency of plant cell. In vitro PTC approaches for the conservation of plant biodiversity and their utilization are of great interest as they offer several advantages such as season and developmental condition independent collection, rapid multiplication using micropropagation, no geographical and climatic barrier for in vitro propagation and maintenance, production and maintenance of clones of desired elite genotypes, production of disease-free plants, storage of plant germplasm and the scope of genetic manipulation. PTC protocols are very useful for in vitro conservation of various endangered, rare and threatened plant species with slow growth, low abundance, recalcitrant seeds and high susceptibility to replanting diseases. De novo organ development is possible in in vitro culture from mature differentiated cells via direct or indirect organogenesis or somatic embryogenesis by modifying the concentration of exogenous plant growth regulators (PGRs) in the culture medium.

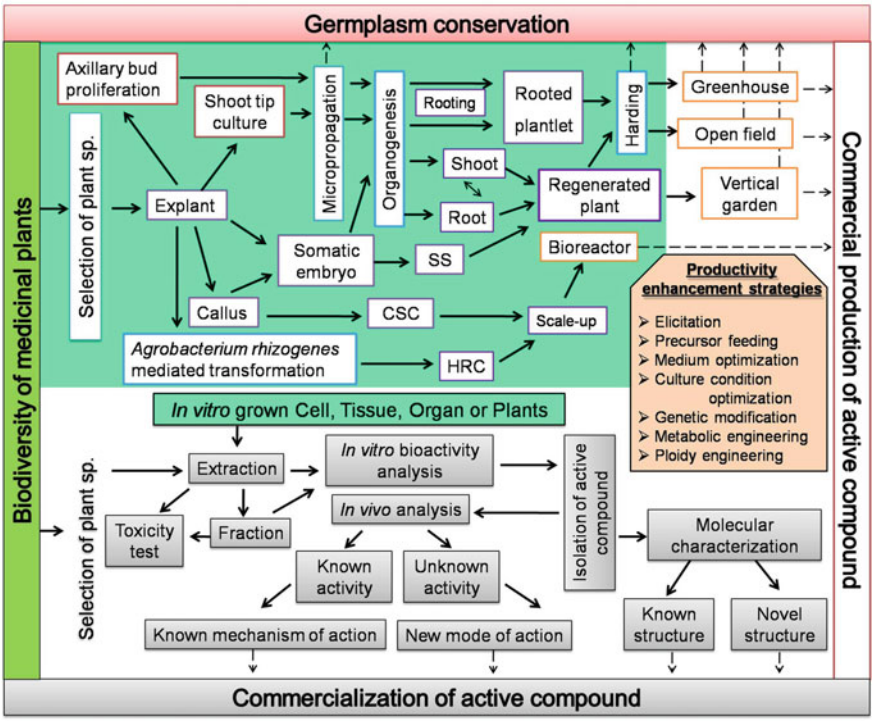


Fig. 16.4 Schematic representation of possible routes of germplasm conservation, commercial production of SMs and discovery of new phytochemical with bioactivity through the application of different in vitro plant tissue culture-based techniques (CSC = cell suspension culture, HRC= hairy root culture, SS = synthetic seed)

16.4.1 Micropropagation of Medicinal Plants and in vitro Conservation

Micropropagation is a very effective and useful direct application of PTC with a strong potential to conserve endangered, disease-prone and recalcitrant species by rapid multiplication of these plants within a comparatively short time period and limited space. It is concerned with the production of numerous disease-free genetically identical plants for medicinal, conservation, reforestation and commercial purposes. The technique of micropropagation is usually divided into several stages, namely pre-propagation, initiation of explants, subculture of explants for proliferation, shooting, rooting and hardening, which are universally utilized for the mass-scale production of MPs. Recently, the application of computational methods such as artificial neural network and image processing helps researchers to better understand the complex relationship among in vitro micropropagation and different multi-variable factors such as the selection of plant genotype; type, size and age of explants; type and concentration of PGRs; composition and pH of the medium; source

of carbohydrate; gelling agent and culture conditions (temperature, light intensity and photoperiod) in a holistic manner (Niazian 2019; Ray et al. 2020).

In vitro rapid proliferation has been achieved successfully in an array of MPs through micropropagation using nodes, internodes, shoot tips, axillary buds as explants in different tissue culture media and aseptic controlled micro-environmental conditions (Table 16.1). Micropropagated plants are usually with similar genetic characters with respect to plant growth and yield of the target molecule. Genetic homogeneity of micropropagated plants ensures product uniformity which is the primary requirement for commercial production. Additionally, it can be applied for the development of disease-free MPs through meristem culture and can be grown in the fields of the pharmaceutical industries. The report of successful in vitro micropropagation of MPs is attenuated due to the problems associated with the final and critical step of hardening and acclimatization of in vitro raised MPs in natural habitats. Wild grown MPs which have undergone a different growth pathway during in vitro propagation often may show low acclimatization rate (Niazian 2019).

16.4.2 Micropropagation via Organogenesis

Regeneration of whole plants from the explants (cell, tissue or organ) is possible in PTC either directly (emergence of adventitious organs from the explants without callus formation) or indirectly (via formation of callus) through organogenesis that results in the differentiation of monopolar structures, i.e. shoot and/or root, on specific culture medium and in culture conditions. Direct or indirect organogenesis may occur spontaneously on basal culture medium without the application of exogenous PGRs or induced in the culture medium containing exogenous PGRs. Initiation of the development of roots, shoots and complete plants from callus cultures depends on various factors such as type and age of explants, type of callus, type and concentration of PGRs used in the culture medium. Organogenesis and regeneration are most promising tools of PTC-based biotechnology for conservation, improvement and production of desired SMs in MPs.

16.4.3 Micropropagation via Somatic Embryogenesis

Somatic embryogenesis is a cost-effective, high-efficiency, PTC-based useful process for the bulk clonal production of desirable regenerated whole plants from a single or groups of somatic cells/tissues (explants) via a multi-step regeneration process in aseptic condition. The acquisition of embryogenic potentialities of the explants largely depends on dedifferentiation which may be possible either spontaneously or induced by manipulating phytohormone concentration in culture medium that leads to the development of somatic embryo (SE). The differentiation of an embryo occurs either directly with the induction of bipolar SEs (without callus formation)

Table 16.1 Micropropagation of medicinal plants published during 2010 to June 2020

Plant (Family)	Micropropagation using preexisting meristem		Micropropagation via organogenesis		Micropropagation via somatic embryogenesis			References
	Explant	Survival rate (%)	Explant	Survival rate (%)	Explant	Regeneration frequency (%)	Survival rate (%)	
Acorus calamus L. (Acoraceae)	Rh	75	–	–	–	–	–	Verma and Singh (2012)[1]
Aloe barbadensis Mill. (Asphodelaceae)	Shoot apical meristem	100	Inflorescence axis, L	80–100	–	–	–	Sahoo and Rout (2014)[2]; Das et al. (2016)[1]
Aloe peglerae Schönland (Asphodelaceae)	ST	100	–	–	–	–	–	Hlatshwayo et al. (2020)[1]
Alpinia calcarata Rosc. (Zingiberaceae)	Rh	>90	–	–	–	–	–	Bhowmik et al. (2016)[2]
Alpinia galanga L. (Zingiberaceae)	AB	90	–	–	–	–	–	Sahoo et al. (2020)[1]
Amomum subulatum Roxb. (Zingiberaceae)	Rh	100	–	–	–	–	–	Purohit et al. (2017)[1]
Ansellia africana Lindl. (Orchidaceae)	N	87	–	–	–	–	–	Bhattacharyya et al. (2017a)[1]
Artemisia annua L. (Asteraceae)	–	–	S, L	45	–	–	–	Zayova et al. (2020)[2]
Artemisia nilagirica (C.B. Clarke) pamp. (Asteraceae)	–	–	N	~73	–	–	–	Shinde et al. (2016)[2]

(continued)

Table 16.1 (continued)

Plant (Family)	Micropropagation using preexisting meristem		Micropropagation via organogenesis		Micropropagation via somatic embryogenesis			References
	Explant	Survival rate (%)	Explant	Survival rate (%)	Explant	Regeneration frequency (%)	Survival rate (%)	
Bacopa monnieri (L.) Wettst. (Scrophulariaceae)	ST, S, N, IN	100	ST, S, N, IN, L	100	L	100	100	Khilwani et al. (2016)[3]; Saha et al. (2020)[1,2,3]
Betula platyphylla (Miquel) Hara (Betulaceae)	–	–	–	–	Mature seed	88	95	Yang et al. (2020)[3]
Boerhaavia diffusa L. (Nyctaginaceae)	N	85	–	–	–	–	–	Patil and Bhalsing (2015)[1]
Cannabis sativa L. (Cannabaceae)	N with AB, ST, C, E	95	N with AB	100	–	–	–	Lata et al. (2016a; b)[2]; Wróbel et al. (2018)[1]
Castilleja tenuiflora Benth. (Orobanchaceae)	N	95	–	–	–	–	–	Martínez–Bonfil et al. (2011)[1]
Catharanthus roseus (L.) G. Don (Apocynaceae)	ST	80	–	–	H	ND	100	Yuan et al. (2011)[3]; Kumar et al. (2013)[1]
Celosia argentea (Var.) Cristata (Amaranthaceae)	–	–	L, S	90	–	–	–	Bakar et al. (2014)[2]
Ceropegia noorjahaniae (Apocynaceae)	N	85	–	–	–	–	–	Chavan et al. (2014)[1]

(continued)

Table 16.1 (continued)

Plant (Family)	Micropropagation using preexisting meristem		Micropropagation via organogenesis		Micropropagation via somatic embryogenesis			References
	Explant	Survival rate (%)	Explant	Survival rate (%)	Explant	Regeneration frequency (%)	Survival rate (%)	
Chlorophytum borivilianum Sant. et Fernand. (Liliaceae)	Shoot base	93–95	L	80	H	30	60–65	Rizvi et al. (2010)[3]; Samantaray and Maiti (2010)[1]; Basu and Jha (2014)[2]
Cinchona officinalis L. (Rubiaceae)	N with AB	ND	–	–	–	–	–	Armijos–González and Pérez–Ruiz (2016)[1]
Coelogyne cristata Lindl. (Orchidaceae)	–	–	Protocorm–like bodies	ND	–	–	–	Naing et al. (2011)[2]
Coleus forskohlii Briq. (Lamiaceae)	N	70	L	100	ND	ND	ND	Sahai and Shahzad (2013)[1]; Sreedevi et al. (2013)[2]
Coriandrum sativum L. (Apiaceae)	–	–	–	–	R	75	~56	Ali et al. (2017)[3]
Croomia japonica Miq. (Stemonaceae)	–	–	Rh	87	–	–	–	Jiang et al. (2018)[2]
Curcuma amada Roxb. (Zingiberaceae)	–	–	–	–	Leaf sheath	~92	~83	Raju et al. (2016)[3]

(continued)

Table 16.1 (continued)

Plant (Family)	Micropropagation using preexisting meristem		Micropropagation via organogenesis		Micropropagation via somatic embryogenesis			References
	Explant	Survival rate (%)	Explant	Survival rate (%)	Explant	Regeneration frequency (%)	Survival rate (%)	
Curcuma aromatica Salisb. (Zingiberaceae)	Rh with AB	ND	–	–	–	–	–	Parida et al. (2020)[1]
Cymbidium giganteum Wall. Ex Lindl. (Orchidaceae)	–	–	Pseudostem	ND	–	–	–	Roy et al. (2012)[2]
Dendrobium crepidatum Lindl. & Paxton (Orchidaceae)	–	–	N	85	–	–	–	Bhattacharyya et al. (2016b)[2]
D. nobile Lindl. (Orchidaceae)	–	–	Pseudostem with node	84.3	–	–	–	Bhattacharyya et al. (2014)[2]
Digitalis lanata Ehrh. (Plantaginaceae)	–	–	–	–	L, R	~95	65	Bhusare et al. (2020)[3]
D. purpurea L. (Plantaginaceae)	N	80	L	ND	–	–	–	Patil et al. (2013)[1]; Pérez–Alonso et al. (2018)[2]
Dorem ammoniacum D. (Apiaceae)	–	–	R, H, C	60	–	–	–	Irvani et al. (2010)[2]
Eclipta alba (L.) Hassk. (Asteraceae)	N, ST	80	–	–	N, L, ST	~96	95	Bardar et al. (2015)[1]; Salma et al. (2019)[3]

(continued)

Table 16.1 (continued)

Plant (Family)	Micropropagation using preexisting meristem		Micropropagation via organogenesis		Micropropagation via somatic embryogenesis			References
	Explant	Survival rate (%)	Explant	Survival rate (%)	Explant	Regeneration frequency (%)	Survival rate (%)	
Elaeocarpus sphaericus (Gaertn.) K. Schum. (Elaeocarpaceae)	N	80	–	–	–	–	–	Saklani et al. (2015)[1]
Embelia ribes Burm F. (Myrsinaceae)	H	>85	–	–	–	–	–	Annapurna and Rathore (2010)[1]
Gentiana scabra Bunge. (Gentianaceae)	ST	96	–	–	–	–	–	Huang et al. (2014)[1]
Gloriosa superba L. (Colchicaceae)	ST	87	Tuber	ND	–	–	–	Yadav et al. (2013[2]; 2015[1])
Gymnema sylvestre (Retz.) R.Br. ex Sm. (Asclepiadaceae)	N, AB	85	–	–	–	–	–	Sharma and Bansal (2013)[1]
Holarrhena antidysenterica (L.) Wall. (Apocynaceae)	N	90	–	–	–	–	–	Kanungo et al. (2012)[1]
Hypoxis hemerocallidea Fisch., C.A. Mey. & Avé–Lall. (Hypoxidaceae)	–	–	Corm	ND	Corm	87	90	Moyo et al. (2014)[2]; Kumar et al. (2017)[3]
Justicia gendarussa Burm. f. (Acanthaceae)	N	90	–	–	–	–	–	Thomas and Yoichiro (2010)[1]

(continued)

Table 16.1 (continued)

Plant (Family)	Micropropagation using preexisting meristem		Micropropagation via organogenesis		Micropropagation via somatic embryogenesis			References
	Explant	Survival rate (%)	Explant	Survival rate (%)	Explant	Regeneration frequency (%)	Survival rate (%)	
Kelussia odoratissima Mozaff. (Apiaceae)	–	–	–	–	Germinated seeds without hypocotyls and cotyledonary leaves	85	ND	Ebrahimi et al. (2018)[3]
Ledebouria revoluta (L.f.) Jessop Syn. *Scilla indica* (Wight) Baker (Asparagaceae)	–	–	Scale leaf, leaf lamina, R	96	Bulb scale	~58	96	Haque and Ghosh (2016)[3]; Haque et al. (2018)[2]
Nothapodytes nimmoniana (Graham) Mabb. (Icacinaceae)	–	–	R, H, CN, AB, terminal bud	97	–	–	–	Prakash et al. (2016)[2]
Oplopanax elatus Nakai. (Araliaceae)	–	–	–	–	L, P, R	64	80	Moon et al. (2013)[3]
Pelargonium sidoides DC. (Geraniaceae)	–	–	–	–	Inflorescence shoot, P	~86	~71	Duchow et al. (2015)[3]
Picrorhiza kurroa Royel ex Benth. (Scrophulariaceae)	N, ST	80	N, L, R	81	–	–	–	Rawat et al. (2013a)[1,2]
Piper nigrum L. (Piperaceae)	–	–	L	ND	–	–	–	Ahmad et al. (2010b)[2]

(continued)

Table 16.1 (continued)

Plant (Family)	Micropropagation using preexisting meristem		Micropropagation via organogenesis		Micropropagation via somatic embryogenesis			References
	Explant	Survival rate (%)	Explant	Survival rate (%)	Explant	Regeneration frequency (%)	Survival rate (%)	
Pittosporum eriocarpum Royle (Pittosporaceae)	N	73	–	–	–	–	–	Thakur et al. (2016)[1]
Plumbago rosea L. (Plumbaginaceae)	–	–	–	–	In, L, P	ND	ND	Borpuzari and Borthakur (2016)[3]
P. zeylanica L. (Plumbaginaceae)	N	ND	N	99	–	–	–	Jain et al. (2018)[1]; Sharma and Agrawal (2018)[2]
Podophyllum hexandrum Royle (Berberidaceae)	–	–	Rh	ND	ZE	79–91	38	Rajesh et al. (2014b; c)[3]; Tariq et al. (2015)[2]
Rauvolfia serpentina (L.) Benth. ex Kurz., (Apocynaceae)	N	95	–	–	–	–	–	Ahmad et al. (2015)[1]
R. tetraphylla L. (Apocynaceae)	–	–	ST, L, R	86	–	–	–	Rohela et al. (2019)[2]
Rhazya stricta Decne. (Apocynaceae)	N with AB	NM	–	–	–	–	–	Mohamed et al. (2014)[1]

(continued)

Table 16.1 (continued)

Plant (Family)	Micropropagation using preexisting meristem		Micropropagation via organogenesis		Micropropagation via somatic embryogenesis			References
	Explant	Survival rate (%)	Explant	Survival rate (%)	Explant	Regeneration frequency (%)	Survival rate (%)	
Rumex nepalensis Spreng. (Polygonaceae)	N	90	L	97	–	–	–	Ahmad et al. (2010a)[2]; Bhattacharyya et al. (2017b)[1]
Ruta graveolens L. (Rutaceae)	ST	80	–	–	–	–	–	Faisal et al. (2018)[1]
Saussurea involucrata (Kar. et Kir.) (Asteraceae)	Shoot base	100	–	–	–	–	–	Kuo et al. (2015)[1]
Solanum aculeatissimum Jacq. (Solanaceae)	–	–	L	>90	–	–	–	Ghimire et al. (2012)
Spilanthes oleracea L. (Asteraceae)	–	–	N	~77	–	–	–	Dandin et al. (2014)[2]
Stevia rebaudiana Bertoni. (Asteraceae)	N	70	–	–	L, IN	63	ND	Soliman et al. (2014)[1]; Keshvari et al. (2018)[3]
Swertia chirayita (Roxb.) H. Karst. [Gentianaceae]	–	–	L	70–80	–	–	–	Kanwar et al. (2017)[2]
Tylophora indica (Burm. f.) Merr. (Asclepiadaceae)	ST, N, AB	80–100	L, S, N, P, R	62–100	L, S, IN	ND	88–90	Da Silva and Jha (2016)[1,2,3]

(continued)

Table 16.1 (continued)

Plant (Family)	Micropropagation using preexisting meristem		Micropropagation via organogenesis		Micropropagation via somatic embryogenesis			References
	Explant	Survival rate (%)	Explant	Survival rate (%)	Explant	Regeneration frequency (%)	Survival rate (%)	
Uraria picta (Jacq.) DC. (Fabaceae)	N	100	–	–	–	–	–	Rai et al. (2010)[1]
Veronica anagallis–aquatica L. (Scrophulariaceae)	N	80	–	–	–	–	–	Shahzad et al. (2011)[1]
Vitex trifolia L. (Verbenaceae)	N with AB	95	–	–	N	89.6	90	Ahmad et al. (2013)[1]; Alatar et al. (2017)[3]
Withania somnifera (L.) Dunal (Solanaceae)	CN	84	ST, R, N	95	–	–	–	Chakraborty et al. (2013)[2]; Nayak et al. (2013)[1]
Zingiber zerumbet Smith (Zingiberaceae)	–	–	Rhizome bud	80	–	–	–	Faridah et al. (2011)[2]
Zygophyllum potaninii Maxim. (Zygophyllaceae)					C, H	100	55	Bayarmaa et al. (2018)[3]

1 = Reference for micropropagation using preexisting meristem, 2 = Reference for micropropagation via organogenesis, 3 = Reference for micropropagation via somatic embryogenesis
(Axillary bud = AB, Apical node = AN, Cotyledon = C, Cotyledonary node = CN, Epicotyls = E, Hypocotyl = H, Leaflet/Leaf = L, Node = N, Internode = IN, Petiole = P, Rhizome = Rh, Root = R, Shoot tip = ST, Stem = S, Zygotic embryo = ZE; ND = not determined)

or indirectly with the induction of embryogenic callus. Restoration of embryogenic competence via dedifferentiation is followed by the proliferation of the embryogenic material either through adventitious budding on the SEs in secondary embryogenesis or embryogenic suspensions/calli that favour large-scale embryogenic cell proliferation before the subsequent embryo differentiation. SEs undergo various changes at morphological, physiological, metabolic, biochemical and gene expression levels during the maturation phase which are followed by desiccation and plant regeneration phase.

SE has the potential for rapid and large-scale multiplication of a wide range of economically important MP species with non-viable or recalcitrant seeds or has limited and rare seed production capability for the ex-situ conservation of germplasms. Moreover, it can be used for the production of mutants and artificial seeds, genetic engineering and germplasm cryopreservation. The innovation of synthetic seed technique in carrot using alginate encapsulated SE in 1982 significantly enhanced the application of SEs by providing the benefits of rapid pathogen-free clonal propagation by avoiding the heterozygosity problem of natural botanical seeds, their short-term storage and transportation for conservation and restoration purposes. Synthetic seeds or artificial seeds can be produced by encapsulating a suitable (~3–5 mm long) plant propagule such as unipolar vegetative propagules, micro-cuttings, or bipolar vegetative propagules such as SEs and protocorm-like bodies (PLBs) within artificial nutrient-filled soft matrix or hydrogel that is able to germinate and produce a healthy plantlet (Gantait et al. 2015; Bayarmaa et al. 2018; Bhusare et al. 2020).

Several researchers explored and assessed the potential of somatic embryogenesis and artificial seed technology as an effective approach to support conservation and rehabilitation programmes for many MP species. Genetic stability, ease in handling and transportation, effectiveness in terms of space, labour, time and cost are the advantages of synthetic seeds containing SE. In addition to conservation, synthetic seeds are ideal to maintain a screened and selected genotype of MPs that contain a high level of desired SMs. Explant selection, treatment with proper concentration and type of PGRs, manipulations of light, temperature and pH, concentrations of encapsulating agent and matrix often act as key factors that should be optimized for successful application of SE and synthetic seeds in MPs (Gantait et al. 2015). Interestingly, Piątczak and Wysokińska (2013) and Rawat et al. (2013b) extended the conception of synthetic seeds by incorporating hairy roots fragments as a novel approach in synthetic seed development of MPs like *Centaurium erythraea* and *Picrorhiza kurroa* with 86% and 73% regeneration, respectively (Gantait et al. 2015).

16.5 In vitro Approaches for Secondary Metabolite Production

In addition to conservation, PTC-based biotechnological approaches can be used for the successful production of concerned SMs, reduction of toxic compounds and production of novel chemical compounds (Gandhi et al. 2015). Several PTC-based SM production platforms have been successfully developed for several MP species in the last three decades to cope up with different problems associated with traditional field culture including scarcity of raw material, low productivity, geographical, seasonal and environmental variation of SM content, lack of uniformity in product quality and yield, comparatively large production cycles, biotic and abiotic stress, effect of application of pesticides and herbicides and continuous reduction in land availability for large-scale cultivation in contrast to the gradual increase in demand for plant-derived SMs.

The objective of the study and the availability of the plant are two key factors of consideration for the appropriate selection of plant material and tissue culture method for the production of desired SMs from the diverse groups of MPs and the different methods of PTC available. Non-crop wild plants are usually used for the production of pharmaceutically important compounds, whereas crop plants are used more frequently for the production of bioengineered compounds such as vaccines, antibodies, enzyme replacement and albumin serum (Espinosa-Leal et al. 2018).

The eco-friendly PTC-based bioprocessing methods for important pharmacologically active SMs include cell culture, callus culture, organ culture, micropropagation of whole plants and HRC, which have great potential to serve as attractive alternatives to large-scale SM production from field-grown plant materials (Halder et al. 2019). Apart from the traditional PTC approaches, various biotechnological interventions such as meticulous screening and selection of high-yielding lines, optimization of culture media composition and physical parameters, precursor feeding, elicitation, large-scale cultivation in the bioreactor, plant cell immobilization and biotransformation have been assayed to evaluate their effectiveness towards enhancement of SM production utilizing in vitro cell culture and/or organ culture of different MP species (Halder et al. 2018). In spite of several thousand papers that have been published on in vitro SM production, still there is an enormous area to exploit due to massive biodiversity of plants and their diverse biosynthetic potential. Moreover, enhancement in SM productivity and/or de novo synthesis of novel chemical compounds could be made possible in this omics era using different in vitro techniques through metabolic engineering.

16.5.1 Non-transgenic Approaches for Secondary Metabolite Production

16.5.1.1 Unorganized Callus and Cell Suspension Culture (CSC) of Medicinal Plants for Secondary Metabolite Production

Callus is a dedifferentiated state of tissue that can be initiated from various differentiated and organized plant tissue explants on plant species-specific culture medium which generally comprise of nutrient medium supplemented with different PGRs in in vitro cultures (Halder and Jha 2020). Plant CSC is characterized by free cells or small groups of cells that have generally been established by transferring undifferentiated callus in the liquid nutrient medium supplied with constant aeration agitation on a rotary shaker at a specific rpm. Simple, zero seasonal variation, the ability to maintain cells in a non-differentiating condition, infection-free production system with higher mass production per unit of time and area, cost-effective efficient downstream processing, feasibility of continuous culture in a chemostat, application of elicitation and precursor feeding and scope of large-scale production in the bioreactor are the major advantages associated with CSCs. However, genetic and biochemical instability, low synthesis ability, irregular response to elicitors are the main constraints of undifferentiated and unorganized cultures.

The biosynthetic capabilities of unorganized callus and CSC of different plants have been greatly exploited by several researchers during the last decade, and a wide range of plant-derived pharmaceutically important SMs such as ajmalicine, anthocyanins, artemisinin, camptothecin, colchicine, podophyllotoxin, reserpine, resveratrol, shikonin, taxol, withanolides, vinblastine and vincristine have been successfully produced (Table 16.2). Interestingly, spontaneous production of some SMs at a comparable amount to intact plants have been reported in undifferentiated and disorganized cultures, whereas SMs like morphinan alkaloids, tropane alkaloids (e.g. hyoscyamine and scopolamine), quinoline alkaloids, dimeric monoterpene indole alkaloids (e.g. vinblastine and vincristine), which are localized in morphologically specialized tissues or organs of native plants are either detected in very low amounts or not detected in CSCs, which might be due to loss of tissue-specific function during their dedifferentiation. Implementation of a number of strategies such as the selection of a parent plant with an elite genotype that ensures high-yielding capacity, screening and selection of high-yielding cell clones from the heterogeneous cell populations, elicitation, precursor feeding, optimization of medium and/or culture conditions, immobilization on beads can increase productivity by several folds.

16.5.1.2 Root Organ Culture of Medicinal Plants for Production of Secondary Metabolites

Roots of few MPs are of great interest in the pharmaceutical industry and in the traditional pharmacopeias worldwide due to their ability to accumulate some unique

Table 16.2 Unorganized undifferentiated callus culture and cell suspension culture of medicinal plants for production of secondary metabolites published from 2010 to June 2020

Secondary metabolites	Chemical nature	Plant source [Family]	Main use	Culture type; strategy	References
Ajmalicine	TIA	*Catharanthus roseus* (L.) G. Don [Apocynaceae]	Anti-hypertensive	Leaf-derived CSC; Elicitation	Mandagi et al. (2017)
Ajmalicine	TIA	*Rauvolfia serpentina* (L.) Benth. ex Kurz., [Apocynaceae]	Anti-hypertensive	Leaf-derived CSC; Elicitation	Zafar et al. (2020)
Andrographolide	Diterpene lactone	*Andrographis paniculata* (Burm.f.) Nees [Acanthaceae]	Anti-cancer, anti-HIV, anti-inflammatory	Shoot and root-derived CSC; Elicitation	Gandi et al. (2012)
Artemisinin	Sesquiterpene lactone	*Artemisia annua* L. [Asteraceae]	Anti-malarial, Anti-snake venom	Leaf, stem and germinated seedling-derived CSC; Elicitation	Zebarjadi et al. (2018); Salehi et al. (2019)
Asiaticoside, Asiatic acid	Triterpenoid saponin	*Centella asiatica* (L.) Urb. [Apiaceae]	Anti-psoriasis, anti-leprosy, anti-eczema	Leaf-derived CC and CSC; Elicitation	Krishnan et al. (2019)
Azadirachtin	Tetranortriterpenoid limonoid	*Azadirachta indica* A. Juss. [Meliaceae]	Anti-plasmodia, anti-parasitic, anti-diabetic	Leaf-derived CSC; Effect of PGRs	Farjaminezhad and Garoosi (2019)
Bacosides	Triterpenoid saponin	*Bacopa monnieri* (L.) Wettst. [Plantaginaceae]	Memory enhancer, anti-neoplastic, anti-Alzheimer's	Leaf-derived CSC	Bansal et al. (2017)
Camptothecin	MIA	*Nothapodytes nimmoniana* (J. Grah.) Mabb. [Icacinaceae]	Anti-cancer	Leaf, hypocotyl and root-derived CC and CSC; Elicitation	Isah (2017)

(continued)

Table 16.2 (continued)

Secondary metabolites	Chemical nature	Plant source [Family]	Main use	Culture type; strategy	References
Camptothecin	MIA quinoline alkaloid	*Ophiorrhiza mungos* L. [Rubiaceae]	Anti-cancer	Leaf-derived CSC; Elicitation	Deepthi and Satheeshkumar (2017)
Camptothecin	MIA	*Camptotheca acuminata* Decne. [Nyssaceae]	Anti-cancer	Leaf, stem, root-derived CSC; Elicitation	Yang et al. (2017)
Capsaicin	Alkaloid	*Capsicum chinense* Murray, *C. frutescens* L. [Solanaceae]	Counterirritant	Hypocotyl-derived CSC; Elicitation	Kehie et al. (2016)
Catharanthine, Vindoline	Indole alkaloid	*Catharanthus roseus* (L.) G. Don [Apocynaceae]	Anti-hypertensive	Cambial meristem-derived CSC; Elicitation	Zhou et al. (2015)
Chicoric, Rutin, Rosmarinic acid. Isoquercetin	Phenolic compounds	*Ocimum basilicum* L. [Lamiaceae]	Antiseptic, analgesic	Leaf-derived CSC; Elicitation	Açıkgöz (2020)
Colchicine	Tropolone alkaloid	*Gloriosa superba* L. [Colchicaceae]	Anti-tumour	Rhizome-derived CSC; Elicitation	Mahendran et al. (2018)
Echitamine, Tubotaiwine, Picrinine, Acetylechitamine	Indole alkaloid	*Alstonia scholaris* (L.) R. Br. [Apocynaceae]	Anti-cancer, anti-tuberculosis	Tender leaf-derived CC; Optimization of medium, elicitation and precursor feeding	Jeet et al. (2020)
Forskolin	Labdane diterpene	*Coleus forskohlii* (Willd.) Briq. [Lamiaceae]	Anti-asthmatic, anti-glaucoma	CSC; Elicitation	Swaroopa et al. (2013)
Ginsenosides	Triterpenes saponin	*Panax ginseng* C. A. Mey. [Araliaceae]	Hepatoprotective, anti-depressant	CSC	Le et al. (2019)

(continued)

Table 16.2 (continued)

Secondary metabolites	Chemical nature	Plant source [Family]	Main use	Culture type; strategy	References
Gymnemic acid	Triterpenoid saponin	*Gymnema sylvestre* (Retz.) R.Br. ex Sm. [Asclepiadaceae]	Anti-diabetes	Leaf-derived CSC; Elicitation	Chodisetti et al. (2015)
Hydroxybenzoic, Hydroxycinnamic acid derivatives	Phenolic acid	*Hypoxis hemerocallidea* Fisch., C.A. Mey. & Avé-Lall. [Hypoxidaceae]	Cardio-protective, anti-impotency, anti-psoriasis	Corm-derived CC	Moyo et al. (2014)
Paclitaxel (taxol), Taxanes	Diterpenoid	*Taxus baccata* L. [Taxaceae]	Anti-cancer	Stem-derived CC	Sarmadi et al. (2019)
Plumbagin	Naphthoquinone	*Plumbago europaea* L., *P. zeylanica* L. [Plumbaginaceae]	Anti-tumour, anti-microbial	Stem and leaf-derived CSC	Patidar et al. (2015); Beigmohamadi et al. (2019)
Podophyllotoxin	Aryl tetralin lignan	*Podophyllum hexandrum* Royle [Berberidacae]	Anti-cancer	Leaf-derived CSC; Precursor feeding	Bhattacharyya et al. (2012); Majumder (2012)
Reserpine	TIA	*Rauvolfia serpentina* (L.) Benth. ex Kurz., [Apocynaceae]	Anti-hypertensive	Leaf-derived CSC; Elicitation	Zafar et al. (2017; 2020)
Resveratrol	Polyhydroxy-stilbene	*Vitis labrusca L.* [Vitaceae]	Cardio-prodective, anti-cancer	CSC	Nivelle et al. (2017)
Rhamnetin	Flavonol	*Vernonia anthelmintica* (L.) Willd. [Asteraceae]	Anthelmintic, diuretic, anti-asthmatic	Leaf-derived CSC; Elicitation	Rajan et al. (2020)
Syringin, Rutin	Flavonoid	*Saussurea involucrate* Matsum. & Koidz. [Asteraceae]	Anti-inflammatory, cardiotonic, anti-fatigue	Petiole, leaf and root-derived CC; Elicitation	Kuo et al. (2015)

(continued)

Table 16.2 (continued)

Secondary metabolites	Chemical nature	Plant source [Family]	Main use	Culture type; strategy	References
Vincristine, Vinblastine	Alkaloid	*Catharanthus roseus* (L.) G. Don [Apocynaceae]	Anti-tumour, Anti-cancer	Leaf and hypocotyl-derived CC and CSC; Elicitation	Fatima et al. (2015); Mekky et al. (2018)
Withanolides	Steroidal lactone	*Withania somnifera* (L.) Dunal [Solanaceae]	Adaptogenic, diuretic, anti-inflammatory, immunomodulatory	Root-derived CSC; Elicitation and precursor feeding	Sivanandhan et al. (2014)

Callus culture (CC), Cell suspension culture (CSC), Monoterpene indole alkaloid (MIA), Terpenoid indole alkaloid (TIA)

root-specific SMs with medicinal applications. Non-transformed adventitious root cultures of various MP species have been developed since early days of PTC as an organ culture system on solid or in liquid medium supplemented with PGRs and used as one of the important alternative source for the production of plant-derived SMs like andrographolide (Singh et al. 2018), chlorogenic acid (Lee et al. 2015), colchicine (Ghosh et al. 2015), eleutheroside B and E (Lee et al. 2015), ginsenoside (Yu et al. 2016; Le et al. 2019; Wang et al. 2019), hypericin (Wu et al. 2014), kaempferide (Han et al. 2019), podophyllotoxin (Rajesh et al. 2014a), steroidal saponins (Basu and Jha 2013), saikosaponins (Kusakari et al. 2012), steviol glycosides (Ahmad et al. 2018), plumbagin (Roy and Bharadvaja 2019), tanshinone (Zaker et al. 2015) and withanolides (Rangaraju et al. 2019). However, the slow growth rate, the requirement of exogenous supply of PGRs in the medium, biochemical and genetic instability in long-term culture and low productivity have limited its broader and commercial applications for SM production.

16.5.1.3 Adventitious Shoot Cultures and Regenerated Medicinal Plants for Production of Secondary Metabolites

Besides the great implementation of shoot culture and shoot culture-derived plants for the conservation of MPs, they also serve as a viable system for PTC-based SM production. Interestingly, few reports confirmed that micropropagated plants retain their ability to produce SMs even after successful field transfer. Non-transformed in vitro shoot cultures or shoot culture-derived micropropagated MPs have been used as an important alternative source for the production of SMs including catharanthine, vindoline and vinblastine from *C. roseus* (Sharma et al. 2019), cardenolides from *D. purpurea* (Pérez-Alonso et al. 2018), bacosides from *B. monnieri* (Saha et al. 2020), tylophorine from *T. indica* (Da Silva and Jha 2016), hypericin from *Hypericum hookerianum* (Sooriamuthu et al. 2013), camptothecin from *C. acuminata* (Sankar-Thomas and Lieberei 2011), reserpine from *R. serpentina* (Panwar and Guru 2015), silymarin from *Silybum marianum* (Sherif et al. 2013), stevioside and rebaudioside A from *Stevia rebaudiana* (Bayraktar et al. 2016), withanolides from *W. somnifera* (Chakraborty et al. 2013), etc.

16.5.2 Transgenic Approaches

16.5.2.1 Transformed Callus and Cell Culture of Medicinal Plants for Secondary Metabolite Production

HRC or Ri- or Ti-transformed plants often showed *rol* gene(s) induced enhancement in SM production due to the over-expression of one or several key regulatory genes involved in biosynthesis pathways. *rol* genes are the potential activators of SMs and cause hypersensitive stress responses. A relatively new biotechnological approach

that relies on *rol* gene specific activation of SM production has been applied successfully in a few plant species to produce transformed callus or cell culture having single or multiple *rol* genes isolated from plasmids of *Agrobacterium* (Sarkar et al. 2018; Halder and Jha 2020). Sarkar et al. (2018) reviewed the effects of *rol* genes of *A. rhizogenes* on morphogenesis and SM accumulation in MPs. Although few reports of transformed CSCs are also available, here only recent reports of the transformed unorganized cultures and their ability to produce SMs have been discussed.

Dubrovina et al. (2010) reported enhancement of 11.9-fold resveratrol accumulation as a result of insertion of *rolC* gene in callus culture of *Vitis amurensis* via *A. tumefaciens*-mediated transformation. Similarly, *rolB*- and *rolC*-transformed callus cultures of *Maackia amurensis* showed production of isoflavonoids at significantly higher levels in comparison with empty vector control callus (Grishchenko et al. 2013; 2016). The *rolC*-transformed calli contained three times higher caffeoylquinic acid in *Cynara cardunculus* var. *altilis*. Stable 3-fold caffeoylquinic acid production was reported in *rolC*-transgenic callus cultures of *C. cardunculus* var. *altilis* compared to the control calli (Vereshchagina et al. 2014). In *rolA*-transformed cultures of *Artemisia dubia* plants, artemisinin and its derived compounds were comparable to that of the non-transformed plant (Amanullah et al. 2016). The potential elicitation capability of *rolA* gene in plant secondary metabolism has been well-observed in *rolA*-transgenic *R. cordifolia* calli, which stimulate anthraquinone yield by the activation of ruberitrinic acid biosynthesis (Veremeichik et al. 2019).

16.5.2.2 Hairy Root Culture of Medicinal Plants for Secondary Metabolite Production

A. rhizogenes-derived HRCs have evolved as a most popular, promising, convenient and extensively used PTC-based biotechnological approach for the long-term production of commercially important pharma molecules in comparison with other forms of conventional in vitro cultures due to the major gifted strengths like rapid growth and large biomass accumulation potential, hormone-autotrophy, similar or greater bio-production capacity for SMs as compared to their parent plants, organ-based PTC method, genetic and biosynthetic stability for long-term culture, feasibility of large-scale production in the bioreactor system and often show de novo synthesis of SMs (Roychowdhury et al. 2017).

Productivity of SMs in HRCs of different plant species can be improved by the application of various biotechnological strategies which include the selection of high-yielding rhizoclone(s), optimization of culture medium and culture conditions such as optimum levels of salt, sugar, nitrogen, phosphate and physical factors such as temperature, illumination, light quality, pH of the medium, agitation, aeration and environmental gas (e.g. oxygen and carbon dioxide), replenishment of nutrient and precursor feeding, elicitation, application of phytohormones in medium and scale-up to the bioreactors (Halder et al. 2019).

Besides the production of valuable SMs of MPs, HRC platform also offers application of elicitation (Gabr et al. 2016; Srivastava et al. 2016a; Akhgari et al. 2019),

large-scale production of desired phytochemicals (Bauer et al. 2015; Patra and Srivastava 2016; Kochan et al. 2017; Thakore et al. 2017), induction of artificial polyploidy (Banerjee 2018) and CRISPR/Cas9 mediated genome editing (Li et al. 2017). Several review articles clearly demonstrated the successful establishment of HRCs of several plant species by utilizing different strains of nature's own genetic engineer *A. rhizogenes* and production of target biopharmaceuticals (Halder et al. 2019; Gutierrez-Valdes et al. 2020; Li and Wang 2020). Table 16.3 enlists few selected recent reports of successful SM production using HRC platform of MPs.

16.5.2.3 Transgenic Medicinal Plants for Secondary Metabolite Production

Agrobacterium rhizogenes mediated genetic manipulation has undergone several developments with adoption of different binary vector system depending upon target of research (Bahramnejad et al. 2019). Different transgenic approaches like transgenic plants with only T-DNA genes of *A. rhizogenes* or *A. tumifaciens* or with novel genes that modify target metabolic pathways or with genes that improve expression of endogenous pathways or with genes not involved in biosynthetic pathways have been applied successfully in MPs to enhance the production of SMs (Kayani et al. 2018).

Regenerated viable Ri- and Ti-transformed plants have been established in a number of plant species such as *T. indica, B. monnieri, Atropa belladonna, Taraxacum platycarpum, P. rosea, Linum usitatissimum, Aesculus hippocastanum*, *Cichorium intybus* through spontaneous direct, spontaneous indirect, induced direct or induced indirect methods (Roychowdhury et al. 2017; Halder and Jha 2020). These Ri- and Ti-transformed plants also showed similar or higher amount of SMs in comparison with the mother plants. Additionally, few reports showed successful hardening and in vivo growth of these transgenic plants with stable potentiality to produce SMs.

16.5.3 Metabolic Pathway Engineering in Medicinal Plants for Production of Secondary Metabolites

PTC not only offers efficient direct or indirect conservation strategies for MPs and enhancement or improvement methods of the production of valuable bioactive phytochemicals, at the same time, it also provides a platform for metabolic engineering, decipherence of biochemical pathways and production of novel compounds through genetic manipulation. PTC can help produce new forms of plant SMs and/or custom-designed MPs valuable for food, pharmaceutical and other industries (Niazian 2019). Metabolic engineering is a promising method to overcome the limitations of conventional PTC for *in planta* overproduction of some pharmaceutically important SMs by the manipulation of endogenous metabolic pathways in an

Table 16.3 List of some selected scientific publications on hairy root culture of medicinal plants for production of secondary metabolites published during 2010 to June 2020

Secondary metabolite	Chemical nature	Plant source [Family]	*Agrobacterium rhizogenes* strain, explant	Content/ fold increased	References
Ajmalicine	TIA	*Rauvolfia serpentina* (L.) Benth. ex Kurz. [Apocynaceae]	A4; Leaf	14.8	Srivastava et al. (2016a)
Anisodine[a], Anisodamine[b]	TA	*Przewalskia tangutica* Maxim [Solanaceae]	ATCC10060, MSU440; Hypocotyl	a = ~14* b = ~5*	Lei et al. (2018)
Artemisinin	Sesquiterpene	*Artemisia annua* L. [Asteraceae]	LBA301; Apical meristem	2.44	Patra and Srivastava (2016)
Atropine	TA	*Przewalskia tangutica* Maxim [Solanaceae]	ATCC10060, MSU440; Hypocotyl	~28*	Lei et al. (2018)
Camptothecin	MIA	*Ophiorrhiza rugosa* var. *decumbens* (Gardner ex Thwaites) Deb & Mondal [Rubiaceae]	LBA 9402; Axenic plant	0.009% DW	Kamble et al. (2011)
Chrysin[c], Baicalein[d], Wogonin[e]	Flavone	*Scutellaria bornmuelleri* Hausskn. ex Bornm. [Lamiaceae]	A4, A13, MSU440, ATCC15834; Stem, petiole, leaf	e = ~11 c = ~9 d = ~13	Gharari et al. (2020)
Emodin[h], Physcion[i]	Anthraquinone	*Polygonum multiflorum* Thunb. [Polygonaceae]	KCTC 2703; Internode, leaf	h = ~4 i = 3.5	Thiruvengadam et al. (2014)
Eupalitin	Flavonol glycoside	*Boerhaavia diffusa* L. [Nyctaginaceae]	ATCC 15834, A4, SA79; Leaf	1.44	Gupta et al. (2016)

(continued)

Table 16.3 (continued)

Secondary metabolite	Chemical nature	Plant source [Family]	*Agrobacterium rhizogenes* strain, explant	Content/ fold increased	References
Farnesiferol B	Sesquiterpene	*Ferula pseudalliacea* Rech.f. [Apiaceae]	ATCC 15824, 1724; Leaf, stem, cotyledon, embryo	0.7–0.75 mg100 mg^{-1}	Khazaei et al. (2019)
Glycyrrhizin	Triterpenoid saponin	*Glycyrrhiza glabra* L. [Fabaceae]	A4; Leaf	~9	Srivastava et al. (2019)
Gymnemic acid	Triterpenoid saponin	*Gymnema sylvestre* (Retz.) R.Br. ex Sm. [Asclepiadaceae]	KCTC 2703; Cotyledon, leaf	~8	Praveen et al. (2014)
Hyoscyamine[j], Scopolamine[K]	TA	*Hyoscyamus reticulatus* L. [Solanaceae]	A7; Cotyledon	l = 5 k = 5	Moharrami et al. (2017)
Mangiferin	Xanthone	*Swertia chirayita* (Roxb.) H. Karst. [Gentianaceae]	LBA9402; Node, internode	2	Samaddar et al. (2019)
Plumbagin	Naphthoquinone	*Plumbago zeylanica* L [Plumbaginaceae]	LBA9402; Node, internode	~6.69 mgg^{-1} DW	Basu et al. (2015)
Reserpine	TIA	*Rauvolfia serpentina* (L.) Benth. ex Kurz. [Apocynaceae]	LBA9402; Leaf, stem	~3.11 mg g^{-1} DW	Ray et al. (2014a)
Resveratrol	Trihydroxystilbene	*Arachis hypogaea* L [Fabaceae]	LBA9402, A4, R1000; Leaflet, petiole	19	Halder and Jha (2016)
Rosmarinic acid	Phenolic acid	*Ocimum basilicum* L. [Lamiaceae]	A4, ARqua1-pTSC5, 8196, 11325; Leaf, hypocotyl, cotyledon	2	Srivastava et al. (2016b)

(continued)

Table 16.3 (continued)

Secondary metabolite	Chemical nature	Plant source [Family]	*Agrobacterium rhizogenes* strain, explant	Content/ fold increased	References
Rosmarinic acid	Phenolic acid	*Dracocephalum moldavica* L. [Lamiaceae]	A4; Shoot, leaf	10	Weremczuk-Jeżyna et al. (2013)
Sanguinarine	Quaternary benzylisoquinoline alkaloid	*Macleaya cordata* (Willd.) R.Br. [Papaveraceae]	10060; Leaf, stem	2.3	Huang et al. (2018)
Scopolamine	TA	*Przewalskia tangutica* Maxim [Solanaceae]	ATCC10060, and MSU440; Hypocotyl	~3*	Lei et al. (2018)
Silymarin	Flavonolignan	*Silybum marianum* (L.) Gaertn [Asteraceae]	A4; Cotyledon	13.59 $\mu g g^{-1}$ DW	Gabr et al. (2016)
Solasodine	Steroidal glycoalkaloid	*Solanum khasianum* C.B. Clarke [Solanaceae]	A4; Leaf	4	Srivastava et al. (2016a)
Solasodine	Steroidal glycoalkaloid	*Solanum mammosum* L. [Solanaceae]	ATCC31798, A4; Leaf	12	Ooi et al. (2016)
Stevioside	Diterpene glycoside	*Stevia rebaudiana* Bertoni. [Asteraceae]	A4; Leaf	~1.72 $mg g^{-1}$ DW	Pandey et al. (2016)
Stigmasterol[m], Hecogenin[n]	Sapogenin	*Chlorophytum borivilianum* Sant. et Fernand. [Liliaceae]	MTCC2364, MTCC532, PRT Gus; Rhizome	m = 21 n = ~2	Bathoju et al. (2017)
Swertiamerin[o], Amarogentin[p]	Secoiridoid glycoside	*Swertia chirayita* (Roxb.) H. Karst. [Gentianaceae]	LBA9402; Node, internode	o = 4 p = ~2	Samaddar et al. (2019)
Thebaine[q], Morphine[r], Codeine[s]	Morphinan alkaloid	*Papaver orientale* L. [Papaveraceae]	ATCC15834, R1000, C58C1; Hypocotyl, Shoot, cotyledon	q = ~3 r = ~6 s = ~3	Hashemi and Naghavi (2016)

(continued)

Table 16.3 (continued)

Secondary metabolite	Chemical nature	Plant source [Family]	*Agrobacterium rhizogenes* strain, explant	Content/ fold increased	References
Valerenic acid	Sesquiterpenoid derivative	*Valeriana officinalis* L. [Valerianaceae]	A13; Root, hypocotyl, leaf	7.9	Torkamani et al. (2014)
Vindoline-type alkaloid[f], Ajmalicine[g]	TIA	*Rhazya stricta* Decne. [Apocynaceae]	LBA9402; Leaf	f = 2 g = 1.5	Akhgari et al. (2019)
Withanolides	Steroidal lactone	*Withania somnifera* (L.) Dunal [Solanaceae]	A4, 8196, 1600, 11325; Leaf	Withaferin A = 30* Withanolide A = 2* Withanolide B = 5*	Johny et al. (2018); Sivanandhan et al. 2020

*Fold was calculated on the basis of given data in the paper by content in HRC/content in control
Monoterpene indole alkaloid (MIA), Terpenoid indole alkaloid (TIA), Tropane alkaloid (TA)

organism using modern biological tools like genomics, proteomicsand metabolomics that involve over-expression or down-regulation of metabolic pathways by diverting common precursors, enzymes, regulatory proteins, rate-limiting steps, stopping the catabolism pathway of the desired product and obstruction of other pathways.

The *in planta* biosynthesis of valuable plant metabolites is a very complicated process, regulated by numerous complex, interrelated metabolic pathways like shikimate pathway (the main source of phenylpropanoids and aromatic compounds—coumarins, flavonoids, lignans, stilbenoids, catechins, vanillin, gallic acid, etc.), terpenoid pathway or isoprenoid pathway that uses methylerythritol 4-phosphate pathway (the main source of monoterpenoids, diterpenoids, hemiterpenoids, tocopherols, plastoquinones, PGRs, naphthoquinones, cannabinoids, furanocoumarines, and TIAs), mevalonic acid pathway (main source of sesquiterpenoids, phytosterols, triterpenoids and polyprenols) and polyketide pathway (main source of acetogenins and anthraquinones). Moreover, the understanding of these complex biosynthetic pathways has become more difficult due to the involvement of different cells, tissues and organelles.

The over-expression of specific genes and utilization of transcriptional regulators involved in biosynthetic pathways of SMs are the most common strategies for plant-derived metabolite engineering using *Agrobacterium*-mediated and/or biolistic transformation methods (Naizian 2019). The down-regulation of specific pathways using antisense RNA, RNA interference (RNAi), co-suppression techniques or increasing catabolism and carbon into competitive pathways are also used in metabolic engineering to ensure minimum synthesis of unwanted metabolites that in turn increase the desired SM production. Biosynthesis of the target metabolites is often hindered due to the sharing of common precursor molecule by different enzymes of different metabolite pathways. This problem can be overcome using metabolic engineering by redirecting the flux of common precursors towards the desired biosynthesis pathway, by inducing over-expression of genes in the precursor pathway or by down-regulation of competitive pathway. Coordination between the distribution of enzyme and substrate of different cells, tissues and cellular compartments is essential to optimize the function of secondary metabolic pathways. Removal of the product (metabolite) from the site of biosynthesis to a specific cell compartment/cell type is very important to protect cells from self-toxicity as well as often to increase the forward reaction.

Transfer of the whole biosynthetic pathway of useful SMs from original MPs to another plant with high biomass product is a preferred genetic engineering strategy to produce large-scale SMs. *Agrobacterium* (both *A. tumefaciens* and *A. rhizogenes*) and particle bombardment mediated transformation techniques have been effectively used for metabolic engineering of several MPs to achieve over-expression of SMs or the de novo synthesis of plant-made pharmaceuticals. Low plant-specific transformation efficiency, lack of reproducible regeneration protocol, unstable integration of the gene of interest, limitation of our knowledge regarding the complexity of biosynthesis pathway and their regulation, difficulties in obtaining licensing, high costs of securing regulatory approval and public acceptance towards products derived

from genetically modified systems are the major challenges of this biotechnological approach, which need to be addressed in future.

16.6 Ploidy Engineering of Medicinal Plants

Polyploidy is the possession of three or more complete sets of chromosomes that may provide certain adaptive advantages and serves as an important factor in the speciation and evolution of eukaryotes (Iannicelli et al. 2020). Induction of artificial polyploidy by the application of antimitotic agents such as colchicine in in vitro plants is often known as ploidy engineering and is used as a plant breeding strategy that enables the development of new and improved cultivars through an amazing impact on phenotypical, biochemical and genetic characteristics. Polyploidization of some medicinal aromatic plant species has been explored to assess its effect on superior vigour and phytochemical composition (qualitative and quantitative) in comparison with their diploid genotypes. Type, duration of exposure and concentration of antimitotic agent are the important parameters in this strategy. There are few reports showing enhancement of SMs in tissue culture regenerated polyploid MPs such as *A. annua, Bletilla striata*, *C. roseus, Dioscorea zingiberensis, Hyoscyamus albus, H. muticus, Pfaffia glomerata, S. miltiorrhiza and S. rebaudiana* in contrast to their diploid relatives (Corrêa et al. 2016; Pan-pan et al. 2018; Xia et al. 2018; Iannicelli et al. 2020).

Cichorium intybus, a medicinal herb with anti-hepatotoxic, anti-ulcerogenic and anti-inflammatory effects, as well as appetite enhancing, digestive, liver tonic, cardiotonic, diuretic and tonic properties, showed a significant increase in total phenolic compound (1.9-fold) and chlorogenic acid (10-fold) concentrations in leaves of autotetraploids in comparison with diploid plants (Ravandi et al. 2013). Corrêa et al. (2016) reported that induced polyploidy in *Pfaffia glomerata* resulted 31% increase in 20-hydroxyecdysone production. Enhanced production of antiviral and anti-cancer compound podophyllotoxin by the upregulation of expression level and enzyme activity of genes related to its biosynthesis coupled with morphological changes have been reported in colchicine-induced tetraploid *Linum album* in comparison with the diploid genotype (Javadian et al. 2017). Similarly, colchicine-induced tetraploid of *Bletilla striata* showed 26.7% increase in productivity (Pan-pan et al. 2018). Moreover, colchicine-induced polyploids in *A. annua* showed phenotypic alterations, enhanced photosynthetic capacity, artemisinin level and endogenous contents of indole-3-acetic acid (NAA), abscisic acid (ABA) and jasmonic acid (JA) through differential expression of 8763 genes (Xia et al. 2018).

The polyploidization strategy in combination with/without genetic engineering has been implemented in HRCs of different MPs to study the effect of genome duplication on SM production in HR of *A. annua, Datura stramonium*, *H. muticus* (Dehghan et al. 2012; Banerjee 2018). Interestingly, tetraploid *H. muticus* plants showed 200% higher scopolamine than their diploid counterparts, but this result was not observed in induced stable tetraploid HRCs (Dehghan et al. 2012). According to Banerjee (2018), 70% of the reported research articles on influence of ploidy levels

on the biosynthetic potentials of the HRCs showed advantage of tetraploid over diploid HRCs concerning their metabolite yields, which include research articles with better yield at higher ploidy level, enhancement of initial low yield at higher ploidy level by nutrient optimization, yield improvement of SMs in higher ploidy level and transgenic HR at higher ploidy level with better yield (Banerjee 2018). Only 30% of the reported articles showed low yield in hairy roots of higher ploidy in comparison with diploid hairy roots (Banerjee 2018). Judicious screening and selection of the superior hairy root clones of biomass/biosynthetic potentials based on chromosomal count and higher ploidy, assessment of the individual ploidy-based physiological need of hairy root clonal diversity, harmonizing with the elicitation and polyploidization at inter-and intra-ploidy diversity levels of hairy root clones for maximizing their productivities and optimization of ploidy dependant requirements parameters in bioreactor are the most important steps for maximize the desired effect (Banerjee 2018).

In addition to polyploidy, haploid plants can be produced by the application of conventional PTC-based methods such as androgenesis, gynogenesis and wide hybridization-chromosome elimination (Naizian 2019). Androgenesis pathway is the most used method of haploid induction in different MPs (Sharma et al. 2018).

16.7 Large-Scale Cultivation of Medicinal Plants in Bioreactors

In spite of great achievements in PTC-based production of pharmaceutically useful SMs utilizing in vitro-derived plant materials (suspension cells, hairy roots and micropropagated plantlets) in laboratory scale, sustainability and yield are the major concerns with respect to the commercial demand. The scale-up of the plant in vitro culture from laboratory scale to large-scale bioreactors is the final destination to fulfil huge public demand. Bioreactor technology has emerged as an important eco-sustainable and automated bioprocessing method for continuous and consistent large-scale production of biomass or valuable therapeutic SMs or transgenic proteins from in vitro cultivation of living cells or tissues or organs under the optimum environmental conditions in the broth medium.

This is the most potential alternative source after the wild population to fulfil the gradual increase in the demand of plant-derived SMs due to better rate of product multiplication, high specificity and minimum cost. Cultivation of plant cells and hairy roots in the bioreactors is more challenging than microbial cultivation in bioreactors. Parameters of cultivation of each type of explant (microorganism, cell suspension, hairy root and animal cell) in bioreactors are unique and depend on their size, aggregation, sensitivity to hydrodynamic stress and viscosity of the culture broth.

There is increasing interest to evaluate the feasibility of SM production at industrial scale through the use of bioreactors of varying sizes and features (Isah et al. 2018). SM production in bioreactors is quite difficult and complex as it requires

several essential optimization and suitable modifications during the design and operation of bioreactors for plant cell and hairy root cultivation. Homogeneous mixing of medium (for efficient nutrient transport and air-bubble dispersion), maintenance of optimum shearing force, optimization of aeration, minimum cell aggregation and adhesion, light supply for phototrophic and mixotrophic cultures, and optimization of temperature, pH, nutrients are required for organ culture and efficient mass transfer in bioreactors due to their larger cell size and shape, shear sensitivity, tendency to aggregate, slow growth rate, lower oxygen demand, limitation in mass transfer and product formation. However, novel computational tools based on mathematical and statistical modelling, neural networks, artificial intelligence, and in silico prediction have been applied recently to predict suitable design and operational parameters for optimal production of SMs in the bioreactors which is a prerequisite for the development of economically feasible bioreactor-based production unit.

Development of various configurations of bioreactors including stirred tanks, airlift, bubble column, orbital shaker, gas-phase bioreactors and mist or spray reactors has been possible by the recent advancements in bioreactor design and construction. Such bioreactors have been used for PTC-based in vitro growth and large-scale production of desired SMs in few plant species under different culture conditions that open up a new platform with great potentiality (Table 16.4).

16.8 Application of Nanoparticles in Tissue Culture of Medical Plants

Nanotechnology is a very recent and advanced technology that deals with the production, characterization and application of substances (nanoparticles or nano materials) with a diameter in the nanoscale (10^{-9} m). In recent times, it is a rapidly expanding field of research due to its wide applications in every field of science including plant science. Plant-based nanoparticle synthesis is more popular as it is cost effective, eco-friendly, safe and single-step method when compared to the more complex chemical and physical methods of nanoparticle synthesis. Nanotechnology has been used in PTC as a new elicitor to promote germination efficiency, boost plant growth and SM production. The effects of some important metal oxide nanoparticles like titanium oxide, zinc oxide, iron oxide and copper oxide have been reported for enhancement of SM production in different plants using different PTC systems (Moharrami et al. 2017; Bhardwaj et al. 2018; Ghazal et al. 2018; Chung et al. 2019; Karimzadeh et al. 2019; Moradpour et al. 2019).

Treatment of suspension cells of *Bacopa monnieri* with zinc oxide nanoparticles (ZnONPs) increased around 2-fold total bacoside A possibly through the modification in gene regulation of isoprenoid pathway than mevalonic acid pathway (Bhardwaj et al. 2018). Karimzadeh et al. (2019) demonstrated different effects of nanoparticle on enzyme activity, total phenol and lignan production in CSCs of *L. usitatissimum*, depending on concentration and type of nanoparticles. Improvement in gymnemic

Table 16.4 List of some selected scientific publications on large-scale cultivation of medicinal plants in bioreactors published during 2010 to June 2020

Plant Species	Type of Culture	Objective(s)	Type of bioreactor	Volume (l)	References
Artemisia annua L.	HRC	Artemisinin	BCB, NMB, modified NMB	3–5	Patra and Srivastava (2016)
		Biomass production	Mist bioreactor	20	Sivakumar et al. (2010)
Atropa belladonna L.	HRC	Scopolamine	Bioreactor	1.5	Habibi et al. (2015)
Azadirachta indica A. Juss	CSC	Azadirachtin	Bioreactor	3	Prakash and Srivastava (2011)
	HRC	Azadirachtin	NSB, STB, BCB, Modified NSB	3–4	Srivastava and Srivastava (2012)
Brugmansia candida Pers	HRC	Hyoscyamin, scopolamine	Modified STB	1.5	Cardillo et al. (2010)
Buddleja cordata Kunth	CSC	Biomass and SM	STR	2–3	Vazquez-Marquez et al. (2019)
Bupleurum falcatum L.	ARC	Saikosaponin	STB, BCB, modified airlift reactor	10–200	Kusakari et al. (2012)
Catharanthus roseus (L.) G. Don	CSC	Ajmalicine	Pilot-scale bioreactor	100	Fulzele and Namdeo (2018)
	HRC	Ajmalicine	BCB	3	Thakore et al. (2017)
	SEC	SE mediated mass production	Growtek bioreactor	0.5	Mujib et al. (2014)
Coleus blumei Benth	HRC	Rosmarinic acid	Airlift bioreactor	1	Bauer et al. (2015)
Dendrobium candidum Wall. ex Lindl	PSC	Biomass and SM	BTBB	3	Cui et al. (2014)
Dracocephalum forrestii W. W. Smith	SC	Rosmarinic acid	NSB	10	Weremczuk-Jezyna et al. (2019)
Eleutherococcus koreanum Nakai	ARC	Eleutherosid, chlorogenic acid	Airlift bioreactor	3	Lee et al. (2015)

(continued)

Table 16.4 (continued)

Plant Species	Type of Culture	Objective(s)	Type of bioreactor	Volume (l)	References
Hyoscyamus niger	HRC	Anisodamine scopolamine, hyoscyamine, cuscohygrine	BCB, HB	1.5	Jaremicz et al. (2014)
Hypericum perforatum L.	ARC	Hypericin	BTAB	5	Wu et al. (2014)
Oplopanax elatus Nakai	ARC	Phenolics, flavonoids	BTAB	5	Jiang et al. (2015)
Panax quinquefolius L.	ARC	Ginsenoside	BTAB	5	Yu et al. (2016)
	HRC	Ginsenoside	NSB	1.5	Kochan et al. (2017)
Polygonum multiflorum Thunb	ARC	Phenolic compounds	BTBB, pilot-scale tank bioreactor	3, 5, 20, 500	Ho et al. (2017)
Rauvolfia serpentina (L.) Benth. ex Kurz	HRC	Reserpine	Modified airlift bioreactor	5	Mehrotra et al. (2015)
Salvia officinalis L.	HRC, SC	Rosmarinic acid	NSB	5	Grzegorczyk and Wysokinska (2010)
Silybum marianum (L.) Gaertn	HRC	Silymarin	STB	2.7	Rahimi et al. (2012)
Vitis amurensis Rupr	SEC	Resveratrol	BTBB	3	Sun et al. (2016)
V. labrusca L.	CSC	Resveratrol	STB	14	Nivelle et al. (2017)
Withania somnifera (L.) Dunal	CSC	Withanolides	Bioreactor	7	Sivanandhan et al. (2014)

Adventitious root culture (ARC), Balloon-type airlift bioreactor (BTAB), Balloon-type bubble (airlift) bioreactor (BTBB), Bubble column bioreactor (BCB), Bubble column/spray hybrid bioreactor (HB), Cell suspension culture (CSC), Drum type airlift bioreactor (DTAB), Hairy root culture (HRC), Nutrient sprinkle bioreactor (NSB), Protocorm suspension culture (PSC), Shoot culture (SC), Stirred tank bioreactor (STB), Somatic embryo culture (SEC)

acid II and phenolic compounds production in CSCs of *G. sylvestre* was reported by using copper oxide nanoparticles (CuONPs) as a new generation of elicitors (Chung et al. 2019). Copper and gold nanoparticles (1:3) application showed a positive effect in enhancing biomass and SM production in adventitious root cultures of *S. rebaudiana* (Ghazal et al. 2018).

Application of Ag-SiO_2 core–shell nanoparticles (AgNPs) with an average size of 101.8 ± 8.9 nm stimulated 3.9-fold artemisinin production over the control in the HRCs of *A. annua* by induction of oxidative stress that result in lipid peroxidation and enhanced catalase activity (Zhang et al. 2013). When HRCs of *Hyoscyamus reticulatus* were elicited with different concentrations of iron oxide nanoparticles (FeNPs)

for different time periods, a 5-fold enhancement of hyoscyamine and scopolamine content was observed in comparison with the control (Moharrami et al. 2017). Application of titanium dioxide (TiO_2) nanoparticles on HRC of *S. rebaudiana* showed a maximum 24.28 mgg^{-1} DW stevioside and 21.28 mgg^{-1} DW rebaudioside production (Moradpour et al. 2019).

16.9 Application of Genome Editing Strategies and Plant Synthetic Biology in Tissue Culture of Medicinal Plants

The efficacy of conventional genetic engineering to change the secondary metabolic pathway in MPs is often limited by the lack of knowledge of the concerned metabolic pathways, unmanageable transcriptional regulation of endogenous genes, copy number variability and random insertion of recombinant DNA construct during conventional gene transformation. In recent times, synthetic promoters, 'tunable' transcription factors, genome editing tools and site-specific recombinases are available that can help plant biotechnology to improve plants faster and more accurately (Niazian 2019). The co-transformation of plant tissues with DNA constructs encoding engineered sequence-specific nucleases like Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats and its associated Cas9 protein (CRISPR/Cas9) which have customizable DNA-binding domain and desired foreigner DNA leads to precise site-specific integration of foreign DNAs (Niazian 2019).

Additionally, CRISPR/Cas9 is one of the newly emerging, suitable, promising, powerful and precise genome editing tools that can be used for gene mutation, deletion, insertion and transcriptional activation/repression of targeted gene of interest in a selected genome. Although the use of CRISPR/Cas9 system in MPs has been limited due to lack of availability of sufficient sequence information in many MPs, the application of it gradually increased in recent years due to its promising potential to regulate plant metabolic networks and improve the quality of MPs (Alok et al. 2018; Shabir 2020). Recently, Shabir (2020) reported the present status of CRISPR/Cas9-mediated genome editing in medicinal and aromatic plants as well as the applications and challenges of this new technique. For example, the expression and metabolomic analysis of edited hairy root lines of *Salvia miltiorrhiza* developed by CRISPR/Cas9-mediated targeted mutagenesis showed suppression of rosmarinic acid synthase gene and decrease in the content of rosmarinic acid and lithospermic acid B (Zhou et al. 2018). Similarly, a rapid and efficient approach for targeted genome modification in *Dioscorea zingiberensis* was also reported using CRISPR/Cas9 system via *A. tumefaciens*-mediated transformation (Feng et al. 2018).

16.10 Morphological, Phytochemical and Genetic Stability of in vitro cultures of Medicinal Plants

In spite of various applications and advantages of the PTC techniques, its broader commercial utilization as an important tool for the germplasm conservation of threatened commercially important MP species via mass proliferation and industrial production of SMs can be limited by genotypic and phytochemical variability developed by somaclonal variations in long-term cultures. Somaclonal variations are mostly useless, uncontrollable, unpredictable and may be developed due to inheritable cryptic genetic defects that include chromosomal aberrations, polyploidy, chromosomal rearrangements, DNA hypomethylation, single-gene mutations, genome adaptation to differential microelement environments and the presence of hot spots (Bhattacharyya et al. 2017a; b). Such genetic changes may result in morphological and biochemical variations in uniform in vitro cultures or regenerants.

The potential chance of development of somaclonal variations may be influenced by several factors like length of culture period, genotype and nature of explants, type and concentration of PGRs applied in culture media, culture conditions and pathway of development of the tissue cultured plants. Although the unorganized cultures such as callus and CSC are used as a platform for both micropropagation and SMs production, in many cases, biochemical and genetic instability have been reported when cultured for long term (Halder and Jha 2020). Moreover, phenotypic, biochemical and genetic instability have also been reported in tissue culture regenerants, though the frequency of such report is very low. Generally, proliferation or regeneration via induced indirect organogenesis is more prone to somaclonal variation in comparison with the clonal propagation or regeneration through proliferation of the axillary buds or direct SEs. Thus, the occurrence of somaclonal variations among the regenerates (clones) derived from the same donor mother plant during in vitro micropropagation and unorganized cultures are a major issue of concern as it disrupts the expected homogeneity of the product which is important for the conservation of elite germplasm as well as industrial production of desired phytochemicals.

Commercial utilizations demand long-term maintenance of the desired phenotypic, biochemical and genetic characters and uniformity among all raw materials (cell, tissue, organ or plant population) utilized in the preparation of medical formulations to achieve the maximum advantage of in vitro culture system. Otherwise, commercial products will slow down consumers' confidence. Thus, to avoid the potential drawback of somaclonal variations, the stringent monitoring and evaluation of the genetic stability/clonal fidelity are the prerequisites for the long-term in vitro cultures and regenerated plants for commercial applications.

Clonal fidelity and genetic stability of in vitro grown callus, cell, organ and micropropagated regenerants of MPs can be assessed by evaluating cytological parameters such as chromosome number and morphology, isozyme profile or PCR-based DNA fingerprinting profiles such as random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR), simple sequence repeats (SSR) and most recently

start codon targeted (SCoT) polymorphism (Thakur et al. 2016; Jiang et al. 2018; Rohela et al. 2019; Jena et al. 2020; Kudikala et al. 2020; Sahoo et al. 2020).

Cytological studies may be used to assess the genetic variability and stability by traditional cytological analysis (determination of chromosome number and morphology) or by flow cytometric analysis (determination of ploidy level and genome size/ DNA content) (Shinde et al. 2016; Ali et al. 2017b). Among molecular markers, RAPD is one of the most common, simple, quick and cost-effective DNA-based molecular markers of analysing the genetic fidelity of tissue culture materials (Rathore et al. 2014; Purohit et al. 2017; Faisal et al. 2018). But in spite of the various advantages, RAPD has a major issue with reproducibility. ISSR markers are polymorphic, reproducible, resolvable, informative, usually developed based on the non-coding regions of the DNA and are widely used in assessing the genetic homogeneity of tissue culture-derived plants (Prakash et al. 2016; Rohela et al. 2019; Jena et al. 2020; Kudikala et al. 2020; Sahoo et al. 2020). SCoT polymorphism is a novel, extremely reliable, cost-effective gene-targeted molecular marker technique derived from flanking ATG translation codon in the plant gene that largely resolved the various limitations of the conventional molecular markers which target a specific region of the genome (Bhattacharyya et al. 2017a). SCoT polymorphism is more sensitive than other conventional molecular markers and can detect even minute degrees of genetic variability. In the recent past, SCoT marker has been widely used in the assertion of clonal fidelity in various MPs and it is considered to be more authentic in assessing genetic homogeneity (Bhattacharyya et al. 2014; Bhattacharyya et al. 2017a; Rohela et al. 2019; Kudikala et al. 2020). It can correlate to functional genes and their corresponding traits (Bhattacharyya et al. 2014, 2017a). Some researchers used molecular markers along with flow cytometry for the assessment of genetic uniformity of in vitro cultures (Jiang et al. 2018; Jena et al. 2020).

Besides the assessment of genetic stability of cell, organ or plant cultures, the biochemical or phytochemical stability assessment is important for commercial production of SMs as genetic stability does not always assure the potential of stable drug yielding traits and stable bioactivities (Pérez-Alonso et al. 2018; Sahoo et al. 2020). Genetic variability originating via somaclonal variations in cultured cells or tissues or plants can disturb the phytoconstituents. Therefore, assessment of the SM yielding potential and bioactivity of long-term in vitro culture is preferred.

16.10.1 Morphological, Biochemical and Genetic Stability of in vitro Non-transformed Culture

From a commercial point of view, higher multiplication rate coupled with enhanced clonal stability and SM production must be ensured by the non-transformed cultures and non-transformed regenerants of important species of MP. Some of the selected studies of genetic fidelity of non-transformed regenerants has been discussed in Table

16.5. Beside these, CSCs or CCs are also evaluated for their biochemical and genetic stability. For unorganized cultures and organ cultures, genetic stability is commonly assessed by cytological and/ or flow cytometric analysis. Use of proper markers and data analysis using a variety of statistical tools provide better insight into g

16.10.2 Morphological, Biochemical and Genetic Stability of Transformed Cultures of Medicinal Plants

Different MPs have been transformed with *A. rhizogenes* or *A. tumifeciens* to develop hairy roots, transformed galls and Ri- or Ti-transformed plants to synthesize important SMs. Similar to the non-transformed cultures, assessment of genetic stability/integrity along with biochemical stability is very important for the long-term transformed cultures. HRCs are usually stable and can be used for long-term culture. Morphological, biochemical and genetic stability of HRCs and Ri-transformed medicinal plants in long-term in vitro cultures have been discussed in the review article of Roychowdhury et al. (2017). Although somaclonal variations and chromosomal abnormalities (including both aneuploidy and polyploidy) are reported in the long-term cultures specially in some unorganized cultures, apart from the variations among different clones of HRC of a single species, the instability in morphology, growth kinetics, biosynthetic potential, chromosome number and loss of integrated T-DNA gene or its expression are very rare in HRCs (Roychowdhury et al. 2017).

rolA-transgenic callus cultures of *R. cordifolia* showed stable and elevated growth with increased anthraquinone production over 14 years (Veremeichik et al. 2019). Similarly, *rolC*-transformed callus cultures of *Maackia amurensis* showed genetic and biochemical stability (Grishchenko et al. 2013) after 4 years of culture.

Long-term stability in biomass and production of TIAs by HRC of *R. serpentina* were reported by Pandey et al. (2014). Cytogenetic characterization *A. rhizogenes* transformed root lines of *R. serpentina* showed genetical stability (Ray et al. 2014b).

Morphological and biochemical stability was also reported in *crypt*-transformed *B. monnieri* plants (Paul et al. 2015) and 6-year-old *T. indica* plants grown in in vitro and ex vitro conditions (Roychowdhury et al. 2013). An interesting study, on 5 years in vitro maintained Ti- and Ri-transformed *C. roseus* plants showed that Ri-transformed plants are more genetically stable than Ti-transformed plants whereas biochemically both are stable (Verma et al. 2015).

Table 16.5 List of some selected scientific publications on stability of in vitro grown or regenerated plants published during 2010 to June 2020

Plant species	Method used (no. primer used)	Plant materials (no. of sample used)	Result of stability Study			References
			Genetic	Biochemical/bioactivity	Morphological	
Aloe barbadensis Mill	RAPD (20), ISSR (15)	In vitro grown leaf-regenerated plantlets (24)	No polymorphism	ND	Normal growth and no sign of morphological variation	Sahoo and Rout (2014)
Alpinia calcarata Rosc	RAPD (20), ISSR (10)	6-month-old hardened plants which derived from rhizome via direct shoot organogenesis (7)	95% and 100 % monomorphism for RAPD and ISSR respectively	ND	ND	Bhowmik et al. (2016)
Alpinia galanga L.	RAPD (25), ISSR (15); GC–MS	6-year-old micropropagated plantlets (295- over a period of 6 years in an interval of 6 months by randomly taking at least 20 plants in every time)	No genetic variations	No significant variations in the essential oil yield, total phenolic and flavonoid contents, and bioactivities	ND	Sahoo et al. (2020)
Amomum subulatum Roxb	RAPD (40)	Tissue culture raised plantlets (10)	3 primers produced polymorphic bands	ND	Morphological variation observed	Purohit et al. (2017)
Annona reticulata L.	SCoT (10), ISSR (10)	Node-derived in vitro regenerated plantlet (6)	Regenerated plantlets are monomorphic and true-to-type with mother plant	ND	ND	Kudikala et al. (2020)

(continued)

Table 16.5 (continued)

Plant species	Method used (no. primer used)	Plant materials (no. of sample used)	Result of stability Study			References
			Genetic	Biochemical/bioactivity	Morphological	
Anoectochilus formosanus Hayata	ISSR (50)	5-year-old micropropagated plantlets derived from axillary bud (20)	Low risk of genetic instability, cluster analysis showed 2.76% polymorphism rate	ND	ND	Zhang et al. (2010)
Ansellia africana Lindl	SCoT (45)	4-month-old tissue culture raised plants (NM)	Polymorphism detected	ND	No morphological abnormalities	Bhattacharyya et al. (2017a)
Artemisia nilagirica (C.B. Clarke) pamp	Cytological analysis, ISSR (15)	5-month-old node-derived regenerated plants (10)	No change in chromosome number and no polymorphism	ND	Morphologically similar	Shinde et al. (2016)
Bacopa monnieri (L.) Wettst	SPAR, ISSR (103), RAPD (35), Flow cytometry	In vitro propagated plants (NM)	No polymorphism	ND	ND	Khilwani et al. (2016); Saha et al. (2020)
Boerhaavia diffusa L.	RAPD (14)	Node-derived regenerated field-grown plants (NM)	No polymorphism	ND	ND	Patil and Bhalsing (2015)

(continued)

Table 16.5 (continued)

Plant species	Method used (no. primer used)	Plant materials (no. of sample used)	Result of stability Study			References
			Genetic	Biochemical/bioactivity	Morphological	
Cannabis sativa L.	ISSR (18); GC-FID	8-month-old in vivo grown regenerated plants after up to 30 passages in culture (11)	No genetic polymorphism	No significant difference in cannabinoids content	Morphologically similar	Lata et al. (2016a, b)
Capparis decidua (Forsk.) Edgew	RAPD (8)	Long-term culture leaf-derived regenerated plantlet (8)	No polymorphism	ND	ND	Tyagi et al. (2010)
Carum copticum L.	Flow cytometry	Regenerated plants via indirect somatic embryogenesis and indirect shoot regeneration (NM)	DNA content, and genome size stability of regenerated plants	ND	ND	Niazian et al. (2017)
Catharanthus roseus (L.) G. Don	RAPD (20)	Node-derived regenerated plants (21)	No polymorphism	ND	Morphologically similar	Kumar et al. (2013)
Chlorophytum borivilianum Sant. et Fernand	RAPD (100), DNA Fingerprinting profiles, Cytology	In vitro and in vivo grown shoot bud-derived micropropagated plants (15)	Monomorphism with the mother plant, somatic chromosome number stable	ND	No gross morphological variation	Samantaray and Maiti (2010); Basu and Jha (2014)

(continued)

Table 16.5 (continued)

Plant species	Method used (no. primer used)	Plant materials (no. of sample used)	Result of stability Study			References
			Genetic	Biochemical/bioactivity	Morphological	
Cleome gynandra L.	RAPD (24)	Node-derived micropropagated plants grown in green house (7)	No polymorphism	ND	ND	Rathore et al. (2014)
Coriandrum sativum L.	Flow cytometry	Root-derived regenerated plants via primary and secondary somatic embryogenesis (NM)	Genetically stable and genome size similar	ND	ND	Ali et al. (2017)
Croomia japonica Miq.	SSR (10), Flow cytometry	In vitro rhizome-derived regenerants (39)	No polymorphism and stable ploidy level	ND	ND	Jiang et al. (2018)
Curcuma zedoaria (Christm.) Roscoe	ISSR (27), Flow cytometry; GC–MS, HPLC	Axillary bud-derived in vitro propagated plants (20) and in vivo grown plants (20)	No polymorphism and low level of difference in their nuclear DNA content	Similarity in phytochemical profile was observed with total of 49 components in leaf and 57 compounds in rhizome	No significant morphological changes	Jena et al. (2020)

(continued)

Table 16.5 (continued)

Plant species	Method used (no. primer used)	Plant materials (no. of sample used)	Result of stability Study			References
			Genetic	Biochemical/bioactivity	Morphological	
Cymbidium giganteum Wall. Ex Lindl	RAPD (40)	Pseudostem-derived micropropagated plantlets (17)	Overall 5.81% change in the regenerants detected, 17 primers produce polymorphism	ND	TDZ derived plants showed phenotypic variation depending TDZ concentrations used	Roy et al. (2012)
Dendrobium nobile Lindl	RAPD (80), SCoT (35); colorimetric method	Pseudostem-derived micropropagated plantlets (NM)	97% genetic fidelity among regenerants	In vitro raised plants exhibited a higher degree of SMs and free radical scavenging activity than the mother plant	ND	Bhattacharyya et al. (2014)
Digitalis purpurea L.	RAPD (8); HPLC	De novo regenerated plantlets (12) via direct organogenesis from leaf	No genetical variation	No differences on cardenolidecon tent	ND	Pérez-Alonso et al. (2018)
Eclipta alba (L.) Hassk	RAPD (40)	In vitro regenerated plant (12)	No genetical variation	ND	ND	Bardar et al. (2015)
Gloriosa superba L.	ISSR (30), RAPD (50)	After 100 days of hardening micropropagated plants (8)	Genetically identical and stable	ND	No morphological variations	Yadav et al. (2013)

(continued)

Table 16.5 (continued)

Plant species	Method used (no. primer used)	Plant materials (no. of sample used)	Result of stability Study			References
			Genetic	Biochemical/bioactivity	Morphological	
Kelussia odoratissima Mozaff	AFLP (10)	Regenerated plantlets via somatic embryogenesis (12)	No polymorphism	ND	ND	Ebrahimi et al. (2018)
Ledebouria revoluta (L.f.) Jessop Syn. *Scilla indica* (Wight) Baker	Cytological analysis	Callus and 1-year-old in vitro and ex vitro plants derived from callus mediated shoot organogenesis (NM)	No variation in ploidy level	All the tissue culture-derived ex vitro field-grown plants have little better anti-microbial activity as compared to naturally propagated in vivo parental plants	Flowering and no morphological variation	Haque et al. (2018)
Nothapodytes nimmoniana (Graham) Mabb	ISSR (60); HPLC	Field transferred mature regenerated plants (10)	Except two primers, no monomorphic band	No significant variation in camptothecin content	Phenotypically similar	Prakash et al. (2016)
Ocimum basilicum L.	RAPD (56), ISSR (17)	Node-derived in vitro-raised plantlets (40)	In vitro-raised plantlets produce 100% monomorphic	ND	ND	Saha et al. (2014)

(continued)

Table 16.5 (continued)

Plant species	Method used (no. primer used)	Plant materials (no. of sample used)	Result of stability Study			References
			Genetic	Biochemical/bioactivity	Morphological	
Pelargonium sidoides DC	HPLC	Roots of 2-year-old plants that were propagated via somatic embryogenesis (NM)	ND	No variation in coumarin content	ND	Duchow et al. (2015)
Phyllanthus fraternus Webster	RAPD (15), ISSR (15)	Acclimatized synseed-derived regenerated plants (16)	Polymorphism were detected with 3 RAPD primers	ND	Flowering and morphologically similar	Upadhyay et al. (2014)
Pittosporum eriocarpum Royle	SCoT (20), ISSR (15), RAPD (15)	Node-derived in vitro-raised plantlets (9)	One RAPD primer showed polymorphic bands in 3 regenerates, UPGMA analysis revealed 97% similarity	ND	ND	Thakur et al, (2016)
Podophyllum hexandrum Royle (Berberidaceae)	RAPD (10)	Rhizome regenerated plants (NM)	No polymorphism detected	ND	ND	Tariq et al. (2015)
Rauvolfia serpentina (L.) Benth. ex Kurz	Flow cytometry	In vitro regenerated plants (NM)	No significant variation in nuclear DNA content	ND	ND	Zafar et al. (2019)

(continued)

Table 16.5 (continued)

Plant species	Method used (no. primer used)	Plant materials (no. of sample used)	Result of stability Study			References
			Genetic	Biochemical/bioactivity	Morphological	
Rauvolfia tetraphylla L.	SCoT (10), ISSR (10), RAPD (10)	Leaf callus-based (4) and stem callus-based (3) acclimated regenerants	No polymorphism and no significant variation in nuclear DNA content	ND	ND	Rohela et al. (2019)
Rhazya stricta Decne	ISSR (16), RAPD (20)	In vitro micropropagated plants	Low frequency of polymorphism in RAPD and ISSR profile	ND	Phenotypically similar	Mohamed et al. (2014)[1]
Rumex nepalensis Spreng	SCoT (36), RAPD (45)	In vitro DSO and IDO mediated regenerated plants (10 each)	DSO-propagated plants showed higher genetic stability that ISO-propagated plants	In both DSO and ISO-regenerated plants the yield of the phytochemicals increased significantly in comparison with the mother plant	Plants developed normal inflorescence and flower morphologically similar	Bhattacharyya et al. (2017b)
Ruta graveolens L.	ISSR (10), RAPD (10)	Shoot tip-derived in vitro plants (10)	No polymorphism	ND	Morphological variation absent	Faisal et al. (2018)
Solanum aculeatissimum Jacq	Flow cytometry	Leaf-derived regenerated plantlets (30)	Ploidy stability	ND	Uniform morphology and normal flowering	Ghimire et al. (2012)

(continued)

Table 16.5 (continued)

Plant species	Method used (no. primer used)	Plant materials (no. of sample used)	Result of stability Study			References
			Genetic	Biochemical/bioactivity	Morphological	
Spilanthes oleracea L.	RAPD (60); HPLC	Node-derived in vitro regenerated plantlets (6)	No polymorphism	No significant difference in scopoletin content	Flowering with no morphologically variation	Dandin et al., (2014)
Stevia rebaudiana Bertoni	ISSR(15)	Tissue culture-derived plants (NM)	Low genetic variations	ND	ND	Soliman et al. (2014)
Tylophora indica (Burm. f.) Merr	RAPD (20), ISSR, chromosomal	In vitro regenerants (14)	Majority reports showed genetic stability, whereas other reported 37.5–62.1% polymorphism	ND	ND	Da Silva and Jha (2016)
Uraria picta (Jacq.) DC	RAPD (20), HPLC	Node-derived in vitro regenerants (NM)	Genetically identical	No significant difference in isoflavonones content	Flowering and no morphological variation	Rai et al. (2010)
Vitex trifolia L.	RAPD (40), ISSR (13)	Acclimatized node-derived regenerated plants (10)	Genetically uniform	ND	No morphologically variation	Ahmad et al. (2013); Alatar et al. (2017)
Withania somnifera (L.) Dunal	RAPD (20), ISSR (10)	In vitro grown regenerated plantlets (14)	No polymorphism	ND	ND	Nayak et al. (2013)

AFLP = Amplified fragment length polymorphism, GC–MS = Gas chromatography–mass spectrometry, HPLC = High-performance liquid chromatography, ISSR = Inter-simple sequence repeat, RAPD= Random amplified polymorphic DNA, SCoT= Start codon targeted, SPAR= single primer amplification reaction, SSR= Simple sequence repeat. All regenerated plants were selected randomly for stability determination.
DSO = Direct shoot organogenesis, ISO = Indirect shoot organogenesis, ND = not determined, NM = not mentioned

16.11 Case Study 1: *Podophyllum hexandrum*

Podophyllum hexandrum Royle (syn. *P. emodi* Wall., family Berberidaceae) commonly known the Himalayan Mayapple or the Indian Podophyllum is a medicinally important herbaceous plant. It is the commercially exploitable plant source of podophyllotoxin ($C_{22}H_{22}O_8$) (Fig. 16.5), a pharmaceutically active lignan. Podophyllotoxin is used as a precursor for the synthesis of important anti-tumour drugs like etoposide (VP-16-213) and teniposide (VM-26) which are used in the treatment of lung cancer, testicular cancer, a variety of leukemias and other solid tumours (Imbert 1998; Gordaliza et al. 2004).

P. hexandrum is an erect, glabrous, succulent herb, 35–60 cm high with creeping perennial rhizome having numerous roots, found in the Himalayan alpine and subalpine zones from Kashmir to Sikkim at altitudes of 2200–4300 m in India (The Wealth of India 1969). From the Indian Himalayas, it has dispersed to Bhutan, Pakistan, Afghanistan, Nepal and China (Chaurasia et al. 2012). The Indian

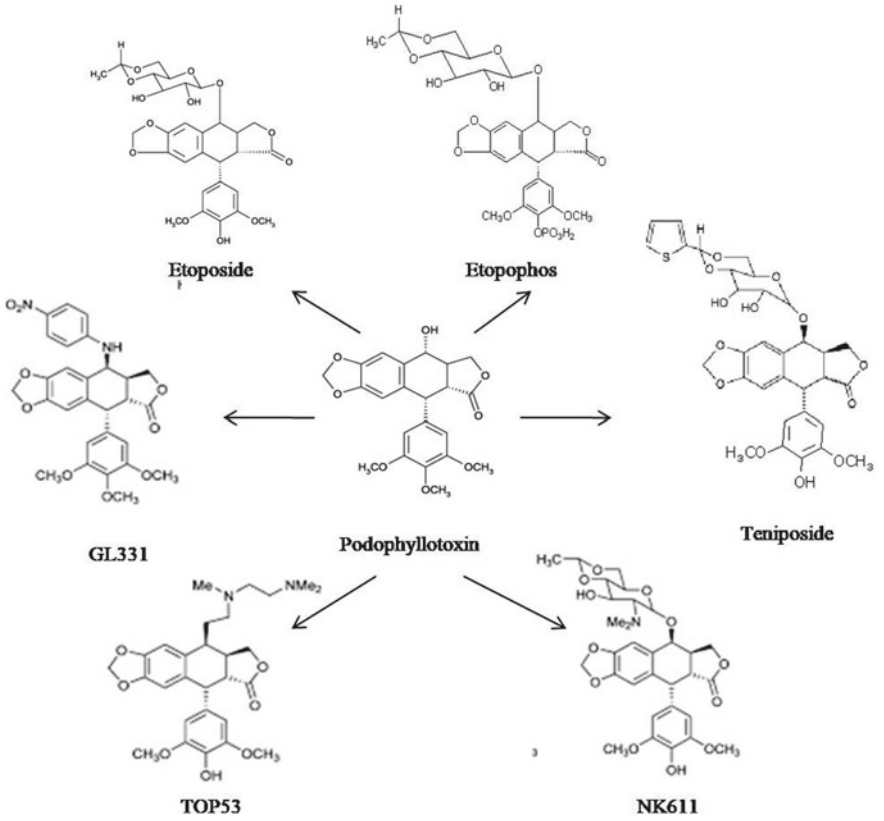


Fig. 16.5 Chemical structure of podophyllotoxin and some of its derivatives

Podophyllum yields 7–15% resin as compared to the American *Podophyllum* or *P. peltatum,* distributed in the Atlantic North America (Chaurasia et al. 2012), which yields only 4–8% resin (Thakur et al. 2010; Qazi et al. 2011). Podophyllotoxin content of rhizomes ranges between 0.36–1.08% DW (Nadeem et al. 2007).

Podophyllotoxin is in high demand in the global market. But *P. hexandrum* is sparse due to its prolonged juvenile stage and poor ability to set fruits. Rhizomes are being indiscriminately collected from the wild to meet the global demand of podophyllotoxin, thus reducing *P. hexandrum* populations. Overexploitation and lack of organized cultivation have made the plant 'critically endangered' (Airi et al. 1997). As podophyllotoxin is immensely important medicinally, new routes for total synthesis of podophyllotoxin have been discovered (Bush and Jones 1995; Berkowitz et al. 2000). But yields are low, and the processes are not viable economically. Thus, the only feasible option is to isolate podophyllotxin from plant sources.

P. hexandrum is a slow growing species (Kushwaha et al. 2008). In nature, the seeds remain dormant for about 10 months, an adaptation to get through the harsh climatic conditions of high altitudes (Badhwar and Sharma 1963). The plant also has a low seed number (Kim et al. 2007) and propagates mostly through the rhizomes (Alam et al. 2009). As the species has already acquired a 'critically endangered' tag and the rate of natural propagation is far less than the harvest of the underground parts, safeguarding the existence of this species is of utmost importance. Thus, efforts are given to develop alternative sources and methods of production of podophyllotoxin and biotechnological approaches, particularly plant cell and tissue cultures of *P. hexandrum* appear to be suitable alternatives for producing this pharmaceutically important lignan, together with conserving this immensely important species.

16.11.1 In vitro Cultures as Means of Podophyllotoxin Production from P. hexandrum

16.11.1.1 Cell Cultures for Podophyllotoxin Production

Large number of studies have been carried out for enhancing the accumulation of podophyllotoxin in in vitro cultures of *P. hexandrum* by optimizing culture conditions and nutrient levels, addition of elicitors and precursors, immobilization, etc. (Majumder and Jha 2009a; Dhiman et al. 2016; Nandagopal et al. 2018).

P.hexandrum is a very recalcitrant species with respect to callus induction in vitro and successful callus cultures of Indian *P. hexandrum* (Fig. 16.6a) were initiated and established from in vitro grown axenic seedling explants and roots and rhizomes isolated from 1-year-old mature plants on B5 and MS media supplemented with growth regulators by Majumder (2008). Cell lines established subsequently differed distinctly in growth rates and podophyllotoxin content and selection of fast-growing cell lines and recloning of such lines led to establishment of cell lines capable of optimum growth, accumulating optimum levels of podophyllotoxin (Majumder and

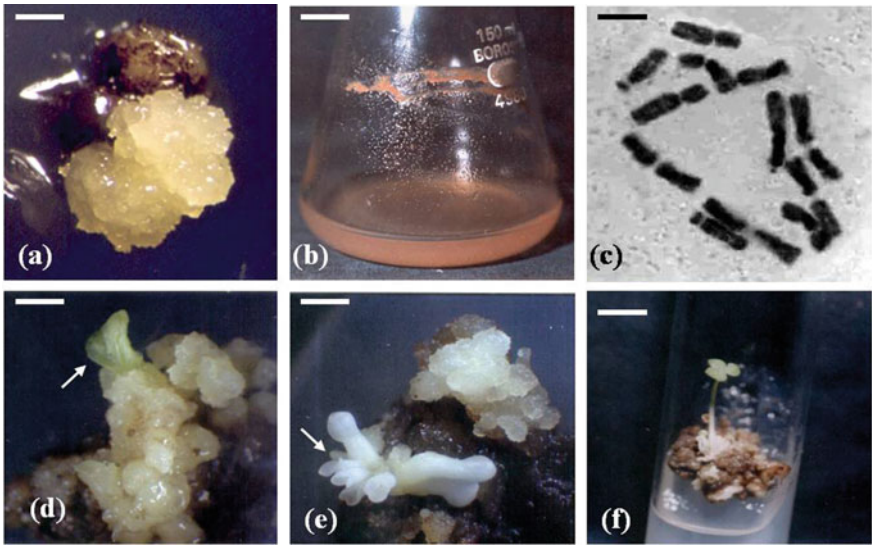


Fig. 16.6 **a** Mature root-derived callus of *Podophyllum hexandrum* (*Bar: 2.5* mm), **b** Cell suspension culture (*Bar: 10* mm), **c** Diploid plate showing 12 chromosomes from 2-year-old cell line (*Bar: 15* μm), **d** Leaf differentiation (arrow) from shoot bud (*Bar: 3* mm), **e** Development of shoot buds from bud primordia on MS medium supplemented with BA and NAA (*Bar: 3* mm), **f** Elongated microshoot (*Bar: 8* mm)

Jha 2007; Majumder and Jha 2009b). Podophyllotoxin was detected from the leaf induced calli by Chakraborty et al. (2010).

Since podophyllotoxin, a lignan, is a product of the phenylpropanoid pathway, the effects of different concentrations of direct precursors from the pathway (phenylalanine, tyrosine, *trans*-cinnamic acid, *para*-coumaric acid) and one indirect precursor (tryptophan) of podophyllotoxin on CSCs (Fig. 16.6b) of the Indian Podophyllum were studied (Majumder 2008; 2012). A maximum increase in podophyllotoxin accumulation of 4.5-fold over untreated control cultures was noted after the addition of *para*-coumaric acid. Content was also enhanced noticeably (2 to 4-fold over control) after adding tyrosine, tryptophan and phenylalanine to CSCs (Majumder 2008; 2012). Addition of precursors for improving the production podophyllotoxin in CSCs of *P. hexandrum* is also reported by previous workers.

Elicitation is an effective strategy to enhance the production of plant SMs in in vitro cultures. Elicitors are signal molecules which trigger the formation of SMs by activating novel genes encoding enzymes in different biosynthetic pathways. Till date there are very few reports on elicitation in cell cultures of *P. hexandrum*. The effect of two well-known elicitors—salicylic acid (SA) and methyl jasmonate (MeJA)—on CSCs of the *P. hexandrum* was analysed in our laboratory, and a remarkable increase in podophyllotoxin accumulation was noted after the addition of SA (Majumder 2008). In contrast, Bhattacharyya et al. (2012) noted 7–8-fold change

in podophyllotoxin accumulation in a 12-day-old CSC of *P. hexandrum* developed from leaf-derived calli after elicitation with 100 μM MeJA.

16.11.1.2 Organ Cultures, in vitro Regeneration and Propagation

In an earlier study, Sagar and Zafar (2005) reported that mother root explants excised from aseptically germinated seedlings of *P. hexandrum* showed induction of lateral roots and calli on Gamborg's B5 and MS media containing auxins and cytokinins. Roots also regenerated from induced calli on Gamborg's B5 and MS media supplemented mostly with auxins and podophyllotoxin could be detected in root cultures. Later on, in vitro root cultures were initiated from rhizomes isolated from in vitro grown plantlets of *P. hexandrum* on MS medium supplemented with different concentrations of IBA, GA_3, hydroquinone and activated charcoal (Li et al. 2009). Podophyllotoxin could be detected in the roots and culture medium. In an attempt to enhance rooting of *P. hexandrum*, in vitro proliferated shoots were cultured on WPM (woody plant medium) supplemented with various auxins (IAA, NAA and IBA) (Guo et al. 2012). The best response was obtained with IAA (1.5 mgl^{-1}) and NAA (0.5 mgl^{-1}). The plantlets could be transplanted successfully. Rajesh et al. (2014a) explored the potentiality of different explants from in vitro germinated seedlings of *P. hexandrum* in generating adventitious roots on MS medium supplemented with various types of auxins. The authors demonstrated that several nutrient parameters (viz. carbon sources, medium strength, initial medium pH, ammonium and nitrate proportion and phosphate ratio) affected higher biomass and podophyllotoxin accumulation in the adventitious root cultures derived from the root segments.

Strategies for in vitro regeneration and propagation of *P. hexandrum* have been developed. In vitro multiplication via multiple shoot formation from zygotic embryos and subsequent rooting was first reported by Nadeem et al. (2000). Sultan et al. (2006) inoculated roots from in vitro germinated seedlings of *P. hexandrum* on ½ strength Gamborg's medium supplemented with 2,4-D (0.5–1.5 mgl^{-1}) and BA (0.2–1.5 mgl^{-1}) for induction of callus cultures. When calli were transferred to MS medium with BA (0.5–5.0 mgl^{-1}) and IAA (0.5–3.0 mgl^{-1}) for shoot regeneration, medium supplemented with 0.5 mgl^{-1} BA and 1.0 mgl^{-1} IAA together with 1% activated charcoal resulted in maximum number of shoots. The regenerated shoots rooted on ½ strength MS medium with activated charcoal, NAA (0.5–2.0 mgl^{-1}) and IAA (1.0–2.0 mgl^{-1}), which were subsequently hardened and transferred to soil.

Chakraborty et al. (2010) developed an efficient protocol for regenerating plantlets of *P. hexandrum* in vitro through direct organogenesis using rhizome explants. MS medium supplemented with 11.42 μM IAA resulted in maximum rate of multiple shoot formation from rhizome explants followed by a combination of NAA and BAP at higher concentrations. Yellowing of leaves was noted when the shoots were maintained in the culture conditions for more than 4 months. The shoots rooted on IBA supplemented ½ strength liquid MS medium.

Regeneration of shoots was observed again from rhizome segments cultured on MS medium supplemented with various concentrations of auxins (viz. IAA and

NAA) and cytokinins (viz. BAP and TDZ), with high concentrations of BAP and NAA exhibiting maximum shoot regeneration (Tariq et al. 2015). The shoots rooted on ½ strength MS medium with varying concentrations of IBA.

Deb et al. (2018) propagated *P. hexandrum* in vitro using excised embryos from mature seeds. The embryos germinated to give rise to prominent cotyledonary tube with multiple leaves on MS medium supplemented with BA (1.0 μM) and GA_3 (0.1 μM). The shoots rooted on IAA (1.0 μM) supplemented MS medium. The in vitro propagated shoots were hardened and transferred to fields.

Plant regeneration via somatic embryogenesis in *P. hexandrum* was first reported by Arumugam and Bhojwani (1990), followed by Nadeem et al. (2000). Recently, Rajesh et al. (2014b) developed another efficient system for in vitro somatic embryogenesis and podophyllotoxin production from seeds collected from three different regions of the Himalayas. SEs were induced from zygotic embryos either directly or through the formation of embryogenic callus. Different types and concentrations of auxins (viz. 2,4-D, NAA, picloram) were used to induce SEs directly from zygotic embryos of which the highest percentage (89.6%) of SE induction was observed in the Milam variety using MS medium supplemented with 1.5 mgl^{-1} 2,4-D. Transfer of the induced SEs to auxin supplemented medium resulted in the formation of embryogenic as well as non-embryogenic callus depending on the type of auxin used. The embryos developed further on 2,4-D free media and germinated on MS basal medium and GA_3 supplemented MS medium. The germinated SEs as well as germinated zygotic embryos produced podophyllotoxin. In continuation with their studies, embryogenic callus initiated from the zygotic embryos was also cultured in liquid MS medium supplemented with different concentrations of 2,4-D and NAA along with PVP (Rajesh et al. 2014c). The effect of variable medium strength and carbon source on induction of SEs and podophyllotoxin accumulation was studied. The embryos matured on ABA supplemented 0.75 strength liquid MS medium and synthesized appreciable amounts of podophyllotoxin. Similar to their previous report, the embryos germinated on GA_3 supplemented liquid medium and synthesized podophyllotoxin. A survival rate of 44% in growth chamber was noted following proper acclimatization of the regenerated plantlets.

Sporadic shoot organogenesis (Fig. 16.6d–f) was observed in callus cultures maintained on MS basal medium supplemented with BA and NAA for 12 weeks, cultures being derived from mature roots by Majumder (2008). The calli developed nodular structures after 12 weeks which transformed into bud primordia like structures within another 3 weeks of culture. Shoot buds developed upto 1–2 cms with leaf differentiation when transferred onto fresh medium under 16/8 h (light/dark) photoperiod but did not grow further even after trials with other combination and concentration of different cytokinins with or without auxin.

16.11.2 Genetic Transformation of P. hexandrum

Initiation of root cultures in *P. hexandrum* through transformation using *A. rhizogenes* was attempted in our laboratory (Majumder 2008). Three wild-type virulent agropine strains of *A. rhizogenes* (A4, LBA9402, 15834), two supervirulent strains (R1600, R1601) and a mannopine strain 8196 were used for infectivity studies by co-culturing *A. rhizogenes* overnight cultures with mature and immature axenic explants of *P. hexandrum* and 2-year-old callus cultures maintained in the laboratory. However, none of the strains of *A. rhizogenes* used could induce roots in callus cultures or mature and immature axenic explants of *P. hexandrum* under any cultural conditions studied. Wound sites of inoculated mature and immature explants necrosed within 2 weeks of inoculation and after another 2 weeks of culture on antibiotic supplemented medium, whole of the explants turned dark brown. Although the inoculated calli continued to proliferate on medium supplemented with antibiotic, roots were not induced from the callus cultures (Majumder 2008). Giri et al. (2001) reported infection of embryos of *P. hexandrum* with *A. rhizogenes* (strains K599, A4, 15834) and induction of callus producing podophyllotoxin. Successful hairy root cultures of *P. hexandrum* has not been reported to date.

In a more recent attempt, Rajesh et al. (2013) studied different factors affecting the efficiency of transformation (viz. *Agrobacterium* strain, co-cultivation duration and acetosyringone concentration) using embryogenic callus of *P. hexandrum.* Of the three different *A. tumefaciens* strains [LBA 4404, EHA 101 and EHA 105 harbouring the binary vector pCAMBIA 2301 (CAMBIA, Canberra, Australia)] used by the authors, EHA 105 had the highest transformation efficiency (10.79%) followed by EHA 101 (5.50%) and LBA 4404 (2.16%). A notable effect of the strain of *A. tumefaciens* and acetosyringone concentration on transformation efficiency was reported. Another important factor controlling transformation efficiency was the duration of co-cultivation of *Agrobacterium* with the explants, with a period of 3 days to be optimum for a transformation efficiency of 29.64%. Bhattacharyya et al. (2016a) transformed *P. hexandrum* cell cultures and callus by using transformed *A. tumefaciens* (GV3101). The transgenic lines overexpressed the four *PhCAD* isoforms (cinnamyl alcohol dehydrogenase or CAD) with *PhCAD3* favouring the highest accumulation of podophyllotoxin as compared to lignin followed by *PhCAD4* and *PhCAD2*, whereas *PhCAD1* favoured both equally.

Thus *P. hexandrum* is a recalcitrant species with respect to morphogenesis as well as genetic manipulation and more research is required for improving efficiency of genetic transformation in the species.

16.11.3 *Genetic and Biochemical Stability in in vitro Cultures of* P. hexandrum

Tariq et al. (2015) could successfully conserve in vitro propagated plants of *P. hexandrum* from rhizome segments, as confirmed by RAPD analysis. The monomorphic banding pattern highlighted the fact that plants propagated in vitro do not necessarily undergo any genetic change.

When cell lines derived following screening and selection of cell suspension cultures of *P. hexandrum* were analysed using 28 random decamer primers (Majumder and Jha 2009b), polymorphic banding patterns were observed, indicating genomic variability among the cell lines. Also, when the cell lines were analysed after 2 years of initiation, considerable variation in podophyllotoxin content was noted between cell lines (0.15–0.45% DW). The content decreased in all the lines till fourth year of initiation (0.03–0.2% DW) after which the lines became stable in producing podophyllotoxin (Majumder and Jha 2009b).

16.11.4 *Recent Developments and Application of "-omics" to in vitro Cultures of* P. hexandrum

Podophyllotoxin is a lignan, a compound containing phenylpropanoid dimers (C_6C_3), connected by C8 carbon located at the side chain of each unit (Umezawa 2003). In plants, its biosynthesis occurs via the phenylpropanoid pathway (Wankhede et al. 2013). The biosynthetic pathway of podophyllotoxin is more or less elucidated and several cDNAs have been reported (Bhattacharyya et al. 2013). In general, mechanisms of enantioselective dimerization of monolignols are involved in the process of lignan biosynthesis (Bhattacharyya et al. 2013, 2016a).

In an attempt to analyse the cell proteome related to enhanced podophyllotoxin accumulation in elicited CSC of *P. hexandrum*, Bhattacharyya et al. (2012) performed 2-DE proteomic profiling of CSC of *P. hexandrum* elicited with MeJA together with untreated control cultures. Of the various functional groups of proteins identified, 13% were related to SMs. Several enzymes related to monolignol/phenylpropanoid biosynthesis [viz. methyl transferases like CCOMT (caffeoyl CoA-O-methyltransferase) and CAOMT (caffeic acid-O-methyltransferase)] were identified. From their study, the authors suggested that the expression of upstream genes (viz. CCOMT and CAOMT) of monolignol pathway could control the biosynthesis of podophyllotoxin.

Next generation sequencing data of de novo transcriptome analysis can be used for identification of unknown genes in non-model organisms. The transcriptome of *P. hexandrum* cell culture was sequenced by Bhattacharyya et al. (2013). The authors reported 454 pyrosequencing from a CSC of *P. hexandrum* to resolve the genes involved in podophyllotoxin biosynthesis, and nearly all the members of the phenylpropanoid pathway were identified using next generation whole transcriptome

sequencing. In addition, the authors also identified EST-SSRs as molecular markers claiming to be applicable in the conservation of this endangered species.

Of the several factors playing crucial roles in gene regulation are microRNAs or miRNAs which are small (~22 nt) single stranded, non-coding RNA molecules (Bartel 2004). These RNAs play vital roles in several developmental processes in plants. In yet another interesting study, Biswas et al. (2016) used 454 pyrosequencing to identify 60 mature miRNAs and 6 pre-miRNAs in *P. hexandrum* CSCs. The authors utilized different bioinformatics tools to show that the targets of these miRNAs are genes involved in primary and secondary metabolism.

Cinnamyl alcohol dehydrogenase or CAD (EC 1.1.1.95) is an NADPH-dependent enzyme in the phenylpropanoid pathway, catalyzing the synthesis of coniferyl alcohol from coniferaldehyde and sinapyl alcohol from sinapaldehyde. Full length cDNA sequences of the CAD isoforms (*PhCAD1, PhCAD2, PhCAD3* and *PhCAD4*) were deduced by Bhattacharyya et al. (2016a) to isolate CAD isoforms in favour of podophyllotoxin from MeJA elicited CSCs of *P. hexandrum*. Major transcripts of podophyllotoxin biosynthesis were identified using next generation sequencing technology. Each isoform had a higher affinity for coniferaldehyde compared to sinapaldehyde, as indicated by in vitro enzyme assays. Their study revealed transcriptome wide identification and characterization of CAD isoforms specific for podophyllotoxin biosynthesis. The transcriptome data also demonstrated the role of MeJA in controlling different genes and transcription factors in relation to the biosynthesis of podophyllotoxin.

In yet another study, Hazra et al. (2017) demonstrated that MeJA induced the production of reactive oxygen species (ROS) which stimulated the accumulation of podophyllotoxin remarkably in cell cultures of *P. hexandrum.* The mRNA stability of three ROS-responsive biosynthetic genes of podophyllotoxin, viz. *PhCAD3*, *PhCAD4* and *NAC3,* was also increased leading to their upregulation. The authors also noted that other genes involved in the podophyllotoxin biosynthesis pathway, unaffected by MeJA induced ROS, were also upregulated by MeJA. Furthermore, MeJA controlled the genes non-responsive to ROS through the down-regulation of five miRNAs (miR172i, miR035, miR1438, miR2275 and miR8291) specific for biosynthesis of SMs. Through their study, the authors suggested that MeJA controls the biosynthesis of podophyllotoxin by two possible modes (a) by increasing the stability of mRNAs of genes responsive to ROS and (b) up regulation of genes non-responsive to ROS through the down-regulation of some miRNAs non-responsive to ROS.

16.12 Case Study 2. *Rauvolfia serpentina*

Rauvolfia serpentina (Linn.) Benth. ex Kurz. is an endangered plant having immense medicinal properties, traditionally used for ages. Pathania et al. (2013) created an extensive compilation of 147 plant-derived molecules (PDMs) reported to be extracted from various plant parts of *R. serpentina.* Terpenoid indole alkaloids

(TIAs) of *R. serpentina* currently in clinical use include ajmalicine and serpentine as anti-hypertensive and ajmaline as anti-arrhythmic (Yang and Stöckigt 2010) while reserpine is a major component of various market drugs. Reserpine (3,4,5-trimethyl benzoic acid ester of reserpic acid) is the most common alkaloid which causes depletion in the catecholamine (epinephrine and norepinephrine) and serotonin levels in the brain and peripheral nervous system, thereby leading to sedative, anti-hypertensive and anti-arrhythmic properties (Rolf et al. 2003).

16.12.1 Micropropagation as a Means of Production of Secondary Metabolites and Stability in Production

Micropropagation of the traditional MP *R. serpentina* is being worked upon extensively. Alkaloid extraction from micropropagated plants is a time saving method for commercialization of medicinally important SMs. Bahuguna et al. (2011) reported that total alkaloid content of in vitro multiple shoots (6 weeks old) was similar to the alkaloid content from leaf and stem of field-grown plants (1 year old). Gantait and Kundu (2017) reported the reserpine content of plantlets germinated from synthetic seeds stored at 25 °C and 8 °C to be 203.38 $\mu g g^{-1}$ DW and 249.37 $\mu g g^{-1}$ DW, respectively, indicating that lower temperature is suitable for maintaining alkaloid content during synthetic seed preservation. In vitro cultures thus retained the alkaloid synthesizing capacity which was often enhanced in comparison with that of the parent plant.

16.12.2 Genetic Transformation

A. rhizogenes mediated genetic transformation has provided an immense potential for generation of genetically stable, fast-growing hairy root lines having high yield of SMs in hormone-free culture media. This technology has been successfully applied in *R. serpentina* since 1993 by Benjamin et al. and Falkenhagen et al. (1993) and since then extensively exploited till date. Explants from in vitro cultures of *R. serpentina* have responded to different virulent strains of *A. rhizogenes* including 15384 (Benjamin et al. 1993), ATCC SV2, SV4 (Sarma et al. 1997), A4 (Mehrotra et al. 2013a), LBA 9402 (Ray et al. 2014a), MTCC 532 and 2364 (Bhagat et al. 2019). Significant variation in reserpine accumulation was observed in LBA9402 transformed hairy root lines with 3.11 $mg g^{-1}$ DW being the overproduction maxima (Ray et al. 2014a; Fig. 16.7). Researchers have focused on the enhanced SM production in the transgenic root lines. Mehrotra et al. (2013a) reported high reserpine content in roots of spontaneously regenerated plantlets grown for 6 months in green house condition (0.0889% DW) and their parent HRCs (0.0882% DW) which was significantly higher than normal roots (0.0180% DW). Interestingly 90% of the regenerated

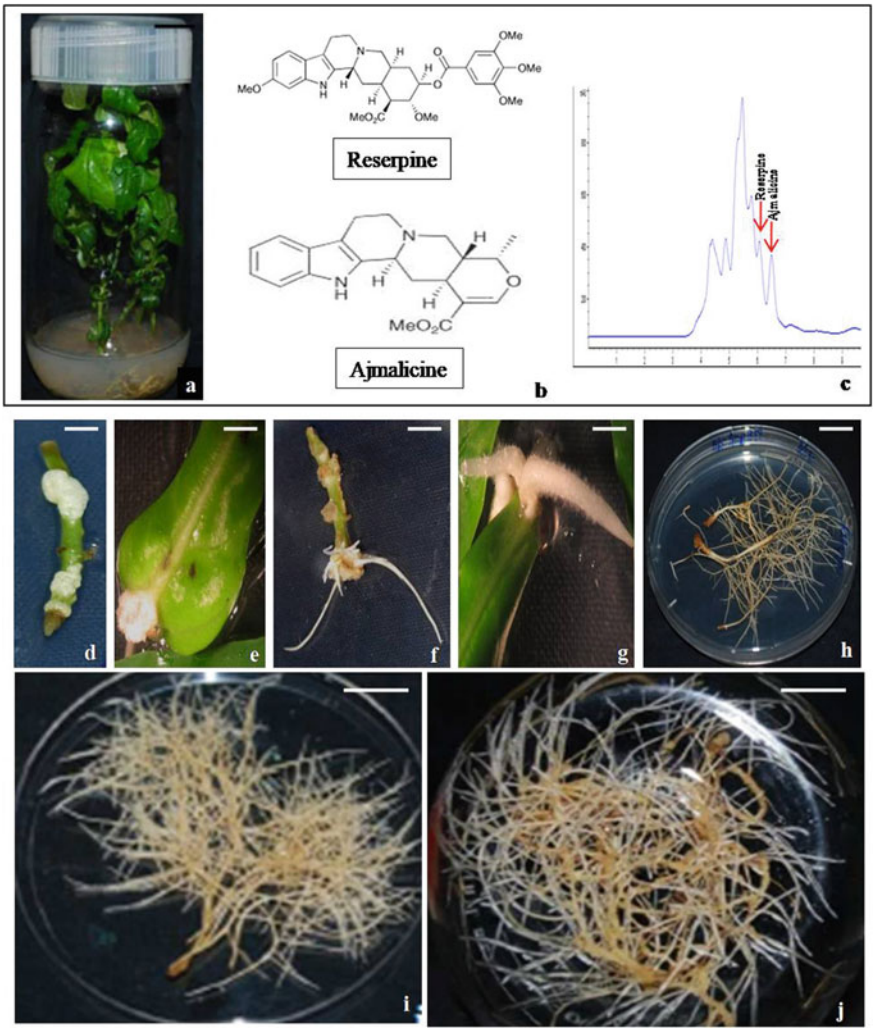


Fig. 16.7 **a** Multiple shoot cultures of *R. serpentina* maintained on MS medium for 8 weeks under 16/8 h photoperiod (*Bar = 2.0* cm), **b** Chemical structures and **c** HPLC chromatogram of valuable secondary metabolites of *R. serpentina*, **d**, **e** Response of internodal and leaf explants to infection by different strains *A. rhizogenes* (*Bar = 0.8* cm), **f**, **g** Root induction from internodal and leaf explants infected with *A. rhizogenes* strain A4 (*Bar = 0.8* cm), **h** Establishment of transformed root culture on modified MS medium (*Bar = 1.6* cm), **i**, **j** Transformed root line cultured on semi-solid and liquid medium (*Bar = 3.0* cm)

plantlets after acclimatization to field conditions showed morphological features similar to non-transformed plants. Insertion of multiple copies of transgene was attributed as a probable cause of the stunted phenotype of some of the regenerated plantlets.

HRCs have been reported to produce novel alkaloids undetected in plant roots as well as cell cultures including a new subgroup of sarpagine alkaloid, namely 19(S), 20(R)-dihydroperaksine, formed by deacetylation of perakine or raucaffrinoline (Sheludko et al. 2002).

16.12.3 Elicitation and Scale-Up

Biotechnological approaches for elicitation of medicinally active SMs have been extended to both untransformed and transformed cultures involving techniques like optimization of culture conditions, precursor feeding, selection of high-yielding lines and modifying biosynthetic pathways. Problems associated with the limited availability of these compounds can be mitigated to a certain extent by this approach.

Zafar et al. (2017) evaluated effect of $AlCl_3$ on reserpine content and callus growth. Under the influence of 0.15 mM $AlCl_3$ enhancement of reserpine content (0.129 mgg^{-1} DW) was noted in comparison with untreated callus cultures (0.083 mgg^{-1} DW). Verma et al. (2012) carried out co-culture of cell lines leading to increase in biomass and alteration of alkaloid profile the underlying mechanism being that metabolite effluents of one plant is up taken by the other plant for downstream processing to a valuable end product. Observations of *Catharanthus* + *Rauwolfia* co-culture resulted in biomass enhancement by 20–25 times in a 7 l stirred tank bioreactor after a 30-day cycle and presence of two new indole alkaloids in the crude extracts of the co-culture previously unidentified in the individual cell cultures. Variation in reserpine content was noted in whole plant cultures under the effect of precursors and elicitors used either singly or in combination. While enhancement of reserpine content was noted after 6 weeks in the presence of ABA, SA and tryptamine; DMSO treatment had a negative effect on reserpine accumulation. Treatment with a combination of SA and tryptamine unregulated reserpine production (58.04 mgg^{-1} DW) against 18.24 mgg^{-1} DW reserpine obtained from untreated whole plant cultures. However, this treatment resulted in decreased biomass (Panwar and Guru 2015).

Significant enhancement of alkaloids upon application of biotic and abiotic elicitors to HRCs was reported by Srivastava et al. (2016a). They reported 14.8- and 3.1-fold increase in ajmalicine content under 100 mM NaCl and 50 mgl^{-1} mannan, respectively. Similarly, 1.9- and 2.9-fold increase in ajmaline content was recorded upon treatment with 200 mM NaCl and 100 mgl^{-1} mannan respectively. Signal transduction triggered by elicitor–receptor binding at the plant cell membren stimulates SM synthesis as a defence response (Halder et al. 2019). Optimization of bioreactor conditions for effective large-scale production of metabolites is the desired outcome of HRC (Gutierrez-Valdes et al. 2020).

16.12.4 Metabolic Engineering

Metabolic engineering of biosynthetic pathways targets the enhancement of medicinally important SMs as well as generation of new compounds within the biological system. As plants produce such chemicals in low amount, industrial demand necessitates synthesis of chemical analogues. Herein lies the necessity for elucidation of the biosynthetic pathway (primarily the enzyme catalyzed reactions along with the regulatory genes) and its engineering.

The amount and types of alkaloids produced as well as their isolation depend primarily on the nutrient medium, culture conditions as well as the nature of in vitro cultures. *R. serpentina* CSCs and HRCs have been suitably used for the production of more than 35 different alkaloids (Yang and Stöckigt 2010). Generation of hybrid somatic cell line RxR17 (*R. serpentina* x *Rhazya stricta*) resulted not only in production and enhancement of indole alkaloids (obtained from methyl jasmonate supplemented culture media) but also generation of novel alkaloids undetected in either of the parental species (Sheludko et al. 1999). Structural biology techniques involving NMR spectroscopy and X-ray crystallography are used to determine 3D structure of biosynthetic enzymes. These techniques have been extended to *R. serpentina* in analysing the structure of strictosidine synthase (STR1), strictosidine glucosidase (SG), raucaffricine glucosidase (RG), polyneuridine aldehyde esterase (PNAE) and vinorine synthase (VS). Understanding the topology of the substrate-binding sites, paves way for enzyme engineering to produce novel medicinal alkaloids; an approach that has been applied in *C. roseus* (Yang and Stöckigt 2010). Characterization of the TIA biosynthetic pathway gene opens up new possibilities of metabolite pathway engineering. Tryptophan decarboxylase (TDC, EC 4.1.1.28) converts tryptophan into tryptamine, the initial step in the pathway leading to the formation of alkaloids such as such as reserpine, ajmaline, ajmalicine, serpentine, vomiline and yohimbine. In order to enhance the biosynthesis of alkaloids, Mehrotra et al. (2013b) over-expressed tryptophan decarboxylase gene (*Crtdc*) from *C. roseus* in HRCs of *R. serpentina.* Enhancement of reserpine and ajmalicine content was noted among hairy root lines obtained by transformation with *A. rhizogenes* strain A4 harbouring pBI121-Crtdc binary construct (reserpine 0.1202%; ajmalicine 0.0064% DW) over those arising from wild-type A4 transformation (reserpine 0.0596% DW; ajmalicine 0.0011% DW). In a novel approach, Corbin et al. (2017) demonstrated that virus induced gene silencing of phytoene desaturase. Such an approach facilitates characterization of genes involved in the metabolic pathway. Thus, metabolic engineering paves way for production of high-value pharmaceuticals in in vitro systems, which can be scaled up for commercial purposes.

16.12.5 Genetic Fidelity of in vitro Cultures of R. serpentina

One of the aims of in vitro cultures is conservation of genetic material of threatened plant species. Somaclonal variations like chromosomal aberrations, polyploidy and mutations arising in response to stress in culture conditions are a setback in germplasm preservation. Hence, the detection of genetic variation among regenerated plantlets has been targeted by researchers. RAPD profiles of regenerated plants showed homogeneity with donor plants (Senapati et al. 2014). ISSR analysis of indirectly and directly regenerated plantlets and parent explant was performed by Saravan et al. (2011). Plantlets obtained by direct organogenesis showed monomorphism, hence genetic stability, whereas callus regenerated plantlets showed polymorphism for one primer. Somatic clones derived from synthetic seed germination showed genetic stability when assessed by RAPD and ISSR (Faisal et al. 2012). Zafar et al. (2019) tested genetic fidelity among plantlets obtained by direct organogenesis and somatic embryogenesis. Comparative analysis of the estimated 2C DNA content of plantlets derived by direct and indirect organogenesis, somatic embryogenesis and field-grown plant reflected genetic stability as no significant variation was detected by the flow cytometric analysis (Zafar et al. 2019). Minor genetic variation was observed in cell lines after four passages through different media. However, transfer from surface culture to submerged culture resulted in significant genetic variation (Spiridonova et al. 2007) as revealed by RAPD analysis. Decreased polymorphism was observed when transfer to submerged culture was preceded by culture on semi-solid medium possibly due to the nature of surface exposure to the nutrient medium.

Genetic stability was also reported in transgenic cultures. A hairy root line without showing tendency of callusing was maintained for >6 years by Pandey et al. (2014). Interestingly, the authors were able to maintain the long-term cultures in both sucrose and table sugar containing media. Increase in productivity of yohimbine, ajmaline and reserpine was noted in the long-term cultures, in both the carbon source, with up to 296% increase in ajmaline production in sucrose containing media.

16.13 Conclusions

MPs with their vast array of pharmacologically active SMs are essential resources of nature that have been used by human beings to maintain and restore good health since ancient times. But, as reviewed, several anthropogenic activities, uneven distribution and availability of MPs all over the earth, inadequately resource management system, lack of public awareness, weakening of biodiversity protection laws, etc., have led to a steady decline in the population of a huge number of these immensely important plants from the wild. Besides, various other factors discussed also affect the availability of phytochemicals from the wild populations of plants. Over the past few decades, a lot of effort has been put by scientists and researchers all over the world to

safeguard the existence of these MPs in their natural habitats either through conventional breeding technologies, ex-situ and in-situ conservation methods or through plant tissue culture approaches. The literature survey demonstrates that various techniques of PTC like micropropagation, somatic embryogenesis, in vitro production of SMs through callus/cell suspension/organ cultures/HRCs or advanced techniques like metabolic pathway/ploidy engineering, nanoparticle application, genome editing have revolutionized the field of MP research. The study further documents the instances of morphological, biochemical and genetic stability/fidelity of non-transformed and transformed in vitro cultures due to their effect on proliferation and SM production. The two case studies presented are examples of the applications of PTC methodologies towards conserving MP biodiversity together with the production of pharmaceutically important SMs. Selection of suitable conventional and upcoming PTC techniques along with screening for stability thus paves way for availability of pharmaceutical SMs from plants with minimal threat to the biodiversity.

Acknowledgements Sumita Jha gratefully acknowledges the contributions of all of her Ph.D. students working over three decades in her laboratory on improvement of indigenous medicinal plans through biotechnological means, and this review is the outcome of all their collective efforts, sincerity, help and dedication. SJ is thankful to the National Academy of Sciences (NASI, Allahabad, India) for the NASI Senior Scientist Platinum Jubilee Fellowship award, and for providing the financial support to continue the research.

References

Açıkgöz MA (2020) Establishment of cell suspension cultures of *Ocimum basilicum* L. and enhanced production of pharmaceutical active ingredients. Ind Crops Prod 148:112278. https://doi.org/10.1016/j.indcrop.2020.112278

Ahmad N, Faisal M, Anis M, Aref IM (2010a) In vitro callus induction and plant regeneration from leaf explants of *Ruta graveolens* L. S Afr J Bot 76(3):597–600

Ahmad N, Fazal H, Abbasi BH, Rashid M, Mahmood T, Fatima N (2010b) Efficient regeneration and antioxidant potential in regenerated tissues of *Piper nigrum* L. Plant Cell Tiss Organ Cult 102(1):129–134

Ahmad N, Javed SB, Khan MI, Anis M (2013) Rapid plant regeneration and analysis of genetic fidelity in micropropagated plants of *Vitex trifolia*: an important medicinal plant. Acta Physiol Plant 35(8):2493–2500

Ahmad N, Alatar AA, Faisal M, Khan MI, Fatima N, Anis M, Hegazy AK (2015) Effect of copper and zinc on the in vitro regeneration of *Rauvolfia serpentina*. Biol Plantarum 59(1):11–17

Ahmad N, Rab A, Ahmad N, Fazal H (2018) Differential pH–induced biosynthesis of steviol glycosides and biochemical parameters in submerge root cultures of *Stevia rebaudiana* (Bert.). Sugar Tech 20(6):734–744

Airi S, Rawal RS, Dhar U, Purohit AN (1997) Population studies on *Podophyllum hexandrum* Royle—a dwindling medicinal plant of the Himalaya. Plant Genet Resour Newsl 110:20–34

Akhgari A, Laakso I, Maaheimo H, Choi YH, Seppänen-Laakso T, Oksman-Caldentey KM, Rischer H (2019) Methyl jasmonate elicitation increases terpenoid indole alkaloid accumulation in *Rhazya stricta* hairy root cultures. Plant 8(12):534. https://doi.org/10.3390/plants8120534

Alam MA, Gulati P, Gulati AK, Mishra GP, Naik PK (2009) Assessment of genetic diversity among *Podophyllum hexandrum* genotypes of the North–western Himalayan region for podophyllotoxin production. Indian J Biotechnol 8:391–399

Alatar AA, Ahmad N, Javed SB, Abdel-Salam EM, Basahi R, Faisal M (2017) Two–way germination system of encapsulated clonal propagules of *Vitex trifolia* L.: an important medicinal plant. J Hortic Sci Biotech 92(2):175–182

Ali M, Mujib A, Tonk D, Zafar N (2017) Plant regeneration through somatic embryogenesis and genome size analysis of *Coriandrum sativum* L. Protoplasma 254(1):343–352

Alok A, Kumar J, Upadhyay SK (2018) Engineering in hairy roots using CRISPR/Cas9–mediated editing. In: Srivastava V, Mehrotra S, Mishra S (eds) Hairy roots. Springer, Singapore, pp 329–342

Amanullah BM, Rizvi ZF, Zia M (2016) Production of artemisinin and its derivatives in hairy roots of *Artemisia dubia* induced by *rolA* gene transformation. Pak J Bot 48(2):699–706

Annapurna D, Rathore TS (2010) Direct adventitious shoot induction and plant regeneration of *Embelia ribes* Burm F. Plant Cell Tiss Organ Cult 101(3):269–277

Armijos-González R, Pérez-Ruiz C (2016) In vitro germination and shoot proliferation of the threatened species *Cinchona officinalis* L. (Rubiaceae). J for Res 27(6):1229–1236

Arumugam N, Bhojwani SS (1990) Somatic embryogenesis in tissue cultures of *Podophyllum hexandrum*. Can J Bot 68:487–491

Badhwar RL, Sharma BK (1963) A note on germination of *Podophyllum* seeds. Indian for 89(6):445–447

Bahramnejad B, Naji M, Bose R, Jha S (2019) A critical review on use of *Agrobacterium rhizogenes* and their associated binary vectors for plant transformation. Biotechnol Adv 37(7):107405. https://doi.org/10.1016/j.biotechadv.2019.06.004

Bahuguna RN, Joshi R, Singh G, Shukla A, Gupta R, Bains G (2011) Micropropagation and total alkaloid extraction of Indian snake root (*Rauwolfia serpentina*). Indian J Agric Sci 81(12):1124–1129

Bakar DA, Ahmed BA, Taha RM (2014) In vitro callus induction and plant regeneration of *Celosia argentea*—an important medicinal plant. Braz Arch Biol Technol 57(6):860–866

Banerjee S (2018) Voyaging through chromosomal studies in hairy root cultures towards unravelling their relevance to medicinal plant research: an updated review. Nucleus 61(1):3–18

Bansal M, Reddy MS, Kumar A (2017) Optimization of cell growth and bacoside—A production in suspension cultures of *Bacopa monnieri* (L.) Wettst. using response surface methodology. Vitro Cell Dev Biol Plant 53(6):527–537

Bardar S, Kaul V, Kachhwaha S, Kothari S (2015) Nutrient optimization for improved in vitro plant regeneration in *Eclipta alba* (L.) Hassk and assessment of genetic fidelity using RAPD analysis. Plant Tiss Cult Biotechnol 24(2):223–234

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297

Basu S, Jha TB (2013) In vitro root culture: an alternative source of bioactives in the rare aphrodisiac herb *Chlorophytum borivilianum* Sant. et. Fern. Plant Tiss Cult Biotechnol 23(2):133–146

Basu S, Jha TB (2014) Direct organogenesis, phytochemical screening and assessment of genetic stability in clonally raised *Chlorophytum borivilianum*. Environ Exp Biol 12:167–178

Basu A, Joshi RK, Jha S (2015) Genetic transformation of *Plumbago zeylanica* with *Agrobacterium rhizogenes* strain LBA 9402 and characterization of transformed root lines. Plant Tiss Cult Biotechnol 25(1):21–35

Bathoju G, Rao K, Giri A (2017) Production of sapogenins (stigmasterol and hecogenin) from genetically transformed hairy root cultures of *Chlorophytum borivilianum* (Safed musli). Plant Cell Tiss Organ Cult 131(3):369–376

Bauer N, Vuković R, Likić S, Jelaska S (2015) Potential of different *Coleus blumei* tissues for rosmarinic acid production. Food Technol Biotechnol 53(1):3–10

Bayarmaa GA, Lee NN, Kang HD, Oyuntsetseg B, Moon HK (2018) Micropropagation of the Mongolian medicinal plant *Zygophyllum potaninii* via somatic embryogenesis. Plant Biotechnol Rep 12(3):187–194

Bayraktar M, Naziri E, Akgun IH, Karabey F, Ilhan E, Akyol B, Bedir E, Gurel A (2016) Elicitor induced stevioside production, in vitro shoot growth, and biomass accumulation in micropropagated *Stevia rebaudiana*. Plant Cell Tiss Organ Cult 127(2):289–300

Beigmohamadi M, Movafeghi A, Sharafi A, Jafari S, Danafar H (2019) Cell suspension culture of *Plumbago europaea* L. towards production of plumbagin. Iran J Biotechnol 17(2):46–54 e2169. https://doi.org/10.21859/ijb.2169

Benjamin BD, Roja G, Heble MR (1993) *Agrobacterium rhizogenes* mediated transformation of *Rauwolfia serpentina*: regeneration and alkaloid synthesis. Plant Cell Tiss Organ Cult 35(3):253–257

Berkowitz D, Choi S, Maeng J (2000) Enzyme–assisted asymmetric total synthesis of (–)–podophyllotoxin and (–)–picropodophyllin. J Org Chem 65(3):847–860

Bhagat P, Verma SK, Singh AK, Aseri GK, Khare N (2019) Evaluation of influence of different strains of *Agrobacterium rhizogenes* on efficiency of hairy root induction in *Rauwolfia serpentina*. Indian J Genet 79(4):1–5

Bhardwaj P, Goswami N, Narula P, Jain CK, Mathur A (2018) Zinc oxide nanoparticles (ZnO NP) mediated regulation of bacosides biosynthesis and transcriptional correlation of HMG–CoA reductase gene in suspension culture of *Bacopa monnieri*. Plant Physiol Biochem 130:148–156

Bhattacharyya D, Sinha R, Ghanta S, Chakraborty A, Hazra S, Chattopadhyay S (2012) Proteins differentially expressed in elicited cell suspension culture of *Podophyllum hexandrum* with enhanced podophyllotoxin content. Proteome Sci 10(1):34. https://doi.org/10.1186/1477-5956-10-34

Bhattacharyya D, Sinha R, Hazra S, Datta R, Chattopadhyay S (2013) De novo transcriptome analysis using 454 pyrosequencing of the Himalayan Mayapple Podophyllum Hexandrum. BMC Genomics 14:748. https://doi.org/10.1186/1471-2164-14-748

Bhattacharyya P, Kumaria S, Diengdoh R, Tandon P (2014) Genetic stability and phytochemical analysis of the in vitro regenerated plants of *Dendrobium nobile* Lindl. an endangered medicinal orchid. Meta Gene 2:489–504

Bhattacharyya P, Kumaria S, Job N, Tandon P (2016) En–masse production of elite clones of *Dendrobium crepidatum*: a threatened, medicinal orchid used in traditional Chinese medicine (TCM). J Appl Res Med Aromat Plants 3(4):168–176

Bhattacharyya D, Hazra S, Banerjee A, Datta R, Kumar D, Chakrabarti S, Chattopadhyay S (2016) Transcriptome—wide identification and characterization of CAD isoforms specific for podophyllotoxin biosynthesis from *Podophyllum hexandrum*. Plant Mol Biol 92:1–23

Bhattacharyya P, Kumar V, Van Staden J (2017a) Assessment of genetic stability amongst micropropagated *Ansellia africana*, a vulnerable medicinal orchid species of Africa using SCoT markers. S Afr J Bot 108:294–302

Bhattacharyya P, Kumaria S, Bose B, Paul P, Tandon P (2017b) Evaluation of genetic stability and analysis of phytomedicinal potential in micropropagated plants of *Rumex nepalensis*—a medicinally important source of pharmaceutical biomolecules. J Appl Res Med Aromat Plants 6(5):80–91

Bhowmik SSD, Basu A, Sahoo L (2016) Direct shoot organogenesis from rhizomes of medicinal Zingiber *Alpinia calcarata* Rosc. and evaluation of genetic stability by RAPD and ISSR markers. J Crop Sci Biotechnol 19(2):157–165

Bhusare BP, John CK, Bhatt VP, Nikam TD (2020) Induction of somatic embryogenesis in leaf and root explants of *Digitalis lanata* Ehrh.: direct and indirect method. S Afr J Bot 130:356–365

Biswas S, Hazra S, Chattopadhyay S (2016) Identification of conserved miRNAs and their putative target genes in *Podophyllum hexandrum* (Himalayan Mayapple). Plant Gene 6:82–89

Borpuzari PP, Borthakur M (2016) Effect of plant growth regulators and explants sources on somatic embryogenesis of matured tissue of the anticancerous medicinal plant *Plumbago rosea*. J Med Plants Stud 4(5):165–170

Bush EJ, Jones DW (1995) Asymmetric total synthesis of (–)–podophyllotoxin. J Chem Soc Perkin Trans 1:151–155

Cardillo AB, Otálvaro AÁM, Busto VD, Talou JR, Velásquez LME, Giulietti AM (2010) Scopolamine, anisodamine and hyoscyamine production by *Brugmansia candida* hairy root cultures in bioreactors. Process Biochem 45(9):1577–1581

Chakraborty A, Bhattacharya D, Ghanta S, Chattopadhyay S (2010) An efficient protocol for in vitro regeneration of *Podophyllum hexandrum*, a critically endangered medicinal plant. Indian J Biotechnol 9(2):217–220

Chakraborty N, Banerjee D, Ghosh M, Pradhan P, Gupta NS, Acharya K, Banerjee M (2013) Influence of plant growth regulators on callus mediated regeneration and secondary metabolites synthesis in *Withania somnifera* (L.) Dunal. Physiol Mol Biol Plants 19(1):117–125

Chandran H, Meena M, Barupal T, Sharma K (2020) Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. Biotechnol Rep 26:e00450. https://doi.org/10.1016/j.btre.2020.e00450

Chaurasia OP, Ballabh B, Tayade A, Kumar R, Kumar GP, Singh SB (2012) *Podophyllum*: an endangered and anticancerous medicinal plant—an overview. Indian J Tradit Know 11(2):234–241

Chavan JJ, Nalawade AS, Gaikwad NB, Gurav RV, Dixit GB, Yadav SR (2014) An efficient in vitro regeneration of *Ceropegia noorjahaniae*: an endemic and critically endangered medicinal herb of the Western Ghats. Physiol Mol Biol Plant 20(3):405–410

Chen SL, Yu H, Luo HM, Wu Q, Li CF, Steinmetz A (2016) Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin Med 11(1):37. https://doi.org/10.1186/s13020-016-0108-7

Chodisetti B, Rao K, Suryakala G, Archana G (2015) Gymnemic acid enhancement in the suspension cultures of *Gymnema sylvestre* by using the signaling molecules–methyl jasmonate and salicylic acid. Vitro Cell Dev Biol Plant 51(1):88–92

Chung IM, Rajakumar G, Subramanian U, Venkidasamy B, Thiruvengadam M (2019) Impact of copper oxide nanoparticles on enhancement of bioactive compounds using cell suspension cultures of *Gymnema sylvestre* (Retz) R Br. Appl Sci 9(10):2165. https://doi.org/10.3390/app9102165

Coelho N, Gonçalves S, Romano A (2020) Endemic plant species conservation: biotechnological approaches. Plants 9(3):345. https://doi.org/10.3390/plants9030345

Corbin C, Lafontaine F, Sepúlveda LJ, Carqueijeiro I, Courtois M, Lanoue A, Dugé de Bernonville T, Besseau S, Glévarec G, Papon N, Atehortúa L, Giglioli-Guivarc'h N, Clastre M, St-Pierre B, Oudin A, Courdavault V (2017) Virus-induced gene silencing in *Rauwolfia* species. Protoplasma 254(4):1813–1818

Corrêa JPO, Vital CE, Pinheiro MVM, Batista DS, Saldanha CW, da Cruz ACF, Notini MM, Freitas DMS, DaMatta FM, Otoni WC (2016) Induced polyploidization increases 20-hydroxyecdysone content, in vitro photoautotrophic growth, and ex vitro biomass accumulation in *Pfaffia glomerata* (Spreng.) Pedersen. Vitro Cell Dev Biol–plant 52(1):45–55

Cui HY, Murthy HN, Moh SH, Cui YY, Lee EJ, Paek KY (2014) Production of biomass and bioactive compounds in protocorm cultures of *Dendrobium candidum* Wall ex Lindl. using balloon type bubble bioreactors. Ind Crops Prod 53:28–33

Da Silva JAT, Jha S (2016) Micropropagation and genetic transformation of *Tylophora indica* (Burm f) Merr: a review. Plant Cell Rep 35(11):2207–2225

Dandin VS, Naik PM, Murthy HN, Park SY, Lee EJ, Paek KY (2014) Rapid regeneration and analysis of genetic fidelity and scopoletin contents of micropropagated plants of *Spilanthes oleracea* L. J Hortic Sci Biotech 89(1):79–85

Das A, Haque S, Ghosh B, Nandagopal K, Jha T (2016) Morphological and genetic characterization of micropropagated field grown plants of *Aloe vera* L. Plant Tiss Culture Biotechnol 25(2):231–246

Deb CR, Rout GR, Mao AA, Nandi SK, Singha RKN, Vijayan D, Langhu T, Kikon ZP, Pradhan S, Tariq M, Swain D (2018) In vitro propagation of some threatened plant species of India. Curr Sci 114(3):567–575

Deepthi S, Satheeshkumar K (2017) Cell line selection combined with jasmonic acid elicitation enhance camptothecin production in cell suspension cultures of *Ophiorrhiza mungos* L. Appl Microbiol Biotechnol 101(2):545–558

Dehghan E, Hakkinen ST, Oksman-Caldentey KM, Ahmadi FS (2012) Production of tropane alkaloids in diploid and tetraploid plants and in vitro hairy root cultures of Egyptian henbane (*Hyoscyamus muticus* L.). Plant Cell Tiss Organ Cult 110(1):35–44

Dhiman N, Patial V, Bhattacharya A (2016) In vitro approaches for conservation and sustainable utilization of *Podophyllum hexandrum* and *Picrorhiza kurroa*: endangered medicinal herbs of Western Himalaya. In: Anis M, Ahmad N (eds) Plant tissue culture: propagation, conservation and crop improvement. Springer, Singapore, pp 45–69

Dubrovina AS, Manyakhin AY, Zhuravlev YN, Kiselev KV (2010) Resveratrol content and expression of phenylalanine ammonia–lyase and stilbene synthase genes in *rolC* transgenic cell cultures of *Vitis amurensis*. Appl Microbiol Biotechnol 88(3):727–736

Duchow S, Blaschek W, Classen B (2015) Reproduction of the medicinal plant *Pelargonium sidoides* via somatic embryogenesis. Planta Med 81(12/13):1169–1174

Ebrahimi M, Mokhtari A, Amirian R (2018) A highly efficient method for somatic embryogenesis of *Kelussia odorotissima* Mozaff., an endangered medicinal plant. Plant Cell Tiss Organ Cult 132(1):99–110

El Sherif F, Khattab S, Ibrahim AK, Ahmed SA (2013) Improved silymarin content in elicited multiple shoot cultures of *Silybum marianum* L. Physiol Mol Biol Plants 19:127–136

Espinosa-Leal CA, Puente-Garza CA, García-Lara S (2018) In vitro plant tissue culture: means for production of biological active compounds. Planta 248(1):1–18

Faisal M, Alatar AA, Ahmad N, Anis M, Hegazy AK (2012) Assessment of genetic fidelity in *Rauvolfia serpentina* plantlets grown from synthetic (encapsulated) seeds following in vitro storage at 4 °C. Molecules 17(5):5050–5061

Faisal M, Ahmad N, Anis M, Alatar AA, Qahtan AA (2018) Auxin-cytokinin synergism in vitro for producing genetically stable plants of *Ruta graveolens* using shoot tip meristems. Saudi J Biol Sci 25(2):273–277

Falkenhagen H, Stockigt J, Kuzovkina IN, Alterman IE, Kolshorn H (1993) Indole alkaloids from the hairy roots of *Rauwolfia serpentina*. Can J Chem 71(12):2201–2203

Faridah QZ, Abdelmageed AHA, Julia AA, Hafizah RN (2011) Efficient in vitro regeneration of *Zingiber zerumbet* Smith (a valuable medicinal plant) plantlets from rhizome bud explants. Afr J Biotechnol 10(46):9303–9308

Farjaminezhad R, Garoosi GA (2019) New biological trends on cell and callus growth and azadirachtin production in *Azadirachta indica*. 3 Biotech 9(8):309 https://doi.org/10.1007/s13205-019-1836-z

Fatima S, Mujib A, Tonk D (2015) NaCl amendment improves vinblastine and vincristine synthesis in *Catharanthus roseus*: a case of stress signalling as evidenced by antioxidant enzymes activities. Plant Cell Tiss Organ Cult 121:445–458

Feng S, Song W, Fu R, Zhang H, Xu A, Li J (2018) Application of the CRISPR/Cas9 system in *Dioscorea zingiberensis*. Plant Cell Tiss Organ Cult 135(1):133–141

Fulzele DP, Namdeo AG (2018) Cost effective pilot–scale ajmalicine production by *Catharanthus roseus* cell suspension cultures in a 100 lit bioreactor. Biotechnol J Int 21(4):1–15

Gabr AM, Ghareeb H, El Shabrawi HM, Smetanska I, Bekheet SA (2016) Enhancement of silymarin and phenolic compound accumulation in tissue culture of milk thistle using elicitor feeding and hairy root cultures. J Genet Eng Biotechnol 14(2):327–333

Gandhi SG, Vidushi M, Bedi YS (2015) Changing trends in biotechnology of secondary metabolism in medicinal and aromatic plants. Planta 241(2):303–317

Gandi S, Rao K, Chodisetti B, Giri A (2012) Elicitation of andrographolide in the suspension cultures of *Andrographis paniculata*. Appl Biochem Biotechnol 168(7):1729–1738

Gantait S, Kundu S (2017) Does synthetic seed storage at higher temperature reduce reserpine content of *Rauvolfia serpentina* (L.) Benth. ex Kurz.? Rendiconti Lincei Scienze Fisiche e Naturali 28(4):1–8

Gantait S, Kundu S, Ali N, Sahu NC (2015) Synthetic seed production of medicinal plants: a review on influence of explants, encapsulation agent and matrix. Acta Physiol Plant 37(5):98–109

Gharari Z, Bagheri K, Danafar H, Sharafi A (2020) Enhanced flavonoid production in hairy root cultures of *Scutellaria bornmuelleri* by elicitor induced over—expression of MYB7 and FNSII2 genes. Plant Physiol Biochem 148:35–44

Ghazal B, Saif S, Farid K, Khan A, Rehman S, Reshma A, Fazal H, Ali M, Ahmad A, Rahman L, Ahmad N (2018) Stimulation of secondary metabolites by copper and gold nanoparticles in submerge adventitious root cultures of *Stevia rebaudiana* (Bert). IET Nanobiotechnol 12(5):569–573

Ghimire BK, Yu CY, Chung IM (2012) Direct shoot organogenesis and assessment of genetic stability in regenerants of *Solanum aculeatissimum* Jacq. Plant Cell Tiss Organ Cult 108(3):455–464

Ghosh S, Ghosh B, Jha S (2015) Role of exogenous carbohydrate and amino acid sources on biomass and colchicine production in nontransformed root cultures of *Gloriosa superba*. Plant Tiss Cult Biotechnol 25(2):247–256

Giri A, Giri CC, Dhingra V, Narasu ML (2001) Enhanced podophyllotoxin production from *Agrobacterium rhizogenes* transformed cultures of *Podophyllum hexandrum*. Nat Prod Lett 15:229–235

Gordaliza M, García PA, Miguel del Corral JM, Castro MA, Gómez-Zurita MA (2004) Podophyllotoxin: distribution, sources, applications and new cytotoxic derivatives. Toxicon 44(4):441–459

Government of India, Medicinal Plants Introduction, Indian System of Medicine and Homoeopathy (ISMH), Department of ISMH, Ministry of Health and Family Welfare, Govt. of India (2000) http://indianmedicine.nic.in/html/plants/mimain.htm

Grishchenko OV, Kiselev KV, Tchernoded GK, Fedoreyev SA, Veselova MV, Bulgakov VP, Zhuravlev YN (2013) The influence of the *rolC* gene on isoflavonoid production in callus cultures of *Maackia amurensis*. Plant Cell Tiss Organ Cult 113(3):429–435

Grishchenko OV, Kiselev KV, Tchernoded GK, Fedoreyev SA, Veselova MV, Bulgakov VP, Zhuravlev YN (2016) *RolB* gene-induced production of isoflavonoids in transformed *Maackia amurensis* cells. Appl Microbiol Biotechnol 100(17):7479–7489

Grzegorczyk I, Wysokinska H (2010) Antioxidant compounds in *Salvia officinalis* L. shoot and hairy root cultures in the nutrient sprinkle bioreactor. Acta Soc Bot Pol 79(1):7–10

Guo Q, Zhou J, Wang Z, Yang H (2012) In vitro rooting of *Podophyllum hexandrum* and transplanting technique. Engineering 4(10):142–145

Gupta R, Pandey P, Singh S, Singh DK, Saxena A, Luqman S, Bawankule DU, Banerjee S (2016) Advances in *Boerhaavia diffusa* hairy root technology: a valuable pursuit for identifying strain sensitivity and up-scaling factors to refine metabolite yield and bioactivity potentials. Protoplasma 253(4):1145–1158

Gutierrez-Valdes N, Häkkinen ST, Lemasson C, Guillet M, Oksman-Caldentey KM, Ritala A, Cardon F (2020) Hairy root cultures—a versatile tool with multiple applications. Front Plant Sci 11:33. https://doi.org/10.3389/fpls.2020.00033

Habibi P, Piri K, Deljo A, Moghadam YA, Ghiasvand T (2015) Increasing scopolamine content in hairy roots of *Atropa belladonna* using bioreactor. Braz Arch Biol Technol 58(2):166–174

Halder M, Jha S (2016) Enhanced *trans*—resveratrol production in genetically transformed root cultures of Peanut (*Arachis hypogaea* L.). Plant Cell Tiss Organ Cult 124(3):555–572

Halder M, Jha S (2020) Morphogenesis, genetic stability, and secondary metabolite production in untransformed and transformed cultures. In: Ramawat K, Ekiert H, Goyal S (eds) Plant cell and tissue differentiation and secondary metabolites. Reference Series in Phytochemistry. Springer, Cham https://doi.org/10.1007/978-3-030-11253-0_15-1

Halder M, Roychowdhury D, Jha S (2018) A critical review on biotechnological interventions for production and yield enhancement of secondary metabolites in hairy root cultures. In: Srivastava V, Mehrotra S, Mishra S (eds) Hairy roots. Springer, Singapore, pp 21–44

Halder M, Sarkar S, Jha S (2019) Elicitation: a biotechnological tool for enhanced production of secondary metabolites in hairy root cultures. Eng Life Sci 19(12):880–895

Han L, Piao XC, Jiang J, Jiang XL, Yin CR, Lian ML (2019) A high production of flavonoids and anthraquinones via adventitious root culture of *Oplopanax elatus* and evaluating antioxidant activity. Plant Cell Tiss Organ Cult 137(1):173–179

Haque SM, Ghosh B (2016) High-frequency somatic embryogenesis and artificial seeds for mass production of true–to–type plants in *Ledebouria revoluta*: an important cardioprotective plant. Plant Cell Tiss Organ Cult 127(1):71–83

Haque SM, Chakraborty A, Ghosh B (2018) Callus mediated shoot organogenesis and regeneration of cytologically stable plants of *Ledebouria revoluta*: an ethnomedicinal plant with promising antimicrobial potency. J Genet Eng Biotechnol 16(2):645–651

Hashemi SM, Naghavi MR (2016) Production and gene expression of morphinan alkaloids in hairy root culture of *Papaver orientale* L. using abiotic elicitors. Plant Cell Tiss Organ Cult 125(1):31–41

Hazra S, Bhattacharyya D, Chattopadhyay S (2017) Methyl jasmonate regulates podophyllotoxin accumulation in *Podophyllum hexandrum* by altering the ROS—responsive podophyllotoxin pathway gene expression additionally through the down regulation of few interfering miRNAs. Front Plant Sci 8:1–9. https://doi.org/10.3389/fpls.2017.00164

Hlatshwayo NA, Amoo SO, Olowoyo JO, Doležal K (2020) Efficient micropropagation protocol for the conservation of the endangered *Aloe peglerae*, an ornamental and medicinal species. Plants 9(4):506. https://doi.org/10.3390/plants9040506

Ho TT, Lee KJ, Lee JD, Bhushan S, Paek KY, Park SY (2017) Adventitious root culture of *Polygonum multiflorum* for phenolic compounds and its plot-scale production in 500 L–tank. Plant Cell Tiss Organ Cult 130:167–181

Huang SH, Agrawal DC, Wu FS, Tsay HS (2014) In vitro propagation of *Gentiana scabra* Bunge—an important medicinal plant in the Chinese system of medicines. Bot Stud 55(1):56. https://doi.org/10.1186/s40529-014-0056-4

Huang P, Xia L, Liu W, Jiang R, Liu X, Tang Q, Xu M, Yu L, Tang Z, Zeng J (2018) Hairy root induction and benzylisoquinoline alkaloid production in *Macleaya cordata.* Sci Rep 8(1):1–9

Iannicelli J, Guariniello J, Tossi VE, Regalado JJ, Di Ciaccio L, Van Baren CM, Pitta Álvarez SI, Escandón AS (2020) The "polyploid effect" in the breeding of aromatic and medicinal species. Sci Hortic 260:108854. https://doi.org/10.1016/j.scienta.2019.108854

Imbert TF (1998) Discovery of podophyllotoxins. Biochimie 80(3):207–222

Irvani N, Solouki M, Omidi M, Zare AR, Shahnazi SJPC (2010) Callus induction and plant regeneration in *Dorem ammoniacum* D., an endangered medicinal plant. Plant Cell Tiss Organ Cult 100(3):293–299

Isah T (2017) Production of camptothecin in the elicited callus cultures of *Nothapodytes nimmoniana* (J. Graham) Mabberly. Chem Pap 71(6):1091–1106

Isah T, Umar S, Mujib A, Sharma MP, Rajasekharan PE, Zafar N, Frukh A (2018) Secondary metabolism of pharmaceuticals in the plant in vitro cultures: strategies, approaches, and limitations to achieving higher yield. Plant Cell Tiss Organ Cult 132(2):239–265

IUCN The IUCN red list of threatened species. Version 2019–2. Available online: http://www.iucnredlist.org. Accessed 25 Jan 2020

Jain P, Danwra K, Sharma HP, Mahato D (2018) In vitro tissue culture studies and synthetic seed formation from *Plumbago zeylanica* L. Indian J Exp Biol 56:769–773

Jaremicz Z, Luczkiewicz M, Kokotkiewicz A, Krolicka A, Sowinski P (2014) Production of tropane alkaloids in *Hyoscyamus niger* (black henbane) hairy roots grown in bubble-column and spray bioreactors. Biotechnol Lett 36(4):843–853

Javadian N, Karimzadeh G, Sharifi M, Moieni A, Behmanesh M (2017) In vitro polyploidy induction: changes in morphology, podophyllotoxin biosynthesis, and expression of the related genes in *Linum album* (Linaceae). Planta 245(6):1165–1178

Jeet A, Singh Y, Singh P, Nimoriya R, Bilung CJ, Kanojiya S, Tripathi V, Mishra DK (2020) Strategies for indole alkaloids enrichment through callus culture from *Alstonia scholaris* (L.) R. Br. Plant Growth Regul 90(2):383–392

Jena S, Ray A, Sahoo A, Sahoo S, Dash B, Kar B, Nayak S (2020) Rapid plant regeneration in industrially important *Curcuma zedoaria* revealing genetic and biochemical fidelity of the regenerants. 3 Biotech 10(1):17 https://doi.org/10.1007/s13205-019-2009-9

Jiang YJ, Piao XC, Liu JS, Jiang J, Lian ZX, Kim MJ, Lian ML (2015) Bioactive compound production by adventitious root culture of *Oplopanax elatus* in balloon—type airlift bioreactor systems and bioactivity property. Plant Cell Tiss Organ Cult 123(2):413–425

Jiang W, Hua S, Zhou X, Han P, Lu Q, Qiu Y (2018) Assessment of genetic stability and analysis of alkaloids potential in micropropagated plants of *Croomia japonica* Miquel, an endangered, medicinal plant in China and Japan. Plant Cell Tiss Organ Cult 135(1):1–12

Johny L, Conlan XA, Adholeya A, Cahill DM (2018) Growth kinetics and withanolide production in novel transformed roots of *Withania somnifera* and measurement of their antioxidant potential using chemiluminescence. Plant Cell Tiss Organ Cult 132(3):479–495

Kamble S, Gopalakrishnan R, Eapen S (2011) Production of camptothecin by hairy roots and regenerated transformed shoots of *Ophiorrhiza rugosa* var. decumbens. Nat Prod Res 25(18):1762–1765

Kanungo S, Pradhan C, Sahoo SL, Sahu RK (2012) Role of auxins in the in vitro rooting and micropropagation of *Holarrhena antidysenterica* Wall., a woody aromatic medicinal plant, through nodal explants from mature trees. J Med Plants Res 6(31):4660–4666

Kanwar K, Soni M, Singh A (2017) In vitro propagation and conservation of an endangered high value medicinal herb *Swertia chirayita* of temperate Himalayas. Indian J Plant Physiol 22(2):247–257

Karimzadeh F, Haddad R, Garoosi G, Khademian R (2019) Effects of nanoparticles on activity of lignan biosynthesis enzymes in cell suspension culture of *Linum usitatissimum* L. Russ J Plant Physl 66(5):756–762

Kayani WK, Kiani BH, Dilshad E, Mirza B (2018) Biotechnological approaches for artemisinin production in *Artemisia.* World J Microb Biot 34(4):54. https://doi.org/10.1007/s11274-018-2432-9

Kehie M, Kumaria S, Tandon P (2016) Biotechnological enhancement of capsaicin biosynthesis in cell suspension cultures of Naga King chili (*Capsicum chinense* Jacq.). Bioprocess Biosyst Eng 39(1):205–210

Keshvari T, Najaphy A, Kahrizi D, Zebarjadi A (2018) Callus induction and somatic embryogenesis in *Stevia rebaudiana* Bertoni as a medicinal plant. Cell Mol Biol 64(2):46–49

Khazaei A, Bahramnejad B, Mozafari AA, Dastan D, Mohammadi S (2019) Hairy root induction and Farnesiferol B production of endemic medicinal plant *Ferula pseudalliacea.* 3 Biotech 9(11):407. https://doi.org/10.1007/s13205-019-1935-x

Khilwani B, Kaur A, Ranjan R, Kumar A (2016) Direct somatic embryogenesis and encapsulation of somatic embryos for in vitro conservation of *Bacopa monnieri* (L) Wettst. Plant Cell Tiss Organ Cult 127(2):433–442

Kim YS, Lim S, Choi YE, Anbazhagan VR (2007) High frequency plant regeneration via somatic embryogenesis in *Podophyllum peltatum* L., an important medicinal plant for source of anticancer drug. Curr Sci 92(5):662–666

Kochan E, Szymczyk P, Kuźma Ł, Lipert A, Szymańska G (2017) Yeast extract stimulates ginsenoside production in hairy root cultures of American ginseng cultivated in shake flasks and nutrient sprinkle bioreactors. Molecules 22(6):880. https://doi.org/10.3390/molecules22060880

Krishnan ML, Roy A, Bharadvaja N (2019) Elicitation effect on the production of asiaticoside and asiatic acid in shoot, callus and cell suspension culture of *Centella asiatica.* J Appl Pharm Sci 9(06):067–074

Kudikala H, Jogam P, Sirikonda A, Mood K, Allini V R (2020) In vitro micropropagation and genetic fidelity studies using SCoT and ISSR primers in *Annona reticulata* L.: an important medicinal plant. Vegetos 1–12

Kumar A, Prakash K, Sinha RK, Kumar N (2013) In vitro plant propagation of *Catharanthus roseus* and assessment of genetic fidelity of micropropagated plants by RAPD marker assay. Appl Biochem Biotechnol 169(3):894–900

Kumar V, Moyo M, Van Staden J (2017) Somatic embryogenesis in *Hypoxis hemerocallidea*: an important African medicinal plant. S Afr J Bot 108:331–336

Kuo CL, Agrawal DC, Chang HC, Chiu YT, Huang CP, Chen YL, Huang SH, Tsay HS (2015) In vitro culture and production of syringin and rutin in *Saussurea involucrate* (Kar. et Kir.)—an endangered medicinal plant. Bot Stud 56:12. https://doi.org/10.1186/s40529-015-0092-8

Kusakari K, Yokoyma M, Inomata S, Gozu Y, Katagiri C, Sugimoto Y (2012) Large-scale production of saikosaponins through root culturing of *Bupleurum falcatum* L. using modified airlift reactors. J Biosci Bioeng 113(1):99–105

Kushwaha R, Pandey S, Chanda S, Bhattacharya A, Ahuja PS (2008) Temperature dependent growth and emergence of functional leaves: an adaptive mechanism in the seedlings of the western Himalayan plant *Podophyllum hexandrum.* J Plant Res 121(3):299–309

Lata H, Chandra S, Techen N, Khan IA, ElSohly MA (2016a) Assessment of the genetic stability of micropropagated plants of Cannabis sativa by ISSR markers. Planta Med 76(01):97–100

Lata H, Chandra S, Techen N, Khan IA, ElSohly MA (2016b) In vitro mass propagation of *Cannabis sativa L.*: a protocol refinement using novel aromatic cytokinin meta-topolin and the assessment of eco-physiological, biochemical and genetic fidelity of micropropagated plants. J Appl Res Med Aroma Plants 3(1):18–26

Le KC, Jeong CS, Lee HS, Paek KY, Park SY (2019) Ginsenoside accumulation profiles in long– and short–term cell suspension and adventitious root cultures in *Panax ginseng*. Hortic Environ Biotechnol 60(1):125–134

Lee EJ, Park SY, Paek KY (2015) Enhancement strategies of bioactive compound production in adventitious root cultures of *Eleutherococcus koreanum* Nakai subjected to methyl jasmonate and salicylic acid elicitation through airlift bioreactors. Plant Cell Tiss Organ Cult 120(1):1–10

Lei T, Wang H, Li S, Shen J, Chen S, Cai X, Zhou D (2018) Genetic transformation of the endangered Tibetan medicinal plant *Przewalskia tangutica* Maxim and alkaloid production profiling revealed by HPLC. 3 Biotech 8(3):179. https://doi.org/10.1007/s13205-018-1203-5

Li C, Wang M (2020) Application of hairy root culture for bioactive compounds production in medicinal plants. Curr Pharm Biotechnol. https://doi.org/10.2174/1389201021666200516155146

Li W, Li MF, Yang DL, Xu R, Zhang Y (2009) Production of podophyllotoxin by root culture of *Podophyllum hexandrum* Royle. Electron J Biol 5(2):34–39

Li B, Cui G, Shen G, Zhan Z, Huang L, Chen J, Qi X (2017) Targeted mutagenesis in the medicinal plant *Salvia miltiorrhiza.* Sci Rep 7:43320. https://doi.org/10.1038/srep43320

Mahendran D, Kishor PBK, Sreeramanan S, Venkatachalam P (2018) Enhanced biosynthesis of colchicine and thiocolchicoside contents in cell suspension cultures of *Gloriosa superba* L. exposed to ethylene inhibitor and elicitors. Ind Crops Prod 120:123–130

Majumder A (2008) Biotechnological approaches for the production of cytotoxic anticancerous compounds. Ph.D. thesis, Calcutta University, Calcutta, India

Majumder A (2012) Influence of an indirect precursor on podophyllotoxin accumulation in cell suspension cultures of *Podophyllum hexandrum.* Plant Tiss Cult Biotechnol 22(2):171–177

Majumder A, Jha S (2007) Growth and podophyllotoxin production in callus and cell suspension cultures of *Podophyllum hexandrum.* In: Proceeding of national symposium on plant biotechnology: new frontiers. CIMAP, Lucknow, pp 433–438

Majumder A, Jha S (2009) Biotechnological approaches for the production of potential anticancer leads podophyllotoxin and paclitaxel: an overview. Ejournal Biol Sci 1(1):46–69

Majumder A, Jha S (2009) Characterization of podophyllotoxin yielding cell lines of *Podophyllum hexandrum.* Caryologia 62(3):220–235

Mandagi SJ, Pandiangan D, Tilaar W (2017) Content of ajmalicine on cultured callus *Catharanthus roseus* (L.) G. Don with tryptophan treatment. Int J Sci Res 6(5):1919–1922

Martínez-Bonfil BP, Salcedo-Morales G, López-Laredo AR, Ventura-Zapata E, Evangelista-Lozano S, Trejo-Tapia G (2011) Shoot regeneration and determination of iridoid levels in the medicinal plant *Castilleja tenuiflora* Benth. Plant Cell Tiss Organ Cult 107(2):195–203

Mehrotra S, Goel MK, Rahman LU, Kukreja AK (2013a) Molecular and chemical characterization of plants regenerated from Ri mediated hairy root cultures of *Rauwolfia serpentina*. Plant Cell Tiss Organ Cult 114(1):31–38

Mehrotra S, Srivastava V, Rahman LU, Kukreja AK (2013b) Overexpression of a *Catharanthus* tryptophan decarboxylase (tdc) gene leads to enhanced terpenoid indole alkaloid (TIA) production in transgenic hairy root lines of *Rauwolfia serpentina*. Plant Cell Tiss Org Cult 115(3):377–384

Mehrotra S, Goel MK, Srivastava V, Rahman LU (2015) Hairy root biotechnology of *Rauwolfia serpentina*: a potent approach for the production of pharmaceutically important terpenoid indole alkaloids. Biotechnol Lett 37(2):253–263

Mekky H, Al-Sabahi J, Abdel-Kreem MFM (2018) Potentiating biosynthesis of the anticancer alkaloids vincristine and vinblastine in callus cultures of *Catharanthus roseus*. S Afr J Bot 114:29–31

Mohamed EAM, Dessoky ES, Attia AO, Hassan MM (2014) Evaluation of genetic fidelity of in vitro raised plants of the important medicinal plant Harmal (*Rhazya stricta* Decne) using RAPD and ISSR markers. Int J Agric Sci Res 4(3):115–124

Moharrami F, Hosseini B, Sharafi A, Farjaminezhad M (2017) Enhanced production of hyoscyamine and scopolamine from genetically transformed root culture of *Hyoscyamus reticulatus* L. elicited by iron oxide nanoparticles. Vitro Cell Dev Biol 53(2):104–111

Moon HK, Kim YW, Hong YP, Park SY (2013) Improvement of somatic embryogenesis and plantlet conversion in *Oplopanax elatus*, an endangered medicinal woody plant. Springerplus 2(1):428. https://doi.org/10.1186/2193-1801-2-428

Moradpour N, Garoosi G, Haddad R (2019) Study the effect of heavy metals and Nano TiO_2 on stevioside and rebadioside A production in *Stevia rebaudiana* Bertoni hairy roots. Iran J Genet Plant Breed 8(1):36–44

Moyo M, Amoo SO, Aremu AO, Gruz J, Šubrtová M, Doležal K, Van Staden J (2014) Plant regeneration and biochemical accumulation of hydroxybenzoic and hydroxycinnamic acid derivatives in *Hypoxis hemerocallidea* organ and callus cultures. Plant Sci 227:157–164

Mujib A, Ali M, Isah T (2014) Somatic embryo mediated mass production of *Catharanthus roseus* in culture vessel (bioreactor)—a comparative study. Saudi J Biol Sci 21(5):442–449

Nadeem M, Palni LMS, Purohit AN, Pandey H, Nandi SK (2000) Propagation and conservation of *Podophyllum hexandrum* Royle: an important medicinal herb. Biol Conserv 92(1):121–129

Nadeem M, Palni LMS, Kumar A, Nandi SK (2007) Podophyllotoxin content, above and below ground biomass in relation to altitude in *Podophyllum hexandrum* populations from Kumaun region of the Indian Central Himalaya. Planta Med 73(04):388–391

Naing AH, Chung JD, Park IS, Lim KB (2011) Efficient plant regeneration of the endangered medicinal orchid *Coelogyne cristata* using protocorm–like bodies. Acta Physiol Plant 33(3):659–666

Nandagopal K, Halder M, Dash B, Nayak S, Jha S (2018) Biotechnological approaches for production of anti–cancerous compounds resveratrol, podophyllotoxin and zerumbone. Curr Med Chem 25(36):4693–4717

Nayak SA, Kumar S, Satapathy K, Moharana A, Behera B, Barik DP, Acharya L, Mohapatra PK, Naik SK (2013) In vitro plant regeneration from cotyledonary nodes of *Withania somnifera* (L.) Dunal and assessment of clonal fidelity using RAPD and ISSR markers. Acta Physiol Plantarum 35(1):195–203

Niazian M (2019) Application of genetics and biotechnology for improving medicinal plants. Planta 249(4):953–973

Niazian M, Noori SAS, Galuszka P, Tohidfar M, Mortazavian SMM (2017) Genetic stability of regenerated plants via indirect somatic embryogenesis and indirect shoot regeneration of *Carum copticum* L. Ind Crops Prod 97:330–337

Nivelle L, Hubert J, Courot E, Jeandet P, Aziz A, Nuzillard JM, Renault JH, Clément C, Martiny L, Delmas D, Tarpin M (2017) Anti-cancer activity of resveratrol and derivatives produced by grapevine cell suspensions in a 14 L stirred bioreactor. Molecules 22(3):474. https://doi.org/10.3390/molecules22030474

Ooi CT, Syahida A, Stanslas J, Maziah M (2016) The influence of methyl jasmonate, cholesterol and L-arginine on solasodine production in hairy root culture of *Solanum mammosum*. Eng Life Sci 16(5):432–442

Pandey P, Kaur R, Singh S, Chattopadhyay SK, Srivastava SK, Banerjee S (2014) Long–term stability in biomass and production of terpene indole alkaloids by hairy root culture of *Rauvolfia serpentina* and cost approximation to endorse commercial realism. Biotechnol Lett 36(7):1523–1528

Pandey H, Pandey P, Pandey SS, Singh S, Banerjee S (2016) Meeting the challenge of stevioside production in the hairy roots of *Stevia rebaudiana* by probing the underlying process. Plant Cell Tiss Organ Cult 126(3):511–521

Pan-pan H, Wei–xu L, Hui–hui L (2018) In vitro induction and identification of autotetraploid of *Bletilla striata* (Thunb.) Reichb. f. by colchicine treatment. Plant Cell Tiss Organ Cult 132(3):425–432

Panwar GS, Guru SK (2015) Stimulation of reserpine production in the whole plant culture of *Rauwolfia serpentina* L. by elicitors and precursor feeding. J Plant Biochem Biotechnol 24(1):49–55

Parida R, Mohanty S, Nayak S (2020) Chemical composition and anti–proliferative activity of essential oil from rhizomes of micropropagated *Curcuma aromatica* in Eastern India. J Biol Act Prod Nat 10(1):1–7

Pathania S, Randhawa V, Bagler G (2013) Prospecting for novel plant–derived molecules of *Rauvolfia serpentina* as inhibitors of aldose reductase, a potent drug target for diabetes and its complications. PLoS ONE 8(4):e61327. https://doi.org/10.1371/journal.pone.0061327

Patidar SL, Tiwari G, Tripathi MK, Patel RP, Mishra SN (2015) In vitro biosynthesis and quantification of plumbagin in cell suspension culture of *Plumbago zeylanica*. Med Plants—Int J Phytomed Ind 7(1):60–67

Patil KS, Bhalsing SR (2015) Efficient micropropagation and assessment of genetic fidelity of *Boerhaavia diffusa* L.—high trade medicinal plant. Physiol Mol Biol Plants 21(3):425–432

Patil JG, Ahire ML, Nitnaware KM, Panda S, Bhatt VP, Kishor PBK, Nikam TD (2013) In vitro propagation and production of cardiotonic glycosides in shoot cultures of *Digitalis purpurea* L. by elicitation and precursor feeding. Appl Microbiol Biotechnol 97(6):2379–2393

Patra N, Srivastava AK (2016) Artemisinin production by plant hairy root cultures in gas–and liquid–phase bioreactors. Plant Cell Rep 35(1):143–153

Paul P, Sarkar S, Jha S (2015) Effects associated with insertion of *cryptogein* gene utilizing Ri and Ti plasmids on morphology and secondary metabolites are stable in *Bacopa monnieri* transformed plants grown in vitro and ex vitro. Plant Biotechnol Rep 9:231–245

Pérez-Alonso N, Martín R, Capote A, Pérez A, Hernández-Díaz EK, Rojas L, Jiménez E, Quiala E, Angenon G, Garcia-Gonzales R, Chong-Pérez B (2018) Efficient direct shoot organogenesis, genetic stability and secondary metabolite production of micropropagated *Digitalis purpurea* L. Ind Crops Prod 116:259–266

Piątczak E, Wysokińska H (2013) Encapsulation of *Centaurium erythraea* Rafn—an efficient method for regeneration of transgenic plants. Acta Biol Crac Ser Bot 55(2):37–44

Prakash G, Srivastava AK (2011) Integrated yield and productivity enhancement strategy for biotechnological production of azadirachtin by suspension culture of *Azadirachta indica*. Asia-Pac J Chem Eng 6(1):129–137

Prakash L, Middha SK, Mohanty SK, Swamy MK (2016) Micropropagation and validation of genetic and biochemical fidelity among regenerants of *Nothapodytes nimmoniana* (Graham) Mabb. employing ISSR markers and HPLC. 3 Biotech 6(2):171. https://doi.org/10.1007/s13205-016-0490-y

Praveen N, Thiruvengadam M, Yang YS, Kim SH, Murthy HN, Chung IM (2014) Production of gymnemic acid from hairy root cultures of *Gymnema sylvestre* R. Br. as influenced by polyunsaturated fatty acids (PUFAs) and their antioxidant activity. Ind Crops Prod 54:54–61

Purohit S, Nandi SK, Paul S, Tariq M, Palni LMS (2017) Micropropagation and genetic fidelity analysis in *Amomum subulatum* Roxb.: a commercially important Himalayan plant. J Appl Res Med Aromat Plants 4:21–26

Qazi P, Rashid A, Shawal SA (2011) *Podophyllum hexandrum*: a versatile medicinal plant. Int J Pharm 3:261–268

Rahimi S, Hasanloo T, Najafi F, Khavari-Nejad RA (2012) Methyl jasmonate influence on silymarin production and plant stress responses in *Silybum marianum* hairy root cultures in a bioreactor. Nat Prod Res 26(18):1662–1667

Rai SK, Sharma M, Jain M, Awasthi A, Purshottam DK, Nair NK, Sharma AK (2010) Rapid in vitro production of cloned plants of *Uraria picta* (Jacq.) DC—a rare medicinal herb in long–term culture. Appl Biochem Biotechnol 162(7):1929–1937

Rajan M, Feba KS, Chandran V, Shahena S, Mathew L (2020) Enhancement of rhamnetin production in *Vernonia anthelmintica* (L.) Willd. cell suspension cultures by eliciting with methyl jasmonate and salicylic acid. Physiol Mol Biol Plants 26(7):1531–1539

Rajesh M, Jeyaraj M, Sivanandhan G, Subramanyam K, Mariashibu TS, Mayavan S, Dev GK, Anbazhagan VR, Manickavasagam M, Ganapathi A (2013) *Agrobacterium*-mediated transformation of the medicinal plant *Podophyllum hexandrum* Royle (syn. *P. emodi* Wall. ex Hook.f. & Thomas). Plant Cell Tiss Organ Cult 114(1):171–182

Rajesh M, Sivanandhan G, Arun M, Vasudevan V, Theboral J, Girija S, Manickavasagam M, Selvaraj N, Ganapathi A (2014a) Factors influencing podophyllotoxin production in adventitious root culture of *Podophyllum hexandrum* Royle. Acta Physiol Plant 36(4):1009–1021

Rajesh M, Sivanandhan G, Jeyaraj M, Chackravarthy R, Manickavasagam M, Selvaraj N, Ganapathi A (2014b) An efficient in vitro system for somatic embryogenesis and podophyllotoxin production in *Podophyllum hexandrum* Royle. Protoplasma 251(5):1231–1243

Rajesh M, Sivanandhan G, Subramanyam K, Kapildev G, Jaganath B, Kasthurirengan S, Manickavasagam M, Ganapathi A (2014c) Establishment of somatic embryogenesis and podophyllotoxin production in liquid shake cultures of *Podophyllum hexandrum* Royle. Ind Crop Prod 60:66–74

Raju CS, Aslam A, Shajahan A (2016) Germination and storability of calcium–alginate coated somatic embryos of mango ginger (*Curcuma amada* Roxb.). Hortic Environ Biotech 57(1):88–96

Rangaraju S, Lokesha AN, Aswath CR (2019) Improved production of withanolides in adventitious root cultures of *Withania somnifera* by suspension culture method. Biosci Biotechnol Res Commun 12(1):73–79

Rathore NS, Rai MK, Phulwaria M, Rathore N, Shekhawat NS (2014) Genetic stability in micropropagated *Cleome gynandra* revealed by SCoT analysis. Acta Physiol Plant 36(2):555–559

Ravandi EG, Rezanejad F, Zolala J, Dehghan E (2013) The effects of chromosome–doubling on selected morphological and phytochemical characteristics of *Cichorium intybus* L. J Hortic Sci Biotech 88(6):701–709

Rawat JM, Rawat B, Mehrotra S (2013a) Plant regeneration, genetic fidelity, and active ingredient content of encapsulated hairy roots of *Picrorhiza kurrooa* Royle ex Benth. Biotechnol Lett 35(6):961–968

Rawat B, Rawat JM, Mishra S, Mishra SN (2013b) *Picrorhiza kurrooa*: current status and tissue culture mediated biotechnological interventions. Acta Physiol Plant 35(1):1–12

Ray S, Majumdar A, Bandyopadhyay M, Jha S (2014a) Genetic transformation of sarpagandha (*Rauvolfia serpentina*) with *Agrobacterium rhizogenes* for identification of high alkaloid yielding lines. Acta Physiol Plant 36(6):1599–1605

Ray S, Samanta T, Majumder A, Bandyopadhyay M, Jha S (2014b) Cytogenetic characterization of *Agrobacterium rhizogenes* transformed root lines of *Rauvolfia serpentina*. Nucleus 57:105–112

Ray A, Halder T, Jena S, Sahoo A, Ghosh B, Mohanty S, Mahapatra N, Nayak S (2020) Application of artificial neural network (ANN) model for prediction and optimization of coronarin D content in *Hedychium coronarium*. Ind Crops Prod 146:112186. https://doi.org/10.1016/j.indcrop.2020.112186

Rizvi MZ, Kukreja AK, Bisht NS (2010) In vitro propagation of an endangered medicinal herb *Chlorophytum borivilianum* Sant. et Fernand. through somatic embryogenesis. Physiol Mol Biol Plants 16(3):249–257

Rohela GK, Jogam P, Bylla P and Reuben C (2019) Indirect regeneration and assessment of genetic fidelity of acclimated plantlets by SCoT, ISSR, and RAPD markers in *Rauwolfia tetraphylla* L.: an endangered medicinal plant. BioMed Res Int. https://doi.org/10.1155/2019/3698742

Rolf S, Bruns HJ, Wichter T, Kirchhof P, Ribbing M, Wasmer K, Paul M, Breithardt G, Haverkamp W, Eckardt L (2003) The ajmaline challenge in Brugada syndrome: diagnostic impact, safety and recommended protocol. Eur Heart J 24(12):1104–1112

Roy A, Bharadvaja N (2019) Establishment of root suspension culture of *Plumbago zeylanica* and enhanced production of plumbagin. Ind Crops Prod 137:419–427

Roy AR, Sajeev S, Pattanayak A, Deka BC (2012) TDZ induced micropropagation in *Cymbidium giganteum* Wall. Ex Lindl. and assessment of genetic variation in the regenerated plants. Plant Growth Regul 68(3):435–445

Roychowdhury D, Ghosh B, Chaubey B, Jha S (2013) Genetic and morphological stability of six-year-old transgenic *Tylophora indica* plants. Nucleus 56:81–89

Roychowdhury D, Halder M, Jha S (2017) *Agrobacterium rhizogenes*-mediated transformation in medicinal plants: genetic stability in long-term culture. In: Jha S (ed) Transgenesis and secondary metabolism. Springer, Cham, pp 323–345

Sagar BPS, Zafar R (2005) In vitro-enhanced production of podophyllotoxin in phytohormonal-induced and regenerated roots of *Podophyllum hexandrum*. Pharm Biol 43(5):404–410

Saha S, Sengupta C, Ghosh P (2014) Evaluation of the genetic fidelity of in vitro propagated *Ocimum basilicum* L. using RAPD and ISSR markers. J Crop Sci Biotechnol 17(4):281–287

Saha PS, Sarkar S, Jeyasri R, Muthuramalingam P, Ramesh M, Jha S (2020) In vitro propagation, phytochemical and neuropharmacological profiles of *Bacopa monnieri* (L) Wettst: A review. Plants 9(4):411. https://doi.org/10.3390/plants9040411

Sahai A, Shahzad A (2013) High frequency in vitro regeneration system for conservation of *Coleus forskohlii*: a threatened medicinal herb. Acta Physiol Plant 35(2):473–481

Sahoo S, Rout GR (2014) Plant regeneration from leaf explants of *Aloe barbadensis* Mill. and genetic fidelity assessment through DNA markers. Physiol Mol Biol Plants 20(2):235–240

Sahoo S, Singh S, Sahoo A, Sahoo BC, Jena S, Kar B, Nayak S (2020) Molecular and phytochemical stability of long term micropropagated greater galanga (*Alpinia galanga*) revealed suitable for industrial applications. Ind Crops Prod 148:112274. https://doi.org/10.1016/j.indcrop.2020.112274

Saklani K, Singh S, Purohit VK, Prasad P, Nautiyal AR (2015) In vitro propagation of Rudraksha (*Elaeocarpus sphaericus* (Gaertn.) K. Schum): a biotechnological approach for conservation. Physiol Mol Biol Plants 21(4):611–615

Salehi M, Karimzadeh G, Naghavi MR (2019) Synergistic effect of coronatine and sorbitol on artemisinin production in cell suspension culture of *Artemisia annua* L. cv. Anamed. Plant Cell Tiss Organ Cult 137(3):587–597

Salma U, Kundu S, Ali MN, Mandal N (2019) Somatic embryogenesis–mediated plant regeneration of *Eclipta alba* (L.) Hassk and its conservation through synthetic seed technology. Acta Physiol Plant 41(6):103. https://doi.org/10.1007/s11738-019-2898-6

Samaddar T, Sarkar S, Jha S (2019) Agrobacterium rhizogenes mediated transformation of the critically endangered species, *Swertia Chirayita*. Plant Tiss Cult Biotechnol 29(2):231–244

Samantaray S, Maiti S (2010) An assessment of genetic fidelity of micropropagated plants of *Chlorophytum borivilianum* using RAPD markers. Biol Plant 54(2):334–338

Sankar-Thomas YD, Lieberei R (2011) Camptothecin accumulation in various organ cultures of *Camptotheca acuminata* Decne grown in different culture systems. Plant Cell Tiss Organ Cult 106(3):445–454

Sarkar S, Ghosh I, Roychowdhury D, Jha S (2018) The effects of *rol* genes of *Agrobacterium rhizogenes* on morphogenesis and secondary metabolite accumulation in medicinal plants. In: Kumar

N (ed) Biotechnological approaches for medicinal and aromatic plants. Springer, Singapore, pp 27–51

Sarma D, Kukreja AK, Baruah A (1997) Transforming ability of two *Agrobacterium rhizogenes* strains in *Rauvolfia serpentina* (L.) leaves. Indian J Plant Physiol 2(2):166–168

Sarmadi M, Karimi N, Palazón J, Ghassempour A, Mirjalili MH (2019) Improved effects of polyethylene glycol on the growth, antioxidative enzymes activity and taxanes production in a *Taxus baccata* L. callus culture. Plant Cell Tiss Organ Cult 137(2):319–328

Senapati SK, Lahere N, Tiwary BN (2014) Improved in vitro clonal propagation of *Rauwolfia serpentina* L. Benth—an endangered medicinal plant. Plant Biosyst 148(5):885–888

Shabir PA (2020) CRISPR/Cas9–mediated genome editing in medicinal and aromatic plants: developments and applications. In: Aftab T, Hakeem KR (eds) Medicinal and aromatic plants. Academic Press. https://doi.org/10.1016/B978-0-12-819590-1.00009-4

Shahzad A, Parveen S, Fatema M (2011) Development of a regeneration system via nodal segment culture in *Veronica anagallis–aquatica* L.—an amphibious medicinal plant. J Plant Interact 6(1):61–68

Sharma U, Agrawal V (2018) In vitro shoot regeneration and enhanced synthesis of plumbagin in root callus of *Plumbago zeylanica* L.—an important medicinal herb. Vitro Cell Dev Biol–Plant 54:423–435

Sharma B, Bansal YK (2013) In vitro propagation of *Gymnema sylvestre* Retz. R. Br through apical bud culture. J Med Plant Res 4(14):1473–1476

Sharma N, Pandey R (2013) Conservation of medicinal plants in the tropics. In: Normah MN, Chin HF, Reed BM (eds) Conservation of tropical plant species. Springer, New York, pp 437–487

Sharma S, Satardekar KV, Barve SS (2018) Genetic improvement of medicinal and aromatic plants through haploid and double haploid development. In: Kumar N (ed) Biotechnological approaches for medicinal and aromatic plants. Springer, Singapore, pp 523–556

Sharma A, Mathur AK, Ganpathy J, Joshi B, Patel P (2019) Effect of abiotic elicitation and pathway precursors feeding over terpenoid indole alkaloids production in multiple shoot and callus cultures of *Catharanthus roseus*. Biology 74(5):543–553

Sheludko Y, Gerasimenko I, Unger M, Kostenyuk I, Stöckigt J (1999) Induction of alkaloid diversity in hybrid plant cell cultures. Plant Cell Rep 18:911–918

Sheludko Y, Gerasimenko I, Kolshorn H, Stockigt J (2002) New alkaloids of the sarpagine group from *Rauvolfia serpentina* hairy root culture. J Nat Prod 65(7):1006–1010

Shinde S, Sebastian JK, Jain JR, Hanamanthagouda MS, Murthy HN (2016) Efficient in vitro propagation of *Artemisia nilagirica* var. *nilagirica* (Indian wormwood) and assessment of genetic fidelity of micropropagated plants. Physiol Mol Biol Plant 22(4):595–603

Singh S, Pandey P, Ghosh S, Banerjee S (2018) Anti-cancer labdane diterpenoids from adventitious roots of *Andrographis paniculata*: augmentation of production prospect endowed with pathway gene expression. Protoplasma 255(5):1387–1400

Sivakumar G, Liu C, Towler MJ, Weathers PJ (2010) Biomass production of hairy roots of *Artemisia annua* and *Arachis hypogaea* in a scaled-up mist bioreactor. Biotechnol Bioeng 107(5):802–813

Sivanandhan G, Selvaraj N, Ganapathi A, Manickavasagam M (2014) Enhanced biosynthesis of withanolides by elicitation and precursor feeding in cell suspension culture of *Withania somnifera* (L.) Dunal in shake-flask culture and bioreactor. PLoS One 9(8):e104005. https://doi.org/10.1371/journal.pone.0104005

Sivanandhan G, Selvaraj N, Ganapathi A, Lim YP (2020) Up-regulation of Squalene synthase in hairy root culture of *Withania somnifera* (L.) Dunal yields higher quantities of withanolides. Ind Crops Prod 154:112706

Soliman HIA, Metwali EMR, Almaghrabi OAH (2014) Micropropagation of *Stevia rebaudiana* Betroni and assessment of genetic stability of in vitro regenerated plants using inter simple sequence repeat (ISSR) marker. Plant Biotechnol 14–0707. https://doi.org/10.5511/plantbiotechnology.14.0707a

Sooriamuthu S, Varghese RJ, Bayyapureddy A, John SST, Narayanan R (2013) Light-induced production of antidepressant compounds in etiolated shoot cultures of *Hypericum hookerianum* Wight & Arn.(Hypericaceae). Plant Cell Tiss Organ Cult 115(2):169–178

Spiridonova KV, Adnof DM, Andreev IO, Kunakh VA (2007) Stability of the genome of highly productive *Rauwolfia serpentina* Benth K–27 cell line at changing maintenance conditions. Biopolym Cell 23(2):86–92

Sreedevi E, Anuradha M, Pullaiah T (2013) Plant regeneration from leaf-derived callus in *Plectranthus barbatus* Andr. [Syn.: *Coleus forskohlii* (Wild.) Briq.]. Afr J Biotechnol 12(18):2441–2448

Srivastava S, Srivastava AK (2012) In vitro azadirachtin production by hairy root cultivation of *Azadirachta indica* in nutrient mist bioreactor. Appl Biochem Biotechnol 166(2):365–378

Srivastava S, Conlan XA, Adholeya A, Cahill DM (2016) Elite hairy roots of *Ocimum basilicum* as a new source of rosmarinic acid and antioxidants. Plant Cell Tiss Organ Cult 126(1):19–32

Srivastava M, Sharma S, Misra P (2016) Elicitation based enhancement of secondary metabolites in *Rauwolfia serpentina* and *Solanum khasianum* hairy root cultures. Pharmacogn Mag 12:315–320

Srivastava M, Singh G, Sharma S, Shukla S, Misra P (2019) Elicitation enhanced the yield of glycyrrhizin and antioxidant activities in hairy root cultures of *Glycyrrhiza glabra* L. J Plant Growth Regul 38(2):373–384

Sultan P, Shawl AS, Ramteke PW, Jan A, Chisti N, Jabeen N, Shabir S (2006) In vitro propagation for mass multiplication of *Podophyllum hexandrum*: a high value medicinal herb. Asian J Plant Sci 5(2):179–184

Sun D, Li C, Qin H, Zhang Q, Yang Y, Ai J (2016) Somatic embryos cultures of *Vitis amurensis* Rupr in air-lift bioreactors for the production of biomass and resveratrol. J Plant Biol 59(5):427–434

Swaroopa G, Anuradha M, Pullaiah T (2013) Elicitation of forskolin in suspension cultures of *Coleus forskohlii* (Willd.) Briq. using bacterial elicitors. J Indian Bot Soc 92:97–100

Tariq A, Naz S, Shahzadi K, Ilyas S, Javed S (2015) Study of genetic stability in in vitro conserved *Podophyllum hexandrum* using RAPD markers. J Anim Plant Sci 25(4):1114–1120

Thakore D, Srivastava AK, Sinha AK (2017) Mass production of ajmalicine by bioreactor cultivation of hairy roots of *Catharanthus roseus*. Biochem Eng J 119:84–91

Thakur A, Thakur PS, Dutt V, Thakur CL (2010) Conservation of *Podophyllum hexandrum* through seeds. Indian J Plant Physiol 15(2):110–116

Thakur J, Dwivedi MD, Sourabh P, Uniyal PL, Pandey AK (2016) Genetic homogeneity revealed using SCoT, ISSR and RAPD markers in micropropagated *Pittosporum eriocarpum* Royle—an endemic and endangered medicinal plant. PLoS ONE 11(7):e0159050

The Wealth of India (1969) Volume VIII. Publications and information directorate, CSIR, New Delhi, India

Thiruvengadam M, Praveen N, Kim EH, Kim SH, Chung IM (2014) Production of anthraquinones, phenolic compounds and biological activities from hairy root cultures of *Polygonum multiflorum* Thunb. Protoplasma 251(3):555–566

Thomas TD, Yoichiro H (2010) In vitro propagation for the conservation of a rare medicinal plant *Justicia gendarussa* Burm. f. by nodal explants and shoot regeneration from callus. Acta Physiol Plant 32(5):943–950

Torkamani MRD, Abbaspour N, Jafari M, Samadi A (2014) Elicitation of valerenic acid in the hairy root cultures of *Valeriana officinalis* L (Valerianaceae). Trop J Pharm Res 13(6):943–949

Tyagi P, Khanduja S, Kothari SL (2010) In vitro culture of *Capparis decidua* and assessment of clonal fidelity of the regenerated plants. Biol Plant 54(1):126–130

Umezawa T (2003) Diversity in lignan biosynthesis. Phytochem Rev 2:371–390

Upadhyay R, Kashyap SP, Singh CS, Tiwari KN, Singh K, Singh M (2014) Ex situ conservation of *Phyllanthus fraternus* Webster and evaluation of genetic fidelity in regenerates using DNA—based molecular marker. Appl Biochem Biotechnol 174(6):2195–2208

Vazquez-Marquez AM, Zepeda-Gómez C, Burrola-Aguilar C, Bernabe-Antonio A, Nieto-Trujillo A, Cruz-Sosa F, Rodriguez-Monroy M, Estrada-Zúñiga ME (2019) Effect of stirring speed on the production of phenolic secondary metabolites and growth of *Buddleja cordata* cells cultured in mechanically agitated bioreactor. Plant Cell Tiss Organ Cult 139(1):155–166

Veremeichik GN, Bulgakov VP, Shkryl YN, Silantieva SA, Makhazen DS, Tchernoded GK, Mischenko NP, Fedoreyev SA, Vasileva EA (2019) Activation of anthraquinone biosynthesis in long-cultured callus culture of *Rubia cordifolia* transformed with the *rolA* plant oncogene. J Biotechnol 306:38–46

Vereshchagina YV, Bulgakov VP, Grigorchuk VP, Rybin VG, Veremeichik GN, Tchernoded GK, Gorpenchenko TY, Koren OG, Phan NHT, Minh NT, Chau LT, Zhuravlev YN (2014) The *rolC* gene increases caffeoylquinic acid production in transformed artichoke cells. Appl Microbiol Biotechnol 98(18):7773–7780

Verma S, Singh N (2012) In vitro mass multiplication of *Acorus calamus* L.-an endangered medicinal plant. Am Eur J Agric Environ Sci 12:1514–1521

Verma P, Mathur AK, Singh A, Srivastava A, Masood N, Luqman S, Mohita Upadhyaya M, Mathur A (2012) Co-culturing of *Catharanthus roseus*, *Vinca major* and *Rauwolfia serpentina* cell suspensions in shake flask and bioreactor: production of a novel alkaloid with antioxidant potential. J Med Plant Res 6(36):4978–4988

Verma P, Sharma A, Khan SA, Mathur AK, Shanker K (2015) Morphogenetic and chemical stability of long-term maintained *Agrobacterium*-mediated transgenic *Catharanthus roseus* plants. Nat Prod Res 29(4):315–320

Wang S, Wang J, Liang W, Yao L, Gao W (2019) Promotion of ginsenosides production in a co-cultivation system of *Panax ginseng* adventitious roots and immobilized *Aspergillus niger*. Ind Crops Prod 140:111564. https://doi.org/10.1016/j.indcrop.2019.111564

Wankhede DP, Biswas DK, Rajkumar S, Sinha AK (2013) Expressed sequence tags and molecular cloning and characterization of gene encoding pinoresinol/lariciresinol reductase from *Podophyllum hexandrum*. Protoplasma 250:1239–1249

Weremczuk-Jeżyna I, Grzegorczyk-Karolak I, Frydrych B, Królicka A, Wysokińska H (2013) Hairy roots of *Dracocephalum moldavica*: rosmarinic acid content and antioxidant potential. Acta Physiol Plant 35(7):2095–2103

Weremczuk-Jezyna I, Kochan E, Szymczyk P, Lisiecki P, Kuźma Ł, Grzegorczyk-Karolak I (2019) The antioxidant and antimicrobial properties of phenol rich extracts of *Dracocephalum forrestii* W. W. Smith shoot cultures grown in the nutrient sprinkle bioreactor. Phytochem Lett 30:254–260

Willis KJ (2017) State of the world's plants 2017. Royal Botanic Gardens, Kew, London

Wróbel T, Dreger M, Wielgus K, Słomski R (2018) The application of plant in vitro cultures in cannabinoid production. Biotechnol Lett 40(3):445–454

Wu SQ, Yu XK, Lian ML, Park SY, Piao XC (2014) Several factors affecting hypericin production of *Hypericum perforatum* during adventitious root culture in airlift bioreactors. Acta Physiol Plant 36(4):975–981

Xia J, Ma YJ, Wang Y, Wang JW (2018) Deciphering transcriptome profiles of tetraploid *Artemisia annua* plants with high artemisinin content. Plant Physiol Biochem 130:112–126

Yadav K, Aggarwal A, Singh N (2013) Evaluation of genetic fidelity among micropropagated plants of *Gloriosa superba* L. using DNA-based markers—a potential medicinal plant. Fitoterapia 89:265–270

Yadav K, Groach R, Aggarwal A, Singh N (2015) A reliable protocol for micropropagation of *Gloriosa superba* L. (Colchicaceae). Asia Pac J Mol Biol Biotechnol 23(1):243–252

Yang L, Stöckigt J (2010) Trends for diverse production strategies of plant medicinal alkaloids. Nat Prod Rep 27:1469–1479

Yang Y, Pu X, Qu X, Chen F, Zhang G, Luo Y (2017) Enhanced production of camptothecin and biological preparation of N^1-acetylkynuramine in *Camptotheca acuminata* cell suspension cultures. Appl Microbiol Biotechnol 101(10):4053–4062

Yang J, Yang D, Lü W, Zhang X, Ma M, Liu G, Jiang J, Li C (2020) Somatic embryogenesis and plant regeneration in *Betula platyphalla*. J For Res 1–8. https://doi.org/10.1007/s11676-020-01131-9

Yu Y, Zhang WB, Li XY, Piao XC, Jiang J, Lian ML (2016) Pathogenic fungal elicitors enhance ginsenoside biosynthesis of adventitious roots in *Panax quinquefolius* during bioreactor culture. Ind Crops Prod 94:729–735

Yuan F, Wang Q, Pan Q, Wang G, Zhao J, Tian Y, Tang K (2011) An efficient somatic embryogenesis based plant regeneration from the hypocotyl of *Catharanthus roseus*. Afr J Biotechnol 10:14786–14795

Zafar N, Mujib A, Ali M, Tonk D, Gulzar B (2017) Aluminum chloride elicitation (amendment) improves callus biomass growth and reserpine yield in *Rauvolfia serpentina* leaf callus. Plant Cell Tiss Organ Cult 130:357–368

Zafar N, Mujib A, Ali M, Tonk D, Gulzar B, Malik M, Sayeed R, Mamgain J (2019) Genome size analysis of field grown and tissue culture regenerated *Rauvolfia serpentina* (L) by flow cytometry: Histology and scanning electron microscopic study for in vitro morphogenesis. Ind Crops Prod 128:545–555

Zafar N, Mujib A, Ali M, Tonk D, Gulzar B, Malik MQ, Mamgain J, Sayeed R (2020) Cadmium chloride ($CdCl_2$) elicitation improves reserpine and ajmalicine yield in *Rauvolfia serpentina* as revealed by high-performance thin-layer chromatography (HPTLC). 3 Biotech 10(8):1–14

Zaker A, Sykora C, Gössnitzer F, Abrishamchi P, Asili J, Mousavi SH, Wawrosch C (2015) Effects of some elicitors on tanshinone production in adventitious root cultures of *Perovskia abrotanoides* Karel. Ind Crops Prod 67:97–102

Zayova E, Nedev T, Petrova D, Zhiponova M, Kapchina V, Chaneva G (2020) Tissue culture applications of *Artemisia annua* L. callus for indirect organogenesis and production phytochemical. Plant Tiss Cult Biotechnol 30(1):97–106

Zebarjadi A, Dianatkhah S, Mohammadi PP, Qaderi A (2018) Influence of abiotic elicitors on improvement production of artemisinin in cell culture of *Artemisia annua* L. Cell Mol Biol 64(9):1–5

Zhang F, Lv Y, Dong H, Guo S (2010) Analysis of genetic stability through intersimple sequence repeats molecular markers in micropropagated plantlets of *Anoectochilus formosanus* Hayata, a medicinal plant. Biol Pharm Bull 33(3):384–388

Zhang B, Zheng LP, Yi Li W, Wen Wang J (2013) Stimulation of artemisinin production in *Artemisia annua* hairy roots by Ag–SiO_2 core–shell nanoparticles. Curr Nanosci 9(3):363–370

Zhou P, Yang J, Zhu J, He S, Zhang W, Yu R, Zi J, Song L, Huang X (2015) Effects of β–cyclodextrin and methyl jasmonate on the production of vindoline, catharanthine, and ajmalicine in *Catharanthus roseus* cambial meristematic cell cultures. App Microbiol Biotechnol 99(17):7035–7045

Zhou Z, Tan H, Li Q, Chen J, Gao S, Wang Y, Chen W, Zhang L (2018) CRISPR/Cas9–mediated efficient targeted mutagenesis of RAS in *Salvia miltiorrhiza*. Phytochemistry 148:63–70

Chapter 17
Higher Plant Sources of Cancer Chemotherapeutic Agents and the Potential Role of Biotechnological Approaches for Their Supply

Peter J. Blanco Carcache, Ermias Mekuria Addo, and A. Douglas Kinghorn

Abstract There are five major structural groups of cancer chemotherapeutic agents derived from higher plants approved clinically in the USA. These are used either in their unmodified naturally occurring form, as semi-synthetic derivatives, or as a conjugate as part of a larger drug molecule. In this chapter, those compounds used currently as approved oncolytic drugs will be described, following by a brief mention of selected plant-derived compounds in current clinical trials as potential cancer chemotherapeutic agents. Next, details on the methods proposed for the production for the oncology drug market of three examples of different structural groups of plant-derived anticancer agents will be given [*viz.*, bisindole (Vinca) alkaloids, podophyllotoxin lignan analogs, and taxane diterpenoid derivatives]. The plant natural products surveyed represent sustainable sources of specialized pure chemicals that are valuable in treating cancer.

Keywords Higher plants · Cancer chemotherapeutic agents · Clinical trials · Biotechnological methods of production · Secondary metabolites

17.1 Introduction

Cancer continues to be the second-leading cause of death, and more than 1.8 million people will be diagnosed with this disease in the USA alone in 2020, with the mortality rate for this same year projected to be above 9 million worldwide (Siegel et al. 2020; WHO 2018). This disease condition has many initial symptoms and complications that lead to a negative impact on an afflicted person's quality of life. Such symptoms include pain, bleeding, extreme fatigue, neurological problems, and dangerous weight gain or loss (National Cancer Institute 2019). The leading cancer types in new estimated cases in the USA for 2020 include those of the prostate or breast, lung and bronchus, colon and rectum, urinary corpus or bladder, melanoma

P. J. Blanco Carcache · E. M. Addo · A. D. Kinghorn (✉)
Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA
e-mail: kinghorn.4@osu.edu

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_17

of the skin, kidney and renal pelvis, non-Hodgkin's lymphoma, oral cavity, pharynx or thyroid, and pancreas, as well as leukemias and lymphomas (Siegel et al. 2020). However, the availability of cancer treatments is still somewhat limited, and many are not easily affordable. Therefore, additional treatments for cancer from sustainable resources need to be found and utilized.

Nature is a rich source of potential therapeutic agents obtained from various different life forms (Butler et al. 2014; Newman and Cragg 2020a). In particular, plant-based traditional medicine continues to play an essential role in the primary health care of approximately 80% of the world's population (Agarwal et al. 2020; Cragg and Newman 2005). Furthermore, nature continues to produce viable effective anticancer agents that are sustainable, such as those in clinical use that come from diverse origins, including plants, terrestrial microbial, and marine organism sources (Agarwal et al. 2020; Basmadjian et al. 2014; Butler et al. 2014; Cragg and Newman 2005; Cragg and Pezzuto 2016; Khazir et al. 2014; Li and Vederas 2009). Moreover, natural products have been established as being chemically diverse with optimal chirality to interact with different drug targets (Crüsemann et al. 2016; Pietra 2002).

Plant constituents have served as an important group of naturally occurring anticancer drugs. A particularly notable example is that of taxol (paclitaxel), a potent antineoplastic taxane diterpenoid isolated from *Taxus brevifolia* Nutt. of very wide clinical use that first reached the clinic in the USA to treat ovarian cancer in 1992 (Donehower 1996; Wani et al. 1971). There are also members of other compound classes of higher plant origin that have afforded anticancer compounds, namely the bisindole alkaloids, the podophyllotoxin lignans, the camptothecin derivatives, and the cephalotaxine analog, omecetaxine mepesuccinate (Agarwal et al. 2020; Butler et al. 2014; Henkin et al. 2018; Newman and Cragg 2020a). Over the past few decades, natural products have been used in various forms to treat various cancer types more efficiently. In more recent years, such natural products have evolved to incorporate some of the modern approaches of drug delivery used to develop safer, more efficaceous anticancer drugs. One type of approach is the use of antibody drug conjugates (ADCs), which are targeted therapeutics composed of three components (a cytotoxic drug, a linker, and an antibody) and are designed for selective delivery of very potent anticancer drugs (Agarwal et al. 2020). In December 2019, the first example of a higher plant-derived ADC was approved by the US FDA, namely famtrastuzumab deruxtecan-nxki (also known as DS-8201a, Enhertu®; Daiichi Sankyo), for the treatment of unresectable or metastatic HER2-positive breast cancer (US Food and Drug Administration 2019). DS-8201a is an ADC derived from the conjugation of the camptothecin derivative DXd with the monoclonal antibody trastuzumab via an enzyme cleavable peptide linker (Nakada et al. 2019).

Thus, higher plants have afforded numerous purified compounds of promise in treating cancer, which can be utilized in not only their unmodified naturally occurring forms, but also as semi-synthetic derivatives, or as conjugates in larger molecules. In this chapter, the major pure compounds of plant origin used currently as approved oncolytic drugs will be surveyed briefly, following by a selection of such compounds in clinical trials as potential cancer chemotherapeutic agents. After this, information on different approaches proposed of the production for the oncology drug market of

three selected groups of plant-derived anticancer agents (viz., bisindole alkaloids, podophyllotoxin analogs, and taxane derivatives) will be provided. Overall, plant natural products may be seen to have afforded sustainable sources of valuable rare compounds that now have major use in treating cancer.

17.2 Approved Plant-Derived Anticancer Drugs

In the paragraphs below, the plant-derived anticancer agents obtained from renewable resources that have been developed as sustainable anticancer agents for the market in Western medicine will be described (Fig. 17.1). These clinically used agents may be classified into several major groups.

The first group of plant-derived anticancer drugs introduced to the US market nearly 60 years ago were the bisindole alkaloids. This class of bisindole alkaloid drugs includes two compounds isolated from *Catharanthus roseus* G. Don (Apocynaceae), namely vinblastine (**1**) and vincristine (**2**). Additionally, three synthetic derivatives vindesine (**3**), vinflunine (**4**), and vinorelbine (**5**) have been developed. The two natural product drugs **1** and **2** have antimitotic effects and antimicrotubule properties. The US FDA approved these for the treatment of different cancer types, including certain forms of breast and lung cancer, leukemia, and lymphoma (Panda et al. 1996; Rowinsky and Donehower 1991; Tafur et al. 1975). Recent optimization of the formulation of vincristine has improved both its pharmacokinetic and pharmacodynamic profiles, to produce the FDA-approved vincristine sulfate liposome injection (Silverman and Deitcher 2013). The synthetic bisindole alkaloid derivatives also are marketed as anticancer drugs for clinical use. Vindesine (**3**) has been approved for childhood acute lymphocytic leukemia. Vinflunine (**4**) is approved in Europe as monotherapy for the treatment of metastatic bladder cancer and vinorelbine (**5**) also is approved for use against non-small cell lung cancer (Ianniello 1996; Roussi et al. 2012).

The second group of plant-derived anticancer drugs that was introduced commercially includes compounds of the podophyllotoxin lignan class. The epipodophyllotoxin-type lignan derivatives, etoposide (**6**), etoposide phosphate (**7**), and teniposide (**8**), were chemically optimized from the natural compound, podophyllotoxin, isolated from *Podophyllum peltatum* L. (Berberidaceae) (Chen et al. 2013; Clark and Slevin 1987). The main mechanism of action of these chemical derivatives of podophyllotoxin is by inhibiting topoisomerase II (Hartmann and Lipp 2006). Etoposide (**6**) and its close chemical analog etoposide phosphate (**7**), a more water-soluble version, have been used against non-small cell lung, small cell lung, and testicular cancers. Teniposide (**8**) has been utilized in various combination chemotherapy regimens, against neuroblastoma, acute lymphoblastic leukemia, and small cell lung cancer (Lee and Xiao 2012).

The third group of plant-derived anticancer drugs that were introduced to the market are representatives of the taxane diterpenoid class. This group comprises the taxane derivative paclitaxel (**9**) and its semi-synthetic derivatives docetaxel (**10**)

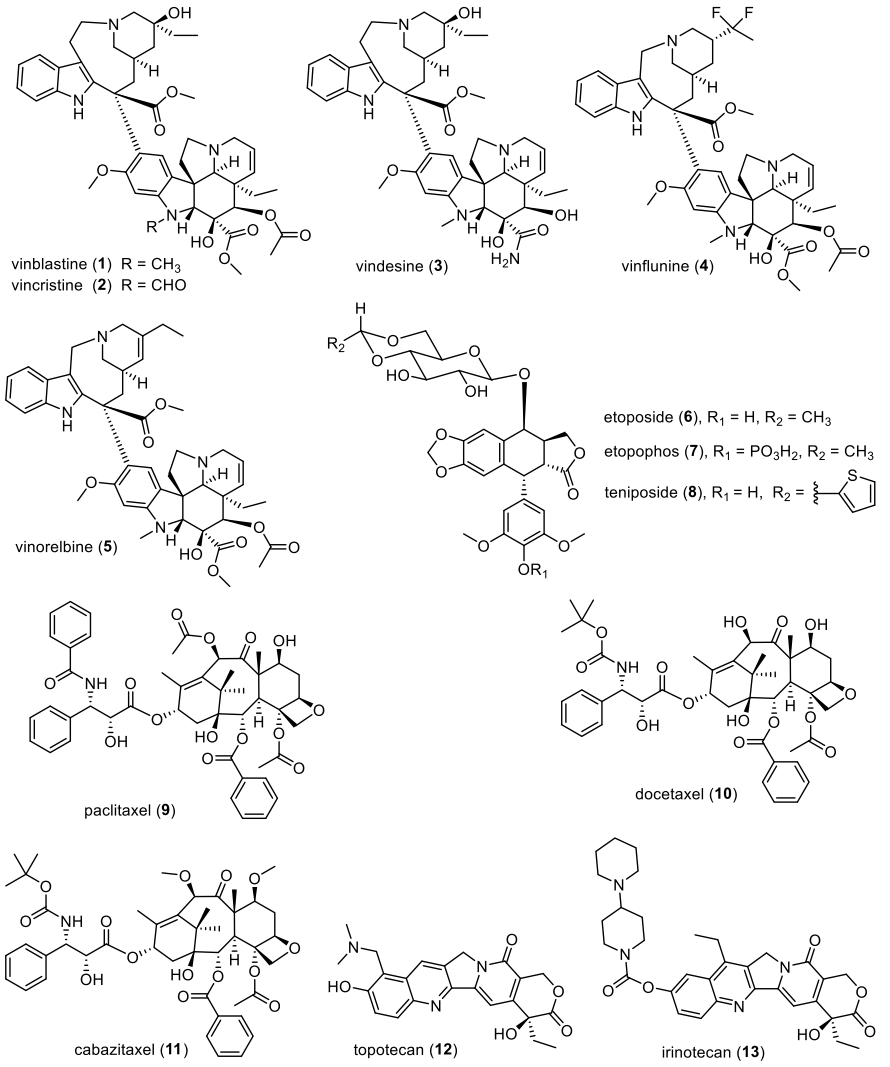


Fig. 17.1 Structures of plant-derived natural products used as anticancer agents

and cabazitaxel (**11**). In 1971, the parent compound, paclitaxel, was isolated and structurally characterized under the trivial name "taxol" from *Taxus brevifolia* Nutt. (Taxaceae) (Wani et al. 1971). Approved initially by the FDA in 1992, paclitaxel was used for the treatment of refractory ovarian cancer (Donehower 1996).

The limited water solubility of paclitaxel led to its chemical optimization as the semi-synthetic derivative, docetaxel (**10**) (Rowinsky and Donehower 1991). The primary mechanisms of action described for these taxane drugs are based on stabilizing the assembly of microtubules as well as inhibiting depolymerization of tubulin

during cell division. At present, both paclitaxel and docetaxel are used for different types of cancer, including breast, ovarian, and non-small cell lung cancer, while a newer analog, cabazitaxel (**11**), is prescribed for the treatment of hormone-refractory prostate cancer (Kingston 2012; Liu et al. 2016; Weaver 2014). Abraxane® is a nanoformulation of paclitaxel, and it has been approved for the treatment of advanced forms of breast cancer, non-small cell lung cancer, and pancreatic cancer (Hare et al. 2017).

A fourth group of plant-derived anticancer drugs to be introduced comprises several camptothecin alkaloid derivatives. In 1966, a new quinolone alkaloid from *Camptotheca acuminata* Decne. (Nyssaceae) was isolated and named camptothecin (Wall et al. 1966). Among camptothecin derivatives of interest for cancer treatment are topotecan (**12**) and irinotecan (**13**). Camptothecin proved to have suboptimal solubility and toxicity; hence, the chemical analogs **12** and **13** were developed successfully to improve its efficiency, by enhancing its bioavailability, and thus gained FDA approval about 25 years ago (Ciardiello et al. 1999; Hörmann et al. 2012). Members of the camptothecin compound class act as topoisomerase I inhibitors, with topotecan utilized for metastatic ovarian cancer (Ciardiello et al. 1999; Hörmann et al. 2012). Irinotecan, on the other hand, is used for metastatic colorectal cancer (Takeba et al. 2007; Villalona-Calero and Kolesar 2002). The very recently US FDA-approved ADC, fam-trastumazab deruxitecan-nxki (Enhertu®, Fig. 17.2) (**14**), is also a camptothecin derivative (Nakada et al. 2019).

The initial member of a fifth group of plant-derived anticancer drugs is categorized under the *Cephalotaxus* alkaloid class. Homoharringtonine was first isolated from an alkaloid fraction of *Cephalotaxus harringtonia* Kitam. (Taxodiaceae) (Powell et al. 1970). This compound, as the derivative, omacetaxine mepesuccinate (**15**), has been shown to exert antitumor and antiangiogenic activity, apoptotic induction, and protein synthesis inhibition. Omacetaxine mepesuccinate (**15**) is the first member of a new class of FDA-approved anticancer agents that acts as a protein translation inhibitor. Omacetaxine mepesuccinate (**15**) has been approved for the treatment of chronic

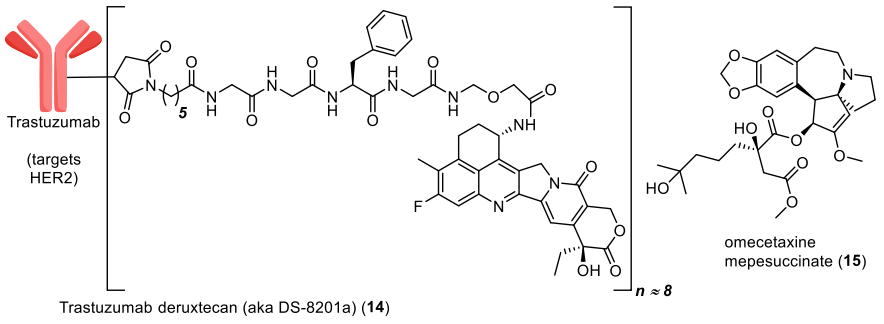


Fig. 17.2 Structures of fam-trastumazab deruxitecan-nxki (Enhertu®; **14**) [Adapted from (Nakada et al. 2019)] and omacetaxine mepesuccinate (**15**)

myeloid leukemia, particularly for adult patients with resistance or intolerance to two or more tyrosine kinase inhibitors (Alvandi et al. 2014).

Thus, higher plants have been shown as outstanding and sustainable sources of therapeutically useful cancer chemotherapeutic agents and hence may be expected to continue to provide new drug leads for the development of new anticancer drugs in the future.

17.3 Examples of Plant-Derived Anticancer Agents Undergoing Clinical Evaluation

In the paragraphs following, a selection of compounds isolated from higher plants and their derivatives that are undergoing human clinical studies is covered (Table 17.1 and Fig. 17.3). These are included in a relevant database of the US National Cancer Institute (NCI) (National Cancer Institute 2020).

A plant-derived natural product that has reached both phase III and IV level clinical trials is the camptothecin derivative karenitecin (**16**). Karenitecin is a topoisomerase I inhibitor intended for the potential treatment of melanoma (Munster and Daud 2011). The tubulin-binding agent combretastatin A-4 analog, fosbretabulin (**17**), has also reached both phase III and IV level clinical trials for the treatment of

Table 17.1 Potential plant-derived anticancer agents in ongoing clinical trials[a]

Chemotherapeutic agent	Compound type[b]	I[c]	II[c]	III[c]
Karenitecin (**16**)	NP-derived	X	X	X (IV)
Fosbretabulin (**17**)	SS NP		X	X (IV)
Combretastatin A-1 (**18**)	SS NP	X	X	
BNC105 (**19**)	NP-derived		X	X (IV)
AT-101 (**20**)	NP-derived	X	X	
Picropodophyllotoxin (**21**)	NP	X	X	
trans-Resveratrol (**22**)	NP	X	X (IV)	
ME-344 (**23**)	NP-derived	X (IV)	X	X
ARQ 761 (**24**)	NP-derived	X (IV)		
TPI 287 (**25**)	NP-derived	X	X	X (IV)
Ortataxel (**26**)	NP-derived	X	X	X (IV)
Riviciclib (**27**)	NP-derived	X	X (IV)	
Minnelide (**28**)	NP	X (IV)		

[a]Adapted from a recent review article and https://www.cancer.gov/about-cancer/treatment/clinical-trials/search (Butler et al. 2014; National Cancer Institute 2020)
[b]NP = natural product; SS = semi-synthetic
[c]I, II, III (IV) correspond to phase I, phase II, and phase III trials; phase IV trials are a continuation of phase III trials on a larger population

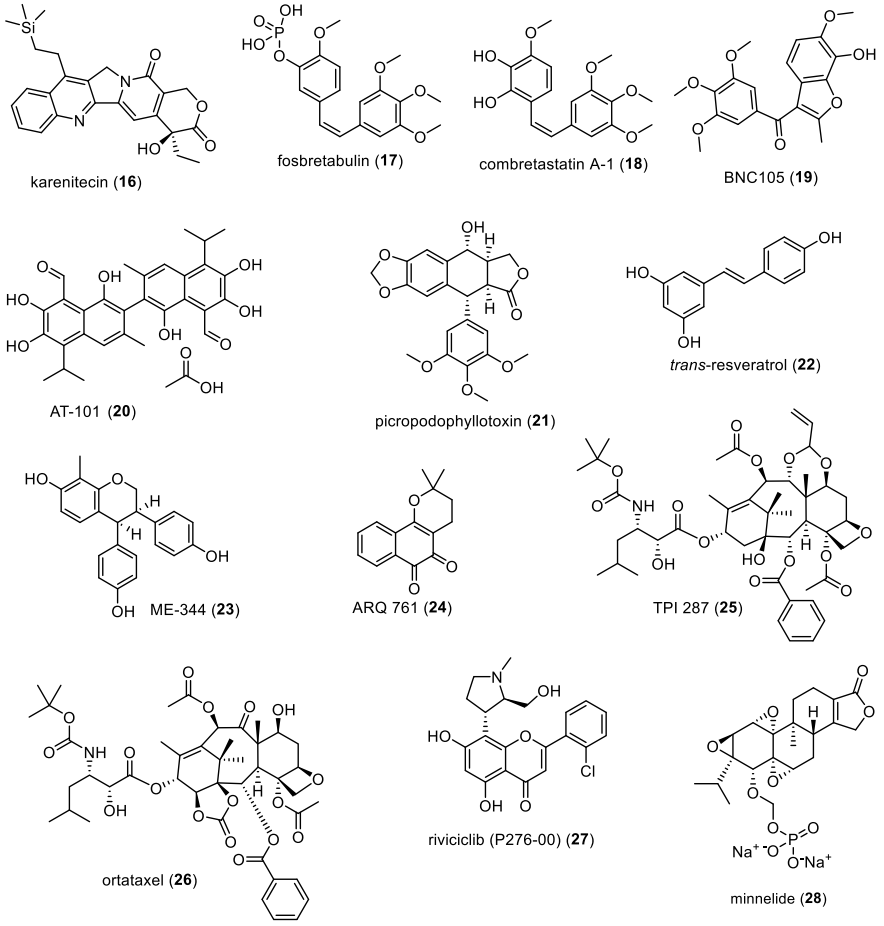


Fig. 17.3 Structures of some plant-derived anticancer agents undergoing clinical trials. [Adapted from (Butler et al. 2014)]

leukemia (Siemann et al. 2009). The structurally related compounds, combretastatin A-1 (**18**) and an additional derivative of combretastatin A-4, BNC105 (**19**), have also reached clinical trials (Butler et al. 2014; Rischin et al. 2011). AT-101 (**20**), a gossypol analog and a Bcl-2 inhibitor, has completed a phase II clinical trial for small cell lung carcinoma (Baggstrom et al. 2011). An insulin-like growth factor 1 receptor pathway modulator, picropodophyllotoxin (**21**), also known as AXL1717, has completed phase II trials against local and metastatic non-small cell lung cancer (Bergqvist et al. 2017; Butler et al. 2014). A naturally occurring plant stilbenoid, *trans*-resveratrol (**22**), has successfully completed phase II trials for relapsed multiple myeloma patients (Tomé-Carneiro et al. 2013).

ME-344 (**23**), a genistein derivative, is being studied as a mitochondrial inhibitor in phase I and IV clinical trials (Butler et al. 2014; Diamond et al. 2017; Scarfò and

Ghia 2013). ARQ-761 (**24**), a beta-lapachone derivative, has entered phase I and IV clinical trials as a reversible Bruton's tyrosine kinase (BTK) inhibitor. It has also been in phase I clinical trials for NAD(P)H:quinone oxidoreductase 1 (NQO1) cancer cell necrosis (Butler et al. 2014; Gerber et al. 2018). The tubulin-stabilizing agents, TPI 287 (**25**) and ortataxel (**26**), are paclitaxel derivatives that have been in phase II and IV clinical trials for glioblastoma (Butler et al. 2014; Khazir et al. 2014; McQuade et al. 2016; Silvani et al. 2019). Riviciclib (P276-00) (**27**), a rohitukine derivative, is under study in phase II and IV as a cyclin-dependent kinase modulator (Joshi et al. 2007). The transcriptional activator, minnelide (**28**), is currently in phase I and IV trials for non-small cell lung carcinoma as a prodrug of triptolide (Rousalova et al. 2013).

Therefore, from the examples given, plant-derived compounds representative of quite wide structural diversity are presently being investigated in human clinical trials as potential anticancer agents.

17.4 Production Methods for Selected Plant-Derived Cancer Chemotherapeutic Agents

In this section, the role of biotechnological methods in solving the supply issue of plant-derived anticancer natural products is presented. Discussion is limited to three examples of anticancer drugs representing three different structural classes of plant secondary metabolites: monoterpene bisindole alkaloids (Vinca alkaloids), a lignan (podophyllotoxin), and a diterpenoid (paclitaxel). Furthermore, emphasis is given on either a lead compound (podophyllotoxin) that subsequently was modified by chemical synthesis, or on those natural products directly used as drugs themselves without modification (vinblastine and vincristine, and paclitaxel).

17.4.1 Vinca Alkaloids

17.4.1.1 Overview and Problems with Supply

The Madagascar periwinkle *Catharanthus roseus* (L) G. Don (Apocynaceae) is currently the only commercial source of the anticancer monoterpene bisindole alkaloids, vinblastine (formerly vincaleukoblastine, **1**), and vincristine (formerly leurocristine, **2**) (Duge de Bernonville et al. 2015). These were isolated, respectively, in 1957 (vinblastine, **1**) and 1961 (vincristine, **2**) from the leaves of *C. roseus* by two independent research groups (Noble et al. 1958; Svoboda 1961). Biosynthetically, these alkaloids are heterodimers produced by the oxidative coupling of catharanthine and vindoline, which each in turn arise from the coupling of tryptamine and secologanin (Fig. 17.4). While tryptamine is biosynthesized from the amino acid trypto-

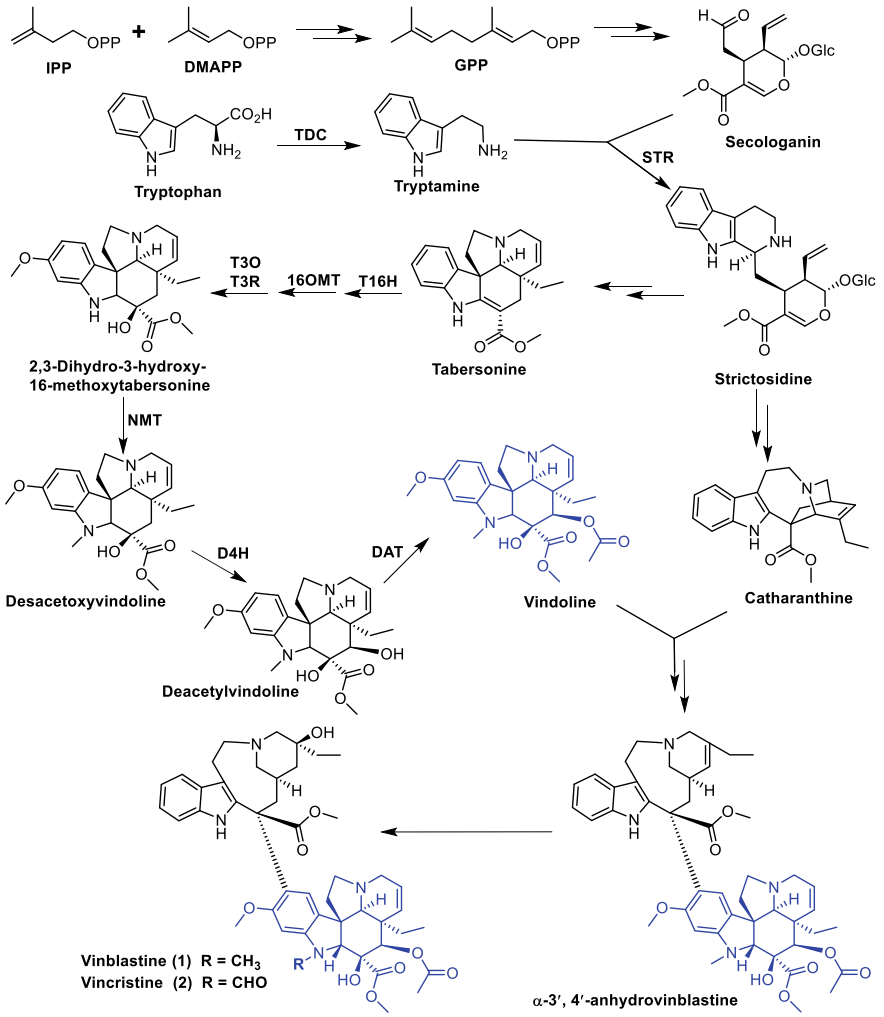


Fig. 17.4 Biosynthesis of vinblastine (**1**) and vincristine (**2**) [TDC: tryptophan decarboxylase; STR, strictosidine synthase; T16H: tabersonine 16-hydroxylase; 16OMT: 16-*O*-methyltransferase; T3O: tabersonine 3-oxygenase; T3R: tabersonine 3-reductase; NMT: *N*-methyltransferase; D4H: desacetoxyvindoline 4-hydroxylase; DAT: acetyl CoA:deacetylvindoline-4-*O*-acetyltransferase; Adapted from (Courdavault et al. 2014; Duge de Bernonville et al. 2015)]

phan in the leaf epidermis, the iridoid secologanin is derived from the monoterpenoid geranyl diphosphate (GPP) via several reactions in the internal phloem-associated parenchyma and epidermis cells (Pan et al. 2016a). Despite their very close structural similarity, these two compounds show remarkably different tumor specificities and toxicities (Noble 1990). Even though these bisindole alkaloids were found to be very effective in the anticancer chemotherapy field and were useful molecular probes in the

elucidation of the microtubule inhibitory mechanism of action of anticancer drugs, their amounts obtainable from their producing plant are extremely small (Ishikawa et al. 2009). Thus, a series of studies have been reported aimed at solving the supply problems of vinblastine (**1**) and vincristine (**2**) via various methods such as synthesis and biotechnological methods.

Factors Affecting Yield

As mentioned above, vinblastine (**1**) and vincristine (**2**) occur in the plant in very small concentration levels. For example, it required nearly 485 kg of dried leaves of *C. roseus* to provide approximately 1 g of vinblastine, representing a yield of 0.0002% dry weight (Noble 1990). This has led to major accessibility issues and, because of this, the prices of these drugs are very high. In addition, approximately 300 tons of *C. roseus* leaves are required to provide 3 kg of these drugs to meet their global demand (Newman and Cragg 2020b). These low yields result from an interplay of environmental, genetic, geography, developmental stage, plant part, and age factors. Most importantly, only the aerial parts of *C. roseus*, mainly the leaves, are able to produce the monoterpene bisindole alkaloids. These are not produced by the roots, as this organ lacks some of the essential enzymes involved in the biosynthesis of vindoline, one of the monomers in the bisindole alkaloids (Mahroug et al. 2007). Additionally, the biosynthesis of these and other monoterpenoid indole alkaloids is under strict regulation and involves more than 30 catalyzed steps with more than 35 intermediates, at least four cell types and at least five cellular compartments (Pan et al. 2016a). For example, Dutta et al. (2005) showed that differential expression levels of three important enzymes involved in the biosynthesis of catharanthine, vindoline, vinblastine, and vincristine correlated with the levels of these alkaloids in different parts, cultivars, mutants, and varieties of *C. roseus*. Plant age and leaf maturation are two of the other factors that affect the content of these alkaloids in the leaves. As an example, the content of α-3′,4′-anhydrovinblastine, the precursor of both vinblastine and vincristine, was found to be dependent on leaf maturation, which increased with leaf age and wounding. However, the content of the monomers decreased with aging (Naaranlahti et al. 1991). Similar trends were also observed for vinblastine, which increased with leaf age and plant maturation (Pan et al. 2016b). In addition, extraction methods, conditions, and solvents used as well as the isolation and purification procedures utilized also affect the yields of these alkaloids. For example, the use of ultrasound-assisted extraction with the ionic liquid 1-allyl-3-methylimidazolium bromide resulted in a higher extraction efficiency of vinblastine from the leaves of *C. roseus* compared to the conventional extraction solvents (85% ethanol and 0.15% sulfuric acid in 50% methanol) and extraction methods (heat reflux extraction and maceration) (Yang et al. 2011).

17.4.1.2 Biotechnological Methods to Improve the Supply of the Vinca Alkaloids

Cultivation and In Vitro Propagation

Catharanthus roseus can be propagated either from seeds or by in vitro propagation methods such as organ formation by either direct or indirect methods and somatic embryogenesis from various explants (Das et al. 2020). In each case, several factors determine the biosynthesis and thus the yields of the monoterpene indole alkaloids, including both the monomers and the bisindoles. For example, the cotyledons and hypocotyls of eight-day seedlings were found to contain variable levels of vindoline and catharanthine when compared to young leaves collected from 15-week old plants. The content of vindoline was also affected by the light-cycle used for cultivation (Magnotta et al. 2006). Production can also be improved using elicitors and physiological stressors. As an example, *C. roseus* plants inoculated with or without arbuscular mycorrhizal fungi and challenged with NaCl and $KHCO_3$ alone or in combination were studied in terms of the accumulation of vinblastine (**1**) and other metabolites. Significant improvement in vinblastine accumulation was observed in those plants inoculated with arbuscular mycorrhizal fungi without any stressors and from plants stressed with $KHCO_3$ alone. NaCl alone or in combination with $KHCO_3$ had no effect on the accumulation (De la Rosa-Mera et al. 2011). Vinblastine production was also shown to be elevated when *C. roseus* was supplemented with nitrate and then irradiated with ultraviolet B (UVB) light (Guo et al. 2014), and when grown under red light and irradiated with ultraviolet A (UVA) light (Fukuyama et al. 2017), and when supplemented with an equal mixture of nitrate and ammonium (Guo et al. 2011).

Plant Cell Culture

One of the problems encountered with the use of undifferentiated plant cell and hairy root cultures for improving the production of vinblastine (**1**) and vincristine (**2**) is the failure of these systems to produce these alkaloids reliably. This arises as their biosynthesis involves both the roots and above-ground parts, particularly since the biosynthesis of vindoline only occurs in the aerial parts of *C. roseus* (Hisiger and Jolicoeur 2007; Kidd et al. 2019). More specifically, the pathway that converts the branching intermediate tabersonine to vindoline is absent in these systems and thus represents a major obstacle in the production of vinblastine and vincristine in cell and hairy root cultures (Sun et al. 2018). These failures of undifferentiated plant cell cultures to biosynthesize vindoline have been correlated with the lack of expression and activity of the enzymes *N*-methyltransferase (NMT), desacetoxyvindoline 4-hydroxylase (D4H), and acetyl CoA:deacetylvindoline 4-*O*-acetyltransferase (DAT), the last three enzymes involved in the vindoline pathway (Fig. 17.4) (St-Pierre et al. 1998; Vázquez-Flota et al. 2002). Furthermore, these enzymes are localized in a cell- (e.g., D4H and DAT in the cytosols of laticifer and idioblast cells) and organelle- (e.g., NMT in thylakoids) specific manner in only the aerial parts and are under strict control (e.g., light is needed to activate *D4H* and *DAT* genes), explaining the lack

of accumulation of vindoline and the bisindoles in plant cell cultures (Salim and De Luca 2013; St-Pierre et al. 1998).

However, some reports have indicated the accumulation of vindoline and vinblastine (**1**) in various systems of *C. roseus* plant cell cultures such as vindoline in transformed (O'Keefe et al. 1997) and elicited (Ramani and Jayabaskaran 2008) cells and vinblastine in cell cultures containing elicitors and inhibitors (Guo et al. 2012). In addition, cell suspension cultures of *C. roseus* have been shown to biotransform vinblastine to vincristine (Hamada and Nakazawa 1991). Furthermore, cambial meristematic cells treated with several methods such as biotic (*Aspergillus flavus*) (Liang et al. 2018) and abiotic (β-cyclodextrin and methyl jasmonate) (Zhou et al. 2015b) elicitors are able to accumulate vindoline.

Plant Tissue and Organ Cultures

As indicated above, the plant roots as well as hairy root cultures of *C. roseus* fail to accumulate vindoline, vinblastine (**1**), and vincristine (**2**). This has been shown due to an alternative pathway that transforms tabersonine to other metabolites such as lochnericine, hörhammericine, and catharanthine instead of vindoline (Facchini and De Luca 2008; Magnotta et al. 2007; Rodriguez et al. 2003). Particularly, the *D4H* and *DAT* genes, the last two genes in the vindoline pathway, are not expressed in either the roots of the intact plant or in hairy root cultures. Furthermore, vindoline biosynthesis involves three different cells in the aerial parts, and these coupled with the light dependence of vindoline biosynthesis explains why these two systems are unable to produce vindoline, vinblastine, and vincristine (Mahroug et al. 2007; Thamm et al. 2016). Additionally, some hairy root metabolic engineering efforts by overexpression of certain of the enzymes involved in the vindoline and bisindole alkaloids biosynthesis have failed to accumulate these alkaloids. For example, even though *A. rhizogenes* transformed hairy roots of *C. roseus* overexpressing either the geraniol 10-hydroxylase (G10H) gene alone or with the ORCA3 transcription factor were able accumulate catharanthine, they were unable to accumulate vindoline, vinblastine, and vincristine (Wang et al. 2010). To add one more example, *C. roseus* hairy root metabolic engineering by overexpressing tabersonine 16-hydroxylase (T16H) and 16-hydroxytabersonine-*O*-methyltransferase (16OMT), the first two enzymes in the tabersonine to vindoline pathway, along with the regulators, failed to produce vindoline (Sun et al. 2018). However, similar to plant cell cultures, there are a few reports (e.g., Hanafy et al. 2016) that indicate the production of vincristine and vindoline in hairy root cultures.

As opposed to hairy root cultures, organ cultures of the above-ground parts such as shoots have been shown to produce vindoline, vinblastine (**1**), and vincristine (**2**) (Wink et al. 2007), depending on several factors. Thus, near-ultraviolet light stimulation was shown to increase the production of vinblastine in multiple shoot cultures of *C. roseus* (15 μg/g fresh weight) (Hirata et al. 1992). In embryos and leaves regenerated from protoplasts, yeast extract elicitation was found to improve the production of vinblastine and vincristine. The best result (15.5 and 4.1 μg/g dry weight, respectively), however, was obtained with 1.5 g/L of yeast extract from protoplast-regenerated leaves (Maqsood and Abdul 2017). In addition, different development

stages of somatic embryos of *C. roseus* initiated from embryogenic callus, and which were elicited by the fungus *Aspergillus flavus*, were shown to accumulate different levels of vinblastine and vincristine, with the most enhanced being observed in the maturation and germination stages (Tonk et al. 2016). Multiple shoot cultures treated with various elicitors and precursors were also able to accumulate vinblastine, with the highest being observed (approximately 0.03% DW), when supplied with the precursor tryptamine (100 mg/L). However, calli induced from the leaves of in vitro grown *C. roseus* were unable to produce vinblastine (Sharma et al. 2019).

Metabolic Engineering

Studies to improve the yields of monoterpene indole alkaloids using metabolic engineering nowadays are being facilitated by continuous elucidation of the biosynthetic machinery in *C. roseus* (Pan et al. 2016a). Such metabolic engineering is performed either in homologous (*C. roseus*) or heterologous (such as *Escherichia coli*, *Saccharomyces cerevisiae,* and *Nicotiana benthamiana*) systems, not only for the biosynthetic genes, but also for the transcription factors that regulate such genes (Sharma et al. 2020). Clearly, success with these approaches has been facilitated greatly by the ever-increasing number of research groups reporting the elucidation of genes, enzymes, regulatory mechanisms, and cellular and organ compartments of monoterpene indole biosynthesis (Caputi et al. 2018; Guirimand et al. 2020; Miettinen et al. 2014; Qu et al. 2015, 2018, 2019). Relevant technical progress made has been reviewed recently (Courdavault et al. 2014; De Luca et al. 2014; Duge de Bernonville et al. 2015; Thamm et al. 2016). Based on this accumulated knowledge of the monoterpene indole biosynthetic pathways, attempts have been made to improve the yields of vinblastine (**1**), vincristine (**2**), vindoline, catharanthine, and other monoterpene indole alkaloids. For example, Kumar et al. (2018) studied the effect of overexpression of geranyl(geranyl) diphosphate synthase [G(G)PPS] and its co-expression with geraniol synthase on the accumulation of the monomers and vinblastine using *Agrobacterium tumefaciens*-transformed *C. roseus* whole plants. Even though both systems showed improvement in the accumulation of the monomers, it was found that only those overexpressing G(G)PPS resulted in the improvement of vinblastine accumulation in the transgenic leaves (Kumar et al. 2018). In another study, the effects of *A. tumefaciens*-mediated overexpression of tryptophan decarboxylase and strictosidine synthase were studied in leaf-regenerated intact *C. roseus* plants. This led to the generation of four transgenic plants showing better accumulation of vinblastine [maximum 0.014% dry weight compared to the control (0.003% dry weight)] (Sharma et al. 2018).

In addition to the above and other homologous expression studies, several heterologous expression experiments with suitable hosts have been studied with respect to their ability to produce the target compounds, particularly the production of some important intermediates in the biosynthesis of the bisindole alkaloids. For example, Qu et al. (2015) studied the heterologous expression of the seven genes involved in the biosynthesis of vindoline from tabersonine in a yeast. They found that this expression was able to biotransform successfully tabersonine into vindoline, producing vindoline at a rate of 0.092 mg/L/h. The authors were able to achieve this by first

identifying the remaining two enzymes [tabersonine 3-oxygenase (T3O) and tabersonine 3-reductase (T3R)] involved in the tabersonine-to-vindoline pathway (Qu et al. 2015). To add a further example of a heterologous expression study, Brown et al. (2015) produced strictosidine in a yeast through the introduction of 21 genes and the deletion of three genes. After several optimization experiments, this resulted in the production of 0.5 mg/L of strictosidine (Brown et al. 2015).

17.4.2 Podophyllotoxin

17.4.2.1 Overview and Problems in Supply

The aryltetralin lignan podophyllotoxin (**29**) was isolated in 1880 from Podophyllin, a water-insoluble resin extracted by alcohol from the rhizomes of several species of *Podophyllum* (Berberidaceae), mainly *Podophyllum hexandrum* Royle [Himalayan mayapple, Indian mayapple; synonym: *Sinopodophyllum hexandrum* (Royle) T.S. Ying, *Podophyllum emodi*] and *Podophyllum peltatum* L. (American mandrake, American mayapple) (Stähelin and von Wartburg 1989). Its structure was completely elucidated in 1951 (Hartwell and Schrecker 1951). Podophyllotoxin (**29**) serves as the starting material for the semi-synthesis of etoposide (**6**), etopophos (**7**), and teniposide (**8**) (Fig. 17.5), which were developed in order to synthesize more potent and less toxic derivatives of podophyllotoxin (Stähelin and von Wartburg 1991). Still today, the supply of etoposide (**6**) and other analogs is met by partial synthesis from podophyllotoxin extracted from the Himalayan mayapple (*P. hexandrum*) (Schultz et al. 2019). However, this species is considered to be endangered due to the uncontrolled harvesting of the wild plants, requiring alternative methods of supply of this WHO-listed essential medicine (Li et al. 2018b; Schultz et al. 2019). To circumvent this problem, other viable options such as large-scale farming, which is based on the

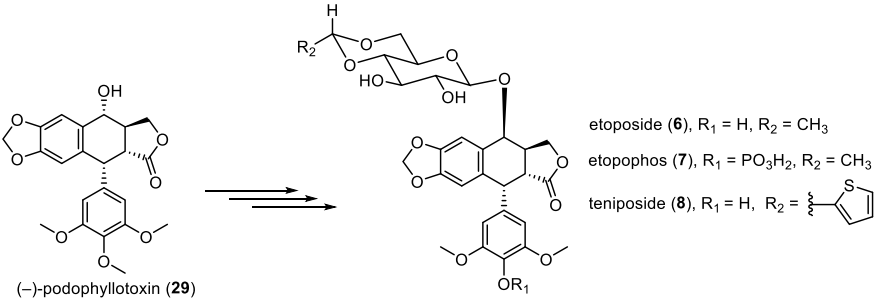


Fig. 17.5 Chemical structures of podophyllotoxin (**29**) and its derivatives [Adapted from (Stähelin and von Wartburg 1991)]

fact that wild varieties can provide high yields of podophyllotoxin, and biotechnological methods, such as those based on plant cell culture, have been suggested (Li et al. 2018b).

Factors Affecting Yield

In a similar manner to other secondary metabolites, the yield of podophyllotoxin (**29**) is affected by several factors. One of these is the plant source used for the isolation of this lignan. Podophyllotoxin has been isolated from several genera belonging to different plant families. Some of these, in addition to *Podophyllum* (Berberidaceae), include *Linum* (Linaceae) (Broomhead and Dewick 1990a), *Juniper* (Cupressaceae) (Renouard et al. 2011), *Diphylleia* (Berberidaceae) (Broomhead and Dewick 1990b), and several other genera (Newman and Cragg 2020b). Furthermore, each botanical source provides different amounts of podophyllotoxin. As an example, among the 12 species of Cupressaceae belonging to five genera (*Chamaecyparis*, *Cryptomeria*, *Cupressus*, *Juniperus*, and *Thuja*) studied for podophyllotoxin content, the needles (leaves) of Eastern red cedar (*Juniperus virginiana* L. "Canaertii") were found to contain the largest yield (0.34% dry weight) (Cantrell et al. 2013). Within the genus *Podophllyllum*, two plants are recognized as the major of sources of podophyllotoxin, viz., *P. hexandrum* and *P. peltatum* (Malik et al. 2014). The roots/rhizomes of the former have provided the largest amount of podophyllotoxin compared to the latter (e.g., 4.3% vs. 0.25% dry weight, respectively) (Broomhead and Dewick 1990b). Other *Podophyllum* species such as *P. sikkimensis* R. Chatterjee and Mukherjee (Paul et al. 2013) and *P. versipelle* Hance (0.32% dry weight) (Broomhead and Dewick 1990b) are usually found to contain lower levels of podophyllotoxin compared to *P. hexandrum*. However, the leaves of *P. peltatum* have been demonstrated to accumulate large amounts of podophyllotoxin, up to 5.6% dry weight (Moraes et al. 2000).

As mentioned above, the current major source of podophyllotoxin (**29**) is *P. hexandrum*, but this plant has been shown to contain variable amounts of podophyllotoxin dependent on several factors. For example, a study on the effects of altitude, the number of leaves, plant age, and collection season within the same collection region on podophyllotoxin production showed that higher yields were observed at elevated altitudes, and in those plants containing only one leaf rather than four, and in aged plants (four years), and those collected during May and June (Purohit et al. 1999). The effect of altitude on podophyllotoxin production has also been shown in other several studies. For example, the podophyllotoxin content of the roots of *P. hexandrum* collected from the Lahaul forest (4300 m) was higher (8.8 to 9.5% dry weight vs. 3.0 to 4.7% dry weight) than those collected from the Parvati forest (1300–1500 m) (Naik et al. 2010). The combined effects of several ecological factors such as temperature, soil pH, altitude, rainfall, sunshine, and nutrients have been shown to affect the content of podophyllotoxin. Thus, among eight sampling sites from seven provinces of mainland China studied for podophyllotoxin and other "active" substances produced by the roots and rhizomes of *P. hexandrum*, those collected from Jingyuan (Ningxia province) provided the largest amount (6.71% dry weight) followed by Yongdeng (Gansu province) and Huzhu (Qinghai province) (Liu et al. 2015). Genetic variability coupled with geographical and environmental

factors might contribute to these differences (Sultan et al. 2008, 2010). In addition to the above environmental and genetic factors, extraction methods can also affect the yield of podophyllotoxin. For example, Canel et al. (2001) found that extraction of the roots, rhizomes, and leaves of *P. peltatum* with an aqueous solvent increased the yield of podophyllotoxin by several times than when extracted with ethanol. This was due to the β-glucosidase enzymes present in tissues that catalyzed the conversion of podophyllotoxin 4-*O*-β-D-glucopyranoside to podophyllotoxin, which usually occurs when the tissues are damaged or treated with aqueous solvents (Canel et al. 2001).

17.4.2.2 Biotechnological Methods to Improve the Supply of Podophyllotoxin

Cultivation and In Vitro Propagation

In large-scale cultivation procedures, several factors may be varied, including temperature, soil pH, elevation, and nutrients, to enhance the biosynthesis and thus the resultant yield of podophyllotoxin (**29**). In one ex situ cultivation study, for example, plant age and hence collection time were the most significant factors to affect the yield of podophyllotoxin, when compared to other factors (Kushwaha et al. 2012). In another investigation, a higher production of podophyllotoxin was observed when *P. hexandrum* was cultivated at 3300 m than when compared with 2300 m, both in the leaves and rhizomes (Li et al. 2018a). Furthermore, the effect of elicitors on podophyllotoxin production has been studied. For example, statistically significant results were obtained when *P. hexandrum* leaves were treated with 3 mM methyl jasmonate, resulting in a 21% increase in podophyllotoxin production in the roots (Seegers et al. 2017). A temperature dependence of podophyllotoxin production has also been shown, with relatively higher accumulations obtained at lower temperatures. This was accompanied by changes in the expression levels of enzymes thought to be involved in the biosynthesis of podophyllotoxin, as determined by transcriptome analysis (Kumari et al. 2014). Micropropagation from various parts of podophyllotoxin-producing plants has been shown also to be successful for such plants that grow slowly and has endangered; one such example is for *Dysosma versipellis* (Hance) M. Cheng (Jiang et al. 2011).

Plant Cell Culture

Plant cell cultures, either using callus and/or suspension cultures, have been studied to optimize the production of podophyllotoxin (**29**). These investigations were performed on several plant species through the consideration and optimization of various factors such as biotransformation, elicitation, growth conditions, nutrients, and solvent extraction methods, in order to increase the yield. For example, when biotransformation using desoxypodophyllotoxin as the substrate was utilized to improve the yield of podophyllotoxin in *P. hexandrum* and *Linum flavum* cell suspension cultures, the highest conversion was observed in cell cultures of *P. hexandrum*

(van Uden et al. 1995). Furthermore, improvement of the yield of podophyllotoxin through co-culture of the hairy roots of *L. flavum* and cell suspension cultures of *P. hexandrum* has been observed. This was due to the uptake of coniferin produced by the hairy roots of *L. flavum* by cell suspension cultures of *P. hexandrum*, which used it to biosynthesize podophyllotoxin (Lin et al. 2003). In addition, improved production of podophyllotoxin in cell cultures derived from various plant species has been demonstrated by the use of elicitors. For example, salicylic acid was shown to increase the biosynthesis of podophyllotoxin (333 μg/g dry weight after 72 h elicitation with this compound) in cell cultures of *L. album*, despite showing non-variable outcomes on the growth, survival, and dry mass of the elicited cells, when compared to a control (Yousefzadi et al. 2010). Both a 15-and a 3.5-fold improvement in podophyllotoxin production were observed when callus cultures of *Juniperus chinensis* were treated with chito-oligosaccharide and laminaran enzyme-hydrolyzate elicitors, respectively (Muranaka et al. 1998).

Despite its lower podophyllotoxin (**29**) content compared to *Podophyllum hexandrum* (Broomhead and Dewick 1990b), *P. peltatum* has also been used to improve the yield of podophyllotoxin using cell cultures. Since an initial report (Kadkade 1981), several studies have shown this species can be used as an alternative to increase the yield of podophyllotoxin. For example, the effect of growth regulators, natural growth factors, carbon sources, callus age, light quality, and plant part used for callus induction was studied in callus cultures of *Podophyllum peltatum*. The highest production of podophyllotoxin was observed when 2,4-dichlorophenoxyacetic acid (2,4-D), casamino acids, sucrose, a duration of eight weeks, red light, and rhizomes and roots, respectively, were used (Kadkade 1982). Cell and adventitious root cultures were also shown to increase the yield of podophyllotoxin from *P. peltatum* by varying medium conditions (e.g., half *vs.* full Murashige and Skoog medium), hormones (e.g., indole-3-butyric acid) and elicitors (e.g., methyl jasmonate) (Anbazhagan et al. 2008).

Plant Tissue and Organ Cultures

Hairy root cultures induced by various strains of *Agrobacterium rhizogenes* have been studied to improve the production of podophyllotoxin (**29**) from various plants such as several species belonging to the genus *Linum* (Malik et al. 2014), *Hyptis suaveolens* (L.) Piot. (Lamiaceae) (Bazaldua et al. 2019), and *P. hexandrum* (Giri et al. 2001). With these systems, several factors such as the use of exogenous hormones (Farkya and Bisaria 2008), biotic elicitors (Bahabadi et al. 2014; Tashackori et al. 2016), and precursor feeding (Chashmi et al. 2016) were adjusted to improve the yield of podophyllotoxin. For example, of the various exogenous phytohormones (auxins, cytokinins and gibberellins) and their combinations tested, the highest production levels of podophyllotoxin, 14.9 and 15.0 mg/g dry weight, were obtained from a medium supplied with a specified concentration, 2 and 3 mg/L, respectively, of indole-3-acetic acid (IAA) (Farkya and Bisaria 2008). Furthermore, yields were improved by manipulation of extraction methods, culture composition, the addition of vitamins, and the use of various strains of *A. rhizogenes* or a combination of the aforementioned factors (Bazaldua et al. 2019; Chashmi et al. 2013; Renouard et al.

2018; Samadi et al. 2014). Recently, tetraploidy induction was used to increase the yield of podophyllotoxin in the shoots derived from *L. album* (Javadian et al. 2017). Furthermore, the role of plant growth regulators, carbon and nitrogen sources at different concentrations/ratios, culture medium strength, pH, and phosphate ratio on podophyllotoxin production in adventitious root cultures derived from the roots of *P. hexandrum* has been studied (Rajesh et al. 2014). In addition, roots derived from the callus and root explant of *P. hexandrum* were shown to produce similar (10.5% and 11.6% dry weight, respectively) amounts of podophyllotoxin compared to that produced by the roots and rhizomes of the original plant (9.3% dry weight) (Sagar and Zafar 2008).

Metabolic Engineering

Recently, Schultz et al. (2019) reported the use of metabolic engineering in *Nicotiana benthamiana* to increase the production of (−)-deoxypodophyllotoxin, a precursor of the etoposide aglycone (–)-4′-desmethylepipodophyllotoxin. (–)-4′-Desmethylepipodophyllotoxin is a more direct precursor of etoposide (**6**) than podophyllotoxin (**29**) and, thus, can be directly synthetically derivatized to the glycoside etoposide (Fig. 17.6) (Lau and Sattely 2015). Schultz et al. (2019) achieved this by the *Agrobacterium tumefaciens*-facilitated introduction of 16 genes involved in the transformation of phenylalanine → coniferyl alcohol → (–)-4′-desmethylepipodophyllotoxin in the leaves of *N. benthamiana*. This provided initially 3.5 mg/g dry weight of (−)-deoxypodophyllotoxin, which was 680 times higher than

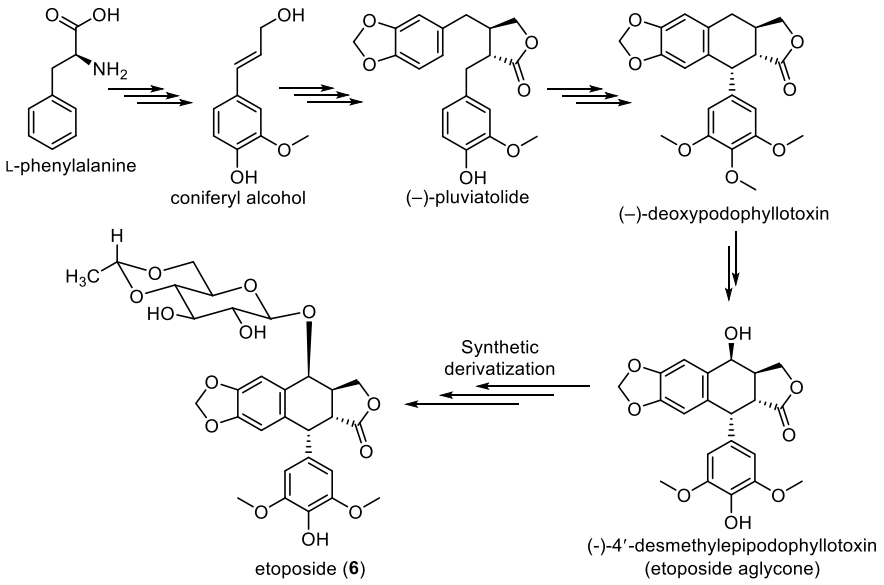


Fig. 17.6 Biosynthesis of the etoposide aglycone (–)-4′-desmethylepipodophyllotoxin and its synthetic derivatization to etoposide (**6**) [Adapted from (Lau and Sattely 2015; Schultz et al. 2019)]

the control (5.2 μg/g dry weight). Further optimization by varying the level of *A. tumefaciens* and harvest time (the optimum being *A. tumefaciens* OD_{600} of 3 and harvest time of 7–9 days, respectively), resulted in a (−)-deoxypodophyllotoxin yield of 4.3 mg/g dry weight. A scale-up procedure using 15–20 plants based on these optimized conditions led to the isolation of 0.7 mg/g (dry weight) of (−)-deoxypodophyllotoxin (Schultz et al. 2019). This is based on the results of the same group who identified the six enzymes that catalyze the conversion of (–)-pluviatolide to the etoposide aglycone (–)-4′-desmethylepipodophyllotoxin (Fig. 17.6) (Lau and Sattely 2015).

17.4.3 Paclitaxel

17.4.3.1 Overview and Problems in Supply

The isolation of the diterpene paclitaxel (**9**, Taxol®, Bristol-Myers Squibb) from the stem of the bark of the Pacific yew or western yew (*Taxus brevifolia* Nutt., Taxaceae) was reported in 1971 (Wani et al. (1971). In their report, the authors indicated that "*Taxol has potent antileukemic and tumor inhibitory properties…*" (Wani et al. 1971) and this was confirmed by several reports both in preclinical and clinical settings as reviewed by several authors (Foa et al. 1994; Kohler and Goldspiel 1994; Rose 1992; Rowinsky et al. 1990; Rowinsky and Donehower 1995), including its unique mechanism of action on tubulin first reported in 1979 by Horwitz and colleagues (Schiff et al. 1979). These and other factors made paclitaxel (**9**) of major interest, which drew attention from several diverse groups including physicians, environmentalists, and organic chemists, of whom the latter embarked on the partial and total synthesis of the drug (Nicolaou and Guy 1995). However, even though paclitaxel was the first taxane diterpenoid to demonstrate antitumor (Wani et al. 1971) and broad-spectrum anticancer activity, more than 20 years elapsed before this natural product was approved by the US FDA (December 1992), as a drug for the treatment of ovarian cancer. One of the factors for such a delay was the extremely small amount of the drug present naturally in the plant source, which required the need for a tedious large-scale isolation procedure (Cragg et al. 1993).

Biosynthetically, as a derivative of the isoprenoid class of natural products, the taxane diterpene skeleton of paclitaxel (**9**) is derived from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). These undergo several reactions to form the diterpene precursor geranylgeranyl diphosphate (GGPP). Starting from GGPP, there are at least 19 steps involved in the biosynthesis of paclitaxel that include the formation of the first committed product, taxa-4(5),11(12)-diene, from GGPP catalyzed by the enzyme taxadiene synthase (TS) (Croteau et al. 2006). Taxa-4(5),11(12)-diene then undergoes several oxidation steps, including hydroxylation, acylation, benzoylation, and oxetane ring formation, to provide 10-deacetylbaccatin III, which can be acetylated by the enzyme 10-deacetylbaccatin III-10β-*O*-acetyltransferase (DBAT), to produce baccatin III. The latter then condenses

with β-phenylalanoyl-CoA derived from α-phenylalanine via β-phenylalanine to form paclitaxel via several reactions including the last benzoylation step (Fig. 17.7) (Croteau et al. 2006; Yu et al. 2017).

Factors Affecting Yield

In the initial structure elucidation report, paclitaxel (**9**) was isolated with a yield of 0.02% (Wani et al. 1971). Such a low concentration level, along with slow growth of the producing plant, posed a major restriction on the early development of paclitaxel. Initially, the supply issue was circumvented by the large-scale collection of *T. brevifolia*, but this raised issues with regard to the possible extinction of this plant

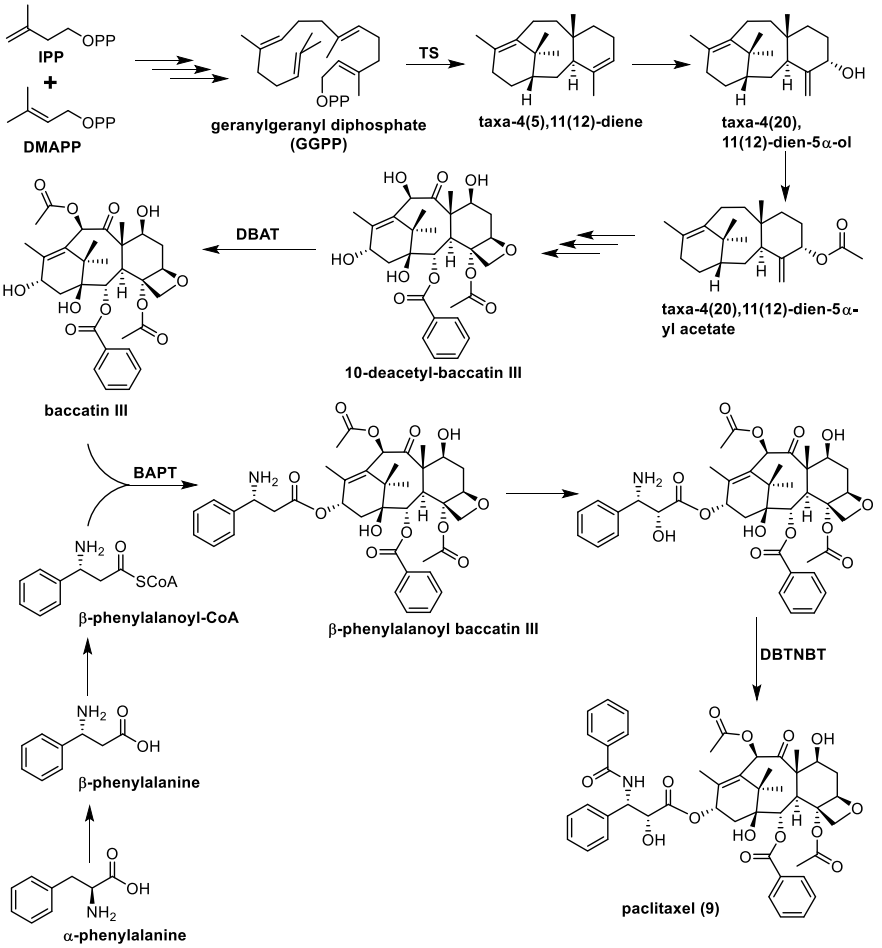


Fig. 17.7 Biosynthesis of paclitaxel (**9**) [TS: taxadiene synthase; DBAT: 10-deacetylbaccatin III-10β-*O*-acetyltransferase; BAPT: baccatin III 13-*O*-(3-amino-3-phenylpropanoyl) transferase; DBTNBT: 3′-*N*-debenzoyl-2′-deoxytaxol-*N*-benzoyltransferase. Adapted from (Liu et al. 2016)]

(Kingston and Newman 2007). As a result, paclitaxel has been the subject of many efforts to solve the supply crisis via various methods such as partial and total synthesis (Nicolaou and Guy 1995), plant tissue culture, and the search for high-yielding *Taxus* species/cultivars or other sources. For example, one of the methods used to solve the paclitaxel supply issue in early work was by semi-synthesis of paclitaxel from the more abundant (e.g., 1 g/kg) precursor 10-deacetylbaccatin III (Denis et al. 1988; Kingston and Newman 2007).

Similar to podophyllotoxin (**29**) and as discussed in the previous section, paclitaxel (**9**) has been isolated from other plant sources, in addition to species of the Taxaceae, with each providing a variable yield dependent on several factors. Even though paclitaxel is biosynthesized by all *Taxus* species that have been investigated, its concentration levels are dependent on several factors such as species, season, and plant part (Croteau et al. 2006). For example, the needles of *T. brevifolia* were shown to yield lower amounts of paclitaxel compared to the bark (Cragg et al. 1993). However, the needles of other *Taxus* species were found to contain similar amounts of paclitaxel (0.008–0.01%) to the bark of *T. brevifolia* (0.01%) (Witherup et al. 1990). This variation of paclitaxel (**9**) with respect to plant part has been shown for other *Taxus* species as well. For example, the roots of *T. mairei* contain larger amounts of paclitaxel (0.19%) than the stem bark, heartwood, twigs, and needles (Liu et al. 2001). In another study, the bark of the Himalayan yew (*T. wallichiana*) was found to contain the largest amounts of paclitaxel when compared to the stems and needles (Mukherjee et al. 2002). This variation in tissue accumulation has been shown to be due to the differential expression of some of the important biosynthetic genes and enzymes in the tissues (Mubeen et al. 2018).

Another important factor is the variability of paclitaxel (**9**) across different species of *Taxus*, which was confirmed in several phytochemical studies (ElSohly et al. 1997a; Mattina and Paiva 1992; Poupat et al. 2000; van Rozendaal et al. 2000; Zhou et al. 2019). For example, of the ten *Taxus* species needles that were studied for paclitaxel and other taxanes, the highest amount of paclitaxel was obtained from *T. floridana* (516 μg/g) and *T. globosa* (433 μg/g). However, the needles of some of the cultivars of the same species (e.g., *T. baccata* "Imperialis," *T. cuspidata* "Henry" and *T.* × *media* "Dutweileri") were devoid of any paclitaxel content (van Rozendaal et al. 2000). This variability of paclitaxel between species might result from genetic diversity related to biosynthetic enzymes and regulators (Yu et al. 2017). For example, the variation in paclitaxel and 10-deacetylbaccatin III observed between *T.* × *media* and *T. mairei* was accompanied by fluctuations in some of the important enzymes involved in the biosynthesis of these taxanes, as revealed by transcriptome analysis (Yu et al. 2017). In addition to their interspecific variation, cultivars of the same species are known to accumulate variable amounts of paclitaxel. For example, of the 17 cultivars of *T.* × *media* with different growth characteristics, needles of the cultivars "Coleana," "Hicksii," and "Stovekenii" contained the highest amount of paclitaxel (378, 322 and 309 μg/g dry weight, respectively) (Wang et al. 2006).

The content of paclitaxel has also been shown to depend on plant age (Mukherjee et al. 2002; Nadeem et al. 2002). As an example, old trees of *T. baccata* (126–161 years) contained more paclitaxel on average than young (40–57 years) and

mature trees (96–108 years) (0.1 vs. 0.05 and 0.04%, respectively) (Nadeem et al. 2002). Environmental factors such as altitude and temperature also affect the content of paclitaxel (Ballero et al. 2003; Mukherjee et al. 2002; Wheeler et al. 1992; Xi et al. 2014). For example, the needles of wild *T. baccata* collected from various altitudes (700–1200 m) in Sardinia were devoid of paclitaxel (Ballero et al. 2003). The collection time and season are other important factors that affect paclitaxel yield (ElSohly et al. 1997b; Glowniak et al. 1999; Veselá et al. 1999; Wheeler et al. 1992). Finally, the selection of extraction methods and of other parameters (e.g., temperature and extraction time) might have significant effects on the overall yield and time required for extraction of paclitaxel and other important taxanes (Kawamura et al. 1999; Talebi et al. 2004).

In addition to the Taxaceae, which are gymnosperms, paclitaxel has also been isolated from angiosperms of the family Betulaceae, in particular from the bark, shells, and leaves of *Corylus avellana* (hazelnut tree). However, the amounts obtained have been usually smaller than those found in *Taxus* species (Gallego et al. 2017). Furthermore, compound yields vary with, for example, plant part (Hoffman and Shahidi 2009) and collection site (Ottaggio et al. 2008). Another important potential general paclitaxel source that has attracted much attention is endophytic fungi. Starting from the first report in 1993 by Stierle et al. (1993), paclitaxel has been shown to be produced by more than 200 endophytic fungi, even those obtained from plants that do not produce paclitaxel, and this topic has been reviewed recently (Newman and Cragg 2020b).

17.4.3.2 Biotechnological Methods to Improve the Supply of Paclitaxel

Cultivation and In vitro Propagation

To meet the increasing global demand and to avoid the extinction of Pacific yew trees as a result of the collection of their bark, much attention has been given to the cultivation of *Taxus* plants in nurseries in several geographic regions. Furthermore, preference has been given to paclitaxel (**9**) extraction from the aerial plant parts (twigs/needles) to avoid damaging these slowly growing trees (Liu et al. 2016). Thus, from the above discussion, any cultivation and in vitro propagation efforts should take these and other factors into consideration in order to succeed as an alternative source of paclitaxel. For example, a negative correlation was observed between minimum and maximum temperature and paclitaxel production in *T. wallichiana* var. *mairei* grown and collected in Ningbo, China (Yang et al. 2016). In another study, bark samples collected from *T. brevifolia* grown under shade conditions contained more paclitaxel than those collected from sun-exposed trees (Kelsey and Vance 1992). Furthermore, several in vitro propagation methods have been studied in terms of enhancing paclitaxel and other taxane production and the germination, regeneration, and conservation of yew trees (Majada et al. 2000; Tafreshi et al. 2011). For example, for one-year old in vitro grown plantlets of *T. baccata*, the aerial parts contained more total taxanes than the roots. However, the roots contained more paclitaxel

and other taxane derivatives with ester side chains than the roots. In both cases, the amount of each type obtained was plant age-dependent. These differences were attributed to differences in the expression of some of the early and late biosynthetic genes, where some were found to be rate-limiting (Onrubia et al. 2011). In another example, the roots of various hydroponically grown cultivars and species of *Taxus* were shown to contain higher or similar amounts of paclitaxel compared to the aerial parts (Wickremesinhe and Arteca 1994), and the concentration levels attained could be manipulated by the use of plant growth regulators (Wickremesinhe and Arteca 1996). Finally, since yew trees require large areas and a long time to grow, which makes their collection labor intensive, cultivation efforts need to take these factors into account (Anterola et al. 2009).

Plant Cell Culture

Several species of *Taxus* such as *T. cuspidata*, *T. chinensis, T. baccata, T. globosa, T. media,* and *T. wallichiana* have been studied for the production of paclitaxel (**9**) in plant cell cultures (Malik et al. 2011; Navia-Osorio et al. 2002a, 2002b; Osuna et al. 2015; Roberts et al. 2003; Tabata 2006; Zhang and Fevereiro 2007). One of the effective methods used to enhance the production of paclitaxel and other taxanes in these systems has been the use of various elicitors such as coronatine, jasmonic acid, methyl jasmonate, and cyclodextrins (Cusido et al. 2014). For example, Sabater-Jara et al. (2014) showed synergistic effects of the combined use of various forms of cyclodextrins and methyl jasmonate on paclitaxel and other taxane derivative production in suspension cultures of *T.* × *media.* The combined use of both elicitors led to higher accumulations of paclitaxel compared to when either one was used alone (65.0 mg/L *vs.* 5.9 and 13.9 mg/L, respectively). This was accompanied by increased levels of the genes involved in the biosynthesis (*TXS*, *T7βOH*, *DBAT*, *BAPT,* and *DBTNBT*) and the ABC genes involved in the transport of paclitaxel (Sabater-Jara et al. 2014).

In addition to elicitation, numerous other methods such as immobilization (Bentebibel et al. 2005; Bonfill et al. 2007), two-stage systems (Khosroushahi et al. 2006), media optimization (Kajani et al. 2012), precursor feeding (Syklowska-Baranek and Furmanowa 2005), and two-phase systems (Wang et al. 2001) have been shown to enhance the production of paclitaxel (**9**) and other taxanes in *Taxus* plant cell cultures. Furthermore, Lee et al. (2010) developed cambial meristematic cells that overcome several problems associated with dedifferentiated plant cells and hence enhance the production of paclitaxel. Along with the use of elicitors and precursors, cambial meristematic cells showed a superior performance when compared to dedifferentiated cells in both small-scale (125 mL) flasks and large-scale (e.g., 3 and 20 L) bioreactors. For example, while dedifferentiated cells derived from needles or embryos produced 23 mg/kg or 39 mg/kg fresh weight of paclitaxel, respectively, cambial meristematic cells were able to produce 102 mg/kg fresh weight of this diterpenoid (Lee et al. 2010).

In fact, production of paclitaxel (**9**) by plant cell culture represents one of the success stories through the use of this biotechnological method in the production of a valuable pharmaceutical. Using Chinese yew (*T. chinensis*) cell cultures in 75,000 L

capacity bioreactors, Phyton Biotech, Inc., now a subsidiary of DFB Pharmaceuticals, produces paclitaxel commercially. Another company that uses cell culture to supply paclitaxel (Genexol®) for the global market is the Korean company Samyang Genex (Leone and Roberts 2013; Wilson and Roberts 2012).

As mentioned in the preceding section, paclitaxel (**9**) and other taxanes have also been isolated from hazelnut tree (*C. avellana*) and thus, several *C. avellana* cell cultures have been studied for enhancing paclitaxel production, as recently reviewed (Gallego et al. 2017).

Plant Tissue and Organ Cultures

In addition, plant cell cultures, hairy root cultures of a number of *Taxus* species have been evaluated for their ability to produce and enhance the production of paclitaxel (**9**). For example, Furmanowa and Syklowska-Baranek (2000) studied paclitaxel production in hairy root cultures of *T.* × *media* var. *hicksii* Rehd. induced by *A. rhizogenes* strain LBA 9402. They noted that elicitation with 100 μM methyl jasmonate produced the highest paclitaxel levels when compared with non-elicited hairy roots (210 vs. 69 μg/g dry weight, respectively) (Furmanowa and Syklowska-Baranek 2000). The same research group increased the production of paclitaxel by the use of various concentrations of the precursors L-phenylalanine and *p*-aminobenzoic acid supplemented alone or with 100 μM methyl jasmonate. The highest paclitaxel production was observed when 100 μM of either of these precursors was used in combination with 100 μM methyl jasmonate [319.7 μg/g (568.2 μg/L) and 130.5 μg/g (221.8 μg/L), respectively] (Syklowska-Baranek et al. 2009). In addition to the above species, hairy root cultures of other *Taxus* species such as *T. cuspidata* (maximum of 52.2 mg/L with 100 μM methyl jasmonate) (Kim et al. 2009) and *T. brevifolia* (the highest being 0.48 mg/g dry weight) (Huang et al. 1997) were shown to produce paclitaxel.

Furthermore, transgenesis and two-phase systems are additional recent methods studied for enhancing the production of paclitaxel (**9**) in hairy root cultures of *T.* × *media* var. *hicksii* (Syklowska-Baranek et al. 2015a, 2015b, 2018). For example, Syklowska-Baranek et al. (2019) examined the effect of several parameters (such as with or without the *TXS* gene, with or without and single *vs.* twice-elicitation, single *vs.* two-phased system) on paclitaxel production and some of its biosynthetic gene profiles in the hairy root cultures of *T.* × *media* var. *hicksii*. They found that the hairy root line containing the *TXS* gene and elicited with a single methyl jasmonate treatment produced a higher paclitaxel concentration level than a line without the *TXS* gene, even with elicitation (maximum of approximately 2.5 and 0.5 mg/g dry weight, respectively). These differences were accompanied by differences in the expression profiles of the *TXS*, *BAPT,* and *DBTNBT* genes (Syklowska-Baranek et al. 2019).

Metabolic Engineering

In similar work on other effective plant-derived anticancer agents, one of the limitations of the application of metabolic engineering procedures in reconstructing the whole paclitaxel (**9**) biosynthetic pathway in heterologous hosts is the fact that not of all of the biosynthetic enzymes of taxanes are yet discovered. However, several

engineering attempts have made to enhance the production of early intermediates and precursors (Courdavault et al. 2020), using numerous heterologous hosts, such as *Arabidopsis thaliana* (Besumbes et al. 2004), *Saccharomyces cerevisiae* (a yeast) (Dejong et al. 2006), *Escherichia coli* (Huang et al. 2001), tomato (Kovacs et al. 2007), a species of moss (Anterola et al. 2009), an endophytic fungus (Bian et al. 2017), and *Bacillus subtilis* (Abdallah et al. 2019). For example, Ajikumar et al. (2010) were able to produce approximately 1 g/L of taxadiene in *E. coli* using a method they developed called "multivariate modular pathway engineering." Using this procedure, they divided the taxadiene biosynthetic pathway into two separate modules, which allowed them to identify factors that affected metabolic flux and thus optimize these toward increasing the yield of taxadiene (Ajikumar et al. 2010). In another study, Zhou et al. (2015a) took metabolic engineering further by co-culturing engineered *E. coli* and *S. cerevisiae* to overcome several limitations of engineering both microbes alone, to produce ferruginol, nootkatone and precursors of paclitaxel. In this microbial consortium, for example, taxadiene produced by engineered *E. coli* was used by *S. cerevisiae* to produce oxygenated taxanes (e.g., taxadien-5α-ol) and a monoacetylated dioxygenated taxane putatively assigned as taxadien-5α-acetate-10β-ol. After several optimization experiments (e.g., changing the carbon source from glucose to xylose), this team of investigators was able to produce 33 and 1 mg/L of taxanes, respectively (Zhou et al. 2015a).

As alternatives to these microbes, plants have been studied as heterologous hosts as they possess some significant advantages over microbial hosts, such as improved cytochrome P450 chemistry (Li et al. 2019). For example, expression of taxadiene synthase in yellow-fruited tomato resulted in the production of taxadiene at a yield of 471 μg/g dry weight (Kovacs et al. 2007). In another more recent example, Li et al. (2019) were able to produce taxadiene and taxadiene-5α-ol (56.6 and 1.3 μg/g fresh weight, respectively), in the leaves of engineered *N. benthamiana.* They achieved this by increasing the availability of isoprenoid precursors such as geranylgeranyl diphosphate through the engineering of the corresponding enzymes. More importantly, the initial failure of the conversion of taxadiene to taxadiene-5α-ol was overcome by chloroplast compartmentalization of the enzymes taxadiene synthase, taxadiene-5α-hydroxylase, and cytochrome P450 reductase (Li et al. 2019).

17.5 Conclusions

Scientific interest in plant secondary metabolites to treat various forms of human cancer has continued unabated for nearly 60 years since the two bisindole alkaloids, vinblastine (**1**), and vincristine (**2**) became approved oncolytic agents. Such interest was heightened after the discovery of the diterpene derivative, paclitaxel (**9**), due in large part to its unprecedented mode of cellular action on tubulin, and its subsequent very wide clinical use on approval. It is not unreasonable that new examples of plant-derived derivatives with potential anticancer activity still remain to be discovered, so there is a worldwide search for such compounds, as exemplified

by relevant studies by our own research team (e.g., Henkin et al. 2018). A number of plant-derived compounds are in clinical trials as potential cancer chemotherapeutic agents, and these represent a quite diverse range of structural types. A number of ingenious methods have been applied to enhancing the available supply of plant-derived anticancer agents already on the market, including procedures for enhanced cultivation and in vitro propagation. Also, biotechnological methods such as plant cell tissue and organ culture methods have been examined in detail, in addition to metabolic engineering. Future refinement of all of these methods may be anticipated in the future.

Acknowledgements Research conducted in our own laboratory directed toward the discovery of new anticancer agents from higher plants and other organism has been funded through program project P01 CA125066 by the U.S. National Cancer Institute, NIH, Bethesda, MD, USA.

References

Abdallah II, Pramastya H, van Merkerk R, Sukrasno QWJ (2019) Metabolic engineering of *Bacillus subtilis* toward taxadiene biosynthesis as the first committed step for Taxol production. Front Microbiol 10:218

Agarwal G, Blanco Carcache PJ, Addo EM, Kinghorn AD (2020) Current status and contemporary approaches to the discovery of antitumor agents from higher plants. Biotechnol Adv 38:107337

Ajikumar PK, Xiao WH, Tyo KE, Wang Y, Simeon F, Leonard E et al (2010) Isoprenoid pathway optimization for Taxol precursor overproduction in *Escherichia coli*. Science 330:70–74

Alvandi F, Kwitkowski VE, Ko CW, Rothmann MD, Ricci S, Saber H et al (2014) U.S. Food and Drug Administration approval summary: omacetaxine mepesuccinate as treatment for chronic myeloid leukemia. Oncologist 19:94–99

Anbazhagan VR, Ahn CH, Harada E, Kim YS, Choi YE (2008) Podophyllotoxin production via cell and adventitious root cultures of *Podophyllum peltatum*. In Vitro Cell Dev Biol Plant 44:494–501

Anterola A, Shanle E, Perroud PF, Quatrano R (2009) Production of taxa-4(5),11(12)-diene by transgenic *Physcomitrella patens*. Transgenic Res 18:655–660

Baggstrom MQ, Qi Y, Koczywas M, Argiris A, Johnson EA, Millward MJ et al (2011) A phase II study of AT-101 (gossypol) in chemotherapy-sensitive recurrent extensive-stage small cell lung cancer. J Thorac Oncol 6:1757–1760

Bahabadi ES, Sharifi M, Ahmadian Chashmi N, Murata J, Satake H (2014) Significant enhancement of lignan accumulation in hairy root cultures of *Linum album* using biotic elicitors. Acta Physiol Plant 36:3325–3331

Ballero M, Loi MC, van Rozendaal ELM, van Beek TA, van de Haar C, Poli F et al (2003) Analysis of pharmaceutically relevant taxoids in wild yew trees from Sardinia. Fitoterapia 74:34–39

Basmadjian C, Zhao Q, Bentouhami E, Djehal A, Nebigil CG, Johnson RA et al (2014) Cancer wars: natural products strike back. Front Chem 2:20

Bazaldua C, Cardoso-Taketa A, Trejo-Tapia G, Camacho-Diaz B, Arellano J, Ventura-Zapata E et al (2019) Improving the production of podophyllotoxin in hairy roots of *Hyptis suaveolens* induced from regenerated plantlets. PLoS One 14:e0222464

Bentebibel S, Moyano E, Palazon J, Cusido RM, Bonfill M, Eibl R et al (2005) Effects of immobilization by entrapment in alginate and scale-up on paclitaxel and baccatin III production in cell suspension cultures of *Taxus baccata*. Biotechnol Bioeng 89:647–655

Bergqvist M, Holgersson G, Bondarenko I, Grechanaya E, Maximovich A, Andor G et al (2017) Phase II randomized study of the IGF-1R pathway modulator AXL1717 compared to docetaxel

in patients with previously treated, locally advanced or metastatic non-small cell lung cancer. Acta Oncol 56:441–447

Besumbes O, Sauret-Güeto S, Phillips MA, Imperial S, Rodríguez-Concepción M, Boronat A (2004) Metabolic engineering of isoprenoid biosynthesis in *Arabidopsis* for the production of taxadiene, the first committed precursor of Taxol. Biotechnol Bioeng 88:168–175

Bian G, Yuan Y, Tao H, Shi X, Zhong X, Han Y et al (2017) Production of taxadiene by engineering of mevalonate pathway in *Escherichia coli* and endophytic fungus *Alternaria alternata* TPF6. Biotechnol J 12:1600697

Bonfill M, Bentebibel S, Moyano E, Palazón J, Cusidó RM, Eibl R et al (2007) Paclitaxel and baccatin III production induced by methyl jasmonate in free and immobilized cells of *Taxus baccata*. Biol Plant 51:647–652

Broomhead AJ, Dewick PM (1990a) Aryltetralin lignans from *Linum flavum* and *Linum capitatum*. Phytochemistry 29:3839–3844

Broomhead AJ, Dewick PM (1990b) Tumour-inhibitory aryltetralin lignans in *Podophyllum versipelle*, *Diphylleia cymosa* and *Diphylleia grayi*. Phytochemistry 29:3831–3837

Brown S, Clastre M, Courdavault V, O'Connor SE (2015) De novo production of the plant-derived alkaloid strictosidine in yeast. Proc Natl Acad Sci USA 112:3205–3210

Butler MS, Robertson AA, Cooper MA (2014) Natural product and natural product derived drugs in clinical trials. Nat Prod Rep 31:1612–1661

Canel C, Dayan FE, Ganzera M, Khan IA, Rimando A, Burandt CL Jr et al (2001) High yield of podophyllotoxin from leaves of *Podophyllum peltatum* by in situ conversion of podophyllotoxin 4-*O*-β-D-glucopyranoside. Planta Med 67:97–99

Cantrell CL, Zheljazkov VD, Osbrink WLA, Castro A, Maddox V, Craker LE et al (2013) Podophyllotoxin and essential oil profile of *Juniperus* and related species. Ind Crops Prod 43:668–676

Caputi L, Franke J, Farrow SC, Chung K, Payne RME, Nguyen TD et al (2018) Missing enzymes in the biosynthesis of the anticancer drug vinblastine in Madagascar periwinkle. Science 360:1235–1239

Chashmi AN, Sharifi M, Behmanesh M (2016) Lignan enhancement in hairy root cultures of *Linum album* using coniferaldehyde and methylenedioxycinnamic acid. Prep Biochem Biotechnol 46:454–460

Chashmi NA, Sharifi M, Yousefzadi M, Behmanesh M, Rezadoost H, Cardillo A et al (2013) Analysis of 6-methoxy podophyllotoxin and podophyllotoxin in hairy root cultures of *Linum album* Kotschy ex Boiss. Med Chem Res 22:745–752

Chen J-Y, Tang Y-A, Li W-S, Chiou Y-C, Shieh J-M, Wang Y-C (2013) A synthetic podophyllotoxin derivative exerts anti-cancer effects by inducing mitotic arrest and pro-apoptotic ER stress in lung cancer preclinical models. PLoS Biol 8:e62082

Ciardiello F, Bianco R, Damiano V, De Lorenzo S, Pepe S, De Placido S et al (1999) Anti-tumor activity of sequential treatment with topotecan and anti-epidermal growth factor receptor monoclonal antibody C225. Clin Cancer Res 5:909–916

Clark PI, Slevin ML (1987) The clinical pharmacology of etoposide and teniposide. Clin Pharmacokinet 12:223–252

Courdavault V, O'Connor SE, Oudin A, Besseau S, Papon N (2020) Towards the microbial production of plant-derived anticancer drugs. Trends Cancer 6:444–448

Courdavault V, Papon N, Clastre M, Giglioli-Guivarc'h N, St-Pierre B, Burlat V (2014) A look inside an alkaloid multisite plant: the *Catharanthus* logistics. Curr Opin Plant Biol 19:43–50

Cragg GM, Newman DJ (2005) Plants as a source of anti-cancer agents. J Ethnopharmacol 100:72–79

Cragg GM, Pezzuto JM (2016) Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. Med Princ Pract 25(Suppl 2):41–59

Cragg GM, Schepartz SA, Suffness M, Grever MR (1993) The taxol supply crisis. New NCI policies for handling the large-scale production of novel natural product anticancer and anti-HIV agents. J Nat Prod 56:1657–1668

Croteau R, Ketchum RE, Long RM, Kaspera R, Wildung MR (2006) Taxol biosynthesis and molecular genetics. Phytochem Rev 5:75–97

Crüsemann M, O'Neill EC, Larson CB, Melnik AV, Floros DJ, da Silva RR et al (2016) Prioritizing natural product diversity in a collection of 146 bacterial strains based on growth and extraction protocols. J Nat Prod 80:588–597

Cusido RM, Onrubia M, Sabater-Jara AB, Moyano E, Bonfill M, Goossens A et al (2014) A rational approach to improving the biotechnological production of taxanes in plant cell cultures of *Taxus* spp. Biotechnol Adv 32:1157–1167

Das A, Sarkar S, Bhattacharyya S, Gantait S (2020) Biotechnological advancements in *Catharanthus roseus* (L.) G. Don. Appl Microbiol Biotechnol 104:4811–4835

De la Rosa-Mera CJ, Ferrera-Cerrato R, Alarcón A, de Jesús S-C, Muñoz-Muñiz OD (2011) Arbuscular mycorrhizal fungi and potassium bicarbonate enhance the foliar content of the vinblastine alkaloid in *Catharanthus roseus*. Plant Soil 349:367–376

De Luca V, Salim V, Thamm A, Masada SA, Yu F (2014) Making iridoids/secoiridoids and monoterpenoid indole alkaloids: progress on pathway elucidation. Curr Opin Plant Biol 19:35–42

Dejong JM, Liu Y, Bollon AP, Long RM, Jennewein S, Williams D et al (2006) Genetic engineering of taxol biosynthetic genes in *Saccharomyces cerevisiae*. Biotechnol Bioeng 93:212–224

Denis JN, Greene AE, Guenard D, Gueritte-Voegelein F, Mangatal L, Potier P (1988) Highly efficient, practical approach to natural taxol. J Am Chem Soc 110:5917–5919

Diamond JR, Goff B, Forster MD, Bendell JC, Britten CD, Gordon MS et al (2017) Phase Ib study of the mitochondrial inhibitor ME-344 plus topotecan in patients with previously treated, locally advanced or metastatic small cell lung, ovarian and cervical cancers. Invest New Drugs 35:627–633

Donehower RC (1996) The clinical development of paclitaxel: a successful collaboration of academia, industry and the National Cancer Institute. Oncologist 1:240–243

Duge de Bernonville T, Clastre M, Besseau S, Oudin A, Burlat V, Glevarec G et al (2015) Phytochemical genomics of the Madagascar periwinkle: Unravelling the last twists of the alkaloid engine. Phytochemistry 113:9–23

Dutta A, Batra J, Pandey-Rai S, Singh D, Kumar S, Sen J (2005) Expression of terpenoid indole alkaloid biosynthetic pathway genes corresponds to accumulation of related alkaloids in *Catharanthus roseus* (L.) G. Don. Planta 220:376–383

ElSohly HN, Croom EM Jr, Kopycki WJ, Joshi AS, ElSohly MA, McChesney JD (1997a) Taxane contents of *Taxus* cultivars grown in American nurseries. J Environ Hortic 15:200–205

ElSohly HN, Croom EM Jr, Kopycki WJ, Joshi AS, McChesney JD (1997b) Diurnal and seasonal effects on the taxane content of the clippings of certain *Taxus* cultivars. Phytochem Anal 8:124–129

Facchini PJ, De Luca V (2008) Opium poppy and Madagascar periwinkle: model non-model systems to investigate alkaloid biosynthesis in plants. Plant J 54:763–784

Farkya S, Bisaria VS (2008) Exogenous hormones affecting morphology and biosynthetic potential of hairy root line (LYR2i) of *Linum album*. J Biosci Bioeng 105:140–146

Foa R, Norton L, Seidman AD (1994) Taxol (paclitaxel): a novel anti-microtubule agent with remarkable anti-neoplastic activity. Int J Clin Lab Res 24:6–14

Fukuyama T, Ohashi-Kaneko K, Hirata K, Muraoka M, Watanabe H (2017) Effects of ultraviolet A supplemented with red light irradiation on vinblastine production in *Catharanthus roseus*. Environ Control Biol 55:65–69

Furmanowa M, Syklowska-Baranek K (2000) Hairy root cultures of *Taxus* × *media* var. *hicksii* Rehd. as a new source of paclitaxel and 10-deacetylbaccatin III. Biotechnol Lett 22:683–686

Gallego A, Malik S, Yousefzadi M, Makhzoum A, Tremouillaux-Guiller J, Bonfill M (2017) Taxol from *Corylus avellana*: paving the way for a new source of this anti-cancer drug. Plant Cell Tiss Org Cult 129:1–16

Gerber DE, Beg MS, Fattah F, Frankel AE, Fatunde O, Arriaga Y et al (2018) Phase 1 study of ARQ 761, a β-lapachone analogue that promotes NQO1-mediated programmed cancer cell necrosis. Br J Cancer 119:928–936

Giri A, Giri CC, Dhingra V, Narasu ML (2001) Enhanced podophyllotoxin production from *Agrobacterium rhizogenes* transformed cultures of *Podophyllum hexandrum*. Nat Prod Lett 15:229–235

Glowniak K, Mroczek T, Zobel AM (1999) Seasonal changes in the concentrations of four taxoids in *Taxus baccata* L. during the autumn-spring period. Phytomedicine 6:135–140

Guirimand G, Guihur A, Perello C, Phillips M, Mahroug S, Oudin A et al (2020) Cellular and subcellular compartmentation of the 2*C*-methyl-D-erythritol 4-phosphate pathway in the Madagascar periwinkle. Plants (Basel) 9:462

Guo X-R, Chang B-W, Zu Y-G, Tang Z-H (2014) The impacts of increased nitrate supply on *Catharanthus roseus* growth and alkaloid accumulations under ultraviolet-B stress. J Plant Interact 9:640–646

Guo X-R, Zu Y-G, Tang Z-H (2011) Physiological responses of *Catharanthus roseus* to different nitrogen forms. Acta Physiol Plant 34:589–598

Guo Z-G, Liu Y, Gong M-Z, Chen W, Li W-Y (2012) Regulation of vinblastine biosynthesis in cell suspension cultures of *Catharanthus roseus*. Plant Cell Tiss Org Cult 112:43–54

Hamada H, Nakazawa K (1991) Biotransformation of vinblastine to vincristine by cell suspension cultures of *Catharanthus roseus*. Biotechnol Lett 13:805–806

Hanafy MS, Matter MA, Asker MS, Rady MR (2016) Production of indole alkaloids in hairy root cultures of *Catharanthus roseus* L. and their antimicrobial activity. S Afr J Bot 105:9–18

Hare JI, Lammers T, Ashford MB, Puri S, Storm G, Barry ST (2017) Challenges and strategies in anti-cancer nanomedicine development: an industry perspective. Adv Drug Deliv Rev 108:25–38

Hartmann JT, Lipp H-P (2006) Camptothecin and podophyllotoxin derivatives: inhibitors of topoisomerase I and II-mechanisms of action, pharmacokinetics and toxicity profile. Drug Saf 29:209–230

Hartwell JL, Schrecker AW (1951) Components of podophyllin. V. The constitution of podophyllotoxin. J Am Chem Soc 73:2909–2916

Henkin JM, Ren Y, Soejarto DD, Kinghorn AD (2018) The search for anticancer agents from tropical plants. Prog Chem Org Nat Prod 107:1–94

Hirata K, Horiuchi M, Asada M, Ando T, Miyamoto K, Miura Y (1992) Stimulation of dimeric alkaloid production by near-ultraviolet light in multiple shoot cultures of *Catharanthus roseus*. J Ferment Bioeng 74:222–225

Hisiger S, Jolicoeur M (2007) Analysis of *Catharanthus roseus* alkaloids by HPLC. Phytochem Rev 6:207–234

Hoffman A, Shahidi F (2009) Paclitaxel and other taxanes in hazelnut. J Funct Foods 1:33–37

Hörmann V, Kumi-Diaka J, Durity M, Rathinavelu A (2012) Anticancer activities of genistein-topotecan combination in prostate cancer cells. J Cell Mol Med 16:2631–2636

Huang Q, Roessner CA, Croteau R, Scott AI (2001) Engineering *Escherichia coli* for the synthesis of taxadiene, a key intermediate in the biosynthesis of taxol. Bioorg Med Chem 9:2237–2242

Huang Z, Mu Y, Zhou Y, Chen W, Xu K, Yu Z et al (1997) Transformation of *Taxus brevifolia* by *Agrobacterium rhizogenes* and taxol production in hairy roots culture. Acta Bot Yunnan 19:292–296

Ianniello GP (1996) Cisplatin, epirubicin, and vindesine with or without lonidamine in the treatment of inoperable nonsmall cell lung carcinoma: a multicenter randomized clinical trial. Cancer 78:63–69

Ishikawa H, Colby DA, Seto S, Va P, Tam A, Kakei H et al (2009) Total synthesis of vinblastine, vincristine, related natural products, and key structural analogues. J Am Chem Soc 131:4904–4916

Javadian N, Karimzadeh G, Sharifi M, Moieni A, Behmanesh M (2017) In vitro polyploidy induction: changes in morphology, podophyllotoxin biosynthesis, and expression of the related genes in *Linum album* (Linaceae). Planta 245:1165–1178

Jiang W, Chen L, Pan Q, Qiu Y, Shen Y, Fu C (2011) An efficient regeneration system via direct and indirect organogenesis for the medicinal plant *Dysosma versipellis* (Hance) M. Cheng and its potential as a podophyllotoxin source. Acta Physiol Plant 34:631–639

Joshi KS, Rathos MJ, Joshi RD, Sivakumar M, Mascarenhas M, Kamble S et al (2007) In vitro antitumor properties of a novel cyclin-dependent kinase inhibitor, P276–00. Mol Cancer Ther 6:918–925

Kadkade PG (1981) Formation of podophyllotoxins by *Podophyllum peltatum* tissue cultures. Naturwissenschaften 68:481–482

Kadkade PG (1982) Growth and podophyllotoxin production in callus tissues of *Podophyllum peltatum*. Plant Sci Lett 25:107–115

Kajani AA, Moghim S, Mofid MR (2012) Optimization of the basal medium for improving production and secretion of taxanes from suspension cell culture of *Taxus baccata* L. DARU J Pharm Sci 20:54

Kawamura F, Kikuchi Y, Ohira T, Yatagai M (1999) Accelerated solvent extraction of paclitaxel and related compounds from the bark of *Taxus cuspidata*. J Nat Prod 62:244–247

Kelsey RG, Vance NC (1992) Taxol and cephalomannine concentrations in the foliage and bark of shade-grown and sun-exposed *Taxus brevifolia* trees. J Nat Prod 55:912–917

Khazir J, Mir BA, Pilcher L, Riley DL (2014) Role of plants in anticancer drug discovery. Phytochem Lett 7:173–181

Khosroushahi AY, Valizadeh M, Ghasempour A, Khosrowshahli M, Naghdibadi H, Dadpour MR et al (2006) Improved Taxol production by combination of inducing factors in suspension cell culture of *Taxus baccata*. Cell Biol Int 30:262–269

Kidd T, Easson ML, Qu Y, De Luca V (2019) Inter-organ transport of secologanin allows assembly of monoterpenoid indole alkaloids in a *Catharanthus roseus* mutant. Phytochemistry 159:119–126

Kim JA, Baek K-H, Son YM, Son SH, Shin H (2009) Hairy root cultures of *Taxus cuspidata* for enhanced production of paclitaxel. J Korean Soc Appl Biol Chem 52:144–150

Kingston DG, Newman DJ (2007) Taxoids: cancer-fighting compounds from nature. Curr Opin Drug Discov Devel 10:130–144

Kingston DGI (2012) Taxol and its analogs. In: Cragg GM, Kingston DGI, Newman DJ (eds) Anticancer agents from natural products, 2nd edn. CRC Press, Boca Raton, FL, pp 123–176

Kohler DR, Goldspiel BR (1994) Paclitaxel (Taxol). Pharmacotherapy 14:3–34

Kovacs K, Zhang L, Linforth RS, Whittaker B, Hayes CJ, Fray RG (2007) Redirection of carotenoid metabolism for the efficient production of taxadiene [taxa-4(5),11(12)-diene] in transgenic tomato fruit. Transgenic Res 16:121–126

Kumar SR, Shilpashree HB, Nagegowda DA (2018) Terpene moiety enhancement by overexpression of geranyl(geranyl) diphosphate synthase and geraniol synthase elevates monomeric and dimeric monoterpene indole alkaloids in transgenic *Catharanthus roseus*. Front Plant Sci 9:942

Kumari A, Singh HR, Jha A, Swarnkar MK, Shankar R, Kumar S (2014) Transcriptome sequencing of rhizome tissue of *Sinopodophyllum hexandrum* at two temperatures. BMC Genomics 15:871

Kushwaha R, Bhattacharya A, Singh B, Singh RD (2012) Factors affecting podophyllotoxin yield in the ex situ grown *Podophyllum hexandrum*, an endangered alpine native of the western Himalayas. J Nat Med 66:1–7

Lau W, Sattely ES (2015) Six enzymes from mayapple that complete the biosynthetic pathway to the etoposide aglycone. Science 349:1224–1228

Lee EK, Jin YW, Park JH, Yoo YM, Hong SM, Amir R et al (2010) Cultured cambial meristematic cells as a source of plant natural products. Nat Biotechnol 28:1213–1217

Lee K-H, Xiao Z (2012) Podophyllotoxins and analogs. In: Cragg GM, Kingston DGI, Newman DJ (eds) Anticancer agents from natural products, 2nd edn. CRC Press, Boca Raton, FL, pp 95–122

Leone LM, Roberts SC (2013) Accessing anti-cancer natural products by plant cell culture. In: Koehn FE (ed) Natural products and cancer drug discovery. Springer, New York, pp 193–211

Li J, Mutanda I, Wang K, Yang L, Wang J, Wang Y (2019) Chloroplastic metabolic engineering coupled with isoprenoid pool enhancement for committed taxanes biosynthesis in *Nicotiana benthamiana*. Nat Commun 10:4850

Li JW, Vederas JC (2009) Drug discovery and natural products: end of an era or an endless frontier? Science 325:161–165

Li M, Ge L, Kang T, Sun P, Xing H, Yang D et al (2018a) High-elevation cultivation increases anticancer podophyllotoxin accumulation in *Podophyllum hexandrum*. Ind Crops Prod 121:338–344
Li M, Sun P, Kang T, Xing H, Yang D, Zhang J et al (2018b) Mapping podophyllotoxin biosynthesis and growth-related transcripts with high elevation in *Sinopodophyllum hexandrum*. Ind Crops Prod 124:510–518
Liang C, Chen C, Zhou P, Xu L, Zhu J, Liang J et al (2018) Effect of *Aspergillus flavus* fungal elicitor on the production of terpenoid indole alkaloids in *Catharanthus roseus* cambial meristematic cells. Molecules 23:3276
Lin HW, Kwok KH, Doran PM (2003) Production of podophyllotoxin using cross-species coculture of *Linum flavum* hairy roots and *Podophyllum hexandrum* cell suspensions. Biotechnol Prog 19:1417–1426
Liu GM, Fang WS, Qian JF, Lan H (2001) Distribution of paclitaxel and its congeners in *Taxus mairei*. Fitoterapia 72:743–746
Liu W, Liu J, Yin D, Zhao X (2015) Influence of ecological factors on the production of active substances in the anti-cancer plant *Sinopodophyllum hexandrum* (Royle) T.S. Ying. PLoS One 10:e0122981
Liu WC, Gong T, Zhu P (2016) Advances in exploring alternative taxol sources. RSC Adv 6:48800–48809
Magnotta M, Murata J, Chen J, De Luca V (2006) Identification of a low vindoline accumulating cultivar of *Catharanthus roseus* (L.) G. Don by alkaloid and enzymatic profiling. Phytochemistry 67:1758–1764
Magnotta M, Murata J, Chen J, De Luca V (2007) Expression of deacetylvindoline-4-*O*-acetyltransferase in *Catharanthus roseus* hairy roots. Phytochemistry 68:1922–1931
Mahroug S, Burlat V, St-Pierre B (2007) Cellular and sub-cellular organisation of the monoterpenoid indole alkaloid pathway in *Catharanthus roseus*. Phytochem Rev 6:363–381
Majada JP, Sierra MI, Sánchez-Tamés R (2000) One step more towards taxane production through enhanced *Taxus* propagation. Plant Cell Rep 19:825–830
Malik S, Bíba O, Grúz J, Arroo RRJ, Strnad M (2014) Biotechnological approaches for producing aryltetralin lignans from *Linum* species. Phytochem Rev 13:893–913
Malik S, Cusidó RM, Mirjalili MH, Moyano E, Palazón J, Bonfill M (2011) Production of the anticancer drug taxol in *Taxus baccata* suspension cultures: a review. Process Biochem 46:23–34
Maqsood M, Abdul M (2017) Yeast extract elicitation increases vinblastine and vincristine yield in protoplast derived tissues and plantlets in *Catharanthus roseus*. Rev Bras Farmacogn 27:549–556
Mattina MJI, Paiva AA (1992) Taxol concentration in *Taxus* cultivars. J Environ Hortic 10:187–191
McQuade JL, Posada LP, Lecagoonporn S, Cain S, Bassett RL Jr, Patel SP et al (2016) A phase I study of TPI 287 in combination with temozolomide for patients with metastatic melanoma. Melanoma Res 26:604–608
Miettinen K, Dong L, Navrot N, Schneider T, Burlat V, Pollier J et al (2014) The seco-iridoid pathway from *Catharanthus roseus*. Nat Commun 5:3606
Moraes RM, Burandt C, Ganzera M, Li X, Khan I, Canel C (2000) The American mayapple revisited—*Podophyllum peltatum*—still a potential cash crop? Econ Bot 54:471–476
Mubeen S, Li ZL, Huang QM, He CT, Yang ZY (2018) Comparative transcriptome analysis revealed the tissue-specific accumulations of taxanes among three experimental lines of *Taxus yunnanensis*. J Agric Food Chem 66:10410–10420
Mukherjee S, Ghosh B, Jha TB, Jha S (2002) Variation in content of taxol and related taxanes in Eastern Himalayan populations of *Taxus wallichiana*. Planta Med 68:757–759
Munster PN, Daud AI (2011) Preclinical and clinical activity of the topoisomerase I inhibitor, karenitecin, in melanoma. Expert Opin Investig Drugs 20:1565–1574
Muranaka T, Miyata M, Ito K, Tachibana S (1998) Production of podophyllotoxin in *Juniperus chinensis* callus cultures treated with oligosaccharides and a biogenetic precursor in honour of Professor G. H. Neil Towers 75th birthday. Phytochemistry 49:491–496

Naaranlahti T, Auriola S, Lapinjoki SP (1991) Growth-related dimerization of vindoline and catharanthine in *Catharanthus roseus* and effect of wounding on the process. Phytochemistry 30:1451–1453

Nadeem M, Rikhari HC, Kumar A, Palni LM, Nandi SK (2002) Taxol content in the bark of Himalayan Yew in relation to tree age and sex. Phytochemistry 60:627–631

Naik PK, Alam MA, Singh H, Goyal V, Parida S, Kalia S et al (2010) Assessment of genetic diversity through RAPD, ISSR and AFLP markers in *Podophyllum hexandrum*: a medicinal herb from the Northwestern Himalayan region. Physiol Mol Biol Plants 16:135–148

Nakada T, Sugihara K, Jikoh T, Abe Y, Agatsuma T (2019) The latest research and development into the antibody-drug conjugate, [fam-] trastuzumab deruxtecan (DS-8201a), for HER2 cancer therapy. Chem Pharm Bull 67:173–185

National Cancer Institute (2019) Symptoms of cancer. https://www.cancer.gov/about-cancer/diagnosis-staging/symptoms Accessed 3 June 2020

National Cancer Institute (2020). Find NCI supported clinical trials. https://www.cancer.gov/about-cancer/treatment/clinical-trials/search Accessed 23 June 20

Navia-Osorio A, Garden H, Cusidó RM, Palazón J, Alfermann AW, Piñol MT (2002a) Production of paclitaxel and baccatin III in a 20-L airlift bioreactor by a cell suspension of *Taxus wallichiana*. Planta Med 68:336–340

Navia-Osorio A, Garden H, Cusidó RM, Palazón J, Alfermann AW, Piñol MT (2002b) Taxol® and baccatin III production in suspension cultures of *Taxus baccata* and *Taxus wallichiana* in an airlift bioreactor. J Plant Physiol 159:97–102

Newman DJ, Cragg GM (2020a) Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod 83:770–803

Newman DJ, Cragg GM (2020b) Plant endophytes and epiphytes: Burgeoning sources of known and "unknown" cytotoxic and antibiotic agents? Planta Med 86:891–905

Nicolaou KC, Guy RK (1995) The conquest of taxol. Angew Chem Int Ed Engl 34:2079–2090

Noble RL (1990) The discovery of the vinca alkaloids–chemotherapeutic agents against cancer. Biochem Cell Biol 68:1344–1351

Noble RL, Beer CT, Cutts JH (1958) Role of chance observations in chemotherapy: *Vinca rosea*. Ann N Y Acad Sci 76:882–894

O'Keefe BR, Mahady GB, Gills JJ, Beecher CWW, Schilling AB (1997) Stable vindoline production in transformed cell cultures of *Catharanthus roseus*. J Nat Prod 60:261–264

Onrubia M, Moyano E, Bonfill M, Palazon J, Goossens A, Cusido RM (2011) The relationship between TXS, DBAT, BAPT and DBTNBT gene expression and taxane production during the development of *Taxus baccata* plantlets. Plant Sci 181:282–287

Osuna L, Tapia N, Cusidó R, Palazón J, Bonfill M, Zamilpa A et al (2015) Taxane production induced by methyl jasmonate in free and immobilized cell cultures of Mexican yew (*Taxus globosa* Schltdl). Acta Physiol Plant 37:199

Ottaggio L, Bestoso F, Armirotti A, Balbi A, Damonte G, Mazzei M et al (2008) Taxanes from shells and leaves of *Corylus avellana*. J Nat Prod 71:58–60

Pan Q, Mustafa NR, Tang K, Choi YH, Verpoorte R (2016a) Monoterpenoid indole alkaloids biosynthesis and its regulation in *Catharanthus roseus*: a literature review from genes to metabolites. Phytochem Rev 15:221–250

Pan Q, Saiman MZ, Mustafa NR, Verpoorte R, Tang K (2016b) A simple and rapid HPLC-DAD method for simultaneously monitoring the accumulation of alkaloids and precursors in different parts and different developmental stages of *Catharanthus roseus* plants. J Chromatogr B 1014:10–16

Panda D, Jordan MA, Chu KC, Wilson L (1996) Differential effects of vinblastine on polymerization and dynamics at opposite microtubule ends. J Biol Chem 271:29807–29812

Paul S, Nandi SK, Palni LMS (2013) Assessment of genetic diversity and interspecific relationships among three species of *Podophyllum* using AFLP markers and podophyllotoxin content. Plant Syst Evol 299:1879–1887

Pietra F (2002) Biodiversity and natural product diversity, 1st edn. Elsevier, Amsterdam

Poupat C, Hook I, Guéritte F, Ahond A, Guénard D, Adeline MT et al (2000) Neutral and basic taxoid contents in the needles of *Taxus* species. Planta Med 66:580–584

Powell RG, Weisleder D, Smith CR Jr, Rohwedder WK (1970) Structures of harringtonine, isoharringtonine, and homoharringtonine. Tetrahedron Lett 815–818

Purohit MC, Bahuguna R, Maithani UC, Purohit AN, Rawat MSM (1999) Variation in podophylloresin and podophyllotoxin contents in different populations of *Podophyllum hexandrum*. Curr Sci 77:1078–1080

Qu Y, Easson M, Simionescu R, Hajicek J, Thamm AMK, Salim V et al (2018) Solution of the multistep pathway for assembly of corynanthean, strychnos, iboga, and aspidosperma monoterpenoid indole alkaloids from 19*E*-geissoschizine. Proc Natl Acad Sci USA 115:3180–3185

Qu Y, Easson ML, Froese J, Simionescu R, Hudlicky T, De Luca V (2015) Completion of the seven-step pathway from tabersonine to the anticancer drug precursor vindoline and its assembly in yeast. Proc Natl Acad Sci USA 112:6224–6229

Qu Y, Safonova O, De Luca V (2019) Completion of the canonical pathway for assembly of anticancer drugs vincristine/vinblastine in *Catharanthus roseus*. Plant J 97:257–266

Rajesh M, Sivanandhan G, Arun M, Vasudevan V, Theboral J, Girija S et al (2014) Factors influencing podophyllotoxin production in adventitious root culture of *Podophyllum hexandrum* Royle. Acta Physiol Plant 36:1009–1021

Ramani S, Jayabaskaran C (2008) Enhanced catharanthine and vindoline production in suspension cultures of *Catharanthus roseus* by ultraviolet-B light. J Mol Signal 3:9

Renouard S, Corbin C, Drouet S, Medvedec B, Doussot J, Colas C et al (2018) Investigation of *Linum flavum* (L.) hairy root cultures for the production of anticancer aryltetralin lignans. Int J Mol Sci 19:990

Renouard S, Lopez T, Hendrawati O, Dupre P, Doussot J, Falguieres A et al (2011) Podophyllotoxin and deoxypodophyllotoxin in *Juniperus bermudiana* and 12 other *Juniperus* species: optimization of extraction, method validation, and quantification. J Agric Food Chem 59:8101–8107

Rischin D, Bibby DC, Chong G, Kremmidiotis G, Leske AF, Matthews CA et al (2011) Clinical, pharmacodynamic, and pharmacokinetic evaluation of BNC105P: a phase I trial of a novel vascular disrupting agent and inhibitor of cancer cell proliferation. Clin Cancer Res 17:5152–5160

Roberts SC, Naill M, Gibson DM, Shuler ML (2003) A simple method for enhancing paclitaxel release from *Taxus canadensis* cell suspension cultures utilizing cell wall digesting enzymes. Plant Cell Rep 21:1217–1220

Rodriguez S, Compagnon V, Crouch NP, St-Pierre B, De Luca V (2003) Jasmonate-induced epoxidation of tabersonine by a cytochrome P-450 in hairy root cultures of *Catharanthus roseus*. Phytochemistry 64:401–409

Rose WC (1992) Taxol: a review of its preclinical in vivo antitumor activity. Anticancer Drugs 3:311–321

Rousalova I, Banerjee S, Sangwan V, Evenson K, McCauley JA, Kratzke R et al (2013) Minnelide: a novel therapeutic that promotes apoptosis in non-small cell lung carcinoma in vivo. PLoS One 8:e77411

Roussi F, Françoise G, Faby J (2012) The vinca alkaloids. In: Cragg GM, Kingston DGI, Newman DJ (eds) Anticancer agents from natural products, 2nd edn. CRC Press, Boca Raton, FL, pp 177–198

Rowinsky EK, Cazenave LA, Donehower RC (1990) Taxol: a novel investigational antimicrotubule agent. J Natl Cancer Inst 82:1247–1259

Rowinsky EK, Donehower RC (1991) The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics. Pharmacol Ther 52:35–84

Rowinsky EK, Donehower RC (1995) Paclitaxel (taxol). N Engl J Med 332:1004–1014

Sabater-Jara AB, Onrubia M, Moyano E, Bonfill M, Palazon J, Pedreno MA et al (2014) Synergistic effect of cyclodextrins and methyl jasmonate on taxane production in *Taxus x media* cell cultures. Plant Biotechnol J 12:1075–1084

Sagar BPS, Zafar R (2008) In vitro.–enhanced production of podophyllotoxin in phytohormonal-induced and regenerated roots of *Podophyllum hexandrum*. Pharm Biol 43:404–410

Salim V, De Luca V (2013) Towards complete elucidation of monoterpene indole alkaloid biosynthesis pathway: *Catharanthus roseus* as a pioneer system. Adv Bot Res 68:1–37

Samadi A, Jafari M, Nejhad NM, Hossenian F (2014) Podophyllotoxin and 6-methoxy podophyllotoxin production in hairy root cultures of *Linum mucronatum* ssp. *mucronatum.* Pharmacogn Mag 10:154–160

Scarfò L, Ghia P (2013) Reprogramming cell death: Bcl-2 family inhibition in hematological malignancies. Immunol Lett 155:36–39

Schiff PB, Fant J, Horwitz SB (1979) Promotion of microtubule assembly in vitro by taxol. Nature 277:665–667

Schultz BJ, Kim SY, Lau W, Sattely ES (2019) Total biosynthesis for milligram-scale production of etoposide intermediates in a plant chassis. J Am Chem Soc 141:19231–19235

Seegers CLC, Setroikromo R, Tepper PG, Horvatovich P, Peters R, Quax WJ (2017) Methyl jasmonate treatment increases podophyllotoxin production in *Podophyllum hexandrum* roots under glasshouse conditions. Plant Soil 417:117–126

Sharma A, Amin D, Sankaranarayanan A, Arora R, Mathur AK (2020) Present status of *Catharanthus roseus* monoterpenoid indole alkaloids engineering in homo- and hetero-logous systems. Biotechnol Lett 42:11–23

Sharma A, Mathur AK, Ganpathy J, Joshi B, Patel P (2019) Effect of abiotic elicitation and pathway precursors feeding over terpenoid indole alkaloids production in multiple shoot and callus cultures of *Catharanthus roseus.* Biologia 74:543–553

Sharma A, Verma P, Mathur A, Mathur AK (2018) Overexpression of tryptophan decarboxylase and strictosidine synthase enhanced terpenoid indole alkaloid pathway activity and antineoplastic vinblastine biosynthesis in *Catharanthus roseus*. Protoplasma 255:1281–1294

Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020. CA Cancer J Clin 70:7–30

Siemann DW, Chaplin DJ, Walicke PA (2009) A review and update of the current status of the vasculature-disabling agent combretastatin-A4 phosphate (CA4P). Expert Opin Investig Drugs 18:189–197

Silvani A, De Simone I, Fregoni V, Biagioli E, Marchioni E, Caroli M et al (2019) Multicenter, single arm, phase II trial on the efficacy of ortataxel in recurrent glioblastoma. J Neurooncol 142:455–462

Silverman JA, Deitcher SR (2013) Marqibo® (vincristine sulfate liposome injection) improves the pharmacokinetics and pharmacodynamics of vincristine. Cancer Chemother Pharmacol 71:555–564

St-Pierre B, Laflamme P, Alarco AM, De Luca V (1998) The terminal *O*-acetyltransferase involved in vindoline biosynthesis defines a new class of proteins responsible for coenzyme A-dependent acyl transfer. Plant J 14:703–713

Stähelin H, von Wartburg A (1989) From podophyllotoxin glucoside to etoposide. Prog Drug Res 33:169–266

Stähelin HF, von Wartburg A (1991) The chemical and biological route from podophyllotoxin glucoside to etoposide: Ninth Cain Memorial Award lecture. Cancer Res 51:5–15

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science 260:214–216

Sultan P, Shawl AS, Ramteke PW, Kour A, Qazi PH (2008) Assessment of diversity in *Podophyllum hexandrum* by genetic and phytochemical markers. Sci Hortic 115:398–408

Sultan P, Shawl AS, Rehman S, Ahmed SF, Ramteke PW (2010) Molecular characterization and marker based chemotaxonomic studies of *Podophyllum hexandrum* Royle. Fitoterapia 81:243–247

Sun J, Zhao L, Shao Z, Shanks J, Peebles CAM (2018) Expression of tabersonine 16-hydroxylase and 16-hydroxytabersonine-*O*-methyltransferase in *Catharanthus roseus* hairy roots. Biotechnol Bioeng 115:673–683

Svoboda GH (1961) Alkaloids of *Vinca rosea* (C*atharanthus roseus*). IX. Extraction and characterization of leurosidine and leurocristine. Lloydia 24:173–178

Syklowska-Baranek K, Furmanowa M (2005) Taxane production in suspension culture of *Taxus x media* var. *hicksii* carried out in flasks and bioreactor. Biotechnol Lett 27:1301–1304

Syklowska-Baranek K, Grech-Baran M, Naliwajski MR, Bonfill M, Pietrosiuk A (2015a) Paclitaxel production and PAL activity in hairy root cultures of *Taxus x media* var. *hicksii* carrying a taxadiene synthase transgene elicited with nitric oxide and methyl jasmonate. Acta Physiol Plant 37:218

Syklowska-Baranek K, Pietrosiuk A, Kokoszka A, Furmanowa M (2009) Enhancement of taxane production in hairy root culture of *Taxus x media* var. *hicksii*. J Plant Physiol 166:1950–1954

Syklowska-Baranek K, Pilarek M, Bonfill M, Kafel K, Pietrosiuk A (2015b) Perfluorodecalin-supported system enhances taxane production in hairy root cultures of *Taxus x media* var. *hicksii* carrying a taxadiene synthase transgene. Plant Cell Tiss Org Cult 120:1051–1059

Syklowska-Baranek K, Rymaszewski W, Gawel M, Rokicki P, Pilarek M, Grech-Baran M et al (2019) Comparison of elicitor-based effects on metabolic responses of *Taxus x media* hairy roots in perfluorodecalin-supported two-phase culture system. Plant Cell Rep 38:85–99

Syklowska-Baranek K, Szala K, Pilarek M, Orzechowski R, Pietrosiuk A (2018) A cellulase-supported two-phase in situ system for enhanced biosynthesis of paclitaxel in *Taxus* × *media* hairy roots. Acta Physiol Plant 40:201

Tabata H (2006) Production of paclitaxel and the related taxanes by cell suspension cultures of *Taxus* species. Curr Drug Targets 7:453–461

Tafreshi SAH, Shariati M, Mofid MR, Nekui MK (2011) Rapid germination and development of *Taxus baccata* L. by in vitro embryo culture and hydroponic growth of seedlings. Vitro Cell Dev Biol Plant 47:561–568

Tafur S, Jones W, Dorman D, Logsdon E, Svoboda G (1975) Alkaloids of *Vinca rosea* L. (*Catharanthus roseus* G. Don). XXXVI: Isolation and characterization of new dimeric alkaloids. J Pharm Sci 64:1953–1957

Takeba Y, Sekine S, Kumai T, Matsumoto N, Nakaya S, Tsuzuki Y et al (2007) Irinotecan-induced apoptosis is inhibited by increased P-glycoprotein expression and decreased p53 in human hepatocellular carcinoma cells. Biol Pharm Bull 30:1400–1406

Talebi M, Ghassempour A, Talebpour Z, Rassouli A, Dolatyari L (2004) Optimization of the extraction of paclitaxel from *Taxus baccata* L. by the use of microwave energy. J Sep Sci 27:1130–1136

Tashackori H, Sharifi M, Ahmadian Chashmi N, Safaie N, Behmanesh M (2016) Induced-differential changes on lignan and phenolic acid compounds in *Linum album* hairy roots by fungal extract of *Piriformospora indica*. Plant Cell Tiss Org Cult 127:187–194

Thamm AMK, Qu Y, De Luca V (2016) Discovery and metabolic engineering of iridoid/secoiridoid and monoterpenoid indole alkaloid biosynthesis. Phytochem Rev 15:339–361

Tomé-Carneiro J, Larrosa M, González-Sarrías A, Tomás-Barberán FA, García-Conesa MT, Espín JC (2013) Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. Curr Pharm Des 19:6064–6093

Tonk D, Mujib A, Maqsood M, Ali M, Zafar N (2016) *Aspergillus flavus* fungus elicitation improves vincristine and vinblastine yield by augmenting callus biomass growth in *Catharanthus roseus*. Plant Cell Tiss Org Cult 126:291–303

U.S. Food and Drug Administration (2019) FDA approves fam-trastuzumab deruxtecan-nxki for unresectable or metastatic HER2-positive breast cancer. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-fam-trastuzumab-deruxtecan-nxki-unresectable-or-metastatic-her2-positive-breast-cancer Accessed: 10 June 2020

van Rozendaal EL, Lelyveld GP, van Beek TA (2000) Screening of the needles of different yew species and cultivars for paclitaxel and related taxoids. Phytochemistry 53:383–389

van Uden W, Bouma AS, Bracht Waker JF, Middel O, Wichers HJ, De Waard P et al (1995) The production of podophyllotoxin and its 5-methoxy derivative through bioconversion of cyclodextrin-complexed desoxypodophyllotoxin by plant cell cultures. Plant Cell Tiss Org Cult 42:73–79

Vázquez-Flota F, De Luca V, Carrillo-Pech M, Canto-Flick A, de Lourdes M-H (2002) Vindoline biosynthesis is transcriptionally blocked in *Catharanthus roseus* cell suspension cultures. Mol Biotechnol 22:1–8

Veselá D, Šaman D, Valterová I, Vaněk T (1999) Seasonal variations in the content of taxanes in the bark of *Taxus baccata* L. Phytochem Anal 10:319–321

Villalona-Calero MA, Kolesar JM (2002) Mitomycin as a modulator of irinotecan anticancer activity. Oncology 16:21–25

Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AT, Sim GA (1966) Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor Inhibitor from *Camptotheca acuminata*. J Am Chem Soc 88:3888–3890

Wang C, Wu J, Mei X (2001) Enhanced taxol production and release in *Taxus chinensis* cell suspension cultures with selected organic solvents and sucrose feeding. Biotechnol Prog 17:89–94

Wang CT, Liu H, Gao XS, Zhang HX (2010) Overexpression of G10H and ORCA3 in the hairy roots of *Catharanthus roseus* improves catharanthine production. Plant Cell Rep 29:887–894

Wang X, Huang Y, Mort AJ, Zeng Y, Tauer CG, Cochran KD (2006) Variation of taxane content in needles of *Taxus x media* cultivars with different growth characteristics. Z Naturforsch C 61:619–624

Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT (1971) Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia.* J Am Chem Soc 93:2325–2327

Weaver BA (2014) How Taxol/paclitaxel kills cancer cells. Mol Biol Cell 25:2677–2681

Wheeler NC, Jech K, Masters S, Brobst SW, Alvarado AB, Hoover AJ et al (1992) Effects of genetic, epigenetic, and environmental factors on taxol content in *Taxus brevifolia* and related species. J Nat Prod 55:432–440

WHO (2018) Cancer. https://www.who.int/news-room/fact-sheets/detail/cancer Accessed 24 Jan 2020

Wickremesinhe ERM, Arteca RN (1994) Roots of hydroponically grown *Taxus* plants as a source of taxol and related taxanes. Plant Sci 101:125–135

Wickremesinhe ERM, Arteca RN (1996) Effects of plant growth regulators applied to the roots of hydroponically grown *Taxus* × *media* plants on the production of taxol and related taxanes. Plant Sci 121:29–38

Wilson SA, Roberts SC (2012) Recent advances towards development and commercialization of plant cell culture processes for the synthesis of biomolecules. Plant Biotechnol J 10:249–268

Wink M, Alfermann AW, Franke R, Wetterauer B, Distl M, Windhövel J et al (2007) Sustainable bioproduction of phytochemicals by plant in vitro cultures: anticancer agents. Plant Genet Resour 3:90–100

Witherup KM, Look SA, Stasko MW, Ghiorzi TJ, Muschik GM, Cragg GM (1990) *Taxus* spp. needles contain amounts of taxol comparable to the bark of *Taxus brevifolia*: Analysis and isolation. J Nat Prod 53:1249–1255

Xi X-J, Guo J, Zhu Y-G, Yang X-L, Yu Y, Cheng Z et al (2014) Genetic diversity and taxol content variation in the Chinese yew *Taxus mairei*. Plant Syst Evol 300:2191–2198

Yang L, Wang H, Zu Y-g, Zhao C, Zhang L, Chen X et al (2011) Ultrasound-assisted extraction of the three terpenoid indole alkaloids vindoline, catharanthine and vinblastine from *Catharanthus roseus* using ionic liquid aqueous solutions. Chem Eng J 172:705–712

Yang L, Zheng ZS, Cheng F, Ruan X, Jiang DA, Pan CD et al (2016) Seasonal dynamics of metabolites in needles of *Taxus wallichiana* var. *mairei.* Molecules 21:1403

Yousefzadi M, Sharifi M, Behmanesh M, Ghasempour A, Moyano E, Palazon J (2010) Salicylic acid improves podophyllotoxin production in cell cultures of *Linum album* by increasing the expression of genes related with its biosynthesis. Biotechnol Lett 32:1739–1743

Yu C, Guo H, Zhang Y, Song Y, Pi E, Yu C et al (2017) Identification of potential genes that contributed to the variation in the taxoid contents between two *Taxus* species (*Taxus media* and *Taxus mairei*). Tree Physiol 37:1659–1671

Zhang C, Fevereiro PS (2007) The effect of heat shock on paclitaxel production in *Taxus yunnanensis* cell suspension cultures: role of abscisic acid pretreatment. Biotechnol Bioeng 96:506–514

Zhou K, Qiao K, Edgar S, Stephanopoulos G (2015a) Distributing a metabolic pathway among a microbial consortium enhances production of natural products. Nat Biotechnol 33:377–383

Zhou P, Yang J, Zhu J, He S, Zhang W, Yu R et al (2015b) Effects of beta-cyclodextrin and methyl jasmonate on the production of vindoline, catharanthine, and ajmalicine in *Catharanthus roseus* cambial meristematic cell cultures. Appl Microbiol Biotechnol 99:7035–7045

Zhou T, Luo X, Zhang C, Xu X, Yu C, Jiang Z et al (2019) Comparative metabolomic analysis reveals the variations in taxoids and flavonoids among three *Taxus* species. BMC Plant Biol 19:529

Chapter 18
Biotechnological Approach to Cultivation of *Rhododendron tomentosum* (*Ledum palustre*) as the Source of the Biologically Active Essential Oil

Anna Jesionek, Adam Kokotkiewicz, and Maria Luczkiewicz

Abstract *Rhododendron tomentosum* (marsh tea, previously *Ledum palustre*), a fragrant shrub with characteristic evergreen leaves and white flowers, grows in Europe, Asia, and North America. It has been used for centuries in folk medicine to treat rheumatic diseases, lung problems, and infections as well as due to its repellent properties. In North America, the tonic beverage known as "Labrador tea", derived from the indigenous tradition and made from *R. tomentosum*, *R. groenlandicum* and *R. neoglandulosum* leaves, is prepared until now. The modern biological research confirm anti-inflammatory, analgesic, antimicrobial and insecticidal effect of the discussed plant material, indicating an important role of the essential oil as an active ingredient. However, obtaining the volatile fraction from *R. tomentosum* ground material for pharmacological studies is difficult because marsh tea is the endangered species in some countries. Moreover, as many as ten chemotypes of *R. tomentosum* on the Eurasian continent have been distinguished, due to the chemical composition of the essential oil. Such heterogeneity of the plant material is problematic, assuming its use for medical purposes. Therefore, the shoot in vitro culture was initiated for the first time for receiving the *R. tomentosum* biomass, being the complex source of biologically active volatile compounds, regardless of environmental conditions. The microshoots were subsequently adapted for large laboratory scale cultivation in commercial and prototype bioreactors. The RITA® temporary immersion system, containing SH medium with 24.60 μM 2-isopentenyladenine and 592.02 μM adenine, provided the highest growth parameters of biomass (Gi = 280%) and the intensified biosynthesis of the essential oil (500 μl 100 g^{-1} dry weight), surpassing the productivity of the aged shoots of the mother plant (300 μl 100 g^{-1} dry weight). The main terpenes of the obtained volatile fraction were ledene oxide (II) (13%), shyobunone (8%), *p*-cymene (7%), and alloaromadendrene (6%). In order to increase the essential oil content in the *R. tomentosum* microshoots, elicitation strategy was applied, using methyl jasmonate and the selected abiotic and biotic elicitors. In response to stress caused by the aphid extract and *Pectobacterium carotovorum* lysate, the accumulation of the volatile fraction increased by

A. Jesionek · A. Kokotkiewicz · M. Luczkiewicz (✉)
Department of Pharmacognosy with Medicinal Plant Garden, Medical University of Gdansk, J. Hallera 107, 80-416 Gdansk, Poland
e-mail: maria.luczkiewicz@gumed.edu.pl

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_18

14%. In addition, the full protocol for micropropagation of marsh tea was developed, including initiation, multiplication, elongation, rooting, hardening, and adaptation of the seedlings to in vivo conditions, for ex situ protection of the discussed endangered species. This article reviews the importance of *R. tomentosum* from a medical point of view as well as the biotechnological approach obtaining an alternative source of this valuable biomass.

Keywords Anti-inflammatory activity · Bioreactor · Elicitation · In vitro cultures · Labrador tea · Micropropagation

Abbreviations

2iP	2-Isopentenyladenine
AD	Adenine
AR	Anderson's *Rhododendron* medium
DW	Dry weight
EO	Essential oil
Gi	Growth index
RAPD	Random DNA
SH	Schenk–Hildebrandt medium
TDZ	Thidiazuron
WP	Woody plant medium

18.1 Introduction

Rhododendron tomentosum Harmaja (previously *Ledum palustre* L.), commonly known also as marsh (Labrador) tea, northern Labrador tea or wild rosemary, is an evergreen shrub growing usually to 50 cm tall. This plant is characterized with the small white five-petaled flowers, gathered into aromatic umbels, as well as the linear leathery leaves that are glabrous on the upper surface and densely covered with rufous hairs beneath (Dampc and Łuczkiewicz 2013). In 1990, the cladistic analysis has revealed that on the basis of the distinct morphological features, such as a secondarily reduced choripetalous corolla, a capsule opening from the base, lepidote scales on the underside of the leaf and the revolute vernation, the species formerly found in the *Ledum* L. genus, i.a. *Ledum palustre*, should be placed actually as a subsection of the *Rhododendron* L. genus (family *Ericaceae*) (Harmaja 1991; Kron and Judd 1990). *R. tomentosum* has been used for ages in traditional medicine in Europe and Canada for the treatment of the following conditions: rheumatism, cold, cough, asthma, diarrhea, infectious diseases and insect bites (Dampc and Łuczkiewicz 2013). The modern researches confirm validity of the application of marsh tea in numerous

ailments, reporting the wide range of its biological activities: antimicrobial (Kim and Nam 2006), antifungal (Judzentiene et al. 2020), antioxidant (Kim and Nam 2006; Jesionek et al. 2018b; Judzentiene et al. 2020), antidiabetic (Harbilas et al. 2009), anti–arthritic (Jesionek et al. 2019b), and insecticidal (Benelli et al. 2020). However, the several chemotypes of *R. tomentosum* exist, which can influence the profile of the pharmacological properties (Jesionek et al. 2019b). Additionally, in some countries the discussed plant is the endangered species due to the drying out of peat bogs and cannot be harvested from natural habitat without the disruption of biodiversity (Dampc and Łuczkiewicz 2013). Therefore, the in vitro cultures of *R. tomentosum* were proposed as the alternative, stable and independent of environmental conditions source of this valuable plant material (Jesionek et al. 2016). Moreover, the biotechnological approach for the large-scale cultivation of the biomass and for the enhancement of terpene secondary metabolites was developed (Jesionek et al. 2017, 2018a).

18.2 Geographic Distribution

R. tomentosum, a species common for arctic and subarctic vegetation types, grows in peatbogs, shrubby areas, heaths, wet pine and boreal forests, moss and lichen tundra, usually in shady or semi-shady positions on a moist acid soil. Its distribution range includes northern Europe, Asia, and North America (Dampc and Łuczkiewicz 2013). Although there is no direct link between longitude of the natural habitat of *R. tomentosum* and its chemotype in terms of the essential oil composition, a general pattern can be observed. As indicated in the literature, in the plant material collected in Europe-ledol, palustrol, γ-terpineol and *p*-cymene dominated, while in Russia and China large amounts of monoterpenes, such as sabinene, *p*-cymene, limonene and *β*-myrcene, were rather identified (Table 18.1). In turn, germacrone was determined as the most abundant component of the volatile fraction of marsh tea native to Alaska (previously *R. tomentosum ssp. subarcticum* or *L. palustre ssp. decumbens*) (Table 18.1). It should be added that two other similar species, which can be easily confused with *R. tomentosum*, can be found throughout northern North America (Flora of North America 2020): widely distributed in this region *R. groenlandicum* (bog Labrador tea, Indian tea, Hudson's Bay Tea) and *R. columbianum* (western Labrador tea) (Table 18.1; Fig. 18.1). The dried leaves from all mentioned above species are traditionally used to prepare the aromatic beverage, known as Labrador tea, which has been drunk by Native Americans, Canadian First Nations and Inuits as well as by modern consumers (Dampc and Łuczkiewicz 2015). However, there is still the lack of data on safety of this tisane. Taking into account the large diversity of the chemical composition of the used plant material, consumption of Labrador tea should be limited to avoid side effects. In Europe, drinking a beverage from *R. tomentosum* is not practiced, probably because of the possible high content of toxic compound, ledol (Dampc and Łuczkiewicz 2015).

Table 18.1 Morphological features* and geographic distribution of various chemotypes** of *Rhododendron tomentosum*, *R. groenlandicum* and *R. columbianum*, the species included in traditional Labrador tea

Species*	Morphology*	Chemotype**	Main distribution	Source
Rhododendron tomentosum (Harmaja) (formerly *Ledum palustre* L., *L. decumbens* (Aiton) Lodd. ex Steud; *L. palustre ssp. decumbens* (Aiton) Hultén; *R. subarcticum* Harmaja; *R. tolmachevii* Harmaja; *R. tomentosum ssp. decumbens* (Aiton) Elven & Murray; *R. tomentosum ssp. subarcticum* (Harmaja) Wallace	To 0.5 m; creeping or prostrate stem, with twigs covered with dense ferruginous, long-crisped hairs and with flattened, glandular scales; coriaceous leaves with linear blade, revolute margins and abaxial surface with ferruginous hairs, sometimes forming dense, uniform mat	γ-terpineol	Poland, Estonia	Jesionek et al. (2019b)
		Ledol, palustrol	Finland, Lithuania, Estonia, western part of Russia	Jesionek et al. (2019b)
		β-myrcene, palustrol	Sweden, middle part of Russia	Jesionek et al. (2019b)
		β-myrcene, *p*-cymene, palustrol	Middle part of Russia	Jesionek et al. (2019b)
		Sabinene	Baikal Lake in Russia, northern part of China, North Korea	Jesionek et al. (2019b)
		p-cymene	Eastern part of Russia	Jesionek et al. (2019b)
		p-cymene, ascaridole	Eastern part of Poland, eastern part of Russia, northern part of China	Jesionek et al. (2019b), Benelli et al. (2020)
		p-cymene, bornyl acetate	Eastern part of Russia, northern part of China	Jesionek et al. (2019b)
		Limonene	Middle part of Russia	Jesionek et al. (2019b)
		α-thujenal	Northern part of China	Jesionek et al. (2019b)

(continued)

Table 18.1 (continued)

Species*	Morphology*	Chemotype**	Main distribution	Source
		Germacrone	Alaska	von Schantz and Hiltunen (1971), Reichardt et al. (1990)
		?	Canada, Greenland	–
Rhododendron groenlandicum (Oeder) Kron & Judd (formerly *Ledum groenlandicum* Oeder)	0.2–1.5 m; erect or prostrate stem with twigs densely covered with ferruginous long-crisped, unbranched hairs and with flattened, glandular scales; coriaceous leaves with ovate–lanceolate blade, revolute margins and abaxial surface with ferruginous, eglandular hairs	Germacrone	Alaska, Canada	von Schantz and Hiltunen (1971), Belleau and Collin (1993)
		α-pinene, sabinene	Province of Quebec, Canada	Belleau and Collin (1993), Collin (2015)
		Limonene, β-selinene		Collin (2015)
		β-bisabolene		Collin (2015)
		Sabinene		Collin (2015)
		?	Greenland, northern part of United States	–
Rhododendron columbianum (Piper) Harmaja (formerly *Ledum columbianum* Piper, *L. glandulosum* Nuttall, *R. neoglandulosum* Harmaja)	To 2 m; erect stem with hairy, papillate twigs with flattened, glandular scales; coriaceous leaves with ovate to lanceolate blade and entire, plane, glabrous margins	?	Western part of United States and Canada	–

*Flora of North America (access: 09.2020)
**Based on the content of the predominant compounds in the essential oil, determined by GC/MS analysis

Fig. 18.1 Morphology of blooming shoots of: **a** *Rhododendron tomentosum* (previously *Ledum palustre*), **b** *R. tomentosum ssp. subarcticum* (previously *Ledum palustre ssp. decumbens*), **c** *R. groenlandicum*, **d** *R. columbianum* (*neoglandulosum*) (*Photo source* https://www.rhododendron.dk, access: 09.2020)

18.3 Phytochemistry

One of the most important, from the pharmacological point of view, secondary metabolites of *R. tomentosum*, is the essential oil, which is responsible to a great extent for the biological activity (Dampc and Łuczkiewicz 2013). The whole aerial organs of the discussed plant have the specific, aromatic fragrance, with slightly narcotic properties causing headache or dizziness during excessive and prolonged inhalation (Dampc and Łuczkiewicz 2013). The yield of the volatile fraction in the marsh tea ranges from 0.2% to even 3.5%, depending on many factors, especially on the age of the shoots and the vegetation phase (Evstratova et al. 1978; Butkiene et al. 2008; Gretsusnikova et al. 2010; Butkiene and Mockute 2011; Raal et al. 2014; Jesionek et al. 2016). Similarly, the chemical composition of the *R. tomentosum* essential oil is very variable. So far, ten different chemotypes of the plant were defined in the Eurasian continent (Table 18.1; Jesionek et al. 2019b). Additionally, the environmental, developmental, cultivation, and genetic conditions influence the chemical composition of the marsh tea volatile fraction, together with the diversified

analytical procedures (Table 18.2). The sesquiterpene alcohols ledol and palustrol, two aromadendrane derivatives, are the most recognizable and characteristic volatile constituents of the *R. tomentosum* plant material. However, in some chemotypes they are completely absent, or other compounds are found in much higher levels (Jesionek et al. 2019b). This considerable variability of the *R. tomentosum* essential oils should be taken into account when designing biological experiments or using the plant material in therapy.

Besides the essential oil, the flavonoid fraction of *R. tomentosum* contributes also to the biological properties of the plant material (Dampc and Łuczkiewicz 2013). So far, many quercetin derivatives were identified in this species, such as quercetin 3-*O*-galactoside (hyperoside), quercetin 3-*O*-glucoside (isoquercitrin), quercetin 3-*O*-rhamnoside (quercitrin), as well as the acetylated compound, unusual in plants of the *Ericaceae* family—quercetin 3-*O*-(6″-*O*-acetyl)-galactoside (Mikhailova and Rybalko 1980; Chosson et al. 1998; Black et al. 2011). Moreover, the *R. tomentosum* plant material contained myricetin, procyanidins, and (+)-catechin, which was one of the most abundant constituents in the whole phenolic fraction of *R. tomentosum ssp. subarcticum* (Black et al. 2011). In addition, the presence of chlorogenic acid, *p*-coumaric acid, and caffeic acid derivatives was confirmed, especially in the flowers of marsh tea (Black et al. 2011). Other groups of compounds recognized in *R. tomentosum* were coumarins: fraxetin, fraxin, esculin, palustroside, esculetin, umbelliferone and scopoletin (Mikhailova and Rybalko 1980; Klokova et al. 1982; Dubois et al. 1990) as well as tannins—methyl gallate and pyrogallol (Gapanenko and Levashova 2015). Among the triterpenoids identified in the discussed plant material, there was ursolic acid (Mikhailova and Rybalko 1980), the compound with proven anti-tumor effect and cytotoxic activity toward various types of cancer cell lines (Dampc and Łuczkiewicz 2013). Phenolic glycoside—arbutin with disinfecting the urinary tract properties was also found (Gapanenko and Levashova 2015).

18.4 Medicinal Properties and Usage

The repellent properties of *R. tomentosum* were well known probably since the Middle Ages. In the eighteenth-century marsh tea was included in Pharmacopoeias of some European countries as the herb widely used in folk medicine (Dampc and Łuczkiewicz 2013). Although traditional therapeutic application of *R. tomentosum* differed a little in various communities (Table 18.3), the extracts of this plant were commonly used in rheumatic ailments, pulmonary diseases, cold symptoms as well as a painkiller. Besides infusions, tinctures and syrups were also prepared. Moreover, the decoction of shoots was used externally, for dressing wounds and as a bath additive in arthritis, especially in Poland (Świejkowski 1950). However, unlike the customs of indigenous people in North America, in Europe, and Asia, *R. tomentosum* was not used to prepare a beverage for everyday consumption, probably due to the high content of ledol, one of the constituents of the essential oil. For the same reason, among European populations the internal use of extracts from marsh tea for

Table 18.2 Influence of the selected factors on the chemical composition of *R. tomentosum* essential oil*

Factor	Investigated variables	Observation	Source
Habitat	Juodupe, Silenai (Lithuania)	Considerable differences between EOs concerned the content of compounds with the menthane carbon skeleton and limonene	Butkiene et al. (2008)
	Kernave marshes near Šulnys Lake (Lithuania) (distances between 3 sampling sites 1 km)	Despite that the plant material was collected from the limited area, significant variability was observed in EOs (different chemotypes)	Judzentiene et al. (2012a)
	Tomsk, Suiga, Tynda, Utesnoe, Lake Baikal (Russia)	Four different chemotypes were identified	Belousova et al. (1990)
	Miszewko, Lubichowo (Poland)	The general profiles of obtained EOs were similar, a few differences in the qualitative and quantitative composition	Jesionek et al. (2019a)
	Four localities in Estonia	Two different chemotypes were identified	Raal et al. (2014)
Part of the plant	Shoots, leaves, and stems	The shoot EO was similar to the leaf EO; the stem EO differed considerably	Gretsusnikova et al. (2010)
	Stems, leaves and flowers	The EOs from different parts revealed big differences in chemical composition	Zhao et al. (2016)
	Seeds and shoots	Some constituents were characteristic only for the seeds	Judzentiene et al. (2012b)
Age of shoots	Young and aged shoots	Young shoots biosynthesized 3–4 times larger amounts of EO and contained larger quantities of terpenoids; the content of palustrol was higher in aged shoots	Butkiene et al. (2008)

(continued)

Table 18.2 (continued)

Factor	Investigated variables	Observation	Source
	Young and aged shoots	Young shoots EO were rich in monoterpenes, while the amounts of ledol, palustrol, γ-terpineol and bornyl acetate were higher in aged shoots	Jesionek et al. (2016)
Period of harvesting (vegetation phase)	The turn of the flowering phase and the formation of seeds (early June) and the vegetative growth stage after seeding (early November)	In June more monoterpene hydrocarbons, sesquiterpene hydrocarbons and some oxygenated sesquiterpenes; in November more oxygenated monoterpenes, higher ledol and palustrol content	Jesionek et al. (2019a)
	From April to October	EOs richest in ledol and palustrol were found in April and October; shoot EO during seed formation in June contained high level of myrcene; the largest amounts of furyl compounds were produced in September and October	Butkiene et al. (2011)
	From bud swelling in April till the vegetation following seeding in November, in 3 consecutive years	Ledol content in EO fluctuated from 23 to 37% throughout the growth season	Evstratova et al. (1978)
	Bloom (July) and non-bloom (April) period	The EOs from different growth period revealed big differences in chemical composition	Zhao et al. (2016)
	From April (at the shoot-growing stage) to October (seed full-ripening phase), during several years	The EOs varied quantitatively and qualitatively, which influenced their biological activities	Judzentiene et al. (2020)

(continued)

Table 18.2 (continued)

Factor	Investigated variables	Observation	Source
Time of day to harvest	7:00 AM, 11:00 AM, 3:00 PM, 7:00 PM, and 11:00 PM within a day	The main components yields were significantly affected by diurnal variation; high levels of major compounds were observed during 11:00 AM and 3:00 PM	Zhang et al. (2017)
Environment	E.g. light (greenhouse, shade), temperature, nutrient availability (foliar C:N, foliar P)	The resource manipulation of light, temperature and nutrients had complex effects on the quantity and quality of EO in the leaves	Baldwin (2003)
Drying of plant material	Shade-drying, oven-drying and freeze-drying	Not many differences in the chemical composition of obtained EOs	Jesionek et al. (2019a)
Method of EO isolation	Hydrodistillation (Deryng and Clevenger apparatus), simultaneous extraction–distillation (Likens-Nickerson apparatus)	The chemical composition of obtained EOs was similar to a great extent	Jesionek et al. (2019a)
	Hydrodistillation (Clevenger apparatus) and supercritical fluid extraction	Higher level of ascaridole (15.1% vs. 4.5%) in SFE samples	Baananou et al. (2015)
Type of cultivation	In vitro cultures, regenerated plants, ground plants	Significant differences in the composition of obtained EOs	Jesionek et al. (2016)

*EO - essential oil

medicinal purposes is nowadays increasingly rare (Dampc and Łuczkiewicz 2013). The chronic and excessive exposure to the discussed toxic compound can lead to impairment of the central nervous system, causing the serious side effects, such as dizziness, nausea, vomiting, loss of consciousness, and breathing problems (Dampc and Łuczkiewicz 2013). In Labrador tea, made mainly from *R. groenlandicum* leaves, the ledol concentration is low, which can probably result in a general stimulating action, like caffeine, after consumption. However, caution is needed (Dampc and Łuczkiewicz 2015).

Modern research confirms multidirectional biological activity of *R. tomentosum*, used in traditional medicine. Anti-inflammatory and analgesic properties of marsh

Table 18.3 Overview of the traditional therapeutic use of *R. tomentosum* in different communities

Community	Traditional use	Source
Polish people	Insect repellent, rheumatism, gout, fever, whooping cough, scabies, lichen, wounds, insect stings, deafness, heel pain, eczema, rashes, night sweats, as diuretic agent	Świejkowski (1950), Kasper-Pakosz et al. (2016)
Lithuanians	Different pains, wounds, lung diseases	Butkiene et al. (2008)
Estonians	Insect repellent, rheumatism, arthrosis, insect bites	Gretšušnikova et al. (2010), Soukand et al. (2010)
Norwegian Sami people	Cold and whooping cough, rheumatism, pain reliever, frost damage of the joints, lowering blood pressure, bladder catarrh, diphtheria, insect repellent	Alm and Iversen (2010)
Swedes	Headache, toothache, pain, shingles	Tunon et al. (1995)
Russians	Respiratory and lung disorders (bronchitis, tuberculosis, whooping cough, asthma), lowering blood pressure, preventing seizures, anthelmintic, ointment for eczema, scabies and insect stings	Shikov et al. (2014)
Chinese people	Cough, asthma, lowering blood pressure, as antifungal agent	Shikov et al. (2014)
Inuit and Cree First Nations of Canada	Pains (stomach ache, toothache, sore throat, headache, back and kidney pain, heart and chest pain), rheumatism and arthritis, inflammation, cold and flu symptoms (cough, nasal congestion), respiratory illnessses (shallow breathing), infection, tuberculosis, abscesses and boils, wounds, diabetes 2, snow blindness and eye problems, canker sores (in the mouth), dry skin, hangovers, thirst, appetite, diarrhea, fainting and weakness, sore or swollen limbs, foot sores and numbness, as emetic agent, tonic beverage	Black et al. (2011)

tea, associated with its anti-arthritic potential, are widely studied in in vitro tests (Wagner et al. 1986; Tunon et al. 1995; Jesionek et al. 2018b), cell line studies (Black et al. 2011; Jesionek et al. 2019b) as well as in animal experiments (Belousov et al. 2006; Zhang et al. 2010). Recently, the influence of *R. tomentosum* essential oils on CD4+ and CD8+ lymphocytes proliferation and apoptosis rates of synoviocytes, involved in the pathogenesis of rheumatoid arthritis, was tested. The results were promising (Jesionek et al. 2019b). The determined antioxidant activity of the marsh tea plant material (Kim and Nam 2006; Black et al. 2011; Jesionek et al. 2018b; Judzentiene et al. 2020) can counteract pathogenesis of rheumatic diseases too, inhibiting signaling pathways caused by oxydative stress and ipso facto reducing inflammatory reactions. It is valued also in the treatment of type 2 diabetes (Fraser et al. 2007; Harbilas et al. 2009). Furthermore, *R. tomentosum* exhibits anticancer properties, which were studied on KB cells (human mouth epidermal carcinoma) (Jin et al. 1999), human lymphoblastoid Raji cells (Spiridonov et al. 2005), and mouse leukemia L1210 cells (Goun et al. 2002). The mixture of extracts from *Archangelica officinalis* and marsh tea was suggested to be used as a radioprotector during the planned and unplanned radiation exposure (e.g. during radiotherapy or in the nuclear industry) (Narimanov et al. 1991). The antifungal (*Candida* sp., *Cryptococcus neoformans, Saccharomyces cerevisiae, Aspergillus niger*), antibacterial (*Streptococcus pneumoniae*, *Clostridium perfringens*, *Mycobacterium smegmatis*, *Acinetobacter lwoffii, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*), and antiviral (tick-borne encephalitis virus) activities of *R. tomentosum* were also examined (Fokina et al. 1991; Jin et al. 1999; Belousov et al. 2006; Kim and Nam 2006; Judzentiene et al. 2020). The well-known insecticidal properties of the marsh tea extracts and its isolated essential oil were confirmed against *Aedes aegypti* and *Culex quinquefasciatus*—mosquitoes, *Spodoptera littoralis*—cotton leafworms, *Musca domestica*—flies, *Ixodus ricinus*—ticks, *Hylobius abietis* and *Phyllodecta laticollis*—phytophagous pests, *Tenebrio molitor*, *Callosobruchus chinensis*, *Sitophilus granarius* and *S. oryzae*—stored pests (Kuusik et al. 1995; Ignatowicz and Wesołowska 1996; Jaenson et al. 2005, 2006; Egigu et al. 2011; Benelli et al. 2020). It is important observation in view of the quick development of the insect pest resistance to synthetic pesticides as well as of searching for non-toxic for humans, environmentally friendly repellents.

As far as modern market is concerned, *R. tomentosum* is the constituent of many homeopathic multi-ingredient preparations, intended mainly for treatment of rheumatism and for relieving mosquito bites (Dampc and Łuczkiewicz 2013). Moreover, the drug "Ledin" for cough, which contained 50 mg of ledol in one tablet, was produced in Russia. *R. tomentosum* plant material was also included in the Russian herbal tea Pectorales Species No 4, for respiratory system diseases (Dampc and Łuczkiewicz 2013).

18.5 Cultivation

The highly variable chemical composition of the *R. tomentosum* essential oils, which can lead to a lack of repeatable and reliable results of biological tests, as well as the status of the endangered species in some countries, making impossible to harvest the plant material for the pharmaceutical industry and research from the natural environment, complicate the use of the discussed herb in modern phytotherapy, e.g. in the treatment of rheumatoid arthritis. Due to the above, in vitro cultures remain the method of choice for production of the *R. tomentosum* biomass, synthesizing biologically active volatile fractions for phytochemical and pharmacological studies. In vitro cultures have been a promising source of secondary plant metabolites already since the late 1960s, ensuring continuous access to biomass, regardless of environmental, geographic and seasonal conditions (Bourgaud et al. 2001). Moreover, the reproducible quality of the in vitro plant material in terms of the content of active substances, often exceeding the productivity of the mother plants, is obtained (Rao and Ravishankar 2002). Therefore, in vitro cultures of marsh tea, optimized for intensive growth and productivity of the selected secondary metabolites, would provide an alternative source of the chemically stable essential oil for medicinal purposes. In turn, the development of the efficient micropropagation protocol would constitute the possibility of the marsh tea reintroduction into the natural environment (Table 18.4).

At the first stages of our biotechnology research, it was decided to initiate from *R. tomentosum* the morphogenically diverse biomasses. As the essential oils are synthesized and then stored in the specialized cell structures (Mulder-Krieger et al. 1988), located in the case of the discussed species in the aerial parts of the plant (Jesionek et al. 2016), the main emphasis was placed on obtaining the microshoots. Additionally, an attempt was made to receive callus, excised and hairy roots cultures, to determine full metabolic potential of in vitro biomasses in terms of volatile fraction biosynthesis. As initial explants the nodal shoot segments, axillary buds and leaves from maternal plant, harvested from Miszewko near Gdańsk in Poland, were used, after sterilization in mercuric chloride for 15 min and transferring onto stationary Schenk–Hildebrandt (SH), Anderson's Rhododendron (AR), and Woody Plant (WP) media, supplemented with 9.84 μM 2-isopentenyladenine (2iP) and 1.0 μM thidiazuron (TDZ). After 60 days since inoculation, the in vitro shoot cultures of *R. tomentosum* were initiated. The most intensive growth (Gi = 343.72%), formation of multiple new shoot primordia, and the morphology similar to the mother plant were shown on SH medium (Jesionek et al. 2016), which confirmed a high demand for nitrates and phosphates of the discussed species, according to the physiological specificity of the family *Ericaceae* (Migas et al. 2006). However, the callus and roots cultures, which were obtained in the further course of biotechnological experiments, showed a tendency to gradual dying out, regardless of the applied medium (data not published).

Table 18.4 Developed micropropagation protocol of the *R. tomentosum* microshoots (Jesionek et al. 2016)

Micropropagation stage	Growth medium	Time of cultivation (days)	Pictures
Shoot culture initiation	SH agar medium (0.6% *w/v*) with 9.84 μM 2iP and 1.0 μM TDZ	60	
Initial stationary culture	SH agar medium (0.6% *w/v*) with 9.84 μM 2iP and 1.0 μM TDZ	30	
Elongated stationary culture	SH agar medium (0.6% *w/v*) with 24.6 μM 2iP	30	
Rooted microshoots	Half strength WP agar medium (0.6% *w/v*) with half–strength of sugar (10 g l^{-1} sucrose) and 4.92 μM IBA	45	
Hardened microshoots	SH agar medium (0.6% *w/v*) without phytohormones	14	
Acclimatized to ex vitro conditions seedlings	De-acidified peat/perlite/gravel	60	

Next step of the biotechnological research was to determine conditions for the continuous and stable multiplication of *R. tomentosum* microshoots, to have a collection of plant matrices to conduct advanced experiments aimed at developing the in vitro system for production of the biologically active essential oil. The elongation stage was important, taking into account further adaptation of microshoots to growth in liquid media (successively shaking cultures and bioreactor) (Jesionek et al. 2017). By modifying the media in terms of the used growth regulators and their concentrations, the SH medium (0.6% *w/v* agar, 30 g l^{-1} sucrose) enriched with 24.6 μM 2iP only, without TDZ addition, was selected for the elongation to over 2 cm of the initial cultures, in order to obtain as many histologically diverse structures, capable of biosynthesis of volatile fraction, as possible (Jesionek et al. 2016). The relatively low growth of microshoots on the above-mentioned medium (Gi = 205.77%) could be

connected with the decrease of the biomass weight through the beneficial reduction of callus at the base (Jesionek et al. 2016).

Subsequently, the stationary shoot cultures of *R. tomentosum* were adapted to growth under shaking conditions in a liquid medium, which provides better oxygenation of biomass and more effective and uniform flow of nutrients in the system. In addition, shaken liquid cultures can be a preparatory stage for introducing plant material into bioreactor installations (Grzegorczyk and Wysokińska 2008; Watt 2012). Among twelve examined variants of SH medium, enriched not only with qualitatively and quantitatively different sets of auxins and cytokinins, but also with the peat filtrate as a source of the minerals and microflora (fungi, bacteria) specific for the mother plant habitat, as well as modified with lowered pH to imitate the natural environmental conditions of marsh tea, the SH medium supplemented with 24.6 μM 2iP and 592.02 μM adenine (AD) was considered as the optimal for cultivation of the *R. tomentosum* shaken microshoots (Gi = 323.64%) (Jesionek et al. 2017). The growth profile, plotted on the basis of the obtained growth parameters, was characterized by the presence of the following phases: logarithmic (0–14 days), linear (14–28 days), *plateau* (28–49 days), and extinction (49–63 days).

Finally, the selected stationary and liquid *R. tomentosum* shoot cultures were used for increasing the scale of the biotechnological experiments in bioreactors (Fig. 18.2a, b). As indicated by the conducted phytochemical studies (hydrodistillation in Deryng apparatus, GC/MS and HPTLC analysis), they retained the ability to biosynthesize essential oil, although with a slightly different chemical composition relative to the ground plant (Table 18.5; Jesionek et al. 2016, 2017, 2018b). Thus, *R. tomentosum* microshoots were suitable as *inoculum* for the development of the in vitro plant growth system for the production of volatile fractions, rich in biologically active terpenes.

The initial and elongated shoot cultures of marsh tea were also included in the developed micropropagation protocol (Table 18.4). In Poland, the studied plant has been the endangered species for almost forty years, due to drying up and transformation for agriculture of wetlands and peat bogs, which constitute the natural habitat of *R. tomentosum.* What's more, the shoots are excessively harvested because of the folk use as a repellent (Dampc and Łuczkiewicz 2013; Kasper-Pakosz et al. 2016). An example of ex situ conservation is micropropagation, that leads to the full regeneration of the plant, preserves the genetic pool, and allows fast multiplication of biomass regardless of environmental factors. As a result of the conducted experiments, a complete micropropagation protocol of *R. tomentosum* was proposed, consisting of the following steps: initiation of shoot culture, multiplication of biomass, preparation of microshoots for in vivo cultivation (elongation, rooting, and hardening), and adaptation of the obtained cuttings to growth in in vivo conditions (Table 18.4). In addition, the preliminary reintroduction of the regenerated plants into the natural environment was also attempted: 66% of the seedlings have survived more than 3 months from the moment of planting (data not published). The genetic similarity of the parent plant and regenerated shoots, estimated by RAPD analysis was determined as high, with little genetic variation (Jesionek et al. 2016).

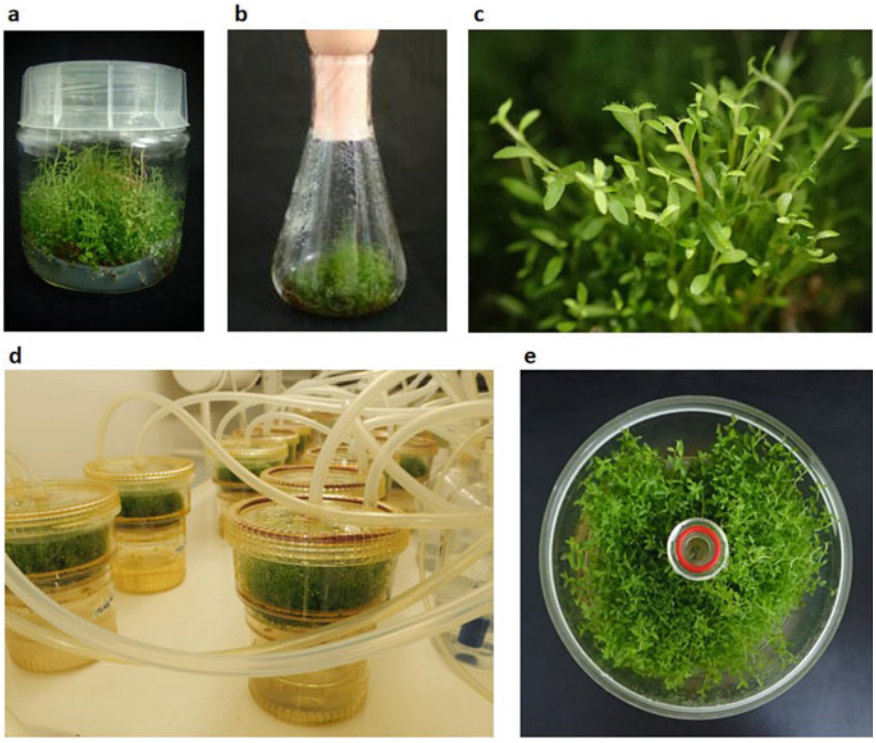


Fig. 18.2 *R. tomentosum* microshoots, cultivated in: **a** elongated stationary culture (SH medium (0.6% *w/v* agar) with 24.6 μM 2iP), **b** shaken liquid culture (SH medium with 24.6 μM 2iP and 592.02 μM adenine), **c**, **d**, **e** RITA® bioreactor (temporary immersion system, SH medium with 24.6 μM 2iP and 592.02 μM adenine) (Jesionek et al. 2017)

18.6 Biotechnological Approach

The aim of the further biotechnological research was to develop the *R. tomentosum* plant-based system for biosynthesis of biologically active volatile terpenes, which could be used for phytotherapy. In vitro cultures of marsh tea were characterized by a relatively low concentration of the essential oil (approx. 50–100 μl 100 g^{-1} DW) (Table 19.5), therefore scaling-up of cultivation was necessary to obtain the measurable, by hydrodistillation, volume of the volatile fraction (Jesionek et al. 2017). The transfer of the plant biomass to the bioreactor is also the final stage of biotechnological procedures for producing the natural products (Bourgaud et al. 2001). In addition, an attempt was made to stimulate accumulation of the *R. tomentosum* essential oil in microshoots growing in the selected installation, using environmental stress in the form of biotic and abiotic elicitors, added to the growth media (Jesionek et al. 2018a).

Table 18.5 Comparison of the main constituents of the essential oils isolated from the *R. tomentosum* microshoots and maternal plant (hydrodistillation in Deryng apparatus, GC/MS analysis) (Jesionek et al. 2016, 2017)

Type of shoot culture	Gi (%)	Essential oil content [μl 100 g^{-1} DW]	The main constituents of the essential oil (>8%)*						
			1	2	3	4	5	6	7
Initial stationary culture	343.72	44±4	4.7	0.1	4.5	5.1	0.2	–	?
Elongated stationary culture	205.77	85±15	4.6	0.2	9.2	7.9	0.3	–	?
Shaken liquid culture	323.64	86±24	7.8	0.4	5.1	8.1	0.1	–	10.2
Magenta™ vessel	97.62	153±49	0.9	–	8.1	15.8	–	–	14.7
Bioreactor RITA®	280.48	498±95	6.9	1.2	5.5	8.2	0.3	–	13.0
Bioreactor PLANTFORM	159.54	192±12	5.6	0.6	5.5	8.1	0.2	–	9.5
Temporary immersion glass bioreactor	214.39	293±100	5.5	0.5	4.4	7.7	0.1	–	9.0
Spray glass bioreactor	247.69	528±55	17.9	2.2	2.9	8.6	–	–	9.0
The maternal plant** (aged shoots)	–	306±16	4.9	18.8	2.3	4.2	15.7	12.1	–
The maternal plant** (young shoots)	–	2412±163	5.1	15.0	1.4	6.3	11.5	9.6	–

*1—*p*-cymene, 2—γ-terpineol, 3— alloaromadendrene, 4—shyobunone, 5—palustrol, 6—ledol, 7—ledene oxide (II)
**Collected in June in Miszewko, Poland
? Indefinite content

At first, the effects of five different types and constructions of culture vessels on morphology (Fig. 18.2c) and growth of shoot cultures of marsh tea as well as the content and chemical composition of volatile fractions were investigated (Table 18.5, Jesionek et al. 2017). Due to the specificity of *R. tomentosum* natural habitat (wetlands), the main focus was placed on the temporary immersion systems, such as RITA® bioreactor (Vitropic, France), PLANTFORM bioreactor (Plant Form AB, Ireland) as well as the prototype glass installation, constructed in Department of Pharmacognosy in Medical University of Gdańsk (Gdańsk, Poland) (Jesionek et al. 2017), enabling periodic flooding of microshoots and their gradual drying in the gas phase. Moreover, the glass spray bioreactor and Magenta™ vessel (Sigma-Aldrich, US-MO), which represented a flood system, were also tested. Taking into account the obtained growth parameters and the essential oil content, RITA® bioreactor was selected as an installation which provided the optimal conditions for the growth of the *R. tomentosum* microshoots (Wp = 280%, DW = 19.98 g l^{-1}) and high biomass viability (Fig. 18.2d, e), together with the intensified volatile fraction biosynthesis (approx. 500 μl 100 g^{-1} DW) (Jesionek et al. 2017). The amount of the essential oil isolated from the biomass cultivated in RITA® bioreactor was five times higher than the concentration of the volatile fraction in stationary and shaken in vitro cultures of marsh tea (85 μl 100 g^{-1} DW), surpassing even the aged shoots of maternal plants in this respect (306 μl 100 g^{-1} DW) (Table 18.5). The advantages of the discussed system include also the possibility of scaling-up the biotechnological process by serial connection of multiple RITA® vessels, which allows for obtaining a large amount of biomass during a single experiment, and therefore for higher essential oil production. Approximately 43 terpene compounds were identified in *R. tomentosum* microshoots cultivated in RITA® bioreactor, generally similar to those found in ground plants, but in various quantitative proportions (Table 18.5). However, terpenoids specific only for in vitro cultures also appeared, such as methyl everninate and ledene oxide (II), with the simultaneous lack of ledol, limonene or linalool, compounds characteristic for the mother shoots. The observed phenomenon may indicate a different course or activity of biochemical pathways involved in the biosynthesis of volatile terpenes under in vitro conditions. When defining a complete growth and production profile of *R. tomentosum* microshoots, it was found that after just four weeks of their cultivation in RITA® system, a high level of essential oil can be achieved (500 μl 100 g^{-1} DW), which remained much about in the same range for next month. The general qualitative profile of the volatile fraction from the marsh tea shoot cultures did not change throughout the experiment (Jesionek et al. 2017). It could be evidence for the economic viability of the developed plant system, opening up the possibility of its use on a commercial scale.

In further biotechnological experiments, strategy for generating stress conditions was applied, in order to improve the essential oil content in the *R. tomentosum* biomass, growing in RITA® bioreactor. Methyl jasmonate and selected abiotic (copper and nickel salts) and biotic (chitosan, ergosterol, lysates of plant and human pathogens: *Candida albicans*, *Escherichia coli*, *Enterobacter sakazaki*, *Pectobacterium carotovorum*, *Dickeya dadanti*) elicitors were added to the growth medium on 21 day of the cultivation, with the exposure time of 7 days. The tested for the first

time elicitor of arthropod origin, ethanol extract from aphids, was also included in the experiment, taking into account well-documented repellent properties of marsh tea (Jesionek et al. 2018a). In response to environmental stress, the increase in the volatile fraction accumulation in *R. tomentosum* microshoots was observed: by 14% after treatment with the aphid extract and *P. carotovorum* lysate (approx. 570 µl 100 g^{-1} DW), and by 8% under the influence of nickel salt and ergosterol (approx. 540 µl 100 g^{-1} DW). The results showed that despite the use of very diverse elicitors, the essential oil content in the studied biomass was rather stable and little susceptible to stressors. However, a positive answer obtained in the case of the elicitors of insect (aphids) and bacterial (*P. carotovorum*) origin encourage to continue these experiments, extending the concentration range and maintaining different time intervals (Jesionek et al. 2018a).

18.7 Conclusions

In conclusion, as a result of biotechnological research, the in vitro plant system was developed, which enabled the continuous production of biologically active essential oil, independently of environmental conditions. The *R. tomentosum* microshoots growing in the RITA® temporary immersion bioreactor were capable of biosynthesis of approx. 500 µl 100 g^{-1} DW of volatile fraction, after 28 days of cultivation, which is an increase of 67% over the amount obtained in the aged shoots of the mother plant. Due to high productivity, the proposed plant system has commercial potential for obtaining the bioactive terpene compounds for medical purposes. However, further biological studies are necessary to confirm the pharmacological properties of *R. tomentosum* microshoots' essential oil, thus economic viability of their large-scale cultivation.

Acknowledgements The conducted research on *Rhododendron tomentosum* received funding from the National Science Center in Poland (decision no. UMO-2014/15/N/NZ7/03027).

References

Alm T, Iversen M (2010) Norway's Rosmarin (*Rhododendron tomentosum*) in past and present tradition. In: Pardo-de-Santayana M, Pieroni A, Puri RK (eds) Ethnobotany in the New Europe: people, health and wild plant resources. Berghahn Books, pp 263–270

Baananou S, Bagdonaite E, Marongiu B, Piras A, Porcedda S, Falconieri D, Boughattas NA (2015) Supercritical CO_2 extract and essential oil of aerial part of *Ledum palustre* L. Chemical composition and anti-inflammatory activity. Nat Prod Res 29:999–1005

Baldwin TA (2003) Effects of resource limitation on the essential oil production in *Ledum palustre ssp decumbens* (Ait.) Hultén. Master thesis, Florida International University, Miami, Florida

Belleau F, Collin G (1993) Composition of the essential oil of *Ledum groenlandicum*. Phytochemistry 33(1):117–121

Belousov MV, Saratikov AS, Akhmedzhanov RR, Berezovskaya TA, Yusubov MS, Dmitruk SE, Basova EV (2006) Pharmacological properties of *Ledum palustre* (*Ericaceae*) from Tomsk region. Rastitelnye Resursy 42:130–140

Belousova NI, Khan VA, Berezovskaya TP (1990) Intraspecies chemical variability of the essential oil of *Ledum palustre*. Chem Nat Compd 26:398–405

Benelli G, Pavela R, Cianfaglione K, Sender J, Urban D, Maślanko W, Canale A, Barboni L, Petrelli R, Zeppa L, Aguzzi C, Maggi F (2020) Ascaridole-rich essential oil from marsh rosemary (*Ledum palustre*) growing in Poland exerts insecticidal activity on mosquitoes, moths and flies without serious effects on non-target organisms and human cells. Food Chem Toxicol 138:111184

Black P, Saleem A, Dunford A, Guerrero-Analco J, Walshe-Roussel B, Haddad P, Cuerrier A, Arnason JT (2011) Seasonal variation of phenolic constituents and medicinal activities of Northern Labrador tea, *Rhododendron tomentosum ssp. subarcticum*, an Inuit and Cree First Nations traditional medicine. Planta Med 77:1655–1662

Bourgaud F, Gravot A, Milesi S, Gontier E (2001) Production of plant secondary metabolites: a historical perspective. Plant Sci 161:839–851

Butkiene R, Mockute D (2011) The variability of the essential oil composition of wild *Ledum palustre* L. shoots during vegetation period. J Essent Oil Res 23:9–13

Butkiene R, Sakociute V, Latvenaite D, Mockute D (2008) Composition of young and aged shoot essential oils of the wild *Ledum palustre* L. Chemija 19:19–24

Chosson E, Chaboud A, Chulia AJ, Raynaud J (1998) Dihydroflavonol glycosides from *Rhododendron ferrugineum*. Phytochemistry 49:1431–1433

Collin (2015) Aromas from Quebec. IV. Chemical composition of the essential oil of *Ledum groenlandicum*: a review. Am J Essent Oil Nat Prod 2(3):06–11

Dampc A, Łuczkiewicz M (2013) *Rhododendron tomentosum* (*Ledum palustre*): a review of traditional use based on current research. Fitoterapia 85:130–143

Dampc A, Łuczkiewicz M (2015) Labrador tea: the aromatic beverage and spice: a review of origin, processing and safety. J Sci Food Agric 95:1577–1583

Dubois MA, Wierer M, Wagner H (1990) Palustroside, a coumarin glucoside ester from *Ledum palustre*. Phytochemistry 29:3369–3371

Egigu MC, Ibrahim MA, Yahya A, Holopainen JK (2011) *Cordeauxia edulis* and *Rhododendron tomentosum* extracts disturb orientation and feeding behavior of *Hylobius abietis* and *Phyllodecta laticollis*. Entomol Exp Appl 138:162–174

Evstratova RI, Kabanov VS, Krylova IL, Prokosheva LI (1978) Content of essential oil and of ledol in leaves of marsh rosemary *(Ledum palustre* L.) during different phases of vegetation. Pharm Chem J 12:1468–1473

Flora of North America (2020) 8:459–460. Sept 2020. Available online at: https://eFloras.org

Fokina GI, Frolova TV, Roikhel VM, Pogodina VV (1991) Experimental phytotherapy of tick-borne encephalitis. Vopr Virusol 36:18–21

Fraser MH, Cuerrier A, Haddad PS, Arnason JT, Owen PL, Johns T (2007) Medicinal plants of Cree communities (Quebec, Canada): antioxidant activity of plants used to treat type 2 diabetes symptoms. Can J Physiol Pharmacol 85:1200–1214

Gapanenko W, Levashova O (2015) Phytochemical study of phenolic compounds of labrador tea (*Ledum palustre* L.). ScienceRise 11(4):14–19

Goun EA, Petrichenko VM, Solodnikov SU, Suhinina TV, Kline MA, Cunningham G, Nguyen Ch, Miles H (2002) Anticancer and antithrombin activity of Russian plants. J Ethnopharmacol 81:337–342

Gretsusnikova T, Jarvan K, Orav A, Koel M (2010) Comparative analysis of the composition of the essential oil from the shoots, leaves and stems the wild *Ledum palustre* L. from Estonia. Procedia Chem 2:168–173

Grzegorczyk I, Wysokińska H (2008) Liquid shoot culture of *Salvia officinalis* L. for micropropagation and production of antioxidant compounds; effect of triacontanol. Acta Soc Bot Pol 77:99–104

Harbilas D, Martineau LC, Harris CS, Adeyiwola-Spoor DC, Saleem A, Lambert J, Caves D, Johns T, Prentki M, Cuerrier A, Arnason JT, Bennett SAL, Haddad PS (2009) Evaluation of the antidiabetic potential of selected medicinal plant extracts from the Canadian boreal forest used to treat symptoms of diabetes: part II. Can J Physiol Pharmacol 87:479–492

Harmaja H (1991) Taxonomic notes on *Rhododendron* subsection *Ledum* (*Ledum, Ericaceae*), with a key to its species. Ann Bot Fenn 28:171–173

Ignatowicz S, Wesołowska B (1996) Repellency of powdered plant material of the Indian neem tree, the Labrador tea, and the sweet-flag, to some stored product pests. Pol Pismo Entomol 65:61–67

Jaenson TG, Palsson K, Borg-Karlson AK (2005) Evaluation of extracts and oils of tick-repellent plants from Sweden. Med Vet Entomol 19:345–352

Jaenson TG, Palsson K, Borg-Karlson AK (2006) Evaluation of extracts and oils of mosquito (*Diptera: Culicidae*) repellent plants from Sweden and Guinea-Bissau. J Med Entomol 43:113–119

Jesionek A, Kokotkiewicz A, Włodarska P, Filipowicz N, Bogdan A, Ochocka R, Szreniawa-Sztajnert A, Zabiegała B, Buciński A, Łuczkiewicz M (2016) In vitro propagation of *Rhododendron tomentosum*: an endangered essential oil bearing plant from peatland. Acta Biol Crac Ser Bot 58:29–43

Jesionek A, Kokotkiewicz A, Włodarska P, Zabiegała B, Buciński A, Łuczkiewicz M (2017) Bioreactor shoot cultures of *Rhododendron tomentosum* (*Ledum palustre*) for a large-scale production of bioactive volatile compounds. Plant Cell Tiss Org Cult 131:51–64

Jesionek A, Kokotkiewicz A, Królicka A, Zabiegała B, Łuczkiewicz M (2018a) Elicitation strategies for the improvement of essential oil content in *Rhododendron tomentosum* (*Ledum palustre*) bioreactor-grown microshoots. Ind Crops Prod 123:461–469

Jesionek A, Pobłocka-Olech L, Zabiegała B, Buciński A, Krauze-Baranowska M, Łuczkiewicz M (2018b) Validated HPTLC method for determination of ledol and alloaromadendrene in the essential oil fractions of *Rhododendron tomentosum* plants and in vitro cultures and bioautography for their activity screening. J Chromatogr B 1086:63–72

Jesionek A, Zabiegała B, Buciński A, Łuczkiewicz M (2019a) From harvesting to distillation—effect of analytical procedures on the yield and chemical composition of *Rhododendron tomentosum* (*Ledum palustre*) essential oil. Acta Pol Pharm Drug Res 76:83–92

Jesionek A, Kokotkiewicz A, Mikosik-Roczyńska A, Ciesielska-Figlon K, Łuczkiewicz P, Buciński A, Daca A, Witkowski JM, Bryl E, Zabiegała B, Łuczkiewicz M (2019b) Chemical variability of *Rhododendron tomentosum* (*Ledum palustre*) essential oils and their proapoptotic effect on lymphocytes and rheumatoid arthritis synoviocytes. Fitoterapia 139:104402

Jin C, Strembiski W, Kulchytska Y, Micetich RG, Danesstalab M (1999) Flavonoid glycosides from *Ledum palustre* L. *subsp. decumbens* (Ait.) Hulton. DARU J Pharm Sci 7(4):5–8

Judzentiene A, Budiene J, Misiunas A, Butkiene R (2012a) Variation in essential oil composition of *Rhododendron tomentosum* gathered in limited population (in Eastern Lithuania). Chemija 23:131–135

Judzentiene A, Butkiene R, Budiene J, Tomi F, Casanova J (2012b) Composition of seed essential oils of *Rhododendron tomentosum.* Nat Prod Commun 7:227–230

Judzentiene A, Budiene J, Svediene J, Garjonyte R (2020) Toxic, radical scavenging, and antifungal activity of *Rhododendron tomentosum* H. essential oils. Molecules 25:1676

Kasper-Pakosz R, Pietras M, Łuczaj Ł (2016) Wild and native plants and mushrooms sold in the open-air markets of south-eastern Poland. J Ethnobiol Ethnomed 12:45

Kim D-M, Nam B-W (2006) Extracts and essential oil of *Ledum palustre* L. leaves and their antioxidant and antimicrobial activities. J Food Sci Nutr 11:100–104

Klokova MV, Serykh EA, Berezovskaya TP (1982) *Ledum* genus coumarins. Khim Prirodnykh Soedin 4:517

Kron KA, Judd WS (1990) Phylogenetic relationships within the *Rhodoreae* (*Ericaceae*) with specific comments on the placement of *Ledum.* Syst Bot 15:57–68

Kuusik A, Harak M, Hiiesaar K, Metspalu L, Tartes U (1995) Studies on insect growth regulating (IGR) and toxic effects of *Ledum palustre* extracts on *Tenebrio molitor* pupae (*Coleoptera*, *Tenebrionidae*) using calorimetric recordings. Thermochim Acta 251:247–253

Migas P, Łuczkiewicz M, Cisowski W (2006) The influence of auxins on the biosynthesis of isoprene derivatives in callus cultures of *Vaccinium corymbosum* var. bluecrop. Z Naturforsch C 61:565–570

Mikhailova NS, Rybalko KS (1980) Chemical constitution of *Ledum palustre*. Chem Nat Compd 16:131–135

Mulder-Krieger T, Verpoorte R, Svendsen AB, Scheffer JJC (1988) Production of essential oils and flavours in plant cell and tissue cultures. A review. Plant Cell Tissue Organ Cult 13:85–154

Narimanov AA, Miakisheva SN, Kuznetsova SM (1991) The radioprotective effect of extracts of *Archangelica officinalis* Hoffm. and *Ledum palustre* L. on mice. Radiobiologiia 31:391–393

Raal A, Orav A, Gretchushnikova T (2014) Composition of the essential oil of the *Rhododendron tomentosum* Harmaja from Estonia. Nat Prod Res 28:1091–1098

Rao SR, Ravishankar GA (2002) Plant cell cultures: chemical factories of secondary metabolites. Biotechnol Adv 20:101–153

Reichardt PB, Bryant JP, Anderson BJ, Phillips D, Clausen TP, Meyer M, Frisby K (1990) Germacrone defends Labrador tea from browsing by snowshoe hares. J Chem Ecol 16(6):1961–1970

Shikov AN, Pozharitskaya ON, Makarov VG, Wagner H, Verpoorte R, Heinrich M (2014) Medicinal plants of the Russian Pharmacopoeia; their history and applications. J Ethnopharmacol 154:481–536

Soukand R, Kalle R, Svanberg I (2010) Uninvited guests: traditional insect repellents in Estonia used against the clothes moth *Tineola bisselliella*, human flea *Pulex irritons* and bedbug *Cimex lectularius*. J Insect Sci 10:150

Spiridonov NA, Konovalov DA, Arkhipov VV (2005) Cytotoxicity of some Russian ethnomedicinal plants and plant compounds. Phytother Res 19:428–432

Świejkowski L (1950) Właściwości trujące polskich roślin leczniczych. Wydawnictwo Polskiego Związku Zielarskiego, Kraków, Poland, pp 60–61

Tunon H, Olavsdotter C, Bohlin L (1995) Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. J Ethnopharmacol 48:61–76

von Schantz M, Hiltunen R (1971) Über die Zusammensetzung des ätherischen Öles von *Ledum palustre* L. einschließlich der geographischen Rassen *ssp. groenlandicum* (Oeder) Hult. und *ssp. decumbens* (Ait.) Hult Sci Pharm 39(3):137–146

Wagner H, Wierer M, Bauer R (1986) In vitro Hemmungder Prostaglandin-Biosynthese durch etherische Ole und phenolische Verbindungen. Planta Med 3:184–187

Watt MP (2012) The status of temporary immersion system (TIS) technology for plant micropropagation. Afr J Biotechnol 11:14025–14035

Zhang M, Zhao J-J, Wu W-B, Zhao Y, Zhu X-L, Qu W-J (2010) Analgesic and anti-inflammatory activities of extracts from *Ledum palustre* L. in mice. Nat Prod Res Dev 22:326–329

Zhang LL, Wang HX, Wang YM, Xu M, Hu XY (2017) Diurnal effects on chinese wild *Ledum palustre* L. essential oil yields and composition. J Anal Sci Meth Instr 7:47–55

Zhao Q, Ding Q, Yuan G, Xu F, Li B, Wang J, Ouyang J (2016) Comparison of the essential oil composition of wild *Rhododendron tomentosum* stems, leaves, and flowers in bloom and non-bloom periods from Northeast China. J Essent Oil Bear Plants 19:1216–1223

Chapter 19
Biology, Phytochemistry, Pharmacology, and Biotechnology of European Ferns, Club Mosses, and Horsetails: A Review

Wojciech J. Szypuła and Agnieszka Pietrosiuk

Abstract Pteridophytes found in Europe have been used for centuries for a variety of ailments but compared to angiosperms they constitute a relatively small group of medicinal plants. The term pteridophytes refer to a polyphyletic group of taxa that consists of club mosses, horsetails, psylophytes and ferns. Now, according to the Peridophyte Phylogeny Group they are members of the monophyletic class of Lycopodiopsida comprising some 1388 species and of the Polypodiopsida class which includes most of all pteridophytes (some 10,597 species). This review presents historical and updated information on pteridophyte taxonomy, secondary metabolites isolated from species screened for medicinal properties, their pharmacological activities, and the use of in vitro plant tissue culture techniques for the conservation of pteridophyte biodiversity and for the biosynthesis of their secondary metabolites. The analysis is based on a comprehensive review of the literature with the relevant papers retrieved from online databases (PubMed, Web of Science, Wiley, Science Direct, Elsevier's Scopus, Google Scholar) and print sources (ACS Publications, SpringerLink and Elsevier journals). The analysis demonstrates that numerous pteridophyte species are a source of popular and valued herbal medicines used for the treatment of a variety of health problems and diseases. The major secondary metabolites isolated from pteridophytes and reported for their medicinal properties include alkaloids, polyphenols and flavonoids, although secondary metabolites belonging to other compound classes also may have a therapeutic value or they may modify the pharmacological activity of the major secondary metabolites. Numerous studies have demonstrated that pteridophyte extracts and their isolated secondary metabolites have a broad spectrum of pharmacological activity including anti-inflammatory, anticancer, cytotoxic, antibacterial, antiparasitic, antifungal, antiviral, and acetyl- and butyrylcholinesterase inhibitory effects. Importantly, of the 205 European pteridophyte species, only some 40 species have been investigated phytochemically and/or

W. J. Szypuła (✉) · A. Pietrosiuk
Department of Pharmaceutical Biology and Medicinal Plants Biotechnology, Faculty of Pharmacy, Medical University of Warsaw, ul. Banacha 1, 02-097 Warsaw, Poland
e-mail: wszypula@wum.edu.pl

A. Pietrosiuk
e-mail: agnieszka.pietrosiuk@wum.edu.pl

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_19

biologically. For some, protocols for in vitro micropropagation have been developed to aid ex-situ species conservation. Further studies are necessary to determine the composition of secondary metabolites and investigate biologically and pharmacologically the remaining 80% of the European pteridophyte flora.

Keywords Medicinal plants · Pteridophytes · Ferns · Club mosses · Horsetails · Secondary metabolites · In vitro cultures

19.1 Introduction

It has been estimated that approximately 40,000 described vascular plant species out of a total of over 400,000 species have medicinal properties. Of these only a few thousand, i.e. 1.5% have been thoroughly investigated (Lamer-Zarawska et al. 2010). Medicinal plants and plant-derived medicines have been widely used by traditional (folk) and modern medicine all over the world. Natural products and their derivatives (including antibiotics) account for over 50% of all drugs currently used in clinical practice (Lamer-Zarawska et al. 2010), but vascular plants are a source of less than 25% of herbal medicines. Quinine, morphine, codeine, atropine, reserpine, and digoxin are good examples of commonly prescribed plant-derived medicines. Newer plant-derived pharmaceuticals include such clinically important anticancer drugs as paclitaxel and vincristine or acetylcholinesterase inhibitors used for Alzheimer's disease, e.g. galantamine and huperzine A.

Interest in natural products derived from plants has greatly increased in the past decades. It has been estimated that herbal medicines, mostly plant extracts or their active ingredients, are used by approximately 80% of the world's population (Lamer-Zarawska et al. 2010), which is associated with increasing popularity of a healthy lifestyle, self-medication, and natural products derived from plants. The global herbal medicine market value is approximately 18 billion dollars and it accounts for nearly a half of the dietary supplements market (Wyk and Wink 2004; Lamer-Zarawska et al. 2010). As phytotherapy is flourishing, phytochemistry is developing fast while biochemical and pharmacological studies of medicinal plants are undertaken to elucidate the mechanisms of action of their active constituents and the effects on the human physiology in health and disease (Wyk and Wink 2004; Lamer-Zarawska et al. 2010). Plant raw materials are studied extensively in research centers all over the world and the number of pre-clinical and clinical studies of medicinal plants and herbal medicinal products is growing.

In 1996, the European Scientific Cooperative on Phytotherapy (ESCOP) defined a herbal medicine as any medicinal product containing as active ingredients exclusively plants parts of plants (raw materials), or plant substances or their combinations in herbal preparations. Phytotherapy is the study of products of botanical origin based on the benefit-risk assessment of their use to protect, restore or improve health. In Europe, the term herbal medicine traditionally refers to any medicinal preparation which contains plant raw materials or a galenic, whether the plant ingredient is an

active substance or an excipient. Herbal medicines may be also defined as these pharmaceuticals in which at least 60% of the ingredients are of plant origin (Wyk and Wink 2004; Lamer-Zarawska et al. 2010). Before such substances and products of natural botanical origin are approved as potential drugs, a detailed phytochemical analysis is performed which includes assessment of the qualitative and quantitative composition and identification of possible combination effects (synergy and antagonism), side-effects and toxicity, indications for use and contraindications, followed by rigorous phase-1 and phase 2 pharmacological studies to confirm their therapeutic properties (Wyk and Wink 2004; Lamer-Zarawska et al. 2010). Research into new plant species or botanical raw materials with potential medicinal properties but hitherto not used in medicine is also strictly regulated. Under Article 1 of Council Directive EEC of, a herbal preparation can be registered as a conventional medicine and obtain a marketing authorization in the European Union. The essential information on plant raw materials and their galenic preparations authorized in Europe is given in the Complete German Commission E Monographs: Therapeutic Guide for Herbal Medicines and the ESCOP monographs (Wyk and Wink 2004; Lamer-Zarawska et al. 2010). All clinical studies of herbal medicines are monitored and coordinated by the EMEA—Europen Medicines Agency (Wyk and Wink 2004; Lamer-Zarawska et al. 2010).

Focusing on specific plant substances or their complexes characterized by evidence-based stability, bioavailability to humans, effectiveness, and safety of use is the future of phytotherapy or herbal medicine (Wyk and Wink 2004; Lamer-Zarawska et al. 2010). A question then arises on the current and potential uses of pteridophytes as a source of medicinal products. A wider utilization of most species of medicinal plants for the production of medicines poses a threat to their natural populations which is now difficult to estimate. Pteridophytes, considering their ecology and evolution are especially at risk. Historically, according to the traditional classification pteridophytes represented the taxonomic rank of class and included ferns, psylophytes, horsetails and club mosses. Today, pteridophytes represent a small number of all vascular plant species of any practical importance for medicine and economy.

The present chapter presents the most important aspects of current and potential medicinal uses of pteridophytes based on the literature review as well as their phylogeny and taxonomy, major secondary metabolites and perspectives for their biotechnological synthesis.

19.2 Short Characteristics of Pteridophytes

Traditionally pteridophytes were considered to represent one taxon which included ferns, psylophytes, horsetails and club mosses. That classification was based on two features which are common to all representatives of the group, i.e. alteration of generations with a dominant sporophyte generation and the free-living, mycotrophic, usually achlorophyllous gametophyte, and the production of haploid spores in special

structures known as sporangia. The sporangia disperse the spores which germinate away from the sporangia.

Using modern phylogenic methods and molecular data a new comprehensive classification founded on the principle of monophyly has been proposed by The Pteridophyte Phylogeny Group (PPG) for club mosses, horsetails and ferns down to the genus level. In total, this classification treats an estimated 11,916 species in 337 genera, 51 families, 14 orders, and two classes and offers the most synthetic approach to the phylogeny of plants earlier referred to as pteridophytes (Schuettpelz et al. 2016). The PPG classification recognizes two pteridophyte classes: Lycopodiopsida Bartl. (approximately 1388 species) and Polypodiopsida Cronquist, Takht. & W. Zimm (approximately 10,597 species, i.e. most of all pteridophytes).

The monophyletic class of Lycopodiopsida (Kenrick and Crane 1997; Pryer et al. 2001; Rai and Graham 2010; Wickett et al. 2014) represents a group of extant vascular plants which early diverged from an ancient, diverse group of fossil plants noted over 420 million years ago (Garrat 1984; Bateman et al. 1992; Bateman 1996; Ruggiero et al. 2015). That ancient group dominant in the Devonian and Carboniferous periods was subsequently replaced by ferns and coniferous plants and later by seed plants (Angiospermae). Nowadays lycophytes account for less than 1% of the world's flora (Kenrick and Davis 2004) and include three extant orders: Lycopodiales DC. ex Bercht. & J. Presl, (16 genera and approximately 388 species), Isoëtales Prantl, (approximately 250 species), and Selaginellales Prantl, (approximately 700 species) (Schuettpelz et al. 2016). The monophyletic class of Polypodiopsida Cronquist, Takht. & W. Zimm. is the most varied and numerous pteridophyte group with four subclasses, 11 orders, 48 families, 319 genera, and an estimated 10 578 species.

The total number of pteridophyte species is estimated at 10,000–15,000 (Akeroyd and Synge 1992; Roos 1996) and their biodiversity increases from the poles towards the equator (Moran 2004). Regions known for particular diversity of pteridophyte species include the Andes (approximately 2500 species), New Guinea (approximately 2000 species), Borneo (1200 species), Ecuador (1250 species), Costa Rica (1165 species) (Moran 2008); China (2600 species); the Philippines (1000 species), Malaysia (550 species), Thailand (700 species), and India (600 species) (Schneider et al. 2004; Soare and Şuţan 2018).

A total of 205 pteridophyte species have been identified in Europe, 30–38 club moss species, 11 horsetail species and approximately 156 fern species (Valentine 1964; Garcia et al. 2017) (Fig. 19.1). Of 194 species of ferns and club mosses found in Europe, 53 species (27.3%) are considered endemic (Garcia et al. 2017) and they account for approximately one-third of the pteridophyte flora of Europe. Typical endemic species have relatively small geographical ranges and are restricted to their characteristic habitats. In Europe, conditions which favor speciation and ecotypic differentiation in ferns are found in very small areas on the continent and in the subtropical laurisilva forests of Macaronesia (Mehltreter et al. 2010; Garcia et al. 2017). This greater species diversity may be explained by geographical isolation and partially subtropical climate. In continental Europe, high species diversity in ferns is observed in the Alps and the Carpathians.

Fig. 19.1 Exemplary Species of the European pteridophyte. **A** *Spinulum annotinum* (L.) A. Haines (Lycopodiaceae), **B** *Huperzia selago* (L.) Bernh. ex Schrank et Mart. (Lycopodiaceae), **C** *Lycopodium clavatum* L. (Lycopodiaceae), **D** *Ophioglossum vulgatum* L. (Ophioglossaceae), **E** *Equisetum telmateia* Ehrh. (Equisetaceae), **F** *Equisetum sylvaticum* L. (Equisetaceae), **G** *Marsilea quadrifolia* L. (Marsileaceae), **H** *Asplenium scolopendrium* L. (Aspleniaceae), **I** *Polypodium vulgare* L. (Polypodiaceae), **J** *Osmunda regalis* L. (Osmundaceae), **K** *Struthiopteris spicant* (L.) Weiss (Blechnaceae), **L** *Thelypteris palustris* Schott (Thelypteridaceae), **M** *Dryopteris affinis* agg. (Lowe) Fraser-Jenk. (Dryopteridaceae), **N** *Asplenium ruta-muraria* L. (Aspleniaceae), **O** *Matteuccia struthiopteris* (L.) Tod. (Onocleaceae), **P** *Pteridium aquilinum* (L.) Kuhn (Dennstaedtiaceae) (*Photos* W. Szypuła)

Increasing species richness in ferns is facilitated by alloploidy when a cross between two species produces a stable hybrid with additional sets of chromosomes (Garcia et al. 2017). As a result new genetically sterile species is produced. Although alloploidy is common in many plants (Garcia et al. 2017), hybrid speciation is much more frequent in ferns than in seed plants (Wagner and Wagner 1980; Garcia et al. 2017). Some hybrid fern species become fertile in a parasexual process. The generated clonal ferns may interbreed with normal sexual plants creating microspecies, which are however taxonomically problematic. A good example is the apogamic (it produces spores that have the same chromosomes as the parent, and a new sporophyte grows directly from the prothallus without fusion of gametes) species complex *Dryopteris affinis* with numerous microspecies such as *D. affinis* (Lowe) Fraser-Jenk. s. str, *Dryopteris borreri* Oberh. et Travel, *Dryopteris cambrensis* (Fraser-Jenk) Beitel et W. R. Buck. or *D. pseudodisjuncta* (Tavel ex Fraser-Jenk.) Fraser-Jenk. Identification of these microspecies is difficult, if not impossible. The Polish species of the genus *Diphasiastrum* Holub are characterized by a fairly high variability confirmed by biometric studies (Pacyna 1972). Interbreeding of the most common species *D. complanatum* (L.) Holub with some other club moss species has been observed. Interbreeding of *D. complanatum* with a less common species *D. tristachym* (Pursh) Holub has produced the hybrid *D. zeilleri* (Rouy) Holub displaying traits intermediate between the two parental species, but in some individual's certain traits are closer to those of one parental species. *D. complanatum* may also form another hybrid, *D. issleri* (Rouy) Holub, with *D. alpinum* (L.) Holub. Importantly, *D. alpinum,* one of the parental species of *D. issleri,* may interbreed with *D. tristachyum,* producing another taxon of hybrid origin *Diphasiastrum oellgaardii* Stoor, Boudrie, Jérôme, Horn, Bennert (Stoor et al. 1996), confirmed by molecular studies (Stoor et al. 1996; Bennert et al. 2011). Considering the morphological variations in *D. complanatum* (L.) Holub within its entire geographical range, Ivanenko, and Tzvelev (2004) distinguished three weakly differentiated subspecies: *D. complanatum* subsp. *complanatum*, *D. complanatum* subsp. *hastulatum* (Sipl.) Ivanenko et Tzvelev and *D. complanatum* subsp. *montelii* (Kukkonen) Kukkonen. The typical subspecies (subsp. *complanatum*) is found in Central Europe including Poland (Ivanenko and Tzvelev 2004). Importantly, identification of taxa of hybrid origin in the genus *Diphasiastrum* Holub is difficult due to a long-term introgression process with interbreeding of hybrids with one of the parental species. The spectrum of morphotypes of particular species is increased which makes ultimate identification difficult unless molecular markers are used (Bennert et al. 2011).

The Aspleniaceae are the spleenwort family of ferns with some 730 species (Schuettpelz et al. 2016; García et al. 2017). They are terrestrial, epilithic, and epiphytic ferms, that have a nearly worldwide distribution but are most abundant and diverse in the tropics. In Europe, some 58 species occur and *Asplenium* is one of the most species-rich fern genera. The Polypodiaceae is another family of ferns that includes 42 species that occur mostly throughout the temperate Northern Hemisphere. Many species are allopolyploid and of hybrid origin and therefore taxonomically problematic. Some families of ferns contain a large number of endemic species, such as Aspleniaceae (16 species, 27.6% of the total), Polypodiaceae (14 species,

33.3%), and Isoëtaceae (13 species, 65%). The genus Isoëtes has been subject to more recent studies (Troia et al. 2016). The European families of ferns with only a single representative species include Cyatheaceae, Osmundaceae, Psilotaceae and Salviniaceae (García et al. 2017).

19.3 A Short Survey of Traditional Medicinal and Other Uses for Pteridophytes

Medicinal properties of pteridophytes were already known in antiquity. Theophrastus of Erresus, a student of Aristotle, considered the "father of botany", in one of his two surviving botanical works ΠΕΡΙ ΦΥΤΩΝ ΙΣΤΟΡΙΑΣ [Enquiry into Plants or History of Plants] described medicinal uses of two fern species, maidenhair spleenwort (*Asplenium trichomanes*) recommended as a diuretic plant and to prevent hair loss and common polypody (*Polypodium vulgare*) prescribed as a laxative (Jędrzejko et al. 1997). Dioscorides (ca. 40–90 AD) and Pliny the Elder (23–79 AD) mentioned in their works some species of medicinal pteridophytes such as common club moss (*L. clavatum*), field horsetail (*E. arvense*), male fern (*Dryopteris filix-mas*) or *Polypodium vulgare*. Medicinal uses of some pteridophytes were recommended in the ancient Sanskrit treatises on medicine and surgery samhitas of Sushruta and Charaka. Ferns are used by physicians in Unani (Yūnānī) Perso-Arabic system of medicine (Uddin et al. 1998).

Polish botanists of the Renaissance also described medicinal properties of pteridophytes in their works. Herbarz [Herbarium] compiled by Marcin of Urzędów and published in Cracow in 1595 includes descriptions of 14 pteridophyte species with medicinal uses for some of them (Furmanowa et al. 1959).

These are:

1. ***Ophioglossum* L.** (syn. *Lingua serpentina*)
2. ***Botrychium lunaria* (L.) Swartz** (syn. *Lunaria Minor*)
3. ***Dryopteris filix-mas* (L.) Schott**—Filix Mas, (syn. *Thelipteris, Osmunda, Mimpheopteris*): used against parasites of the alimentary tract
4. ***Adiantum capillus-veneris*** L. (syn. *Capillus Veneris, Herba cincinalis, Capillus terrae*): used for breathlessness and difficulty breathing as well as to prevent hair loss
5. ***Phegopteris dryopteris* (L.) Fee** (syn. *Dryopteris*, *Filicula):* recommended against roundworm
6. ***Phyllitis scolopendrium* (L.) Newm**. (syn. *Cervina Lingua, Dioscoridis Philitis*): recommended for bloody diarrhea
7. ***Asplenium trichomanes* L.** (syn. *Capillus Trichomanes*, *Capillaris herba*)
8. ***Asplenium ruta-muraria* L**. (syn. *Asplenon, Scolopendrom, Citarach*, Argentina),
9. ***Pteridium aquilinum* (L.) Kuhn** (syn. *Filix Pteris*, *Filix Fenaria*, *Filix foemina*, *Osmunda Regalis*)

10. ***Polypodium vulgare* L.** (syn. *Polipodium, Filicula*): recommended for contusions and bruises
11. ***Equisetum arvense* L.** (syn. *Cauda Equina altera, Scevola, Salix equinaaltera Hypuris* Dios.): recommended for asthma, breathlessness, and difficulty breathing
12. ***Lycopodium clavatum* L.** (syn. *Spica Sarmatica*): recommended for renal stones and renal disease
13. ***Lycopodium selago* L.** (syn. *Spica Sarmatica*): recommended as a diuretic, a potent emetic, and a remedy counteracting the effects of poisons.

According to the myths and folk beliefs, pteridophytes used to be seen as magical plants (Jędrzejko et al. 1997). For instance, fir club moss (*Huperzia selago*) worn as an amulet or taken as a medication was believed to protect healthy people against evil spells and witches and to evict evil spirits from persons believed to be possessed. Bunches of fir club moss were hung on the doors to cowsheds to protect cattle against witches' spells. Adder's tongue (*Ophioglossum vulgatum*), on the other hand, was considered a love herb, and maidens stitched it in the hems of their skirts to attract suitors. According to Belarusian folk beliefs, the milky infusion of adder's tongue could neutralize the effects of adder venom (Jędrzejko et al. 1997). In folk medical practice, the appearance and morphological traits of some species, often reflected in their common names, informed about the gender of patients they were intended for, e.g. *Athyrium filix-femina* was prescribed exclusively to females and *Dryopteris filix-mass* to males (Jedrzejko et al. 1997). For ages, some pteridopphytes were used as food for animals and humans. Ancient hermits fed themselves with underground parts of field horsetail and edible bracken fern (*Pteridium aquilinum*) (Jedrzejko et al. 1997). Horsetails and club mosses were used in some industries. Horsetails, due to their high silica content, were used to scrub metal objects. Club moss spores, especially of *Lycopodium clavatum,* were sprinkled on inner surfaces of casting molds and to manufacture fireworks and combustible materials (Jedrzejko et al. 1997; Szypuła and Pietrosiuk 2021). From 1664 on, monographs of club moss spores were included in pharmacopoeias as a medicinal remedy such as a safe dusting powder for babies, a wound powder or for dusting pills (Muszyński 1946; Szypuła and Pietrosiuk 2021). Field horsetail was commercially used to dye fabrics greyish-yellow.

Nowadays, numerous pteridophyte species are used as food or in traditional medicine in the regions characterized by their abundance and high biodiversity. It has been estimated that over 20% of the global pteridophyte flora is found in China (Soare and Şuţan 2018). For over 3000 years, 52 fern species have been traditionally consumed as food in China and another 144 species are also edible. Their dry or salted fronds (leaves), starch, or herbal tea are commercially available food products (Liu et al. 2012; Soare and Şuţan 2018).

The centuries-old use of pteridophytes in traditional folk medicine lays the ground for modern pharmacological research. Crude plant extracts, normalized extracts, or purified isolated substances continue to be investigated to document their traditional uses and find new medicinal properties. Some of pteridophyte species and substances

isolated from these plants possess exceptional medicinal properties with current and potential clinical applications (Soare and Şuţan 2018).

To date, several horsetail and club moss species and some 180 fern species are used by folk medicine worldwide. Fourteen of these species are described in the pharmacopoeias in 29 countries (Klama 1992; Jedrzejko et al. 1997). The latest 10th edition of the European Pharmacopoeia contains monographs of raw materials obtained from two pterygoid species, *Drynaria fortunei* and *Equiesetum arvense.*

For a long time, *E. arvense* has been used in traditional medicines for the treatment of brittle fingernails, loss of hair, and for rheumatic diseases. Flavonoids, caffeic acid derivatives, phytoosterols, tannin, triterpenoids and alkaloids are phytochemical compounds which are reported from this plant (Asgarpanah and Roohi 2012; Al-Snafi 2017). Due to the easy collection of the plant and being widespread and also remarkable biological activities, this plant has become medicine in many countries. The pharmacological studies showed that it possessed antioxidant, anticancer, antimicrobial, smooth muscle relaxant effects of the vessels and ileum, anticonvulsant, sedative, anti-anxiety, dermatological immunological, antinociceptive, anti-inflammatory, antidiabetic, diuretic, inhibition of platelet aggregation, promotion of osteoblastic response, anti-leishmanial, and many other effects (Asgarpanah and Roohi 2012; Al-Snafi 2017). *Drynaria fortunei* known as Gu-Sui-Bu, is used in traditional Chinese and Korean medicine. Flavonoid compounds –naringen and its derivatives are the main active constituents of *Drynaria fortunei* (Wong et al. 2013; Gou et al. 2019). The dry stem and root of *D. fortunei* are applied to the clinical medical use and used for the treatment of common injuries, including bone fractures and bruising, in treating inflammation, hyperlipidemia, oxidative damage, arteriosclerosis, rheumatism, and gynecological diseases.

19.4 Short Characteristics of Pteridophyte Secondary Metabolites and Their Medicinal Properties

19.4.1 Phenolic Compounds

Phenolic compounds, phenylpropamide derivatives, glycated or non-glycated, ubiquitously distributed in ferns are their main bioactive substances. Commonly found phenolic compounds include chlorogenic acid, caffeic acid, ferulic acid, hydroxybenzoic acid, hydroxycinnamic acid, vanillic acid, and other compounds possessing functional acid groups. Phenolic compounds have antioxidant and photoprotective properties. They are especially abundant in *Polypodium leucotomos* which is used in skin care products, in particular as a treatment for psoriasis (Lucca 1992; House et al. 1994; Gonzalez et al. 2010). Extracts of sword bracken ferns (*Pteris ensiformis)* appear active against atherosclerosis as they contain the glycosylated phenolic compound 7-*O*-caffeoylhydroxymaltol-3-β-D-glucopyranoside (Wei et al. 2007). Chalcone derivatives are another group of bioactive phenolic compounds

found in ferns. Silver fern (*Pityrogramma calomelanos*) contains dihydrochalcone which is cytotoxic and may be responsible for the anticancer potential of this fern (Martin et al. 2006). Licoagrochalcone D isolated from the roots of *Pteris multifida* is its major bioactive constituent (Hu and Zheng 2005). Some phenol derived compounds (4-vinylphenol, fylligenin and arctigenin) isolated from maidenhair spleenwort (*Asplenium trichomonas*) belong to the class of estrogens (Dall'Acqua et al. 2009) and *Asplenium trichomonas* extracts and isolated constituents were used as an ammenagogue (Negri 1979; Moerman 1998; Pomini 1990).

19.4.2 Phloroglucinol Derivatives

Phloroglucinol is a phenolic compound. Phenols are hyroxy derivatives of aromatic hydrocarbons characterized by a hydroxyl group attached to a carbon atom. They can be classified into mono-, di- or triphenols according to the number of hydroxyl groups (Euw et al. 1980; Widén et al. 1996, 1999, 2015). Phloroglucinol (synonym: 1,3,5-trihydroxybenzene; molecular formula: $C_6H_6O_3$) is a symmetric phenol with hydroxyl groups at the ortho, para and meta positions (Kohlmünzer 2007). All phloroglucinols may be roughly classified according to the number of hexacyclic rings which form their skeleton. Most of the natural phloroglucinols contain two, three, or four hexacyclic rings bound together by a methylene bridge (Euw et al. 1980; Kohlmünzer 2007). To date, only two one-ring compounds have been identified, aspidinol-B and fraginol-B (Euw et al. 1980). Aspidinol-B is most likely an artefact produced as a result of degradation of its oligomers, particularly para-aspidin, trispara-aspidin, and margaspidin during prolonged storage of plant material or during the analytical work (Euw et al. 1980; Widén et al. 1999, 2015). Fraginol later isolated from *Dryopteris fragrans* (L.) Schot is a typical compound of this species.

In the presence of strong acids, in the reaction of phloroglucinol with coniferyl aldehyde, lignified cell walls stain red, which is used in histochemical studies of plants (Kohlmünzer 2007).

There are several published studies on phloroglucinols from different species of the genus *Dryopteris* Adans. (Euw et al. 1980; Widén et al. 1996, 1999, 2015; Wollenweber et al. 1998; Vogler et al. 2012). Phloroglucinols are produced by idioblastic trichomes and secreted into the schizogenous cavities in storage parenchyma (Zenkteler 2000). Phloroglucinol derivatives often collectively referred to as crude filicins or aspidins usually account for some 1.5–2% of the dry mass of plant material. The usual main constituents of filicin are albaspidin (two homologues), filixic acid (three homologues), and flavaspidinic acid (Euw et al. 1980; Wollenweber et al. 1998; Widén et al. 1999, 2015). Hegnauer (1962) lists phloroglucinols (aspidin, aspidinol, flavaspidin, and desaspin) as the main bioactive constituents in *Dryopteris*.

Phloroglucinols are contained in the rhizomes and stipe bases in most species in the genus *Dryopteris* Adans. (Euw et al. 1980; Widén et al. 1999, 2015; Wollenweber

et al. 1998; Vogler et al. 2012). Some populations of *D. expansa* (Presl) Fraser-Jenkins & Jermy (=*D. assimilis* Walker) and a few already investigated species from Asia, *Dryopteris sparsa* (D. Don) Kuntze, *D. polita* Ros., *D. subexaltata* (Christ) C. Chr. and Africa such as *Dryopteris kilemensis* (Kuhn) Kuntze are exceptions totally lacking phloroglucinols or containing only their minute amounts (Euw et al. 1980; Widén et al. 1999, 2015). Apart from the genus *Dryopteris* Adans., phloroglucinols have been found only in a few closely related fern genera *Ctenitis* (C.Chr.) C.Chr. and *Polystichum* Roth as well as in most species of *Arachniodes* Blume (Mehra and Mittal 1961; Widén et al. 1978; Euw et al. 1980), which are not found in Europe. The composition of phloroglucinols is usually constant for each species and dependent on age of the plant, season of collection, or origin (Euw et al. 1980). The qualitative composition of filicins in *D. filix-mas* varies slightly, but does not depend on plant origin while in *D. expansa* (=*D. assimilis*) it is characterized a high variability. Some North American and European populations of *D. expansa* have scant amounts or total lack of phloroglucinols and others contain their large amounts. Ferns from Asia differ in chemical constituents from those in America and Europe as they contain much less phloropyrone and more tridesaspidin (Euw et al. 1980). A pronounced variation in the phloroglucocynol content can be also seen in *D. carthusiana* as some populations are rich in para-aspidin while some totally lack it. Also sporophytes of *D. cristata* from North America and Europe sometimes contain and sometimes lack para-aspidin (Euw et al. 1980). Species of the genus *Dryopteris* Adans. of hybrid origin and allopolyploids generally display an additive composition of phloroglucinols compared to their parental species (Euw et al. 1980). For instance, the composition of phloroglucinols in *D. x pseudoabbreviata* Jermy, a sterile hybrid of *D. aemula* (Aiton) Kuntze x *D. oreades* Fomin is a mixture (the arithmetic sum of the values) of phloroglucinols found for the parental species (Euw et al. 1980). On the other hand, in some species the composition is altered or the biosynthesis of some phloroglucinol(s) present in one is suppressed by the other parental species with the resulting lack of some compounds characteristic of the parent or ancestor (Euw et al. 1980). Studies have also demonstrated that species of hybrid origin and allopolyploids may occasionally contain new compounds which are not present in either of their parental species (Euw et al. 1980).

Phloroglucinol and its derivative flavaspidinic acid have an antibacterial activity (Kim et al. 2006). They are highly active against Gram-positive bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus mutans* and *Bacillus subtilis,* but have no antifungal properties (Lee et al. 2009).

Phloroglucinols are also effective against parasitic infestations, in particular against flatworms (liver fluke, tapeworms) (Magalhães et al. 2009). Phloroglucinol metabolites paralyze the nervous system of parasitic worms and cause muscle contractions. Magalhães et al. (2009) demonstrated in vitro schistosomicidal effects of some phloroglucinol derivatives (aspidin, flavaspidinic acid, methylene-bis-aspidinol, and desaspidinol) isolated from the rhizome of *Dryoptera* species against *Schistosoma mansoni* adult worms.

Phloroglucinol and its derivatives have been found to inhibit oxidative stress and inflammation. Kim et al. (2010, 2017) demonstrated that phloroglucinol inhibits the

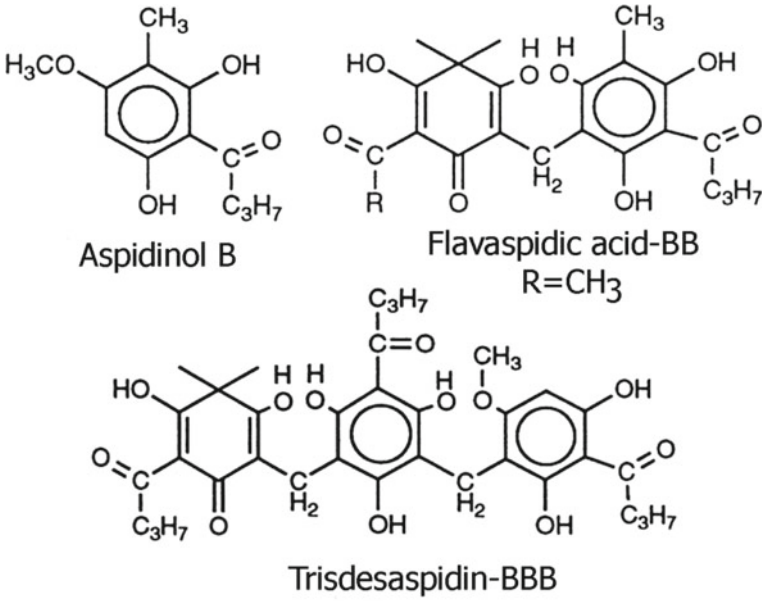


Fig. 19.2 Phloroglucinol isolated from the rhizome of a fern of the genus *Dryopteris* Adans. (after Euw et al. 1980)

production of inflammatory mediators such as interleukin-1β (IL-1β), interleukin-6 (IL-6), prostaglandin E_2 (PGE) and tumor necrosis factor-α (TNF-α). Phloroglucinol also reduced intracellular hydrogen peroxide levels and inhibited oxidation of membrane proteins. Figure 19.2 presents the most characteristic phloroglucinol derivatives isolated from ferns.

19.4.3 Terpenoids

Terpenoids constitute the largest group of chemical compounds in ferns and the main representatives are triterpenoids, diterpenoids and sesquiterpenoids.

Triterpenoids

Triterpenoids from ferns are either hydrocarbons or functional molecules belonging to the class of ecdysteroids (Reddy et al. 2001; Nakane et al. 2002; Bresciani et al. 2003; Singh et al. 2008; Parihar et al. 2010). To date, numerous triterpenoids have been isolated from at least fifty fern species (Lafony et al. 2011). Figure 19.3 presents the most characteristic triterpenoids isolated from ferns.

Hydrocarbons such as "fern-7-en" or filicene are characteristic phytoconstituents of ferns and may be used as markers of their biodiversity (Reddy et al. 2001; Nakane

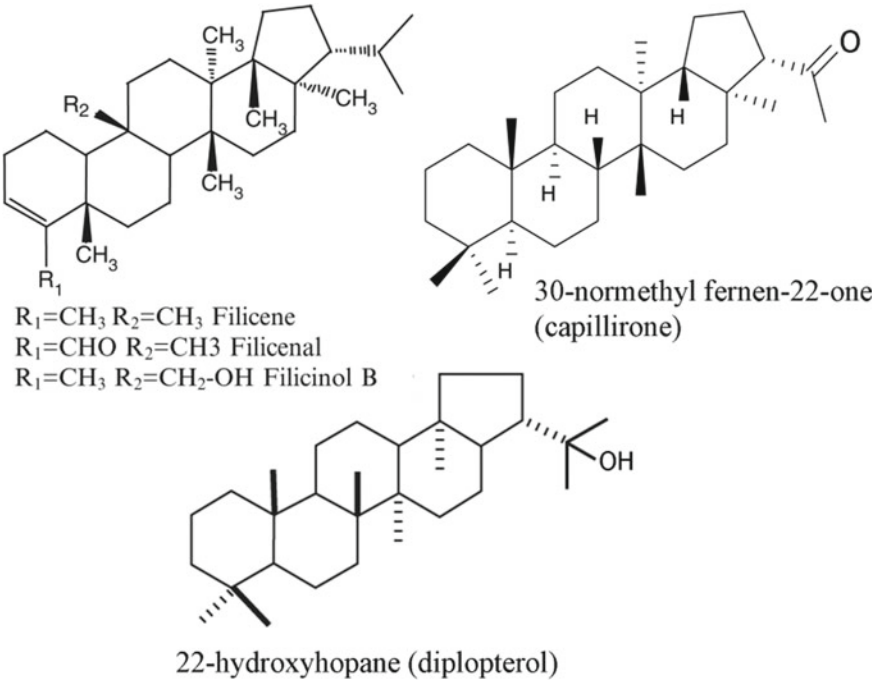


Fig. 19.3 Some triterpenoids isolated from ferns) (after De Souza et al. 2009; Ho et al. 2011; Vetter 2018

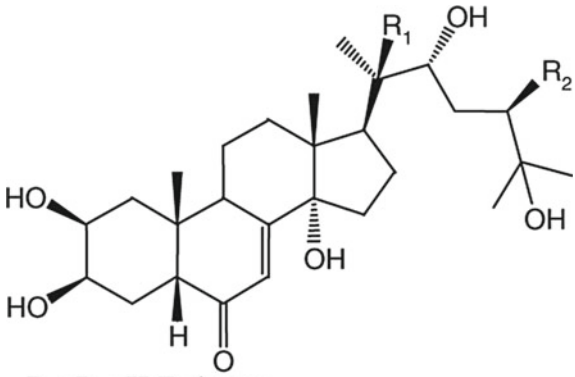
et al. 2002; Bresciani et al. 2003; Singh et al. 2008; Parihar et al. 2010). Ecdysteroids are of great interest to the researchers due to a wide spectrum of biological activities of their metabolites. They have adaptogenic properties seen in their anabolic, hypoglycemic, cholesterol-lowering, tonic, hepatoprotective, and laxative effects (Lafont and Dinan 2003). *Microsorum scolopendria* and *M. membranifolium* are a rich source of ecdysteroids. The main constituents in the leaves and rhizomes are 20-hydroxyecdysone, ecdysone, 2-deoxy-20 hydroxyecdysone, and 2-deoxyecdysone (Fig. 19.4; Ho et al. 2007; Meybeck et al. 2010).

Diterpenoids

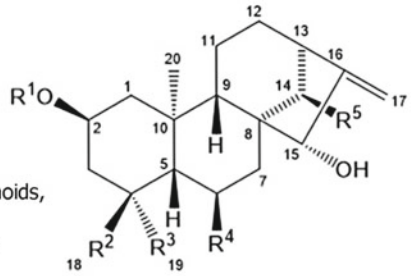
Diterpenoids have been found in some medicinal ferns of the genus *Pteris* and classified into *ent*- kauranes, *ent*-atisanes and *ent*-pimaranes (Alonso-Amelot 2002). Figure 19.5 presents pterokauranes which belong to the type of *ent*-kauranes.

Sesquiterpenoids

Ferns contain indane-type and cadinane-type sesquiterpenoids. The anti-sesquiterpenoids, mostly a sesquiterphenyl with an indanone skeleton named pterosin, and its glycoside pteroside are known for their biological activities (Ben Cao 1999; Hu et al. 2006; Ge et al. 2008; Ouyang et al. 2008; Zheng et al. 2008).

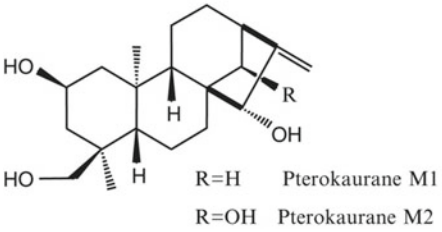


R_1=R_2=H Ecdysone
R_1=H R_2=Me 20-deoxymakisterone
R_1=OH R_2=H 20-hydroxyecdysone
R_1=OH R_2=Me Makisterone A
R_1=OH R_2=Et Makisterone C

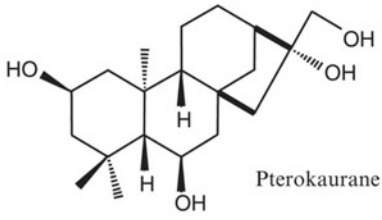


Ent-kaurane diterpenoids, pterokaurane M1 from *Pteris multifida*

R^1	R^2	R^3	R^4	R^5
H	CH_3	CH_3	H	H



R=H Pterokaurane M1
R=OH Pterokaurane M2



Pterokaurane M3

Fig. 19.4 Some ecdysones isolated from ferns (after Ho et al. 2011)

Fig. 19.5 Some pterokauranes isolated from ferns (after Ho et al. 2011 and Kim et al. 2017)

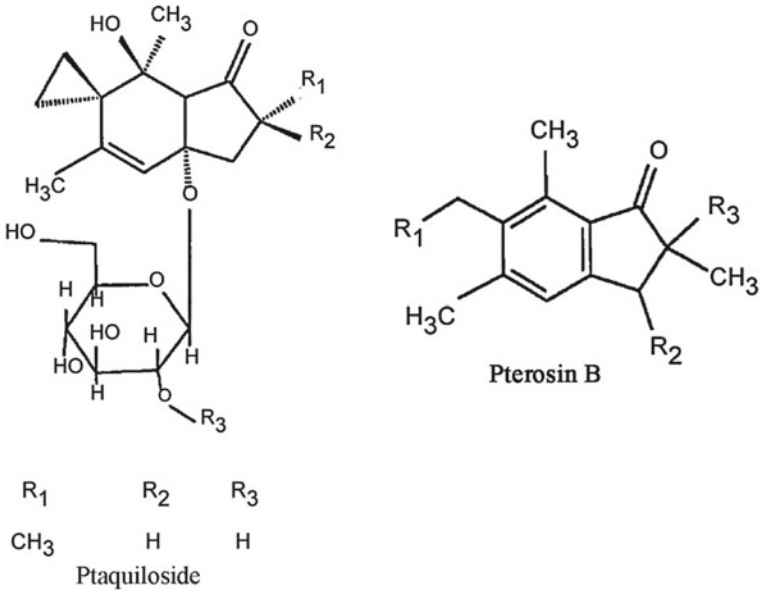


Fig. 19.6 Ptaquiloside isolated from bracken fern (*Pteridium aquilinum*) (after Potter and Barid 2000)

These compounds found mainly in the species of the genera *Pteridium* and *Polypodium,* have been used by traditional medicine. Pterosin-sesquiterpenoids named multifidosides A and B were isolated from *Pteris multifida* and they showed cytotoxity against the HepG2 tumor cell line (hepatic cancer) and the K562 line (human leukemia) (Ge et al. 2008; Ouyang et al. 2008). *Pteridium aquilinium*, bracken fern in some regions traditionally used as a medicinal plant, contains ptaquilosides (PT) (Fig. 19.6) which are, toxic and carcinogenic, and can cause neoplasms of the urinary bladder and stomach (Yamada et al. 2007). Epidemiological data from the Andean regions of Venezuela indicate an association between high gastric cancer incidence rates and consumption of milk from cows that graze bracken fern (Orellana 2001).

Ptaquiloside (PT) alkylates DNA when it is activated to its unstable carbocation dienone form (ATP) under alkaline conditions of the alimentary tract, which produces irreversible DNA damage and is responsible for bracken fern carcinogenicity. Transversions in the codon 61 of H-ras have been identified which transform protein p21 and alter GTPase activity stimulating uncontrolled cell proliferation. PT causes structural chromosome aberrations (chromosome and chromatin breaks) as demonstrated in an in vitro model using human lymphocytes (Matsuoka et al. 1989).

19.4.4 Flavonoids

A large number of flavonoids, glycated and non-glycated, have been isolated from ferns (apigenin, luteolin, naringenin, campherol). They have potent biological activities. They lower lipid levels in the blood, protect the liver, alleviate inflammation, induce relaxation of the coronary arteries and have antibacterial effects. Some, such as violantanin and isoviolontanin from *Angiopteris evecta* (G. Forst.) Hoffm., have hypoglycemic and antidiabetic effects (Nguyen 2005). Flavonoids from *Pteris multifida* Poiret ex Lamarck is used in the treatment of liver disease (Wang and Zhang 2008).

19.4.5 Alkaloids

There are very few published studies on alkaloids in ferns. In the family *Dryopteridaceae*, alkaloids have been confirmed exclusively in *Dryopteris filix-mas* (Zhou et al. 2007), but most of the members of this family have not been investigated for alkaloids. There are reports of pyrrolizidine alkaloids isolated from *Pteridium aquilinium* (Hirono 1986; Jędrzejko et al. 1997). According to Hegnauer (1962) there are no alkaloids in the ferns of the *Polypodiaceae* family. Pyridine alkaloids such pallustrin and nicotine, and nicotine derivatives are found in several horsetail species.

Alkaloids are the most important secondary metabolites in club mosses. Usually, they contain two pyridine rings and are quinolizine, pyridine, or α-pyridone type alkaloids (Ma and Gang 2004). Usually, they contain 16 or 18 carbon atoms and 1 or 2 nitrogen atoms. 18-carbon alkaloids are mostly the acetylated derivatives of 16-carbon alkaloids. Alkaloids with a greater number of carbon atoms have been also identified. As *Lycopodium* alkaloids are not structurally related to any of the earlier described alkaloids, a system of their classification has been developed based on structural similarities and pharmacological activities. Some of the identified alkaloids have been divided into four classes: (1) the lycopodine group; (2) the lycodine group; (3) the fawcettimine group; and (4) a miscellaneous group (Ayer and Trifonov 1994) (Fig. 19.7). The lycopodine-type alkaloids are the most numerous group and they commonly occur in the *Lycopodium* species. They are characterized by four connected six-membered rings, of which two are a quinolizidine ring system. Huperzine A with a chinolizidine skeleton is the most important *Lycopodium* alkaloid isolated from the Chinese species *Huperzia serrata* and from *H. selago,* both belonging to the *Huperziaceae* family (Szypuła and Pietrosiuk 2021). Reviews of the current state of knowledge on the medicinal properties of *Lycopodium* alkaloids have been published by Ma and Gang (2004), Fereira et al. (2016), and Szypuła and Pietrosiuk (2021). Huperzine A easily crosses the blood–brain barrier, is a highly acetylcholinesterase-specific inhibitor and at higher concentrations also inhibits butyrylcholinesterase (BuChE) (Fereira et al. 2016; Szypuła and Pietrosiuk 2021). The pharmacodynamic properties of Huperzine A are superior

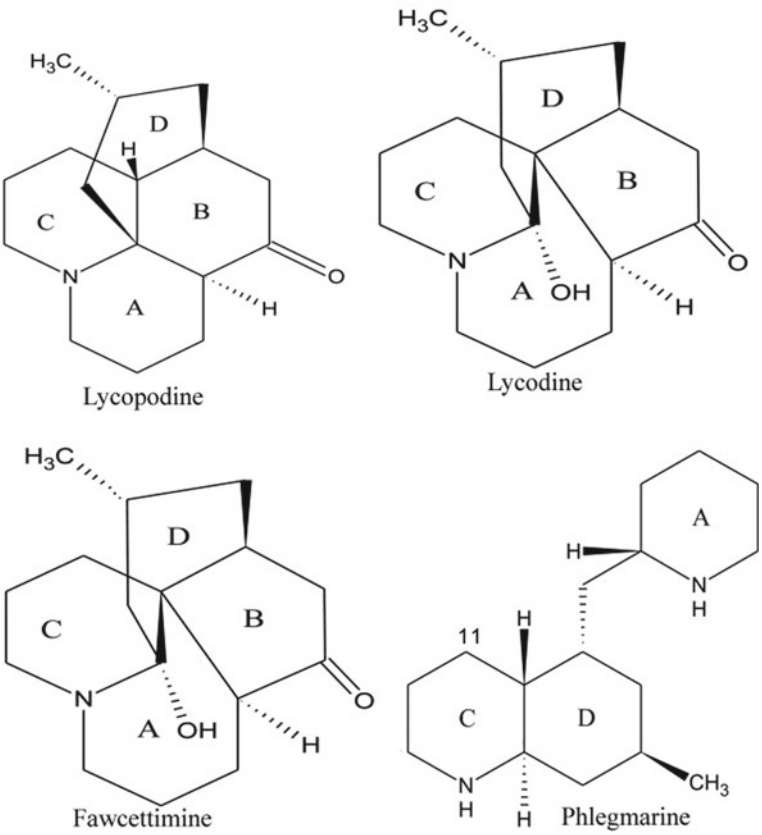


Fig. 19.7 Representative compounds of the four major classes of Lycopodium alkaloids (after Ma and Gang 2004)

to those of drugs currently used in Alzheimer's disease such as donepezil, galantamine or rivastigmine. It is better transported across the blood–brain barrier, its oral bioavailability is higher, and it is less toxic (Ferreira et al. 2016). Recent studies have demonstrated that additionally Huperzine has neuroprotective properties and may be also used as an antidote in cases of poisoning with organophosphate pesticides (Ma and Gang 2004; Fereira et al. 2016).

19.4.6 Other Chemical Compounds Isolated from Ferns

Osladin (Fig. 19.8) has been identified in some ferns. This bidesmosidic saponin is found in the rhizome of the fern *Polypodium vulgare* of the *Polypodiaceae* family (Grzybek 1983). Osladin is intensely sweet, approximately 3 000 times sweeter than

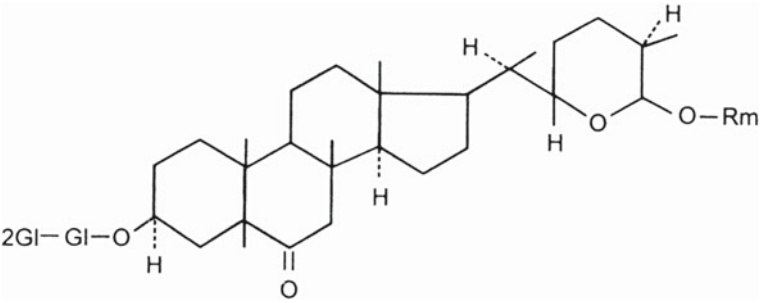


Fig. 19.8 Osladin isolated from rizomes oh *Polypodium vulgare* (after Kohlmünzer 2007)

sucrose. Another saponin, pteridin, has been isolated from the rhizome of the bracken fern, along with other compounds such as cyanogenic glycosides, tannins, essential oils, mucilage or starch. Tannins and essential oils have been also found in the rhizomes of *Athyrium filix-femina* and of *Dryopteris filix-mass*. The ferns of the genus *Asplenium* contain pimelic acid derivatives and the rare amino acid N-acetylornithine. Bracken fern also contains the enzyme thiaminasine which inactivates vitamin B_1.

According to the checklist of pteridophytes in Flora Europea (Valentine 1964) and some more recent data (García et al. 2017), there are approximately 205 pteridophyte species native to Europe. Phytochemical and biological characterization has been performed on a few of them only, including four club moss species (*Huperzia selago, Lycopodium clavatum, Diphasiastrum complanatum, Diphasiastrum alpinum*) and most species of the genus *Dryopteris* Adans., and focused on the content of alkaloids in the club mosses and phloroglucinols in the ferns. There have also been studies, though less detailed, of some species belonging to the genera *Aslplenium, Polypodium, Polystichum* and *Equisetum*. Most pteridophyte species native to Europe seems to have attracted less research interest than angiosperms and the published data, mostly fragmentary, do not allow for more thorough comparative chemotaxonomic studies. In practice, any comparison of chemotaxonomic data with the pteridophyte phylogeny according to The Pteridophyte Phylogeny Group (Schuettpelz et al. 2016) is difficult if not impossible. Scarcity of phytochemical analyses in pteridophytes is most likely due to either a relatively unfamiliarity of a number of species or to difficulties in the identification of enigmatic taxa, especially those of hybrid origin. As there are numerous published studies on the valuable medicinal properties of some pteridophytes, especially plants growing in tropical and subtropical areas, it seemed interesting to check whether some of the pteridophyte species native to Europe have similar medicinal properties and therapeutic potential. The available literature data suggest that some European ferns, club mosses and horsetails have anti-inflammatory, antibacterial, antifungal and anticancer activities. Also described are their immunomodulating, hepatoprotective, diuretic and hypoglycemic effects. Some alkaloids, which may be extracted from *Huperzia selago*, are already used in clinical practice to ameliorate the symptoms of Alzheimer's disease (Szypuła and Pietrosiuk 2021).

Although there is a fair number of published studies describing the medicinal properties of European pteridophyte species, the actual data are scattered across many publications, often incomplete and generally poor compared to the information on angiosperms. Apparently, there are no comprehensive and detailed works on the current state of knowledge on the pteridophyte taxa with confirmed phytochemical composition and biological activities. Table 19.1 presents the literature review and current state of knowledge on the secondary metabolites of most pteridophytes native to Europe and potential uses for their bioactive secondary metabolites. Also included is the information on metabolites whose presence could be possibly expected in these species which have not been yet screened.

19.5 Plant Biotechnology in the Biosyntshesis of Secondary Metabolites and Conservation of Pteridophyte Species

Research into in vitro cultures of plants contains two complementary aspects: theoretical and applied. It produces new knowledge and insights into the biochemical, genetic, and physiological determinants of processes involved in the development, differentiation, and morphogenesis of plants, and the biosynthesis of their secondary metabolites. This knowledge is used in the natural sciences, including plant genetics and breeding, pharmacy, or biotechnology. Techniques of in vitro plant regeneration are now used in plant breeding and cultivation (Villalobos and Engelmann 1995). Many species of crop plants (crops for food, and for medicinal and industrial uses) have been thoroughly investigated and procedures have been developed which allow their production and processing on an industrial scale. Plant biotechnology has contributed to the growing popularity of many ornamental plants sold in pots or as cut flowers. Commercial plant tissue culture (micropropagation) laboratories have been active for over 60 years and are an important sector of economy in many countries, often referred to as 'in vitro industry'. Micropropagation of plants continues to develop making use of many biotechnological improvements. Biotechnology has opened up new perspectives for the biosynthesis of secondary metabolites using tissue culture which would allow propagation and industrial uses of many plant species, often rare and at risk of global extinction, which could not be otherwise preserved.

In the case of pteridophytes as compared to angiosperms, the uses of biotechnological methods are limited due to the low species diversity and hence probable low diversity of secondary metabolites with potential commercial uses. Pteridophytes are evolutionary relics and their biochemical, genetic, and physiological properties have not yet been thoroughly elucidated which may be responsible for the lack of interest among researchers in the in vitro biosynthesis of pteridophyte secondary metabolites. Micropropagation of ferns is an exception because methods used in tissue cultures of angiosperms may be repeated for a number of ferns. Plant material is obtained for research purposes without depleting the natural populations. Micropropagation of most lycopod species is more difficult but not impossible (Szypuła et al. 2005,

Table 19.1 Bioactive molecules of European pteridophytes and their properties

Ferns

Species	Metabolites	Biological activity	Literature
Asplenium adiantum-nigrum	**Procyanidin, prodelphinidin, various kaempferol 3,7-di-*O*-glycosides**, protocatechic acid, gentisic acid, mangiferin and mangiferin glucoside, *p*-hydroxybenzoic acid, aesculin, chlorogenic acid, caffeic acid, *p*-coumaric acid, rosmarinic acid, gallocatechin, epigallocatechin, gallate, catechin, epicatechin, rutin, epigallocatechin, xnthone 2,4-di-c-glycosides,	Contraceptive, diuretic, emmenagogue, expectorant, laxative, ophthalmic, pectoral, antibacterial, anti-oxidant	Imperato (1991c), Mir et al. (2013), Valizadeh et al. (2015), Bahadori et al. (2015a, 2015b), Zivkovic et al. (2020), Umikalsom et al. (1994)
Adiantum capillus-veneris	**More than 130 compounds belonging to triterpenoids, flavonoids, phenyl propanoids, phenolics, coumarins, phytosterols, fatty acids and others** e.g. adiantone, adiantoxide, astragalin, β-sitosterol, caffeic acids, caffeylgalactose, caffeylglucose, campesterol, carotenes, coumaric acids, coumaryl glucoses, diplopterol, epoxyfilicane, fernadiene, fernene, filicanes, hopanone, hydroxy-adiantone, hydroxy-cinnamic acid, isoadiantone, isoquercetin, kaempferols, lutein, mutatoxanthin, naringin, neoxanthin, nicotiflorin, oleananes, populnin, procyanidin, prodelphinidin, quercetins, querciturone, quinic acid, rhodoxanthin, rutin, shikimic acid, violaxanthin, and zeaxanthin	Antiseptic, anti- inflammatory, antidiabetic, antifungal, antidysentric, antiulcer, anticancer, antiviral	Kumar and Kaushik (1999), Mukhopadhyay and Gupta (2005), Ibraheim et al. (2011), Pan et al. (2011), Jiang et al. (2011), Rajurkar and Gaikwad (2012), Yuan et al. (2012, 2013), Ahmed et al. (2012, 2015), Haider et al. (2013), Yuan et al. (2013), Jing et al. (2011), Aulakh et al. (2019)
Adiantum reniforme	No data available. Composition of secondary metabolites probably similar to an Asian species e.g. *Cheilanthes farinose* and other polyphenols, flavonoids as rutin, cinnamic acid, caffeic acid, quinic acid derivatives, chlorogenic acid, quercetin	No data available. Bioactivity probably similar to Asian species	Aulakh et al. (2019)
Allosorus acrosticus	No data available, see *Adiantum reniforme*	No tested	–
Allosorus fragilis	No data available, see *Adiantum reniforme*	No tested	–
Allosorus guanchicus	No data available, see *Adiantum reniforme*	No tested	–
Allosorus hispanicus	No data available, see *Adiantum reniforme*	No tested	–
Allosorus persicus	No data available, see *Adiantum reniforme*	No tested	–
Allosorus pteridioides	No data available, see *Adiantum reniforme*	No tested	–
Allosorus tinaei	No data available, see *Adiantum reniforme*	No tested	–

(continued)

Table 19.1 (continued)

Ferns			
Anogramma leptophylla	**Lipidic derivatives** e.g. nonanal, 6-methoxymellein, 6-hydroxymellein. **Shikimic derivatives**: benzaldehyde, phenylethanal, 2,3-dihydrocoumarin, coumarin, 4-hydroxy-3-methoxyacetophenone, 6,7-dimethoxycoumarin (scoparone), 7-methoxy-6-prenylcoumarin (suberosin)	Antimicrobial and Antifungal	Fons et al. (2018)
Arachniodes webbiana	**Phloroglucinols** e.g. aspidinol-B, flavaspidic acid-BB, paraaspidyn-AB, desaspidyn-BB, albaspidyn-BB, trisparaaspidyn-BBB	Probably antyhelmintic, antibacterial	Gibby et al. (1992)
Asplenium adulterinum	No data available. Composition of secondary metabolites probably similar to *Asplenium ruta-muraria*	Not tested	–
Asplenium aegaeum	No data available	Not tested	–
Asplenium aethiopicum	**Flavonoid derivatives:** rutin, isoquersetin, gallic acid, ferulic acid, chlorogenic acid. resorcinol, coffeic acid	Not tested	Johnson et al. (2020)
Asplenium anceps	No data available	Not tested	-
Asplenium aureum	Polyphenols	Antioxidative, antimicrobal	Lai et al. (2009)
Asplenium auritum	No data available	Not tested	–
Asplenium azoricum	No data available	Not tested	–
Asplenium balearicum	No data available. Composition of secondary metabolites probably similar to *Asplenium adiantum-nigrum*	Not tested	–
Asplenium bourgaei	No data available	Not tested	Durdević et al. (2007), Aulakh et al. (2019)
Asplenium ceterach	**Flavonoid derivatives:** *p*-coumaric, ferulic acid, pterosin b, catechin, quercetin, chlorogenic acid	Astringent, diuretic, emollient, antioxidant, tyrosinase inhibitory activity	Pekgoz and Cinbilgel (2019), Durdevic et al. (2007), Farràs et al. (2019)
Asplenium creticum	No data available	No tested	–
Asplenium fissum	No data available	No tested	–
Asplenium fontanum	**Flavonoid derivatives** kaempferol 3-*O*-gentiobioside, kaempferol 3,7-*O*-glycoside, kaempferol 3-*O*-glycoside	No tested	Iwashina et al. (2000)

(continued)

Table 19.1 (continued)

Ferns			
Asplenium foreziense	**Flavonoid derivatives** kaempferol 3-*O*-gentiobioside, kaempferol 3,7-*O*-glycoside, kaempferol 3-*O*-glycoside	No tested	Iwashina et al. (2000)
Asplenium hemionitis	No data available	No tested	–
Asplenium hispanicum	No data available	No tested	–
Asplenium hybridum	No data available	No tested	–
Asplenium jahandiezii	**Lipidic compounds**: 9-oxononanoic acid, (E)-2-decenal, (E)-2-heptenal, nonanal, octanoic acid, 1-octen-3-ol, tetradecanoic acid, 2,3-octanedione, octanol, (E,Z)-2,4-decadienal, (E,E)-2,4-decadienal, octanal (E)-2-decenol, hexanoic acid	9-oxononanoic acid stimulates the activity of phospholipase A2, the key enzyme of the arachidonate	Froissard et al. (2015)
Asplenium lepidum	No data available	No tested	–
Asplenium lolegnamense	No data available	No tested	–
Asplenium macedonicum	No data available	No tested	–
Asplenium majoricum	No data available	No tested	–
Asplenium marinum	**Flavonoid derivatives:** kaempferol 3-*O*-methyl ether 7-*O*-glucoside, kaempferol 3,4'-di-*O*-methyl ether 7-*O*-glucoside	No tested	Umikalsom et al. (1994)
Asplenium monanthes	No data available	No tested	–
Asplenium obovatum	Laempferol 3-*O*-gentiobioside, kaempferol 3,7-*O*-glycoside, kaempferol 3-*O*-glycoside	No tested	Iwashina et al.2000
Asplenium octoploideum	No data available	No tested	-
Asplenium petrarchae	**Lipidic compounds** with a very high level of octanoic acid, (E)-2-Decenal, (E)-2-heptenal, nonanal, 9-oxononanoic acid, 1-octen-3-ol, benzoic acid, carotenoid derivatives, i.e., 4-hydroxy-β-ionone and 4-hydroxy-5,6-epoxyionol	Antimicrobial	Froissard et al. (2015)

(continued)

Table 19.1 (continued)

Ferns			
Asplenium ruta-muraria	**Phenols derivatives:** gallic acid, protocatechuic acid, aesculin, mangiferin glucoside, epigallocatechin, p-hydroxybenzoic acid, gentisic acid, chlorogenic acid, caffeic acid, epicatechin, gallocatechin gallate, rutin, p-coumaric acid, ferulic acid, rosmarinic acid,epigallocatechin gallate	Antimicrobial, antioxidant	Zivkovic et al. (2020)
Asplenium sagittatum	No data available	No tested	-
Asplenium scolopendrium	**Quercetin glycosides, triterpenoids, polyphenols**	Wound healing activity/ antibacterial	Oniga et al. (2004), Bahadori et al. (2015a, b)
Asplenium seelosii	No data available	No tested	–
Asplenium septentrionale	**Terpenoids, flavonoid:s** kaempferol, quercetin; aldehydes, alcohols, kaempferol 3-sophoroside-4'-glucoside	No tested	Imperato (1990)
Asplenium terorense	No data available	No tested	–
Asplenium trichomanes	**Flavonoid derivatives:** 4-vinyl-phenol-1-*O*-[α-L-rhamnopyranosyl (1 → 6)-D-glucopyranose], 2 kaempferol-3-*O*- α -arabino-(2″acetyl)-furanosylo-7-*O*- α -L-rhamnopyranoside:, kaempferol-3-*O*-α-arabinofuranosyl-7-*O*-α-L-rhamnopyranoside, 4-vinylphenol, 4-(1-metoxy-ethyl)-phenol (5), p-hydroxyacetophenone, phylligenin, arctigenin	Antimicrobial, laxative, expectorant, Emmenagogue, estrogenic	Dall' Acqua et al. (2009)
Asplenium viride	**3-Methylquercetin**	No tested	Voirin and Jay (1974)
Athyrium distentifolium	No data available. Composition of secondary metabolites probably similar to *Athyrium filix-femina*	No tested	–
Athyrium filix-femina	**Phenolic compounds, flavonoids**: procyanidin, kaempferol 3-*O*-rutinoside, keempferol 3-*O*-glucoside, various kaempferol 3,7-di-*O*-glycosides, various uncharacterized quercetin glycosides, N-caffeoyl-phenylalanine, N-caffeoyl-tryptophan, 5-O-caffeoyl-shikimic acid, 3,4-di-*O*-caffeoyl-quinic acid, chlorogenic acid. Essentially polyketides and aromatics (2-phenylethanal, 2-phenylethanol, 1-octen-3-ol, monoterpeness (2-phenylethanal and 3.7-dimethyloctan-3-ol)	Antiparasitic, anthelmintic, antioxidant activity	Umikalsom et al. (1994), Soare et al. (2012), Fons et al. (2018), Adam (1995)

(continued)

Table 19.1 (continued)

Ferns			
Blechnum spicant	**Aromatic compounds**: benzaldehyde, 2-phenylethanal, 2-phenylethanol, ethyl vanillate. **Polyketide compounds**: (*E*)-2-heptenal, 1-octen-3-ol, 3-octanol, (*e*)-2-decenal, nonanoic acid. **Monoterpenic compounds**: α-pinen, 3.7-dimethyloctan-3-ol; **Sesquiterpenic compounds**: (*E*)-nerolidol. **Carotenoid derivatives**: epoxy-α-ionone, 4-hydroxyepoxy-β-ionol. **Other**: ponasterone-a and 22-hopanol	No tested	Fons et al. (2018), Jizba and Herout (1974)
Botrychium boreale	No data available	No tested	–
Botrychium lanceolatum	No data available	No tested	–
Botrychium lunaria	No data available	No tested	–
Botrychium matricariifolium	No data available	No tested	–
Botrychium multifidum	No data available	No tested	–
Botrychium simplex	No data available	No tested	–
Botrychium virginianum	No data available	No tested	–
Cosentinia vellea	No data available	No tested	–
Cryptogramma crispa	**Lipidic derivatives:** 1-Octen-3-ol, nonanal; Shikimic derivatives: benzaldehyde, benzyl alcohol phenylethanal, 2-phenylethanol, 4-ethenyl-2-methoxyphenol, 4-hydroxy-3-methoxybenzaldehyde (vanillin), 4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzoic acid (vanillic acid); **Carotenoid derivatives** e.g 3,4-dehydro-7,8-dihydro-β-ionone 9-methyl-α-ionol, 3-Hydroxy-5,6-epoxy-ionone. **Flavonoid derivatives**: quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-galactoside, hydroxycinnamic esters mainly chlorogenic acid, and 5-*O*-caffeoylshikimic acid		Veit et al. (1996), Fons et al. (2018)
Cryptogramma stelleri	No data available	No tested	–
Culcita macrocarpa	No data available	No tested	–

(continued)

Table 19.1 (continued)

Ferns			
Cystopteris fragilis	**Xantones derivatives**:1,3-dihydroxy-5,6,7-trimethoxyxantho, mangiferin, isomangiferin and their aglycone 1,3,6–7-tetrahydroxyxanthone and 3-*O*-methylisomangiferin		Imperato (1991a, b)
Cystopteris montana	**Xantones derivatives**:1,3-dihydroxy-5,6,7-trimethoxyxantho, mangiferin, isomangiferin and their aglycone 1,3,6–7-tetrahydroxyxanthone and 3-*O*-methylisomangiferin	No tested	Imperato (1991a, b)
Cystopteris sudetica	**Xantones derivatives**:1,3-dihydroxy-5,6,7-trimethoxyxantho, mangiferin, isomangiferin and their aglycone 1,3,6–7-tetrahydroxyxanthone and 3-*O*-methylisomangiferin	No tested	Imperato (1991a, b)
Davallia canariensis	**Triterpene derivatives:** filic-3-ene, 3-isopropyl-3a,5a,7a,8,11b,13a-hexamethyl-2,3,3a,4,5,5a,5b,6,7,7a,10,11,11a,11b,12,13,13a,13b-octadecahydro-1H-cyclopenta[alpha] chrysene, ($C_{30}H_{50}$)	No tested	Gonzalez-Platas et al. (1999)
Diplazium caudatum	No data available	No tested	–
Diplazium sibiricum	No data available	No tested	–
Dryopteris aemula	**Phloroglucinol derivatives**: albaspidin AB, margaspidin BB and homologs BB and AB, trisaemulin PBB, trisaemulin BBP, and trisaemulin PBP	Anthelmintic	Widén et al. (1976)
Dryopteris affinis	**Phloroglucinol derivatives**: flavaspidic acid AB, flavaspidic acid PB trisphloroaspidc acid ABB, trisphloroaspidc acid BBB, albaspidin BB, albaspidin AB albaspidin AA, filixic acid BBB, filixic acid ABB filixic acid ABA, norflavaspidic acid BB, Norflavaspidic acid AB, tetra-flavaspidic acid BBBB, tetra-albaspidin ABBA, tetraalbaspidin BBBB. **Terpenic derivatives** i.e. carota-5,8-diene, aristolene, (*E*)-nerolidol, aristola-1(10),8-diene, α-selinene, β-selinene, eremophilene. **Polyketide derivatives** as filicinic compounds, propionylfilicinic acid, and acetylfilicinic. The main **lipid derivative** is 1-octen-3-ol	Anthelmintic	Widén et al (1996)
Dryopteris aitoniana	**Phloroglucinol derivatives**: hexa-flavaspidic acid BBBBBB, tetra-albaspidin BBBB, tetra-flavaspidic acid BBBB, penta-albaspidin BBBBB, hexa-albaspidin BBBBBB, hexa-flavaspidic acid BBBBBB	Anthelmintic	Euw et al. (1985)

(continued)

Table 19.1 (continued)

Ferns			
Dryopteris ardechensis	**Phloroglucinol derivatives**: flavaspidic acid BB, flavaspidic acid AB, para-aspidin BB, para-aspidin AB, desaspidin BB, albaspidyn AA, albaspidin AB, albaspidin BB, filixix acid BBB, filixix acid ABA, filixix acid ABB, pentherin I, trisflavaspidic acid ABB, tris-flavaspidic acid BBB. Terpenic derivatives: carota-5,8-diene, (*E*)-nerolidol, α-selinene, aristola-1(10),8-diene, linalool. **The polyketide derivatives:** filicinic compounds mainly propionylfilicinic acid, valeroylfilicinic acid, and butyrylfilicinic acid. **Isoprenoid derivatives**:4-hydroxy-β-ionone, 3-oxo-α-ionone, β-ionone; **Lipid derivatives** such as 3-octanol, decanal and 1-octen-3-ol completed	Anthelmintic	Fraser-Jenkins and Widén (1993), Froissard et al. (2014)
Dryopteris borreri	**Phloroglucinol derivatives**: para-aspidin BB, para-aspidin AB, aspidinol-B, tris-para-aspidin BBB, para-aspidin-BB, para-aspidin AB, flavaspidic acid AB, flavaspidic acid PB, albaspidin BB, albaspidin AB, albaspidin AA, filixic acid BB, filixic acid AB, filixic acid AA, tetra-albaspidin ABBA, tris-flavaspidic acid BBB; **Terpenic derivatives** i.e., (*E*)-nerolidol, eremophilene; **Aromatic components** i.e., 4-hydroxy-3-methoxyacetophenone, 1-octen-3-ol. benzaldehyde, cinnamaldehyde, vanillin, vanillic acid, dihydroactinidiolide	Anthelmintic	Widén et al. (1996), Froissard et al. (2014)
Dryopteris cambrensis	**Phloroglucinol derivatives**: flavaspidic acid AB, flavaspidic acid PB, filixic acid (BBB, ABB, ABA), tris-phloroaspidin acid ABB and BBB, albaspidin AA and BB, tetra-albaspidin, tetra-flavaspidic acid BBBB; **Terpenic derivatives**: carota-5,8-diene, (*E*)-nerolidol, eremophilene; **Aromatic components**: mainly 4-hydroxy-3-methoxyacetophenone; **Isoprenoids derivatives**: β-ionone derivatives, i.e., 4-hydroxy-7,8-dihydro-β-ionone and 4-oxo-7,8-dihydro-β-ionone; **Lipid derivative mainly** 1-octen-3-ol	Anthelmintic	Widén et al. (1996)
Dryopteris carthusiana	**Phloroglucinol derivatives**: aspidin BB, aspidin PB, flavaspidic acid, para-aspidin, desaspidin, albaspidin and homologes (AA,BB, PB, PP), floraspiron, trisflavaspidic acid	Anthelmintic	Euw et al. (1980)
Dryopteris caucasica	**Phloroglucinol derivatives**: aspidinol, flavaspidin, para-aspidin, desaspidin, filixaure, tridesaspidin	Anthelmintic	Widén et al. (1973b)

(continued)

Table 19.1 (continued)

Ferns			
Dryopteris corleyi	**Phloroglucinol derivatives**: aspidinol B, flavaspidic acid BB and AB, albaspidin BB and AB, para-aspidin BB and AB, phloraspin BB, margaspidin BB, trisflavaspidic acid BBB	Anthelmintic	Fraser-Jenkins and Widén (1993)
Dryopteris crispifolia	No data available	Probably anthelmintic	-
Dryopteris cristata	No data available	Anthelmintic	Euw et al. (1980), Widén et al. (1999)
Dryopteris dilatata	**Phloroglucinol derivatives**: aspidinol, para-aspidin BB flavaspidin acid BB, filixic acid BBB, trisflavaspidic acid BBB, metyleno-bis-norflavaspdic acid BBBB, aspidin acid AB, aspidin BB acid, albaspidin acid BB, desaspidin acid BB, floraspin acid AB, floraspidin BB, floraspironu BB acid, florpironu-BB, ortodesaspidin BB acid, trisaspidin BBB acid, trisdesaspidin BBB acid, metyleno-bis-desaspidinol **Flavonoids and polyphenols derivatives**: astragalin, isoquercetin, chloragenic acid kafatar acid, kaempferol-3-*O*-*β*-D-glukozydo-7-*O*-*α*-L-ramnopiranozyd; **Isoprenoids:** β-ionone derivatives, i.e., 4-hydroxy-7,8-dihydro-β-ionone and 4-oxo-7,8-dihydro-β-ionone	Anthelmintic	Euw et al. (1980), Vogler et al. (2012), Froissard et al. (2011)
Dryopteris expansa	**Phloroglucinol derivatives**: flavaspidin acid BB, albaspidin BB, para-aspidin, desaspidin, albaspidin BB, floraspiron, trisdesaspidin BBB, filixic acid	Anthelmintic	Euw et al. (1980)
Dryopteris filix-mas	**Phloroglucinol derivatives**: flavaspidin acid BB, filixic acid BBB, PBB, and PBP, desaspidin AB, para-aspidin AB, para-aspidin PB, trispara-aspidin BBB, trisdesaspidin BBB, tris-flavaspidin BBB acid, tetra-flawaspidin BBBB, albaspidin BB **Flavonoids and polyphenols derivatives**: astragalin, izoquercetin, chloragenic acid, rutin, kaempferol -3-*O*-rutynozyd	Anthelmintic	Euw et el. (1980), Widén et al. (1999, 2015), Vogler et al. (2012)

(continued)

Table 19.1 (continued)

Ferns			
Dryopteris fragrans	**Phloroglucinol derivatives**: disflavaspidic acid PB flavaspidic acid AB,PB, BB, albaspidin AP, BB, PB, PP, dryofragin, saroaspidin A, methylene-bis-aspidinol BB **Phenolic glycoside:** dryofragone, dryofracoumarin A and B, chromone glycoside frachromone C, coumarin glycoside dryofracoulin A and undulatoside A; 3,5-dimethyl-6-hydroxy-2-methoxy-4-*O*-D-glucopyranosyl-oxy-acetophenone; **Sesquiterpene and glucoside**: dryofraterpene A (7*S*, 10*S*)-2,3-dihydroxy-calamenene-15-carboxylic acid methyl ester), 3-*O*-β-D-glucopyranosylalbicanol-11-*O*-β-D-glucopyranoside, dihydroconiferylalcohol, (*E*)-3-(4-hydroxyphenyl) acrylic acid, esculetin (4), 5,7-dihydroxy-2-hydroxymethylchromone, eriodictyol, isoorientin, xianglinmaojuesides A-C, 3β,11-dihydroxy-drim-8 (12)-en-11-O-beta-D-glucopyranoside, 3 β,11-dihydroxy-drim-8(12)-en-3-*O*-β -D-glucopyranoside, 11,14-dihydroxy-drim-8(12)-en-11-*O*- β -D-glucopyranosid	Antifungal and fungicidal, anticancer, anti-oxidation, and anti-inflammation activities, immunomodulatory activity, Antibacterial	Kuang et al. (2008, 2009), Huang et al. (2014), Peng et al. (2016), Xueping et al. (2018), Liu et al. (2018), Zhong et al. (2017), Zhang et al. (2018), Hua et al. (2018)
Dryopteris guanchica	No data available	No tested	-
Dryopteris intermedia	No data available	No tested	-
Dryopteris lacunosa	No data available	No tested	-
Dryopteris mindshelkensis	No data available	No tested	-
Dryopteris oligodonta	**Phloroglucinol derivatives** Filixsäure BBB, PBB and PBP	Anthelmintic	Widén et al. (1973a)
Dryopteris oreades	**Phloroglucinol derivatives**: norflavaspidic acid BB, AB, flavaspidic acid BB, Ab, filixic acid BBB, AbA, trisflavaspidic acid BBB, ABB; **Terpenic compounds**: Aristola-1(10),8-diene, Carota-5,8-diene, Eremophilene, (*E*)-Nerolidol	Anthelmintic	Froissard et al. (2014), Widén et al. (1996)
Dryopteris pallida	The Phloroglucinols of the *Dryopteris-villarii* Complex and Some Related Ferns (Dryopteridaceae)	Probably anthelmintic	
Dryopteris pseudodisjuncta	**Phloroglucinol derivatives:** flavaspidic acid BB, BA, albaspidin BB, BA, AA	Probably anthelmintic	Widén et al. (1996)

(continued)

Table 19.1 (continued)

Ferns			
Dryopteris remota	**Phloroglucinol derivatives**: para-aspidin BB, trispara-aspidin BBB, flavaspidic acid, aspidin, desaspidinol, albaspidin, trisdesaspidin, trispara-aspidin PBB, tetraflavaspidic acid **Isoprenoid derivatives**: 4-hydroxy-5,6-epoxyionol, 4-oxo-7,8-dihydro-β-ionone, 3-oxo-α-ionol; **Lipid derivatives**: 1-octen-3-ol, 3-hexenoic, nonanal,3-octanol, benzyl alcohol, benzaldehyde	Probably anthelmintic	Euw et al.(1980), Wollenweber et al. (1998), Froissard et al. (2014)
Dryopteris schorapanensis	No data available	No tested, probably anthelmintic	-
Dryopteris tyrrhena	**Phloroglucinol derivatives:** norflavaspidic acid BB, flavaspidic acid AB, para-aspidin AB, albaspidin BB, BA, filixic acid BBB, ABA, ABB, trispara-aspidin BBB, trisflavaspidic acid BBB, ABB	No tested, probably anthelmintic	Fraser-Jenkins and Widén (1993)
Dryopteris villarii	**Phloroglucinol derivatives**: filixic acid BBB, para-aspidin flavaspidic acid, desaspidin acid BB, albaspidin AB, AP, BB, PB and BB) **Flavonoids and polyphenols derivatives:** 7-*O*-glukoside-4'- apigenin acetyl, 3-*O*-ramnosid-7-*O*-glukoside quercetin	No tested, probably anthelmintic	Euw et al. (1980), Widén et al.(1996, 2015), Euw et al. 1980, Wollenweber et al. (1998), Imperato (2008)
Elaphoglossum semicylindricum	No data available	No tested	–
Grammitis azorica	No data available	No tested	–
Grammitis jungermannioides	No data available	No tested	–
Grammitis quaerenda	No data available	No tested	–
Gymnocarpium dryopteris	**Isoprenoids:** β-ionone derivatives, i.e., 4-hydroxy-7,8-dihydro-β-ionone and 4-oxo-7,8-dihydro-β-ionone	No tested	Froissard et al. (2011)
Gymnocarpium jessoense	No data available	No tested	–
Gymnocarpium robertianum	No data available	No tested	–
Hymenophyllum maderense	No data available	No tested	–
Hymenophyllum tunbrigense	No data available	No tested	–
Hymenophyllum wilsonii	No data available	No tested	–
Marsilea aegyptiaca	No data available	No tested	–

(continued)

Table 19.1 (continued)

Ferns			
Marsilea batardae	No data available	No tested	–
Marsilea quadrifolia	No data available	No tested	–
Marsilea strigosa	No data available	No tested	–
Onoclea (*Matteuccia*) *struthiopteris*	**Flavonoids, phenolics, stilbenes, and steroids, about 20 flavonoid glycosides**: matteflavosides A − G e.g. matteflavoside A, matteflavoside B, kaempferol-3-*O*-*β*-D-glucopyranoside, kaempferol-3-*O*-*β*-D-glucopyranosyl-7-*O*-*α*-L-rhamnopyranoside, kaempferol-3,7-di-*O*-*α*-L-rhamnopyranoside, kaempferol-*3*-*O*-(α-L-3-O-acetylrhamnopyranosyl)-7-*O*-*α*-Lrhamnopyranoside, kaempferol-*3*-*O*-(*α*-L-2-*O*-acetylrhamnopyranosyl)-7-*O*-*α*-L-rhamnopyranoside (12),24 kaempferol-3-*O*-(*α*-L-4-*O*-acetylrhamnopyranosyl)-7-*O*-*α*-L-rhamnopyranoside (13),25 kaempferol-*3*-*O*-[*β*-D-glucopyranosyl-(1 → 2)-*α*-L-rhamnopyranosyl]-7-*O*-*α*-L-rhamnopyranoside, kaempferol-3-*O*-[1,2,4-trihdroxy-3-oxo-5-methyltetrahydropyran-(1 → 2)-*α*-L-rhamnopyranosyl]-7-O-*α*-L-rhamnopyranoside, protoapigenone ophiofolius A apigenin-4′-*O*-*β*-D-glucopyranoside and matteuorien. **About 103 volatile** components, and (E)-phytol (24.8%), nonanal (15.1%), decanal (7.6%) as the main compounds. **Ecdysteroids** e.g. 20E, Pterosterone	Antioxidant, antiviral activity against the influenza A(H1N1) virus, prevention of influenza, Japanese encephalitis, and viral parotitis	Li et al. (2015), Miyazawa al. (2007), Takemoto et al. (1967)
Ophioglossum azoricum	No data available	No tested	–
Ophioglossum lusitanicum	No data available	No tested	–
Ophioglossum polyphyllum	No data available	No tested	–
Ophioglossum vulgatum	**The glycosylated and acylated flavonols**: quercetin-3-*O*-[(6-caffeoyl)-*β*-glucopyranosyl (1 → 3) *α*-rhamnopyranoside]-7-*O*-*α*-rhamnopyranoside, kaempferol-3-*O*-[(6-caffeoyl)-*β*glucopyranosyl (1 → 3) *α*-rhamnopyranoside]-7-*O*-*α*-rhamnopyranoside, quercetin-3-*O*-methyl Ether phenylpropanoid derivatives including *p*-hydroxybenzoate and *p*-coumarate	Extract was found to be active against the bovine viral diarrhoea virus. Also active in scratch-wound healing assays on keratinocytes	Clericuzio et al. (2012), Xue et al. (2020)
Osmunda regalis	Hexahydrofarnesyl acetone, 2,4-di-t-butylphenol, and phytol	Cytotoxic and antiviral	Bouazzi et al. (2018)

(continued)

Table 19.1 (continued)

Ferns			
Paragymnopteris marantae	No data available	No tested	–
Pellaea calomelanos	No data available	No tested	–
Phegopteris connectilis	**Isoprenoids:** β-ionone derivatives, i.e., 4-hydroxy-7,8-dihydro-β-ionone and 4-oxo-7,8-dihydro-β-ionone	No tested	Froissard et al. (2011)
Pilularia globulifera	No data available	No tested	–
Pilularia minuta	No data available	No tested	–
Polypodium cambricum	**Triterpenoids**	Immunomodulatory activity	Lombardi et al. (2005)
Polypodium interjectum	**Triterpenoids, Coumarins, Saponins, Polyphenols,**	Antibacterial	Bahadori et al. (2015a,b)
Polypodium vulgare	Ecydosteroids e.g.ecdysone, ecdysterone and 5β-hydroxyecdysterone. Saponins e.g Osladin, Triterpenoids, Phenolic compound	Immunomodulatory activity, antiepileptic, analgesic activity, protective effect in various neurological and neurodegenerative disorders, stimulatory effect on the adrenoceptors, antioxidant properties, antibacterial, antibiofilm properties	De Souza et al. (1970), Grzybek 1983, Lombardi et al. (2005), Messeguer (1998), Dinan (2001), Yao et al. (2012), Dar et al. (2012), Gleńsk et al. (2019), Bagniewska-Zadworna et al. (2008)
Polystichum aculeatum	**Anthraquinones, Quinones, Cardiac glycosides**	Antibacterial	Bahadori et al. (2015)
Polystichum braunii	**Phenolic and flavonoid**: kaempferol, quercetin and, gallic, coumaric, syringic, sinapic, vanillic, caffeic, and benzoic acids	Anti-inflammatoryand antiarthritic potential	Saleem et al. (2020)
Polystichum drepanum	No data available	No tested	–
Polystichum falcinellum	No data available	No tested	–
Polystichum lonchitis	No data available	No tested	–

(continued)

Table 19.1 (continued)

Ferns			
Polystichum setiferum	**Aromatic compounds**: Benzaldehyde, Acetophenone, Benzoic acid, 4-Amino-2-methoxyphenol, Isovanillin, Methyl vanillate, 4-Hydroxybenzoic acid **Polyketide compounds**: (E)-2-and (E)-3 Hexenal, 1-Octen-3-ol and 1-Octen-3-one, (E)-3-Hexenoic acid, Nonanal, (E)-2-Nonenal, (E)-2-Nonenol. Monoterpenic compounds: α Pinene, trans–*trans*-Linalool oxide (pyran). **Isoprenoid derivatives**: 3-Hydroxy-5,6-epoxy- β -ionone, 3-Oxo- α -ionol and other	No tested	Froissard et al. (2011)
Psilotum nudum	**Arylpyrones, arylpyrone mad biflavonoid glykosides**: 3'-hydroxypsilotin, 3'-hydroxypsilotinin-di-*O*-hexoside, psilotin and psilotinin-di-*O*-hexoside, 3'-hydroxypsilotinin, apigenin-6,8-di-C-glucoside, amentoflavone-di-Hexosideamentoflavone-tri-hexoside, apigenin-7-*O*-glucoside(apigentrin, cosmosin), apigenin-7-*O*-rhamnoglucoside (rhofolin), dihydroamentoflavone-hexoside, amentoflavone-hexoside, hydroxy-amentoflavon, 2,3-dihydro-amentoflavon, amentoflavon, 2" 3"-dihydro-amentoflavon, robustaflavon, dihydro-*O*-methyl-amentoflavon, *O*-methyl-amentoflavone (tentative), binaringenin, dihydrohinokiflavone (tentative), hinokiflavon, hinokiflavone(4',6"-O-biapigenin), robustaflavone(3',6"-biapigenin), amentoflavone(3',8"-biapigenin), 2,3-dihydro-amentoflavon, apigenin, β-sitosterol, campesterol and stigmasterol,	No tested	Šamec et al. (2019)
Pteridium aquilinum	**Monoterpenes, Sesquiterpenes** such as ptaquilosides, pterosins A and B, p-coumaric acid, p-hydroxybenzoic acid, caffeic acid, ferulic acid, vanillic acid, protocatechuic acid, kaempferol, quercetin, and apigenin, p astragalin, **Ecdysteroids**, probably **alkaloids**	Antioxidant activity, carcinogenic and cytotoxic, hepartotoxic (Ptaquilosides), astringent, anthelmintic, useful in diarrhoea	Francisco and Cooper-Driver, (1984), Kovganko et al. (2004), Nwiloh et al. (2014), Vetter (2010, 2018)
Pteridium pinetorum	Since the species was not distinguished before from the *Pteridium aquilinum* species, its chemical composition is likely to be as done	No tested, probably like Pteridium aquilinum	-

(continued)

Table 19.1 (continued)

Ferns			
Pteris cretica	**Pterosin-type sesquiterpenoids** e.g. pterosins, creticolacton A, 13-hydroxy-2(R),3(R)-pterosin L, creticoside A, and spelosin 3-*O*-*β*-d-glucopyranoside **Flavonoids** including rutin, quercitrin, Luteolin, Luteolin 8-C-rhamnoside-7-*O*-rhamnoside, apigenin, and luteolin-7-*O*-glucoside, n luteolin 7-*O*-rutinoside, luteolin 7-*O*-glucoside	Cytotoxic activity against HCT-116 cells, antimicrobial and antioxidant	Lu et al. (2019), Hou et al. (2019), Saleem et al. (2016), Imperato (1994a, b)
Pteris dentata	No data available	No tested	–
Pteris incompleta	No data available	No tested	–
Pteris vittata	**Phenolics**, rutin, kaempferol monoglycoside, kaempferol diglycoside, quercetin monoglycoside, quercetin, diglycoside, maybe alkaloids	Antimicrobial/antioxidant/ antiproliferative	Salatino and Prado (1998), Singh et al. (2008), Vetter (2010, 2018)
Salvinia natans	**Dibenzoyl glycoside** - natansnin, Methylbenzoate 3,4-dihydroxy methylbenzoate, natansnin, hypogallic acid, caffeic acid, paeoniflorin, pikuroside	Antioxidant effect, antibacteroal	Srilaxmi et al. (2010), Al-Maliki et al. (2017)
Thelypteris dentata	**Alkaloids, Phytosterols, Phytosterols, Phenols, Saponins, Tanins**	Antibacterial activity	Manhas et al. (2018)
Thelypteris limbosperma	**Aromatic compounds**: benzaldehyde, 2-phenylethanal, 2-phenylethanol, benzoic acid, cinnamic acid, ethyl vanillate, vanillic acid. **Polyketide compounds**: 1-octen-3-ol, 3-octanone, 3-octanol, (Z)-3-hexenoic acid, (E)-2-hexenoic acid, nonanoic acid, (Z)-6-dodecen-4-olide. **Monoterpenic compounds**: *α*-pinene, *β*-pinene, terpinen-4-ol, limonene, *α*-terpineol, cis-dihydrocarvone, trans-dihydrocarvone, 8.9-dihydrocarveol, *γ*-terpinen-7-al, perillic acid, carvone hydrate. **Sesquiterpenic compounds**: *β*-caryophyllene, (E)-nerolidol	No tested	Fons et al. (2010)
Thelypteris palustris	**Flavonoid and its glycoside**: (2*S*)-5,7-dihydroxy-6-methylflavanone 7-*O*-*β*-D-gluco- pyranoside, cryptostrobin ((2*S*)-5,7-dihydroxy-8-methylf1avanone), kaempferol 3-O-*β*-D-glucopyranoside and kaempferol 3- O-*β* -rutinoside	No tested	Murakami et al. 1986
Thelypteris pozoi	No data available	No tested	–
Trichomanes speciosum	No data available	No tested	–
Woodsia alpina	No data available	No tested	–

(continued)

Table 19.1 (continued)

Ferns			
Woodsia glabella	No data available	No tested	–
Woodsia ilvensis	**Essential oils: monoterpenes, diterpenes, sesquiterpenes, alcohols, aldehydes, acids; tannins**	Antipyretic, wound healing; for kidney diseases	Aibuldinov et al. (2012), Minarchenko et al. (2017)
Woodsia pulchella	No data available	No tested	–
Woodwardia radicans	No data available	No tested	–
Horsetails			
Species	**Metabolites**	**Biological activity**	**Literature**
Equisetum arvense	**Flavonoids, caffeic acid derivatives, phytoosterols, tannin, triterpenoids and phenolics such as:** styrylpyrones and phenolic acids, isoquercetin, quercetin 3-*O*-glucoside, quercetin, 3-*O*-(6"-*O*-malonylglucoside), 5-*O*-caffeoyl, 3'- deoxyequisetumpyrone (3, 4-hydroxy-6-(4'-hydroxy-D-styryl)-2-pyrone-3-*O*- -D-glucopyranoside), 4'-omethylequisetumpyrone (3,4-hydroxy-6-(3'-hydroxy-4'-methoxy-estyryl)-2-pyrone-3-*O*-D-glucopyranoside, kaempferol 3-*O*-sophoroside-7-*O*-glucoside, kaempferol 3-*O*-(6″-O malonylglucoside)-7-*O*-glucoside, kaempferol 3-*O*-sophoroside, quercetin3-*O*-glucoside, apigenin, apigenin 5-*O*-glucoside, luteolin, luteolin 5-*O*-glucoside, genkwanin 5-*O*-glucoside and isoquercitrin (mesotartaric acid, monocaffeoyl meso-tartaris acid dihydroactinidiolide, phenolic glycosides such as equisetumoside A, equisetumoside B and equisetumoside C, onitin, onitin-9-*O*-glucoside, monocaffeoyl meso-tartaris acid, di-*E*-caffeoyl-mesotartaric acid, hexahydrofarnesyl acetone, cis-geranyl **Alkaloids** nicotine	Hepatoprotective activities, antioxidant, anti-inflammatory, analgesic, antibacterial, antifungal, antitumor and neuroprotective effects, anticonvulsant, antibacterial, antifungal, anti haemorrhagy, astringent, diuretic, hepatoprotective, antiviral (HIV), vasorelaxant, cardioprotective and vulnerary effects	Signe et al. (2020), Asgarpanah and Elnaz Roohi (2012), De Monte (2004), Oh et al. (2004), Radulovic et al. (2006), Milovanovic et al. (2007), Aulakh et al.2019, Phillipson and Melville (1960)

(continued)

Table 19.1 (continued)

Horsetails			
Equisetum fluviatile	**Protoflavonoid**s: 2′,3′-dihydroprotogenkwanone, 2′,3′-dihydro-2′-hydroxyprotoapigenone; **Flavonoids and caffeic acid derivatives**: apigenin, apigenin 4'-*O*-glucoside, kaempferol 3-*O*-glucoside, Kaempferol 3-*O*-(6"-*O*-malonylglucoside)-glucoside, kaempferol 3,7-*O*-diglucoside, 5-*O*-caffeoyl shikimic acid, monocaffeoyl *meso*-tartaric acid, monocaffeoylmeso-tartaric acid, Dicaffeoyl *meso*-tartaric acid **Essential oil:** hundred ninety-one compounds -major contributors hexadecanoic acid, (*E*)-phytol, and hexahydrofarnesyl acetone **Alkaloids:** nicotine, palustrine. **Other**: methyl methionin sulfonate, dimethyl sulfide, and dimethyl sulfone. aconitic acid, also known as equsetic acid (horsetail acid), magnesium aconitate, in addition to calcium oxalate, potassium aconitate, saponins	Diuretics, antioxidant, antimicrobial	Radulovic et al. (2008), Pouny et al. (2011), Wright et al. (2007), Milovanovic et al. (2007), Wróbel and Różański (2020), Phillipson and Melville (1960)
Equisetum hyemale	No data available	No tested, probably diuretics, antioxidant, antimicrobial	-
Equisetum palustre	**Flavonoids and caffeic acid derivatives**: Kaempferol, kaempferol diglucoside, Kaempferol 3-*O*-glucoside, Kaempferol 3'-*O*-rutinoside, Kaempferol 3-*O*-rutinoside-7-*O*-sophoroside, Kaempferol 3-*O*-rutinoside-7-*O*-glucoside, Monocaffeoyl *meso*-tartaric acid	No tested, probably diuretics, antioxidant, antimicrobial	Radulovic et al. (2008), Pouny et al. (2011), Wright et al. (2007), Milovanovic et al. (2007)
Equisetum pratense	No data available	No tested, probably diuretics, antioxidant, antimicrobial	–
Equisetum ramosissimum	No data available	No tested, probably diuretics, antioxidant, antimicrobial	–
Equisetum scirpoides	No data available	No tested, probably diuretics, antioxidant, antimicrobial	–
Equisetum sylvaticum	**Flavonoids and caffeic acid derivatives:** quercetin 3-*O*-glucoside, kaempferol 3-*O*-glucoside, kaempferol 3'-*O*-rutinoside, kaempferol 3,7-*O*-diglucoside,kaempferol 3-*O*-glucoside-7-*O*-rhamnoside, kaempferol 3-*O*-rutinoside-7-*O*-glucoside, 5-*O*-Caffeoyl shikimic acid, monocaffeoyl *meso*-tartaric acid	No tested, probably diuretics, antioxidant, antimicrobial	Wright et al. (2007), Milovanovic et al. (2007), Radulovic et al. (2008), Pouny et al. (2011)

(continued)

Table 19.1 (continued)

Horsetails			
Equisetum telmateia	**Flavonoids and caffeic acid derivatives:** kaempferol, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-(6”-*O*-acetylglucoside), kaempferol 3,7-*O*-diglucoside, kaempferol 3-*O*-(6”-*O*-acetylglucoside)-7-*O*-glucoside, kaempferol 3-*O*-glucoside-7-*O*-rhamnoside, kaempferol 3-*O*-(6″-*O*-acetylglucoside)-7-*O*-rhamnoside, kaempferol 3-*O*-rutinoside-7-*O*-glucoside, 5-*O*-caffeoyl shikimic acid, monocaffeoyl *meso*-tartaric acid	No tested, probably diuretics, antioxidant, antimicrobial	Wright et al. (2007), Milovanovic et al. (2007), Radulovic et al. (2008), Pouny et al. (2011)
Equisetum variegatum	No data available	No tested, probably diuretics, antioxidant, antimicrobial	-
Clubmosses			
Species	**Metabolites**	**Biological activity**	**Literature**
Dipasiastrum alpinum	**Alkaloids**: clavolonine (8b-hydroxylycopodine), lycoclavine, lycopodine, des-N-methyl-α-obscurine, 8S-O-acetylepiclavolonine,	Antioxidant/acetylcholinesterase and/or butyrilcholinesterase inhibitors	Ma and Gang (2004), Halldorsdottir et al. (2008, 2013, 2015),
Diphasiastrum complanatum	**Flavones derivatives**: syringic acid, chrysoeriol, apigenin; Terpene derivatives: serratenediol, tohogenol; **Alkaloids**: lycopodine, complanadine A, flabellidine (1,2,3-trihydro-*N*α-Ac-lycodine), 1-hydroxylycodine, N-methyl-lycodine, lyconadine A, lycopladine F, G, H, lycospidine A, dehydroisofawcettiine n-oxide, lyconadins G, H, lycoplanine A, nicotin	Antioxidant, acetylcholinesterase and/or butyrilcholinesterase inhibitors	Manske and Marion (1942), Inubushi et al. (1964), Voirin and Labreton (1967), Voirin and Jay (1978), Ishiuchi et al. (2009b,c, 2011a,b), Cheng et al. (2013), Czapski et al. (2014), Szypuła Pietrosiuk (2021)
Diphasiastrum issleri	**Alkaloid** lycopodine	No tested, probably antioxidant, acetylcholinesterase and/or butyrilcholinesterase inhibitors	Ma and Gang 2004
Diphasiastrum madeirense	No data available, probably **alkaloids**	No tested, probably antioxidant/acetylcholinesterase and/or butyrilcholinesterase inhibitors	-

(continued)

Table 19.1 (continued)

Clubmosses			
Diphasiastrum oellgaardii	No data available, probably **alkaloids** as in *D. alpinum* and *D. tristachyum* (parent species)	No tested, probably antioxidant/acetylcholinesterase and/or butyrilcholinesterase inhibitors	-
Diphasiastrum tristachyum	Lycopodine	No tested, probably antioxidant/acetylcholinesterase and/or butyrilcholinesterase inhibitors	Ma and Gang (2004)
Diphasiastrum zeilleri	No data available, probably **alkaloids** as *D. complanatum* and *D. tristachyum* (parent species)	No tested, probably antioxidant/acetylcholinesterase and/or butyrilcholinesterase inhibitors	–
Huperzia selago	**Alkaloids**: huperzine A (selagine), huperzine B, acrifoline, 12-epilycodoline (pseudoselagine), lycodoline, licopodine, selagoline, serratidinina, 6β-hydroxyhuperzine A, α- and β-obscurine, serratine and lucidolin deacetylfawcettine, fawcettimine, 16-hydroxyhuperzine B, deacetyllycoclavine, annopodine, lycopecurine, des-*N*-methylfastigiatine and flabelline	Anti-oxidation, anti-inflammation, neuroprotective	Ma and Gang (2004), Czapski et al. (2014), Lenkiewicz et al (2016), Szypuła et al. (2020),
Huperzia dentata	No data available	No tested	–
Huperzia suberecta	No data available	No tested	–
Lycopodiella cernua (*Palhinhaea cernua*)	**Alkaloids**: lycopodine, anhydrolycocernuine, cernuine (deoxylycocernuine), cernuine *N*-oxide, lycocernuine (12α-hydroxycernuine)	No tested, propably antioxidant/acetylcholinesterase and/or butyrilcholinesterase inhibitors	Ma and Gang (2004)
Lycopodiella inundata	**Alkaloids**: anhydrolycodoline (D11,12-lycopodine), clavolonine (8β-hydroxylycopodine), lycodoline, lycopodine, lycoflexine, anhydrolycocernuine (deoxy-D12,13-lycocernuine), lycocernuine (12α-hydroxycernuine)	No tested propably antioxidant/acetylcholinesterase and/or butyrilcholinesterase inhibitors	Ma and Gang (2004)

(continued)

Table 19.1 (continued)

Clubmosses			
Lycopodium annotinum (= *Spinulum annotinum*)	**Alkaloids e.g.** acetylfawcettiine, acrifoline [$D^{11,12}$,8-oxo-dihydrolycopodine (5β-OH)], acrifolinol, annotinine, annotine, lycodoline, lycopodine, Annotinolide F, Lycoannotine A-I, Lannotinidines H-J	Antioxidant/acetylcholinesterase and/or butyrilcholinesterase inhibitors	Ma and Gang (2004), Halldorsdottir et al. (2008, 2010) Tang te al. (2017), Ishiuchi et al. (2009a)
Lycopodium clavatum	**Alkaloids**: acetyldihydrolycopodine, acetylfawcettiine, acetyllycoclavine, anhydrolycodoline ($D^{11,12}$-lycopodine), clavolonine (*8β*-hydroxylycopodine), deacetylfawcettiine, deacetyllycoclavine (*O*-des-Ac-lycoclavine), dihydrolycopodine (5*α* -OH), fawcettiine, flabelliformine (4*α*-hydroxylycopodine), lycoclavine, lycodoline, lycopodine, des-*N*-methyl-α-obscurine, lycodine, *α*-obscurine (2,3-dihydro-*β*-obscurine), fawcettimine, lycoflexine, lycopoclavamine A and B, lycoclavatumide, 8*β*,11*α*-dihydroxylycopodine	Antioxidant/acetylcholinesterase and/or butyrilcholinesterase inhibitors	Ma and Gang (2004), Pongpamorn et al. (2016), Katakawa et al. (2011)
Quillworts			
Species	**Metabolites**	**Biological activity**	**Literature**
Isoëtes azorica	No data available	No tested	–
Isoëtes boryana	No data available	No tested	–
Isoëtes creussensis	No data available	No tested	–
Isoëtes delilei	No data available	No tested	–
Isoëtes durieui	No data available	No tested	–
Isoëtes echinospora	No data available	No tested	–
Isoëtes fluitans	No data available	No tested	–
Isoëtes gymnocarpa	No data available	No tested	–
Isoëtes haussknechtii	No data available	No tested	–
Isoëtes heldreichii	No data available	No tested	–
Isoëtes histrix	No data available	No tested	–
Isoëtes iapygia	No data available	No tested	–

(continued)

Table 19.1 (continued)

Quillworts			
Isoëtes lacustris	No data available	No tested	–
Isoëtes longissima	No data available	No tested	–
Isoëtes malinverniana	No data available	No tested	–
Isoëtes phrygia	No data available	No tested	–
Isoëtes sabatina	No data available	No tested	–
Isoëtes tenuissima	No data available	No tested	–
Isoëtes tiguliana	No data available	No tested	–
Isoëtes todaroana	No data available	No tested	–
Spikemosses			
Species	**Metabolites**	**Biological activity**	**Literature**
Selaginella denticulata	**Flavonoid**: robustaflavone 4'-methyl ether, robustaflavone, 4'-dimethyl ether, 2",3"-dihydrorobustaflavone, 4'-dimethyl ether, 2", 3" dihydrorobustaflavone, 4', 7"-trimethyl ether, robustaflavone, amentoflavone, caffeoylquinic acids, 3,5-di-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, chamaecyparin, 2,3-dihydroisocryptomerin, delicaflavone, delicatulines A, B, Cryptomerin B, Hinokiflavone, Isocryptomeri, Sotetsuflavone, Robustaflavone, alkaloids	Anti-tumor	Lin et al. (2000), Lin and Chou (2000), Yao et al. (2018), Lopez-Saez et al. (1994a, 1995)
Selaginella helvetica	**Flavonoids**: ginkgetin, amentoflavone, hinokiflavone, heveaflavone alkaloids, sterols	No tested	Jiang et al. (2018)
Selaginella kraussiana	**Hinokiflavone, Amentoflavone**	No tested	Qasim et al. (1985)
Selaginella selaginoides	**Amentoflavone, Hinokiflavone, Robustaflavone**	anti-inflammation, anti-oxidation, anti-diabetes, and anti-senescence effects	López-Sáez et al. (1994a, b, 1995), Yu et al. (2017)

2013; Szypuła and Pietrosiuk 2020). Below are presented current data on the use of tissue culture methods in biotechnology of selected groups of ferns in Europe.

19.5.1 The Lycopodiaceae

In Europe, 14 club moss species occur and three of these have been studied using tissue culture techniques. As early as the 1950s, first in vitro cultures of the gametophytes of *L. complanatum* i *H. selago* were established (Freeberg i Wetmore 1957; Freeberg 1957). The researchers tested the impact of various methods of scarification on spore germination and next investigated the morphology of the gametophytes they obtained and the effect of some endophytic fungi on the gametophyte development. They were the first to observe apogamous development of sporophytes in the gametophyte cultures of *H. selago.* In recent years, procedures for establishing and maintaining in vitro cultures of *H. selago* sporophytes and gametophytes have been developed which use a variety of starting material: shoot fragments, vegetative propagules, somatic embryos, and spores (Szypuła et al. 2005, 2013, 2020; Szypuła and Pietrosiuk 2021) (Fig. 20.9). Establishing sporophyte cultures using vegetative propagules as the initiating material is a fast and effective method and vegetative propagules seem superior to shoot fragments in this respect (Szypuła et al. 2005). Initiating cultures of *H. selago* sporophytes from shoot fragments procured from sporophytes growing in their natural locations is effective but require collection of large amounts of plant material which subsequently needs time-consuming and complicated decontamination and disinfection (Szypuła et al. 2005) (Fig. 20.9a–c). Obtaining propagules from plants growing in the wild does not demand cutting off parts of rare plants often protected by the law as propagules are formed spontaneously in the apical shoot parts of *H. selago* sporophytes. When the decontamination and disinfection protocol proposed by Szypuła et al. (2013) is followed, up to 90% of explants are free from contaminants. Sporophytes developed from the propagules and grew roots. The optimal results were achieved using Moore medium without plant growth regulators or supplemented with 0.05 μM IBA and 1.4 μM kinetin (Szypuła et al. 2013) which ensured both viability of the propagules and their further development (Fig. 19.9a, c). The biomass growth index for *H. selago* sporophytes grown from propagules, determined at 3 months of culture (1 passage) on Moore medium ensuring optimal growth was 650% and at 6 months the biomass growth index increased to 1114%. Callus proliferation was also observed in the cultures of *H. selago* and *Lycopodiella inundata* sporophytes (Atmane et al. 2000; Szypuła et al. 2013.) Callus was initiated in the apical meristem of shoots exclusively on Moore medium supplemented with plant growth regulators, i.e. 0.05 μM IBA and 1.4 μM kinetin (Atmane et al. 2000). There have been only a few published studies on callus induction in club mosses. De Maggio (1964) described induction of gametophyte callus of *L. obscurum* on culture media without plant growth regulators. Bienaime et al. (2015) established cultures of *Lycopodiella inundata* callus and isolated

alkaloids from the biomass. They determined the effects of plant growth regulators on biomass increase and on alkaloid accumulation which reached 1% (alkaloid weight/dry weight). Apart from tissue cultures of sporophyte callus and cultures of shoots and sporophytes initiated from propagules, somatic embryogenesis in the Lycopodiaceae family has been documented for two species, *Lycopodiella inundata* and *Huperzia selago* (Atmane et al. 2000; Szypuła et al. 2005). In both cases, somatic embryos were obtained which generated sporophytes free from contaminants such as fungi and endophytic bacteria. Long-term commercial uses of plant tissue culture require an effective method of plant material disinfection and elimination of endophytes. The use of plant material, i.e. shoots and propagules, to obtain sporophytes for potential alkaloid synthesis significantly reduces the scale of the process. So far, somatic embryogenesis is the only approach to initiate axenic culture of club moss sporophytes. However, considering a limited number and scope of studies on somatic embryogenesis in Lycopodiaceae and its low-efficiency further research is needed, especially into the mechanisms of embryogenesis induction.

Recently, an effective method has been presented of establishing in vitro axenic cultures of *H. selago* gametophytes using spores from sporophytes procured from natural populations (Szypuła et al. 2020) (Fig. 19.9d-f). Prothalli were obtained after 7–18 months and the optimal results were achieved on the medium originally developed by Whittier and Storchova (2007) and on Moore medium as modified by Freeberg and Wetmore (1957. Cultures on these media yielded from 90 to 100% of viable, rapidly growing gametophytes. The best biomass growth index for prothallus calculated for fresh (FW) and dry weight (DW), determined at 24 weeks of culture was 2500% (FW) and 2200% (DW). The content of the alkaloid huperzine A ranged from 0.74 mg/g DW to 4.73 mg/gDW which is the maximum reported for club mosses. The results obtained by Szypuła et al. (2020) demonstrate that using *H. selago* gametophytes it is possible to increase huperzine A biosynthesis by approximately 42% compared to the sporophyte culture while the yield of this alkaloid is even 35-fold higher than from *H. serrata*, which is used by the pharmaceutical industry.

A literature search yielded no information on in vitro cultures of other club mosses native to Europe. However, the micropropagation of the gametophytes of *Huperzia selago* and *Diphasiastrum complanatum* using spores collected in the wild and initiation of cultures using shoots, propagules or somatic embryos opens up new perspectives for developing protocols for micropropagation of rare, endemic, or endangered taxa, such as *Diphasiastrum madeirense* (J.H.Wilce) Holub (*Lycopodium madeirense* J.H.Wilce) (Szypuła et al. 2020).

19.5.2 The Selaginellaceae

To date, reports on establishing in vitro cultures of two European spike moss species, meadow spike moss *Selaginella apoda* (Schulz et al. 2010), considered a model species, with a short life cycle and *Selaginella kraussiana* (Webster 1979) have been published. The cultures were initiated from megasporess which developed into

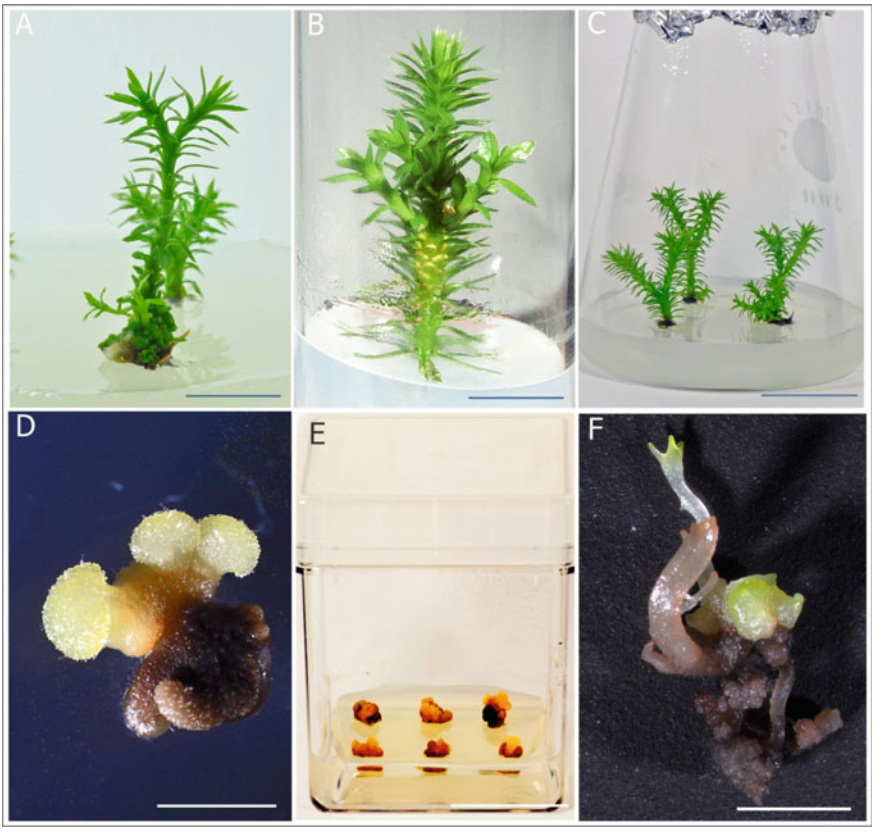


Fig. 19.9 In vitro culture of sporophytes (**A**-**C**) and gametophytes (**D**-**F**) of *Huperzia selago* for multiplication and an alkaloid production. **A** Young sporophyte aster 2 months of culture and callus developing in the base of *H. selago* shoots growing on Moorr medium with addition of IBA (0.015 mg/l) and Kin (0.3 mg/l). **B** The formation of adventitious shoots of *H. selago* on Murashige and Skoog medium with half-strength mineral salt content and full strength of organic componen medium without growth regulators, after the 3 months of culture. **C** Young sporophytes developed from bulbils (vegetative propagules) aster 3 months of culture. **D, E** Mature gametophyte of *H. selago* from culture. **f** Somatic organogenesis in the prothallus callus (*Photos* W. Szypuła)

gametophytes and quickly growing sporophytes. This year Park et al. (2020) have published a protocol for efficient micropropagation of sporophytes of *Selaginella martensi,* an evergreen perennial spike fern that is native to South America and New Zealand. They determined the optimal shoot-tip culture conditions to ensure shoot tip proliferation and sporophyte growth using Murashige and Skoog (MS) medium (1962) with micronutrients at various concentrations. Sporophytes that were grown from shoot-tips in vitro were acclimated in *ex vitro* soil and successfully survived in the greenhouse. With the method, numerous clonal in vitro-grown sporophytes could be obtained and be proliferated *ex vitro* to produce a large number of plants. This protocol can provide a way for developing similar procedures for European spike

moss species like e.g. *Selaginella helvetica,* which in many regions is threatened with extinction (Zarzycki and Kaźmierczakowa 2014).

19.5.3 The Isoetaceae

There are a number of published studies on the micropropagation of different spies of the genus *Isoëtes* (Oh et al. 2013; Caldeira et al. 2019), including *Isoëtes sabatina* which is an aquatic quillwort endemic to Italy (Magrini et al. 2020). *Isoëtes sabatina* is one of the rarest quillworts in Europe and it is critically endangered due to its restricted range and the continuous decline of its population and habitat quality. An optimized protocol was developed for its micropropagation using micro- and megaspores. Water-agar medium (1%) without any plant growth regulators proved to be the most effective with a high percentage of microspore and megaspore germination and gametophyte development. The megagametophytes produced fast-growing sporophytes which grew roots. The developed sporophytes were transferred to water-agar medium. The protocol could be useful for the micropropagation of other European quillworts. Of the 20 quillwort species native to Europe, 12 are endemic, four are threatened with extinction throughout Europe and the remaining ones are critically endangered in particular countries.

19.5.4 The Equisetaceae

Equisetaceae (the horsetail family) is a monotypic family represented by a single order, *Equisetum*, which in Europe comprises 10 species and some taxa of hybrid origin. Regrettably, it has not been adequately investigated by botanists and the occurrence of hybrids often prevents accurate identification of individual plants. Although generally horsetails are not threatened with extinction at the European level, some species are included in the IUCN Red List of Threated Species or species which are at the verge of extinction in some countries of the European Union (García et al. 2017; Wróbel 2020). Some horsetail species have medicinal properties and field horsetail (*Equisetum arvense*) described in the European Pharmacopoeia is procured from its natural locations. Although it is a common plant which is not endangered, protocols for its micropropagation have been developed for fast production of material with such potential uses as biosynthesis of secondary metabolites and their isolation or genetic and biochemical studies. *E. arvense* spores transferred on to Murashige and Skoog (MS) medium (1962) with or without cytokine produced gametophytes from which sporophytes were obtained after 2 months in culture (Kuriyama et al. 1990; Kuriyama and Maeda 1999). Also, a method of cryopreservation has been developed for *Equisetum ramosissimum* spores (Ballesteros et al. 2011). Horsetail spores quickly lose viability, usually in a few weeks, and the only effective method of its

preservation is storage in liquid nitrogen at −80 °C. Cryopreserved spores maintain their viability with rapid germination and normal gametophyte and sporophyte development for several years.

19.5.5 The Aspleniaceae

The Aspleniaceae family includes *Asplenium*, the most species-rich genus of European ferns. Many of them are hybrids, some 16 species are endemic to Europe and 17 species are included in the IUCN Red List of Threated Species or species which are at the verge of extinction at the European level (García et al. 2017). Some species such as *Asplenium adulterinum* Milde, *Asplenium adiantum-nigrum* L. and *Asplenium cuneifolium* Viv. are called 'serpentine ferns' because they are endemic to the serpentine areas which in Europe are found in the Alps, the mountains of the Balkan Peninsula, the south-east region of Portugal, Britain, and in Poland's Lower Silesia region. Most European serpentine species must be protected under the law to prevent dramatic decline in their genetic diversity and size of natural populations. In this situation, in vitro culture techniques may aid in *ex situ* conservation of some species. Sporophyte induction from spore-derived gametophytes has been studied in some fern species: *Asplenium adiantum-nigrum* (Somee et al. 2010), *Asplenium adulterinum, A. cuneifolium* (Marszał-Jagacka and Kromer 2011), *Asplenium trichomanes* (Pangua et al. 1994). Sporulation under in vitro conditions allows recreating the entire developmental cycle, from the sporophyte to the gametophyte. In vitro cultures are also effective in the generative and vegetative reproduction of these fern species. One study from Poland assessed the effects of cryopreservation on the viability of *Asplenium cuneifolium* gametophytes, their regeneration in post-rewarming culture and further micropropagation (Makowski et al. 2020). Valuable medicinal properties of some *Asplenium* species have been confirmed and the micropropagation methods developed for the purposes of their *ex situ* conservation can be used to obtain plant material for the isolation of biologically-active secondary metabolites.

19.6 Conclusions and Prospects

Medicinal uses of pteridophytes, either as whole plants or their parts or as a source of active substances in plant-based pharmaceutical products, are relatively limited when compared to seed plants. Recent advances include extensive research into acetylcholinesterase-inhibiting alkaloids naturally occurring in some club moss species, e.g. huperzine A which is used for Alzheimer's disease and other types of cognitive impairment. Most of the pteridophyte species investigated to date offer a rich source of unique secondary metabolites with great medicinal potential. These phytochemicals have anticancer, antioxidative and anti-inflammatory actions and some are potent inhibitors of enzymes involved in intracellular signaling

pathways. European ferns, psylophytes, horsetails and club mosses have remained grossly understudied. Out of approximately 210 European species, only 37% have been studied phytochemically and biological and medicinal properties have been confirmed in most of the species. However, much remains to be explored as for many species only general biological studies have been performed. Huperzine A, naturally occurring in *H. selago,* is an exception and its use to alleviate the symptoms of Alzheimer's disease has been evaluated in clinical trials. Biotechnology and tissue culture techniques have been used for micropropagation of some fern species, two club moss species and a few horsetails, mainly to aid their in situ conservation. This approach is especially important in the case of these pteridophyte species which are endangered, endemic, and threatened, for instance as a result of uncontrolled harvesting for medicinal uses (some *Asplenium*, *Selaginella* or *Isoetaceae* species). Pteridophytes are phylogenetically diverse and older than seed plants which is reflected in the problems of maintaining pteridophyte in vitro cultures. The rate of their growth in vitro is different and usually slower than in model plants. Pteridophytes also require different media and culture conditions. These difficulties may account for a relatively limited interest of researchers in this group of plants and biotechnological tools for their propagation.

References

Adam KP (1995) Caffeic acid-derivatives in fronds of the Lady Fern (*Athyrium-Filix-Femina*). Phytochemistry 40(5):1577–1578

Ahmed A, Jahan N, Wadud A, Imam H, Hajera S, Bilal A (2012) Physicochemical and biological properties of *Adiantum capillus-veneris* Linn: an important drug of unani system of medicine. Int J Curr Res Rev 4(21):70–75

Ahmed D, Khan M, Saeed R (2015) Comparative analysis of phenolics, flavonoids and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from *Adiantum caudatum* leaves Antioxidants 4(2):394–409

Aibuldinov Ye, Ozek G, Rakhmadiyeva S, Basher KHC (2012) Gas-chromatographic and mass spectrometric evaluation of essential oils of four ferns from Kazakhstan. Newsletter of L.N.Gumilyov Eurasian National University 6: 265–70

Akeroyd J, Synge H (1992) Higher plant diversity. In: Groombridge B (ed) Global biodiversity status of the earth's living resources, 1st edn. Springer, Dordrecht, pp 64–87

Al-Maliki GM, Al-Khafaji KK, Karim RM (2017) Antibacterial activity of two water plants *Nymphaea alba* and *Salvinia natans* leaves against pathogenic bacteria. Int J Fish Aquat Stud 5(5):353–355

Al-Snafi AE (2017) The pharmacology of *Equisetum arvense*—a review. IOSR J Pharm. 7(2):31–42

Alonso-Amelot ME, Avendaño M (2002) Human carcinogenesis and bracken fern: a review of the evidence. Curr Med Chem 9:675–686

Asgarpanah J, Roohi E (2012) Phytochemistry and pharmacological properties of *Equisetum arvense* L. J Med Plants Res 6:3689–3693

Atmane N, Blervacq AS, Michaux-Ferriere N, Vasseur J (2000) Histological analysis of indirect somatic embryogenesis in the Marsh clubmoss *Lycopodiella inundata* (L.) Holub (Pteridophytes). Plant Sci 156:159–167. https://doi.org/10.1016/S0168-9452(00)00244-2

Asgarpanah J, Roohi E (2012) Phytochemistry and pharmacological properties of *Equisetum arvense* L. J. Med Plants Res 6:3689–3693

Aulakh MK, Kaur N, Saggoo MIS (2019) Bioactive phytoconstituents of pteridophytes—a review. Indian Fern J 36:37–79

Ayer WA, Trifonov LS (1994) Lycopodium alkaloids. The alkaloids, vol 45. Academic, San Diego, pp 233–266

Bagniewska-Zadworna A, Zenkteler E, Karolewski P, Zadworny M (2008) Phenolic compound localisation in *Polypodium vulgare* L. rhizomes after mannitol-induced dehydration and controlled desiccation. Plant Cell Rep 7:1251–1259. https://doi.org/10.1007/s00299-008-0548-3

Bahadori M, Mahmoodi Kordi F, Ali Ahmadi A, Bahadori S, Valizadeh H (2015) Antibacterial evaluation and preliminary phytochemical screening of selected ferns from Iran. Res J Pharmacog 2(2):53–59

Bahadori MB, Sonboli A, Kordi FM, Dehghan H, Valizadeh H (2015) Cytotoxicity, antioxidant activity and phenolic content of eight fern species from North of Iran. Pharm Sci 21(1):18–24. https://doi.org/10.15171/PS.2015.12bmc.2010.12.025

Ballesteros D, Estrelles E, Walters C, Ibars AM (2011) Effect of storage temperature on green spore longevity for the ferns *Equisetum ramosissimum* and *Osmunda regalis*. CryoLetters 32:89–98

Bateman RM (1996) An overview of Lycophyte phylogeny. In: Camus JM, Gibby M, Johns RJ (eds) Pteridology in perpective. Royal Bot Gardens Kew, pp 405–417

Bateman RM, Di Michelle WA, Willard DA (1992) Experimental clafdistican analyses of anatomically preserved arborescent Lycopsids from the Carboniferous of Euramerica: an essey in paleobotanical phylogenetics. Ann Mo Bot Gard 79:500–550

Ben Cao ZB (1999) Editorial committee of the administration bureau of traditional Chinese medicine. Shangai Science & Technology Press, Shangai, pp 122–124

Bennert HW, Horn K, Kauth M, Fuchs J et al (2011) Flow cytometry confirms reticulate evolution and reveals triploidy in Central European *Diphasiastrum* taxa (Lycopodiaceae*, Lycophyta*). Ann Bot 108:867–876

Bienaime C, Melin A, Bensaddek L, Attoumbré J, Nava-Saucedo E (2015) Effects of plant growth regulators on cell growth and alkaloids production by cell cultures of *Lycopodiella inundata*. Plant Cell Tissue Organ Cult 123(3):523–533. https://doi.org/10.1007/s11240-015-0856-6

Bouazzi S, Jmii H, ElR M, Faidi K, Falconieri D et al (2018) Cytotoxic and antiviral activities of the essential oils from Tunisian Fern, *Osmunda regalis*. S Afr J Bot 118:52–57. https://doi.org/10.1016/j.sajb.2018.06.015

Bresciani LF, Priebe JP, Yunes RA, Dal Magro J et al (2003) Pharmacological and phytochemical evaluation of *Adiantum ceneatum* growing in Brazil. Zeitschrift Für Naturforschung [c] 58:191–194

Caldeira CF, Abranches CB, Gastauer M, Ramos SJ, Guimarães JTF, Pereira JBS, Siqueira JO (2019) Sporeling regeneration and ex situ growth of *Isoëtes cangae (Isoetaceae): initial steps towards the conservation of a rare Amazonian quillwort*. Aquat Bot 152:51–58

Cheng JT, Liu F, Li XN, Wu XD, Dong LB, Peng LY, Huang SX, He J, Zhao QS (2013) Lycospidine A, a new type of Lycopodium alkaloid from *Lycopodium complanatum*. Org Lett 15:2438–2441. https://doi.org/10.1021/ol400907v

Clericuzio M, Tinello S, Burlando B, Ranzato E, Martinotti S, Cornara L, La Rocca A (2012) Flavonoid oligoglycosides from *Ophioglossum vulgatum* L. having wound healing properties. Planta Med 78(15)1639–1644

Czapski GA, Szypuła W, Kudlik M, Wilenska B, Kania M, Danikiewicz W, Adamczyk A (2014) Assessment of antioxidative activity of alkaloids from *Huperzia selago* and *Diphasiastrum complanatum* using in vitro systems. Folia Neuropathol 4:394–406

Dall'Acqua S, Tomè F, Vitalini S, Agradi E, Innocenti G (2009) In vitro estrogenic activity of *Asplenium trichomanes* L. extracts and isolated compounds. J Ethnopharmacol 122(3):424–429

Dar PA, Sofi G, Jafri MA (2012) *Polypodium vulgare* Linn. A versatile herbal medicine: a review. Int J Pharm Sci Res 3:1616–1620

De Maggio AE (1964) Organization in gametophyte callus of Lycopodium and its morphogenetic implications. Proc Natl Acad Sci U S A 52:854–859

De Monte FHM, JrJg DS, Russi M et al (2004) Antinociceptive and anti-inflammatory properties of the hydroalcoholic extract of stems from *Equisetum arvense* L. in mice. Pharmacol Res 49(3):239–243

De Souza NJ, Ghisalberti EL, Rees HH, Goodwin TW (1970) Studies on insect moulting hormones: Biosynthesis of ecdysone, ecdysterone and 5β-hydroxyecdysterone in *Polypodium vulgare*. Phytochemistry 9(6):1247–1252

De Souza MM, PereiraMA AJV, Mora TC, Bresciani LF, Yunes RA, Delle Monache F, Cechinel-Filho V (2009) Filicene obtained from *Adiantum cuneatum* interacts with the cholinergic, dopaminergic, glutamatergic, GABAergic, and tachykinergic systems to exert antinociceptive effect in mice. Pharmacol Biochem Behav 93:40–46

Dinan L (2001) Phytoecdysteroids: biological aspects. Phytochemistry 57(3):325–339. https://doi.org/10.1016/S0031-9422(01)00078-4

Durdević L, Mitrović M, Pavlović P, Bojović S, Jarić S, Oberan L, Gajić G, Kostić O (2007) Total phenolics and phenolic acids content in leaves, rhizomes and rhizosphere soil under *Ceterach officinarum* D.C., *Asplenium trichomanes* L. and *A. adiantum nigrum* L. in the Gorge of Sićevo (Serbia). Ekol Bratislava 26:164–173

Euw JV, Lounasmaa M, Reichstein T, Widén CJ (1980) Chemotaxonomy in *Dryopteris* and related fern genera. Review and evaluation of analytical methods. Studia Geobot 1(1):275–311

Euw JV, Reichstein T, Widén CJ (1985) The Phloroglucinols of *Dryopteris aitoniana* PICHI SERM. (Dryopteridaceae, Pteridophyta). Helv Chim Acta 68:1251–1275

Farràs A, Másedas G, Les F, Terrado EM, Mitjans M, López V (2019) Evaluation of anti-tyrosinase and antioxidant properties of four fern species for potential cosmetic applications. Forests 10:179

Freeberg JA, Wetmore RH (1957) Gametophytes of Lycopodium as grown in vitro. Phytomorphology 7:204–217

Freeberg JA (1957) The apogamous development of sporelings of *Lycopodium cernuum* L., *L. complanatum* var. *Flabelliforme* Fernald and *L. selago* L. in vitro. Phytomorphology 7:217–229

Ferreira A, Rodrigues M, Fortuna A, Falcão A, Alves G (2016) Huperzine A from *Huperzia serrata*: a review of its sources, chemistry, pharmacology and toxicology. Phytochem Rev 15(1):51–85. https://doi.org/10.1007/s11101-014-9384-y

Fons F, Froissard D, Morel S, Bessiere JM, Buatois B, Sol V, Fruchier A, Rapior S (2018). Pteridaceae fragrant resource and bioactive potential: a Mini-review of aroma compounds. Nat Prod Commun 13(5):651–655

Francisco MS, Driver GC (1984) Anti-microbial activity of phenolic acids in *Pteridium aquilinum*. Am Fern J 743:87–96

Fraser-Jenkins CR, Widén CJ (1993) Phloroglucinol derivatives in *Dryopteris-Ardechensis* and *D-Corleyi* (Pteridophyta, Dryopteridaceae) and their putative ancestors. Ann Bot Fenn 30(1):43–51

Froissard D, Fons F, Bessière JM, Buatois B, Rapior S (2011) Volatiles of French ferns and "fougère" scent in perfumery. Nat Prod Commun 6: 1723–1726

Froissard D, Rapior S, Bessiere JM, Fruchier A, Buatois B, Fons F (2014) Volatile organic compounds of six French dryopteris species: natural odorous and bioactive resources. Nat Prod Commun 9(1):137–140

Froissard D, Rapiorb S, Bessièrec JM, Buatoisc B, Fruchierd A, Sola V, Fonsb F (2015) Asplenioideae species as a reservoir of volatile organic compounds with potential therapeutic properties. Nat Prod Com 10(6):1079–1083

Furmanowa M, Michalska Z, Parczewski A, Zarębska I (1959) Lecznictwo renesansowe w Polsce na podstawie Herbarza Marcina z Urzędowa. Studia i Materiały z Dziejów Nauki Polskiej, Seria B Historia Nauk Biologicznych i Medycznych 2:233–313

García CM, Väre H, Nieto A, Bento ER, Dyer R, et al (2017) European red list of lycopods and ferns. Brussels: IUCN. https://doi.org/10.2305/IUCN.CH.2017.ERL.1.en

Garrat MJ (1984) The appearance of Baragwanathia (Lycophytine) in the Silurian. Bot J Linn Soc 89:355–358

Ge X, Ye G, Li P, Tang W-J, Gao J-L, Zhao W-M (2008) Cytotoxic diterpenoids and sesquiterpenoids from *Pteris multifida*. J Nat Prod 71:227–231

Gibby M, Rasbach H, Reichstein T, Widen CJ, Viane RLL (1992) Micromorphology, chromosome-numbers and phloroglucinols of arachniodes-foliosa and a-webbiana (Dryopteridaceae, Pteridophyta). Bot Helv 102(2):229–245

Gleńsk M, Dudek MK, Ciach M, Włodarczyk M (2019) Isolation and structural determination of flavan-3-ol derivatives from the *Polypodium vulgare* L. rhizomes water extract. Nat Prodt Res. https://doi.org/10.1080/14786419.2019.1655302

González-Platas J, Ruiz-Pérez C, González AG, et al (1999) Filic-3-ene, a pentacyclic triterpene from *Davallia canariensis.* Acta Cryst C55:1835–1837. https://doi.org/10.1107/S0108270199009762

Gonzalez S, Gilaberte Y, Philips N (2010) Mechanistic insights in the use of a *Polypodium leucotomos* extract as an oral and tropical photoprotective agent. Photoch Photobiol Sci 9:559–563

Guo W, Shi K, Xiang G, Lu D, Dou H (2019) Effects of rhizoma drynariae cataplasm on fracture healing in a rat model of osteoporosis. Med Sci Mon Int Med J Exp Clin Res 25:3133–3133

Grzybek J (1983) Phytochemical and biological investigations on *Polypodium vulgare* L. Acta Pol Pharm 40:259–263

Haider S, Kharbanda C, Alam MS, Hamid H, Ali M, Alam M, Nazreen S, Ali Y (2013) Anti-inflammatory and anti-nociceptive activities of two new tri t e rpenoids from *Adiantum capillus-vener is* Linn. Nat Prod Res 27(24):2304–2310

Halldorsdottir ES, Olafsdottir ES, Jaroszewski JW (2008) Alkaloid content of the Icelandic club moss *Lycopodium annotinum*—acetylcholinesterase inhibitory activity in vitro. Planta Med. 74:1043–1043. https://doi.org/10.1055/s-0028-1084398

Halldorsdottir ES, Jaroszewski JW, Olafsdottir ES (2010) Acetylcholinesterase inhibitory activity of lycopodane-type alkaloids from the Icelandic *Lycopodium annotinum ssp alpestre.* Phytochemistry 71:149–157. https://doi.org/10.1016/j.phytochem.2009.10.018

Halldorsdottir ES, Palmadottir RH, Nyberg NT, Olafsdottir ES (2013) Phytochemical analysis of alkaloids from the Icelandic club moss *Diphasiastrum alpinum.* Phytochem Lett 6:355–359

Halldorsdottir ES, Kowal NM, Olafsdottir ES (2015) The genus *Diphasiastrum* and its Lycopodium alkaloids. Planta Med 81:995–1002. https://doi.org/10.1055/s-0035-1546182

Hegnauer R (1962) Chemotaxonomie der Pflanzen, Band 1. Birkhäuser Verlag Basel und Stuttgart. 520 p

Hirono I (1986) Carcinogenic principles isolated from bracken fern. Crit Rev Toxicol 17(1):1–22. https://doi.org/10.3109/10408448609037069

Ho R, Teai T, Loquet D, Bianchini J-P, Girault J-P, Lafont R, Raharivelomanana P (2007) Phytoecdysterios in the genus *Microsorum (Polypodiaceae*) from French Polynesia. Nat Prod Com 2:803–806

Ho R, Teai T, Bianchini J-P, Lafont R, Raharivelomanana P (2011) Ferns: from traditional uses to pharmaceutical development, chemical identification of active principles. In: Fernández H, Kumar A, Revilla MA (eds) Working with ferns. Springer, New York

Hou M, Hu W, Wang A, Xiu Z, Shi Y, et al (2019) Ultrasound-assisted extraction of total flavonoids from *Pteris cretica* L.: process optimization, HPLC analysis, and evaluation of antioxidant activity. Antioxidants (Basel) 8(10):425. https://doi.org/10.3390/antiox8100425

House RP, Lagos S, Ochoa L, Torres C, Mejia T, Rivas M (1994) Plantas medicinales communes de Honduras. Honduras: UNAH-CIMN-HCID/CIIR.GTZ. https://doi.org/10.1248/cpb.57.877

Hu H, Zheng X (2005) Studied on the chemical compositions from the roots of *Pteris multifida* Poir. Tianran Chanwu Yanjiu Kaifa 17:169–171

Hu H, Jian Y, Zheng X, Liu J, Cao H (2006) A new sesquiterpene glycoside from the roots of *Pteris multifida* Poir. Indian J Chem 45B:1274–1277

Hua X, Yang Q, Zhang WJ, Dong ZM, Yu SY, Schwarz S, Liu SG et al (2018) Antibacterial activity and mechanism of action of aspidinol against multi-drug-resistant methicillin-resistant *Staphylococcus aureus.* Front Pharmaco 9:619

Huang Y-H, Zeng W-M, Li G-Y, Liu G-Q, Zhao D-D, Wang J, Zhang Y-L (2014) Characterization of a new sesquiterpene and antifungal activities of chemical constituents from *Dryopteris fragrans* (L.) Schott. Molecules 19(1):507–513

Ibraheim ZZ, Ahmed AS, Gouda YG (2011) Phytochemical and biological studies of *Adiantum capillus-veneris* L. Saudi Pharm J 19(2):65–74
Imperato F (1990) Kaempferol 3-Sophoroside-4'-Glucoside from *Asplenium-septentrionale*. Phytochemistry 29(10):3374–3375
Imperato F (1991) A new xanthone from the Fern *Cystopterys-fragilis*. J Nat Prod 54(2):603–605
Imperato F (1991) Polyphenolics of phylogenetic and biosynthetic interest from the fern *Cystopteris-fragilis*. Can J Bot 69(1):218–221
Imperato F (1991) Xanthone 2,4-di-C-glycosides from asplenium adiantum-nigrum. Phytochemistry 30(11):3839–3840
Imperato F (1994) Luteolin 8-C-rhamnoside-7-O-rhamnoside from *Pteris cretica*. Phytochemistry 37(2):589–590
Imperato F (1994) A new flavone glycoside from the fern *Pteris cretica*. Experientia 50(11–12):1115–1116
Imperato FA (2008) New flavonoid glycoside from the fern *Dryopteris villarii*. Nat Prod Commun 3:1709–1712. https://doi.org/10.1177/1934578X0800301026
Inubushi Y, Sano T, Tsuda Y (1964) Serratenediol: a new skeletal triterpenoid containing a seven member ring. Tetrahedron Let 21:1303–1310
Ishiuchi K, Kodama S, Kubota T, Hayashi S, Shibata T, Kobayashi J (2009a) Lannotinidines HJ, new Lycopodium alkaloids from *Lycopodium annotinum*. Chem Pharm Bull 57:877–881
Ishiuchi K, Kubota T, Hayashi S, Shibata T, Kobayashi J (2009b) Lycopladine H, a novel alkaloid with fused-tetracyclic skeleton from *Lycopodium complanatum*. Tetrahedron Lett 50:6534–6536. https://doi.org/10.1016/j.tetlet.2009.09.035
Ishiuchi K, Kubota T, Hayashi S, Shibata T, Kobayashi J (2009c) Lycopladines F and G, new $C_{16}N_2$-type alkaloids with an additional C_4N unit from *Lycopodium complanatum*. Tetrahedron Lett 50:4221–4224. https://doi.org/10.1016/j.tetlet.2009.04.139
Ishiuchi K, Kubota T, Ishiyama H, Hayashi S, Shibata T, Kobayashi J (2011) Lyconadins C and F, new Lycopodium alkaloids from *Lycopodium complanatum*. Tetrahedron Lett 52:289–292. https://doi.org/10.1016/j.tetlet.2010.11.024
Ishiuchi K, Kubota T, Ishiyama H, Hayashi S et al (2011) Lyconadins D and E, and complanadine E, new Lycopodium alkaloids from *Lycopodium complanatum*. Bioorg Med Chem 19:749–753. https://doi.org/10.1016/j
Ivanenko YuA, Tzvelev NN (2004) O rode *Diphasiastrum* (Lycopodiaceae) w Wostocznoj Evrope. Bot Zhur. 85(1):100–111
Iwashina T, Lopez-Saez JA, Herrero A, Kitajima J, Matsumoto S (2000) Flavonol glycosides from *Asplenium foreziense* and its five related taxa and A-incisum. Biochem Syst Ecol 28(7):665–671
Jędrzejko K, Klama H, Żarnowiec J (1997) Zarys wiedzy o roślinach leczniczych. Śląska Akademia Medyczna w Katowicach, Katowice – Sosnowiec, pp 1–695
Jiang MZ, Yan H, Wen Y, Li XM (2011) vitro and in vivo studies of antioxidant activities of flavonoids from *Adiantum capillus-veneris* L Afr. J Pharm Pharmacol 5(18):2079–2085
Jiang Y, Li D, Ma X, Jiang F, He Q, Qiu S, Li Y, Wang G (2018) Ionic liquid ultrasound-based extraction of biflavonoids from *Selaginella helvetica* and investigation of their antioxidant activity. Molecules 23(12):3284
Jizba J, Herout V (1974) Plant Substances .38. Ponasterone-a and 22-hopanol, characteristic components of fern *Blechnum-Spicant* L. Collection of Czechoslovak. Chem Commun 39(12):3756–3759
Johnson MA, Gowtham J, Janakiraman N, et al (2020) Phytochemical profile of *Asplenium aethiopicum* (Burm. f.) Becherer Using HPTLC. Separations 7,8
Katakawa K, Mito H, Kogure N, Kitajima M et al (2011) Ten new fawcettimine-related alkaloids from three species of Lycopodium. Tetrahedron 67:6561–6567. https://doi.org/10.1016/j.tet.2011.05.107
Kennrick P, Crane P (1997) The origin and early diversification of land plants: Achladistic study. Washington: Smithsonian 389:33–39
Kenrick P, Davis P (2004) Fossil plants. Natural History Museum, London, p 216

Kim HJ, Lim HW, Choi SW, Yoon CS (2006) Antimicrobial effect of ethanol extract of *Dryopteris crassirhizoma* Nakai on *Propionibacterium acnes*. J Soc Cosm Chem 32:201–208
Kim MM, Kim SK (2010) Effect of phloroglucinol on oxidative stress and inflammation. Food Chem Toxicol 48(10):2925–2933
Kim Jung W, Seo Ji Y, Oh Won K, Sung Sang H (2017) Anti-neuroinflammatory ent-kaurane diterpenoids from *Pteris multifida* Roots. Molecules 22(1):27
Klama H (1992) Paprotniki i ich zastosowanie w lecznictwie. Wiadomości Zielarskie 2:18–22
Kohlmünzer S (2007) Farmakognozja. Podręcznik dla studentów farmacji. Wyd. 5. Unowocześnione. Wydawnictwo Lekarskie PZWL. Warszawa, pp 220–225
Kovganko NV, Kashkan ZN, Krivenok SN (2004) Bioactive compounds of the flora of Belarus 4 Pterosins A and B from *Pteridium aquilinum*. Chem Nat Compd 40(3):227–229
Kuang H, Zhang Y, Li L, GuoYu Z, WeiMin W et al (2008) A new phenolic glycoside from the aerial parts of *Dryopteris fragrans*. Fitoterapia 79(4):319–320
Kuriyama A, Sugawara Y, Matsushima H, Takeuchi M (1990) Production of sporophyte structures from gametophytes by cytokinin in *Equisetum arvense*. Naturwissenschaften 77:31–32
Kuriyama A, Maeda M (1999) Direct production of sporophytic plants from spores of *Equisetum arvense*. Plant Cell Tiss Org Cult 58:77–79
Kumar A, Kaushik P (1999) Antibacterial effect of *Adiantum capillus-veneris* Linn. Indian Fern Journal 16:72–74
Kuang H, Sun Chao Z, Yanli Z, Yanlong Ch, Dong Y et al (2009) Three drimane sesquiterpene glucoside from the aerial parts of *Dryopteris fragrans* (L.) Schot. Fitoterapia 80(2):134–137
Lafont R, Dinan L (2003) Practical uses for ecdysteroids in mammals and human: an update. J Insect Sci 3:7
Lafony R, Ho R, Raharivelomanana P, Dinan L (2011) Ecdysteroids in fern: distribution, diversity, biosynthesis and function- In: Fernández H, Kumar A, Revilla MA (eds) Working with ferns. Springer, New York
Lai HY, Lim YY, Tan SP (2009) Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. Biosci Biotechnol Biochem 73(6):1362–1366
Lamer-Zarawska E, Kowal-Gierczak B, Niedworok J (eds) (2010) Fitoterapia i leki roślinne. 2010. Wydawnictwo Lekarskie PZWL, pp 5–30
Lee HB, Kim JC, Lee SM (2009) Antibacterial activity of two phloroglucinols, flavaspidic acids AB and PB, from *Dryopteris crassirhizoma*. Arch Pharm Res 32:655–659
Lenkiewicz A, Czapski GA, Jęśko H, Wilkaniec A, Szypuła W et al (2016) Potent effects of alkaloid-rich extract from *Huperzia selago* against sodium nitroprusside-evoked PC12 cells damage via attenuation of oxidative stress and apoptosis. Folia Neuropathol 2:156–166
Li B, NY, Zhu LJ, Wu FB, Yan F, Zhang X, Yao XS (2015) Flavonoids from *Matteuccia struthiopteris* and their anti-influenza virus (H1N1) activity. J Nat Prod 78: 987–995
Lin LC, Chou CJ (2000) Three new biflavonoids from *Selaginella delicatula*. Chinese Pharm J 52(4):211–218
Lin LC, Kuo YC, Chou CJ (2000) Cytotoxic biflavonoids from *Selaginella delicatula*. J Nat Prod 63(5):627–630
Liu Y, Wujisguleng W, Long C (2012) Food uses of ferns in China: a review. Acta Soc Bot Pol 81(4):263–270. https://doi.org/10.5586/asbp.2012.046
Liu Z-D, Zhao D-D, Jiang S, Xue B, Zhang Y-L, Yan X-F (2018) Anticancer phenolics from *Dryopteris fragrans* (L.) Schott. *Molecules 23*,680
Lombardi VRM, Eetcheverria I, Fernandez-Novoa L, Diaz J, Seoane S, Cacabelos R (2005) In vitro response of rat microglia and human polymorphonuclear cells (PMN) to immunoactive compounds. Orient Pharm Exp Med 5(3):216–230. https://doi.org/10.3742/opem.2005.5.3.216
López-Sáez JA, Pérez-Alonso MJ, Negueruela AV (1994a) Biflavonoids of *Selaginella denticulata* growing in Spain. Z Naturforsch C 49(3/4):267–270
López-Sáez JA, Pérez-Alonso MJ, Velasco Negueruela A (1994b) The biflavonoid pattern of *Selaginella selaginoides*. Zeitschrift Für Naturforschung, C: J Biosci 49:265–355

Lopez-Saez JA, Perez-Alonso MJ, Velasco-Negueruela A (1995) Flavonoids of *Selaginella denticulata* and *S. selaginoides*. Fitoterapia 66(2):188–189
Lu J, Peng C, Cheng S, Liu J, Ma Q, Shu J (2019) Four new pterosins from *Pteris cretica* and their cytotoxic activities. Molecules 24(15):2767. https://doi.org/10.3390/molecules24152767
Lucca DM (1992) Flora medicinal Boliviana: Diccionario Enciclopedico. Editorial Los Amigos del Libro, Bolivia
Ma X, Gang DR (2004) The Lycopodium alkaloids. Nat Prod Rep 21:752–772. https://doi.org/10.1039/b409720n
Magalhães LG, Kapadia GJ, Da Silva Tonuci LR, Caixeta SC, Parreira NA, Rodrigues V, Da Silva Filho AA (2009) In vitro schistosomicidal effects of some phloroglucinol derivatives from *Dryopteris* species against *Schistosoma mansoni* adult worms. Parasito Res 106:395–401
Magrini S, Azzella MM, Bolpagni R, Zucconi L (2020) In Vitro propagation of *Isoëtes sabatina* (Isoetaceae): a key conservation challenge for a critically endangered quillwort. Plants 9(7):887. https://doi.org/10.3390/plants9070887
Makowski D, Mikuła A, Rybczyński JJ (2011) Cryopreservation of *Asplenium cuneifolium* gametophyte and regeneration of plant material in post-rewarming culture. Cryopreservation of Crop Species in Europe. Eur Coop Sci Technol, Angers, pp 151–153
Manhas S, Attri Ch, Seth MK, Seth A (2018) Determination of phytochemical constituents and evaluation of antimicrobial activity of medicinal fern *Christella dentata*. Indian Fern J 35:169–178
Manske RHF, Marion L (1942) The alkaloids of Lycopodium species. *L. complanatum*. Canad J Res 20b (5):87–92
Martin KP, Sini S, Zhang C-L, Slater A, Madhusoodanan PV (2006) Efficient induction of apospory and apogamy in vitro in silver fern (*Pityrogramma calomelanos* L.). Plant Cell Rep 25:1300–1307
Marszał-Jagacka J, Kromer K (2010) In vitro propagation of rare and endangered serpentine fern species. In: Kumar A, Fernández H, Revilla MA (eds) Working with ferns: issues and applications. Springer, New York, NY, pp 149–164
Matsuoka A, Hirosawa A, Natori S, Iwasaki S, Toshio S, Motoi I Jr (1989) Mutagenicity of ptaquiloside, the carcinogen in bracken, and its related illudane-type sesquiterpenes. Mut Res 215(2):179–185
Mehra PN, Tc M (1961) Significance of internal secretory glands in relation to filicin. Planta Med 9:189–199
Mehltreter K, Walker LR, Sharpe JM (eds) (2010) Fern ecology. Cambridge University Press, Cambridge
Messeguer J, Melé E, Reixach N, Irurre-Santilari J, Casas J (1998) *Polypodium vulgare* L. (Wood Fern): In vitro cultures and the production of phytoecdysteroids. In: Bajaj YPS (eds) Medicinal and aromatic plants X. Biotechnology in agriculture and forestry, tom 41. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-58833-4_17
Meybeck A, Ho R, Teai T, Raharivelomanana P (2010) Use of aqueous of organic or aqueous organic extract of *Microsorum*. PCT In. Appl, coden:pixxd2 wo 2010007247(a2)
Milovanovic V, Radulovic N, Todorovic Z et al (2007) Antioxidant, antimicrobial and genotoxicity screening of hydro-alcoholic extracts of five serbian *Equisetum Species*. Plant Food Hum Nutr 62(3):113–119
Minarchenko V, Tymchenko I, Dvirna T, Makhynia L (2017) A review of the medicinal ferns of ukraine Scripta. Sci Pharm 4(1):7–23
Mir SA, Mishra AK, Reshi ZA, Sharma MP (2013) Preliminary phytochemical screening of some Pteridophytes from district Shopian (J&K) Int J Pharm Pharm Sci 5(4):632–637
Miyazawa M, Horiuchi E, Kawata J (2007) Components of the essential oil from *Matteuccia struthiopteris*. J Oleo Sci 56:457–461
Moerman DE (1998) Native American ethnobotany. Timber Press, Oregon
Moran RC (2004) A natural history of ferns Portland. Timber Press, Portland
Moran RC (2008) Diversity, biogeography, and floristics. In: Ranker TA, Haufler CH (eds) Biology and evolution of ferns and lycophytes. Cambridge University Press, Cambridge, pp 367– 394

Mukhopadhyay R, Gupta K (2005) Antifungal activity of the crude extracts and extracted phenols from gametophytes and sporophytes of two species of *Adiantum*. Taiwania 50(4):272–283
Murakami T, Wada H, Tanaka N, Kido T, Iida H, Saiki Y, Chen CM (1986) Chemical and chemotaxonomical studies of Filices. LXV. A few new flavonoid glycosides (2). Yakugaku Zasshi 106:982–988
Muszyński J (1946) Widłaki i ich zastosowanie w lecznictwie. Far Pol 10:309–315
Nakane T, Maeda Y, Ebihara H, Arai Y, Masuda K, Takano A et al (2002) Fern constituents: triterpenoids from *Adiantum capillus-veneris*. Chem Pharm Bull 50:1273–1275
Negri G (1979) Descrizione e proprieta delle plante medicinali e velenose. In Nuovo erbario figurato, Hoepli, Milano: Vitalita, pp 19–20
Nguyen KH (2005) Assessment of anti-diabetic effect of Vietnamese herbal drugs. Endrocrine and Diabetes Unit, Department of Moelcular Medicine and Surgery, Karolinska Institute, Stockholm, Sweden, 53 p
Nwiloh BI, Monago CC, Uwakwe AA (2014) Chemical composition of essential oil from the fiddleheads of *Pteridium aquilinum* L. Kuhn found in Ogoni. J Med Plants Res 8(1):77–80
Oh H, Kim DH, Cho JH, Kim YC (2004) Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from *Equisetum arvense*. J Ethnopharmacol 95:421–424
Oh M, Kim C, Na H, Shin H, Liu J, Choi H, Kim S (2013) High frequency sporophytes regeneration from the spore culture of the endangered aquatic fern *Isoetes coreana* . Am J Plant Sci 4(6A):14–20. https://doi.org/10.4236/ajps.2013.46A003
Oniga I, Toiu A, Mogosan C, Bodoki E (2004) Preliminary investigations of *Phyllitis scolopendr ia* (L) Newman (Polypodiaceae) Farmacia 52:48–54
Orellana C (2001) Ferns and gastric cancer in Venezuelan Andes. Lancet Oncol 2(3):P125. https://doi.org/10.1016/s1470-2045(00)00240-0
Ouyang D, Yang P, Kong D (2008) Chemical constituens from *Pteris multifida* Poir. Zhongguo Yiyao Gongye Zazhi 39:898–900
Pacyna A (972) Biometrics and taxonomy of the polish species of the genus *Diphasium* Presl. Fragm Flor Geobot 18(3–4):255–277
Pan C, Chen YG, Ma XY, Jiang JH, He F, Zhang Y (2011) Phytochemical constituents and pharmacological activities of plants from the genus *Adiantum*: a review. Trop J Pharm Res 10(5):681–692
Pangua E, Lindsay S, Dyer A (1994) Spore germination and gametophyte development in three species of *Asplenium*. Ann Bot 73:587–593
Parihar P, Parihar L, Bohra A (2010) In vitro antibacterial activity of fronds (leaves) of some important pteridophytes. J Microbiol Antimicrobials 2:19–22
Park K, Jang BK, Lee HM, Cho JS, Lee CH (2020) An efficient method for in vitro shoot-tip culture and sporophyte production using *Selaginella martensii* spring sporophyte. Plants 9:235. https://doi.org/10.3390/plants9020235
Pekgoz AK, Cinbilgel I (2019) Phytochemical contents and antioxidant activities of fern, *Asplenium ceterach* L. Different Altitudes. Bangladesh J Bot 48(2):315–320
Peng B, Bai Rui-Feng L, Ping H, Xu-Yang W et al (2016) Two new glycosides from *Dryopteris fragrans* with anti-inflammatory activities. J Asian Nat Prod Res 18(1):59–64
Phillipson JD, Melville C (1960) An investigation of the alkaloids of some British species of *Equisetum*. J Pharm Pharmacol 12:506–508. https://doi.org/10.1111/j.2042-7158.1960.tb12699.x
Pomini L (1990) Erborista Italiana. Italy, Ed. Vitalità, 1140 p
Pongpamorn P, Wanerlor S, Ruchirawat S, Thasana N (2016) Lycoclavatumide and 8 β,11 α-dihydroxylycopodine, a new fawcettimine and lycopodine-type alkaloid from *Lycopodium clavatum*. Tetrahedron 72:7065–7069. https://doi.org/10.1016/j.tet.2016.09.046
Pouny I, Etievant C, Marcourt L, Huc-Dumas I et al (2011) Protoflavonoids from Ferns Impair Centrosomal Integrity of Tumor Cells. Planta Med 77(5):461–466

Potter DM, Baird MS (2000) Carcinogenic effects of ptaquiloside in bracken fern and related compounds. Br J Cancer 83(7):914–920. https://doi.org/10.1054/bjoc.2000.1368
Pryer KM, Schneider H, Smith AR, Cranfill R, Wolf PG, Hunt JS (2001) Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature 409:618–621
Qasim MA, Roy SK, Kamil M, Ilyas M (1985) Phenolic constituents of S*elaginellaceae*. Indian J Chem 24B(2):220
Radulović N, Stojanović G, Palić R (2006) Composition and antimicrobial activity of *Equisetum arvense* L. essential oil. Phytother Res 20:85–88
Radulovic N, Stojanovic G, Milovanovic V, Dokovic D, Randelovic V (2008) Volatile constituents of *Equisetum fluviatile* L. J Essent Oil Res 20:437–441
Rai HS, Graham SW (2010) Utility of a large, multigene plastid data set in inferning higher-order relatiomships in ferns and relatives (monilophytes). Am J Bot 96:1444–1456
Rajurkar NS, Gaikwad K (2012) Evaluation of phytochemicals, antioxidant activity and elemental content of *Adiantum capillus-veneris* leaves. J Chem Pharm Res 4(1):365–374
Reddy VL, Ravikanth V, Rao TP, Diwan PV, Venkateswarlu Y (2001) A new triterpenoid from the fern *Adiantum lunulatum* and evaluation of antibacterial activity. Phytochemistry 56:173–175
Ranker TA, Haufler CH (eds) (2008) Biology and evolution of ferns and lycophytes. Cambridge University Press, Cambridge, 480 p
Roos M (1996) Mapping the world's pteridophyte diversity - systematics and floras. In: Camus JM, Gibby M, Johns RJ (eds) Pteridology in perspective. Royal Botanic Gardens, Kew, pp 29–42
Ruggiero MA, Gordon DP, Ornell TM, Bailly N, et al (2015) A hogher level classification of all living prganisms. PloS ONE 10: eo119248.
Salatino MLF, Prado J (1998) Flavonoid glycosides of Pteridaceae from Brazil. Biochem Syst Ecol 26(7):761–769
Saleem F, Khan MTJ, Saleem H, Azeem M, Ahmed S, Shahid N, Altaf H (2016) Phytochemical, antimicrobial and antioxidant activities of *Pteris cretica* L. (*Pteridaceae*) extracts. Acta Pol Pharm 73:1397–1403
Saleem A, Saleem M, Akhtar MF, Rasul A, Baig MMFA (2020) Chemical characterisation, in vitro antioxidant, cytotoxicity and safety evaluation of *Polystichum braunii* (Spenn.) Fee roots. Nat Prod Res Aug 3:1–6. https://doi.org/10.1080/14786419.2020.1797727
Šamec D, Pierz V, Srividya N, Wüst M, Lange BM (2019) Assessing chemical diversity in *Psilotum nudum* (L.) Beauv., a pantropical whisk fern that has lost many of its fern-like characters. Front Plant SciJul 10:868. https://doi.org/10.3389/fpls.2019.00868.
Schneider H, Schuettpelz E, Pryer KM, Cranfill R, Magallón S, Lupia R (2004) Ferns diversified in the shadow of angiosperms. Nature 428(6982):553–557. https://doi.org/10.1038/nature02361
Schuettpelz E et al (2016) A community – derived classification for extant lycophytes and ferns. J Syst Evol 54(6):563–603
Schulz C, Little DP, Stevenson DW, Bauer D, Moloney C, Stützel T (2010) An overview of the morphology, anatomy, and life cycle of a new model species: the lycophyte *Selaginella apoda* (L.) Spring. Int J Plant Sci 171:693–712
Signe JK, Aponglen GA, Ajeck JM, Taiwe GS (2020) Anticonvulsant activities of friedelan-3-one and n-dotriacontane both isolated from *Harungana madagascariensis* Lam (Hypericaceae) seeds extracts. J Med Plant Res 14(10):509–517
Singh M, Singh N, Khare PB, Rawat AKS (2008) Antimicrobial activity of some important *Adiantum* species used traditionally in indigenous system of medicine. J Ethnopharmacol 115:327–329
Soare LC, Ferdes M, Stefanov S, Denkova Z, Nicolova R, Denev P, Bejan C, Paunescu A (2012) Antioxidant activity, polyphenols content and antimicrobial activity of several native pteridophytes of Romania. Not Bot Horti Agrobot Cluj Napoca 40(1):53–57
Soare LC, Şuţan NA (2018) Current trends in pteridophyte extracts: from plant to nanoparticles. In: Fernández H (ed) Current advances in fern research. Springer, Cham, pp 329–357
Somer M, Arbesú R, Menéndez V, Revilla MA, Fernández H (2010) Sporophyte induction studies in ferns in vitro. Euphytica 171(2):203–210

Srilaxmi P, Sareddy GR, Kavi Kishor PB, Setty OH, Babu PP (2010) Protective efficacy of natansnin, a dibenzoyl glycoside from *Salvinia natans* against CCl4 induced oxidative stress and cellular degeneration in rat liver. BMC Pharmacol 10:13. https://doi.org/10.1186/1471-2210-10-13

Stoor AM, Boudrie M, Jérôme C, Horn K, Bennert HW (1996) *Diphasiastrum oellgaardii* (Lycopodiaceae, Pteridophyta), a new Lycopod species from Central Europe and France. Feddes Repert 107(3–4):149–157

Szypuła W, Pietrosiuk A, Suchocki P, Olszowska O, Furmanowa M, Kazimierska O (2005) Somatic embryogenesis and in vitro culture of*Huperzia selago* shoots as a potential source of Huperzine A. Plant Sci 168:1443–1452. https://doi.org/10.1016/j.plantsci.2004.12.021

Szypuła WJ, Mistrzak P, Olszowska O (2013) A new and fast method to obtain in vitro cultures of *Huperzia selago* (Huperziaceae) sporophytes, a club moss which is a source of Huperzine A. Acta Soc Bot Pol 82:313–320. https://doi.org/10.5586/asbp.2013.034

Szypuła WJ, Wileńska B, Misicka A, Pietrosiuk A (2020) Huperzine A and Huperzine B Production by Prothallus Cultures of *Huperzia selago* (L.) Bernh. ex Schrank et Mart. *Molecules* 25(14):3262. https://doi.org/10.3390/molecules25143262u

Szypuła WJ, Pietrosiuk A (2021) Production of cholinesterase-inhibiting compounds in in vitro cultures of club mosses. In: Ramawat KG, Ekiert HM, Goyal S (eds) Plant cell and tissue differentiation and secondary metabolites. Reference series in phytochemistry. Springer, Cham, pp 921–960. https://doi.org/10.1007/978-3-030-30185-9_30

Takemoto T, Hikino Y, Arai T, Kawahara M, Konno C, Arihara S, Hikino H (1967) Isolation of insect moulting substances from *Matteuccia struthiopteris*, *Lastrea thelypteris*, and *Onoclea sensibilis*. Chem Pharm Bull 15:1816

Tang Y, Xiong J, Zou YK, Wang W, Huang C, Zhang HY, Hu JF (2017) Annotinolide F and lycoannotines A-I, further Lycopodium alkaloids from *Lycopodium annotinum* Phytochemistry 143:1–11

Troi, A, Pereira JB, Kim C, Taylor WC (2016) The genus *Isoetes* (Isoetaceae): a provisional checklist of the accepted and unresolved taxa. Phytotaxa 277:101–145. https://doi.org/10.11646/phytotaxa.277.2.1

Uddin MG, Mirza MM, Pasha MK (1998) The medicinal uses of pteridophytes of Bangladesh. Bangladesh J Plant Taxon. 5(2):29–41

UmiKalsom Y, Grayerbarkmeijer RJ, Harborne JB (1994) A Comparison of the Flavonoids in *Athyriaceae* and *Aspleniaceae*. Biochem Syst Ecol 22(6):587–594

Valentine DH (1964) Lycopodiaceae L. In: Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM, WEBB DA (eds) Flora Europea, vol I. Cambridge University Press, Cambridge, pp 3–4

Valizadeh H, Sonboli A, Kordi FM, Dehghan H, Bahadori MB (2015) Cytotoxicity, antioxidant activity and phenolic content of eight fern species from North of Iran. Pharm Sci 21(1):18

Veit M, Bilger T, Muhlbauer T, Brummet W, Winter K (1996) Diurnal changes in flavonoids. J Plant Physiol 148(3–4):478–482

Vetter J (2010) Toxicological medicinal aspects of the most frequent fern species, *Pteridium aquilinum* (L.) Kuhn. In: Kumar A, Fernandez H, Revilla MA (eds) Working with ferns: issues and applications. Springer, New York, pp 361–375

Vetter J (2018) Secondary metabolites of ferns. In: Fernandez H (eds) Current advances in fern research. Springer, Berlin,pp 307–327

Villalobos VM, Engelmann F (1995) *Ex situ* conservation of plant germplasm using biotechnology. World J Microbiol Biotechnol 11(375):382

Vogler G, Donath O, Saukel J, Rauch AW, Kählig H, Krenn L. (2012) Polar phenolic compounds in *Dryopteris filix-mas* and *Dryopteris dilatata*. Verh Zool-Bot Ges Ã–sterreich. 148/149:279–289

Voirin B, Jay M (1974) Presence of 3-Methylquercetin in *Asplenium-Viride*. Phytochemistry 13(1):275–276

Voirin B, Jay M (1978) Contribution of flavone biochemistry to systematics of the *Lycopodiales* order *Lycopodium* genus. Bioch Sys Ecol 6:95–97

Voirin B, Lebreton P (1967) Recherches chimiotaxinomiques sur les plantles vascularies. Sur la presence de métyl-6 chrysine chez la fougére *Lonchitis tisserantii* Alston et Tardieu.Buletin de la Société de Chimie Biologique 49(10):1402–1405

Wagner WH Jr, Wagner F (1980) Polyploidy in pteridophytes. Basic Life Sci 13:199–214. https://doi.org/10.1007/978-1-4613-3069-1_11

Wang G, Zhang L-M (2008) Study of the extract from *Pteris multifida* Poir. on antitumor activity. Hebeisheng Kexueyuan Xuebao 25:52–54

Webster TR (1979) An artificial crossing technique for Selaginella. Am Fern 69:913

Wei H-A, Lian T-W, Tu Y-C, Hong J-T, Kou M-C, Wu M-J (2007) Inhibition of low-density lipoprotein oxidation and oxidative burst in polymorphonuclear neutrophils by caffeic acid and hispidin derivates isolated from sword brake ferns (*Pteris ensiformis* Burm.). J Agri Food Chem 55:10579–10584

Wickett NJ., Mirarab S, Nguyen N, Warnow Ts et al (2014) Phylotranscriptsomia analysis of the orogon and early diversification of land plants. Procaed Nat Acad Sc USA 11:E 4859–4868

Widén CJ, Faden RB, Lounasmaa M, Vida G, Euw JV, Reichstein T (1973) Phloroglucides of Nine Dryopteris Species from Kenya and *Dryopteris-Oligodonta* (Desv) Pinc-Scerm and *Dryopteris-Dilatata* from Canary Islands. Helv Chim Acta 56(7):2125–2151

Widén CJ, Fraser-Jenkins CR, Lounasma M, Euw JV, Reichste T (1973b) Phloroglucides of *Dryopteris-Caucasica* (A.Br.) Fraser-Jenkins and Corley. Helv Chim Acta 56(3):831–838

Widén CJ, Lounasmaa M, Jermy AC, Euw JV, Reichstein T (1976) Phloroglucinols of 2 Fern Hybrids from England and Scotland, of Authentic Aspidium-Remotum Braun, a and of *Dryopteris-Aemul*a (Aiton) Kuntze from Ireland. Helv Chim Acta 59(5):1725–1744

Widén C-J, Huurre S, Iwatsuki K (1978) Chemotaxonomic studies on Aranchniodes (Dryopteridaceae) II. Phloroglucinol Derivatives and Taxonomic Evaluation. Bot Mag Tokyo 91:247–254

Widén C-J, Fraser-Jenkins CR, Reichstein T, Gibby M, Sarvela J (1996) Phloroglucinol derivatives in *Dryopteris* sect. *Fibrillosae* and related taxa (Pteridophyta, Dryopteridaceae). Ann Bot Fenn 33:69–100

Widén C-J, Cr F-J, Reichstein T, Sarvela J (1999) A survey of phenolic compounds in *Dryopteris* and related fern genera. Part II. Phloroglucinol derivatives in subgenus *Dryopteris* (Pteridophyta, Dryopteridaceae). Acta Bot Fenn 164:16–54

Widén CJ, Fraser-Jenkins CR, Roux JP (2015) A survey of phenolic compounds in *Dryopteris* and related fern genera. Part IV. Phloroglucinol derivatives and morphology in the section Marginatae (Pteridophyta, Dryopteridaceae) Ann. Bot Fen. 52(1–2):53–83

Whittier DP, Storchova H (2007) The gametophyte of *Huperzia selago* in culture. Am Fern J 97(3):149–154

Wollenweber E, Stevens JF, Ivanic M, Ml D (1998) Acylphloroglucinols and flavonoid aglycones produced by external glands on the leaves of two *Dryopteris* ferns and *Currania robertiana.* Phytochemistry 48(6):931–939. https://doi.org/10.1016/S0031-9422(97)01003-0

Wong KC, Pang WY, Wang XL, Mok SK et al (2013) *Drynaria fortunei*-derived total flavonoid fraction and isolated compounds exert oestrogen-like protective effects in bone. Br J Nutr 110:475–485

Wright CI, Van-Buren L, Kroner CI, Koning MMG (2007) Herbal medicines as diuretics: A review of the scientific evidence. J Ethnopharmacol 114(1):1–31

Wróbel D (2020) Proposition of a key to *Equisetum* genus. Herbalism 1(6):95–99

Wróbel D, Różański H (2020) *Equisetum fluviatile* L. water horsetail (*Equisetaceae* Michx. ex DC.). Herbalism 1(6)100–112

Wyk B-E, Wink M (2004) Medicinal Plants of the World. CABI, Nosworthy Way Wallingford Oxfordshire OX10 8DE UK, 520 pp

Xue JS, Zhang B, Zhan HD, et al (2020) Phenylpropanoid derivatives are essential components of sporopollenin in vascular plants. Mol. Plant S1674–2052–2056

Xueping L, Jiayuan L, Tao J, Lili Z, Yixi H et al (2018) Analysis of chemical composition and in vitro antidermatophyte activity of ethanol extracts of *Dryopteris fragrans* (L.) Schott. J Ethnopharmacol 226:36–43

Yamada K, Ojika M, Kigoshi H (2007) Ptaquiloside, the major toxin of bracken, and related terpene glycosides: chemistry, biology and ecology. Nat Prod Rep 24:798–813

Yao H, Duan J, Ai F, Li Y (2012) Chemical constituents from a Chinese fern *Polypodium hastatum* Thunb. Bioch Syst Ecole 44:275–278. https://doi.org/10.1016/j.bse.2012.06.013

Yao CP, Zou ZX, Zhang Y, Li J, Cheng F et al (2018) New adenine analogues and a pyrrole alkaloid from *Selaginella delicatula.* Nat Prod Res 4:1–7

Yu S, Yan H, Zhang L, Shan M, Chen P, Ding A, Li SFY (2017) A Review on the Phytochemistry, Pharmacology, and Pharmacokinetics of Amentoflavone, a Naturally-Occurring Biflavonoid. Molecules 22(2):299

Yuan Q, Wang J (2012) Ruan J (2012) Screening for bioactive compounds from *Adiantum capillus-veneris* L. J Chem Soc Pak 34:207–216

Yuan Q, Zhang X, Liu Z, Song S, Xue P, Wang J, Ruan J (2013) Ethanol extract of *Adiantum capillus-veneris* L suppresses the production of inflammatory mediators by inhibi t ing NF-κB act ivat ion. J Ethnopharmacol 147(3):603–611

Zarzycki K, Kaźmierczakowa R (eds) (1993) Polska czerwona księga roślin. Paprotniki i rośliny kwiatowe. Inst. Botaniki im. W. Szafera PAN, Instytut Ochrony Przyrody PAN. Kraków pp 305

Zenkteler E (2000) Systemy wegetatywnego rozmnażania paproci in vivo oraz in vitro. Wydawnictwo Naukowe Uniwersytetu im. A. Mickiewicza w Poznaniu, pp 88–109

Zhang T, Wang L, Duan D-H, Zhang Y-H, Huang S-X, Chang Y (2018) Cytotoxicity-Guided Isolation of Two New Phenolic Derivatives from *Dryopteris fragrans* (L.) Schott. *Molecules 23*,1652

Zheng X-D, Hu H-B, Hu H-S (2008) A new Neolignan Glycoside from *Pteris multifida* Poir. Bull Korean Chem Soc 29(6)

Zhong Z-C, Zhao D-D, Liu Z-D, Jiang S, Zhang Y-L (2017) A New Human Cancer Cell Proliferation Inhibition Sesquiterpene, Dryofraterpene A, from Medicinal Plant *Dryopteris fragrans* (L.) Schott. *Molecules 22,*180

Zhou X-Ch, Abulimit M, Ababakril S, He Ch-X (2007) Study on Extraction and Anti-microbial Activities of Total Alkaloids from *Dryopteris filix-mas* (L.) Schott. Bull Disease Control & Prevention, 22 (5):14

Zivkovic S, Milutinovic M, Maksimov V, Ciric A, Ivanov M, Bozunovic J, Banjanac T, Misic D (2020) Antioxidant and antimicrobial activity of two *Asplenium* species. S Afr J Bot 132:180–187

Chapter 20
Solidago virgaurea L.—Chemical Composition, Traditional and Medicinal Use, Pharmaceutical Properties, Potential Application, and Biotechnological Studies—A Review

Jaromir Budzianowski, Barbara Thiem, and Małgorzata Kikowska

Abstract *Solidago virgaurea* L.—a herbaceous perennial found in Europe, Asia and North America, is a source of several important secondary metabolites, which possess great potential for medicinal purposes. The raw material contains a wide spectrum of bioactive compounds including bisdesmosidic phenol glycosides, flavonoids, phenolic acids and their depsides, *cis*-clerodane-type diterpenes, oleanane-type triterpenoid saponins, the essential oil, and anthocyanins. At present, *S. virgaurea* has the application as a diuretic, an antispasmodic, an analgesic, and an anti-inflammatory fact in diseases of the urinary system. Earlier, the plant was widely used in traditional medicine. *Solidaginis virgaureae herba* is used as a diuretic and a disinfectant of the urinary tract. Due to the herb overexploitation from its natural habitats, this taxon has become a rare species in Europe. Nowadays European goldenrod is cultivated in some European countries as a result of agronomic scientific research. Therefore, the method of plant micropropagation and other in-vitro growth systems allow plant biomass to be multiplied for phytochemical and biological research. This review presents the unique chemical and pharmaceutical properties of *Solidago virgaurea* L., a species that differs from other *Solidago* taxa.

Keywords European goldenrod · Secondary metabolites · Biological activity · In-vitro cultures · Cultivation · Utilization

20.1 Botanical Description and Distribution

Solidago virgaurea L. (synonymous: *Amphiraphis leiocarpa*, *Amphiraphis pubescens*, *Dectis decurrens*, *Doria virgaurea*; also called European goldenrod, Aaron's rod or woundwort) is a magnificent herbaceous perennial (Fig. 20.1) of the family of Asteraceae (formerly Compositae).

J. Budzianowski · B. Thiem (✉) · M. Kikowska
Department of Pharmaceutical Botany and Plant Biotechnology, Poznan University of Medical Sciences, 14 Św. Marii Magdaleny St., 61-861 Poznań, Poland
e-mail: bthiem@ump.edu.pl

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_20

Fig. 20.1 *Solidago virgaurea* L. in the pharmacognostic garden (*Photo* M. Nowak)

Its striated stem, reddish-violet at the lower part, reaching a height of up to 1 m, is abundantly sprinkled with leaves of various shapes. Basal leaves are obovate or oblanceolate with a serrate margin and a long petiole, while cauline leaves are alternate with a toothed margin and a short petiole. The plant has a woody rhizome, which is cylindrical, nodded and short. Its yellow flowers are gathered in capitula inflorescences forming a tightly packed panicle. Each capitulum contains 6–12 female florets and about 10–30 hermaphrodite, tubular florets. It blossoms from July to October, but flower shoots appear only in the second year. The fruit is a cylindrical achene with a white pappus composed of bristle hairs. The appearance of this plant depends largely on the conditions in which it grows; the phenotypic variation reflects its

habitat heterogeneity—it looks different in the mountains, where it produces special low forms, and different in the lowlands (Hirano et al. 2017; European Pharmacopeia 9.0; PDR 2000).

European goldenrod is widespread across Europe as well as North Africa and Northern, Central and Southwest Asia (China, Russia, India, Turkey, Kazakhstan, etc.). It grows wild or is cultivated as a garden flower in many different cultivars for decorative purposes. This species is not as common as it was once believed, its resources are decreasing. Nowadays, this taxon is displaced by the species of foreign origin, which are characterized by faster growth, greater habitat tolerance and better crossing conditions. *S. virgaurea* is a polymorphic taxon within several closely related taxa, which have been described as various ranks from variety and the subspecies to the species (Kiełtyk and Mirek 2014; Różański et al. 2016). In Poland, the genus *Solidago* is mainly represented by four species that is *S. canadensis*, *S. gigantea*, *S. graminifolia*, and *S. virgaurea.* In Europe and Asia, the native species is *S. virgaurea*, which crossed with *S. canadensis* known as *S. niederederi*. In higher mountainous locations, European goldenrod gradually changes from a lowland to mountain form – *S. virgaurea* ssp. *alpestris.* For many years the plant has been found as a ruderal at the edges of forests, scrubs, roadside, and wasteland (Rola and Rola 2010).

20.2 Use of Raw Material

According to European Pharmacopeia 9.0, the definition of the raw plant material (herbal substance) of *Solidaginis virgaureae herba* is: "whole or fragmented, dried, flowering aerial parts of *Solidago virgaurea* L." with minimum 0.5% and maximum 1.5% of flavonoids, expressed as hyperoside (European Pharmacopeia 9.0). There must be no confusion with other adulterations, despite the differences in quality and quantity of compounds and their actions, drugs containing *Solidago gigantea* or *Solidago canadensis* are exchanged with *Solidago virgaurea* on the market (PDR 2000). Some pharmacopeial studies (for example, Polish Pharmacopeia 11) distinguish between the two raw materials. *Solidaginis herba*—goldenrod herb, these are flowering aerial parts of *Solidago gigantea* or *Solidago canadensis*, their varieties or hybrids and/or their mixtures. Such a herb should contain not less than 2.5% of flavonoids, calculated as hyperoside. Whereas *Solidaginis virgaureae herba* – European goldenrod herb, these are flowering aerial parts of *Solidago virgaurea.* Such a herb should contain not less than 0.5% and not more than 1.5% of flavonoids in terms of hyperoside (Polish Pharmacopeia 11). Regardless of whether it is *Solidaginis virgaureae herba* or *Solidaginis herba*, upper parts of goldenrod shoots are cut during the harvest, which in practice means that inflorescences dominate the weight of the material. The more leaves and flowers are there in the raw material, the more valuable raw material is for healing. If stalks with a negligible content of active substances are dominant, they reduce quality of the raw material and its pharmacognostic value (Różański et al. 2016).

S. virgaurea has been a medicinal plant known in Europe since the thirteenth century (Madaus 1938). The traditional use of *Solidago virgaurea* L. is well-documented in whole Europe. In the Czech Republic, the herbal substance is only available in combination products. The comminuted substance and combination products in the form of tea are mostly used as adjuvant therapy in inflammations of the urinary tract and prevention of cysto- and nephrolithiasis. In Germany and Sweden, traditional herbal medicinal products, the ethanolic extracts of the fresh herb, are used for supporting the elimination function of the kidneys. Most of products on Polish and Spanish markets are herbal teas with *Solidaginis virgaureae herba* being one of components. No authorized herbal medicinal products containing *Solidago virgaurea* are on the Bulgarian, Finnish, Irish, Latvian, Slovenian, and English markets (EMEA 2008).

The raw material has a diuretic effect due to increased filtration in the renal glomeruli and the decrease in renal resorption in the renal tubules (leiocarposide, flavonoids and saponosides); it lowers blood pressure, also has the spasmodic, anti-inflammatory and weak analgesic effects (leiocarposide). Polyphenols present in the raw material form water-soluble complexes with toxic metabolic products, which facilitate their excretion in urine. The diuretic effect is due to inhibition of the enzyme converting angiotensin (ACE) and inhibition of inert endopeptidase activity (NEP). Flavonoids seal the walls of small blood vessels (EMEA 2008).

The raw material is used for increasing the amount of urine excreted in bacterial infections and inflammations of the kidneys and urinary tract, as well as for preventing the formation of stones and kidney sand, and supporting treatment of rheumatism and certain dermatoses. American phytotherapists pay attention to the use of goldenrod in treatment of catarrh of the respiratory system, which is justified due to the content of triterpene saponins in the raw material (Skidmor-Roth 2010).

Due to its astringent and antibacterial properties (tannins), the raw material is also used in treatment of inflammation of the gastrointestinal tract, the upper respiratory tract and topically in some skin diseases (EMEA 2008).

Goldenrod herb is found in herbal mixtures, lotions, pastes, drops, and tablets. According to Polish Pharmacopeia 8.0, it is used as a diuretic and a disinfectant of the urinary tract (orally, 6–12 g of the raw material daily). The raw material is not used in the case of oedema caused by the impaired heart or kidney function. This is a product for use in specified indications exclusively based upon long-standing use. The side effects occur in the case of allergy/hypersensitivity to plants of Compositae (Asteraceae) family (Polish Pharmacopeia 8.0). According to the European Medicines Agency/Evaluation of Medicines for Human Use interactions with other medicinal products, the influence on the ability to drive, and overdose were not reported (EMEA 2008).

Dietary supplements aimed at influencing the function of the urinary system most often, apart from the fruit extract of large cranberry (*Vaccinium macrocarpon*), contain also the goldenrod herb extract, which contributes to proper functioning of the urinary system and urine output as well as supports the maintenance of the healthy bladder and the urinary tract (pharmacy on-line).

20.3 Biological and Pharmacological Activity

There are several in-vitro studies on the antimicrobial activity of *Solidago virgaurea* L. The alcoholic extracts were studied for their activity against the following bacteria strains: *Bacillus subtilis*, *Bacillus pumilus*, *Proteus mirabilis*, *Proteus vulgaris*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* (Thiem and Goslińska 2002), *S. aureus*, *Enterobacter faecalis*, *E. coli, P. aeruginosa, Bacillus cereus, B. subtilis, Klebsiella pneumonia*e (Demir et al. 2009), *S. aureus*, *S. faecalis*, *B. subtilis*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa* (Kołodziej et al. 2011). The experiments conducted by Brantner resulted in the observations that the extracts of *S. virgaurea* exhibited higher activity against *S. aureus* and *S. epidermidis* than those from *S. gigantea* and *S. canadensis* (Brantner 1999). In all the studies, the extracts demonstrated moderate bactericidal activity and were more potent against Gram-positive bacteria than Gram-negative. The most sensitive strains of bacteria were *B. subtilis* and *P. aureus* (Thiem and Goslińska 2002; Demir et al. 2009; Kołodziej et al. 2011).

The latest research on *S. virgaurea* ssp. *virgaurea* activity against *Candida albicans* was conducted in 2020 by the team of French scientists. The plant extract inhibited adherence and the hyphal formation of *C. albicans.* Even though the extract from the studied plant reduced biomass of *Candida*-bacteria biofilm, it did not reduce growth of bacteria and fungi. As the authors of the study concluded, the effectiveness of toothpaste containing *Solidago* extract in the randomized, double-blind clinical study could result from interactions between lipids, iron and solidagosaponins, which showed detergent and the iron chelator activity (Precheur et al. 2020). The antimycotic activity against dermatophytes - *Trichophyton mentagrophytes*, *Microsporum gypseum*, and *Microsporum canis* was shown for the ethanolic extract of *S. virgaurea* (Pepeljnjak et al. 1998).

In another study, *S. virgaurea* displayed the amoebicidal and amoebistatic activity. As shown by the authors, the extract had chemotherapeutic property in-vitro at the concentration of approximately 0.01 mg extract/mL. Moreover, the application of the extract extended the survival time for mice infected with *Acanthamoeba castellanii* (Derda et al. 2009).

The antioxidant activity for the methanolic and aqueous extracts from *Solidago virgaurea* was proved in DPPH free radical scavenging activity and reducing power assays. The activity of the methanolic extract was higher than of the aqueous extract (Demir et al. 2009).

The phenolic acid fraction of *Solidago virgaurea* aerial part as well as the isolated compounds were tested to assess the anti-inflammatory activity. The carrageen-induced rat paw oedema model was applied, while the level of the pro-inflammatory cytokines was measured. The isolated compound—3,4,5-*O*-tricaffeoyl-quinic acid—exhibited significant activity in inhibiting oedema. The phenolic acid fraction and the isolated compounds (also 3,4-*O*-dicaffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid and 4,5-*O*-dicaffeoylquinic acid) inhibited the production of inflammatory mediators (Motaal et al. 2016). Additionally, the activity of triterpenoid saponin complex

of European goldenrod was studied in-vivo and the results from the experiments conducted on the oedema model in rats showed significant reduction in the volume of oedema (Jacker et al. 1981). The anti-inflammatory activity of the combined preparation Phytodolor® composed of *Solidago virgaurea*, *Fraxinus excelsior* and *Populus tremula* was shown in-vitro in an experiment on the activity of TNF-α and COX-2 and in-vivo in the oedema model in rats (Okpanyi et al. 1989; El-Ghazaly et al. 1992).

The aqueous-ethanolic extracts (from 0 to 100%) from *S. virgaurea* var. *gigantea* were investigated in order to assess the anti-obesity effect. Among all the extracts, 10% extract exhibited the strongest inhibitory effect on adipogenesis in in-vitro study of lipid accumulation in 3T3-L1 cells; the extract exhibited high anti-adipogenic effect. Supplementation with the studied extract decreased the body weight gain and food intake. *Solidago* extract reduced adipocytes size and the expression of genes related to adipogenesis in white adipose tissue and genes of markers related to lipogenesis. The authors concluded that *S. virgaurea* could be a potential food supplement for preventing obesity (Wang et al. 2017).

The ethanolic extract of *S. virgaurea* showed the spasmolytic activity of isolated smooth muscles of intestines of guinea pig (Westendorf and Vahlensieck 1981). The aqueous extract of leaves inhibited muscarinic M_2 and M_3 receptor-mediated contraction of rat and human bladder muscle strips. The authors of these studies wanted to investigate the effect of the extract on muscarinic receptors as they are the main pharmacological target in treatment of the bladder dysfunction. However, the therapeutic effects of *S. virgaurea* were not demonstrated in the controlled clinical studies (Borchert et al. 2004).

Many studies were performed on the diuretic activity of *S. virgaurea* extracts and the isolated compounds. The main property of *S. virgaurea* allowing for its long-lasting medicinal use in treatment of urinary tract diseases is the diuretic activity with a complex action spectrum, including the anti-inflammatory, analgesic, anti-spasmodic, and antibacterial effects (Melzig 2004). Leiocarposide showed the anti-inflammatory activity in the carrageen oedema test in rats. Oedema inhibition was 20% and 27% at the doses of 100 mg and 200 mg/kg, respectively, and appeared after 5 h. The reference drug—phenylbutazone, reduced oedema by 66% at the dose of 50 mg/kg, already after 2 h (Metzner et al. 1984). Leiocarposide exhibited also the analgesic activity in mice, in two tests, and it was 70% and 100% at the dose of 200 mg/kg and was comparable to that of the reference drug—aminophenazone, 60% and 100% at the doses of 100 and 50 mg/kg, respectively (Metzner et al. 1984). The components considered to be responsible for the diuretic activity, such as leiocarposide, the flavonoid fraction and the saponin fraction, were isolated from aerial parts of *S. virgaurea* and subjected to the extensive pharmacological studies (Chodera et al. 1985, 1986, 1988, 1991). The results of those investigations were summarized (Budzianowski 1999). Leiocarposide at the dose of 25 mg/kg and furosemide at the dose of 6 mg/kg increased diuresis in rats by 110% and 125%, respectively, after intraperitoneal (i.p.) administration, and by 80% and 100%, respectively, after peroral (p.o.) administration. The diuretic activity of leiocarposide appeared after 5 h (i.p. and p.o.) after treatment and lasted up to 24 h, while that of furosemide

started after 1 h (i.p.) or 3 h (p.o.) and lasted up to 2 h (Chodera et al. 1985, 1986). A part of a leiocarposide molecule obtained by its degradation that is 3,6-dihydroxy-6-methoxybenzoic acid, exerted no diuretic activity. The activity of leiocarposide (25 mg/kg) was decreased by the flavonoid fraction (25 mg/kg) or the saponin fraction (1 mg/kg i.p. or 20 mg/kg p.o.) by 30% and 10%, respectively. Leiocarposide appeared to be less toxic than furosemide–LD_{50} values were 1.55 mg/kg and 0.80 mg/kg, respectively (Chodera et al. 1985). The compound did not influence the urinary excretion of potassium, sodium, and calcium ions (Chodera et al. 1985). Leiocarposide at the dose of 25 mg/kg administered p.o. as 1.0% water solution decreased growth of calculi (stones) by *ca.* 55% in the experimental urolithiasis induced by surgical implementation of human urinary calculi of known chemical composition into the rat bladder (Chodera et al. 1988). In turn, the flavonoid fraction from *S. virgaurea* (25 mg/kg, p.o.) exhibited the diuretic activity of 88% after 24 h in rats, and was stronger than that of the flavonoid fractions isolated from *S. gigantea*, *S. canad*ensis var. *canadensis* and *S. canadensis* var. *scabra*—82%, 57% and 60%, respectively. Moreover, the overnight decrease in the potassium and sodium excretion, and the increase in the calcium excretion were observed (Chodera et al. 1991). After administration to rats, leiocarposide was either found unchanged in urine (Chodera et al. 1986) or unchanged fecally excreted; in urine, it was found less than 10% as metabolites (Fötsch et al. 1989).

20.4 Biotechnological Studies

The availability of raw materials may be limited due to specificity of climate and habitat requirements, progressive environmental degradation, slow plant growth, and sometimes long development of constitutive organs as the valuable raw material. Increasing pollution and adverse changes occurring in the natural environment result in shrinkage of plant resources and collection of raw materials from natural sites in such areas becomes problematic. The yield and the content of bioactive compounds depend on climatic factors and locality. An alternative solution to these restrictions may be the production of plant biomass using biotechnological methods. In-vitro cultures create the possibility of producing equal and homogeneous biomass in a continuous production process, regardless of the changing climatic and environmental conditions. The method of plant micropropagation and other in-vitro growth systems allow for plant biomass multiplication for phytochemical and biological research. In addition, there may appear certain problems related to collection, transport and storage of the plant material for the extraction and due to the uniform material available throughout the year they may be avoided. Micropropagation is aimed at obtaining a large number of homogeneous plants using only a small fragment of the parent plant. This technique can provide the renewable amount of the raw material, allowing for phytochemical profile assessment, quantitative analyzes and studying the activity of the biological extracts, the fractions or the isolated compounds, which

is especially relevant for the rare species, endemics and protected plants (Debnath et al. 2006).

The first indications of the introduction of *Solidago virgaurea* into in-vitro cultures conditions date back to the late 1990s. The seed germinated with the efficiency greater than 50% on gibberellic acid-enriched MS media. The next step of micropropagation—shoot multiplication with employment of shoot tips as initial explants via lateral buds development, was carried out on the medium supplemented with a single cytokinin (BAP) or combination of a cytokinin with an auxin (kinetin and IAA) (Fig. 20.2). The presence of BAP in the medium generated higher induction of new shoots. Rooting occurred spontaneously on the previously mentioned media, which

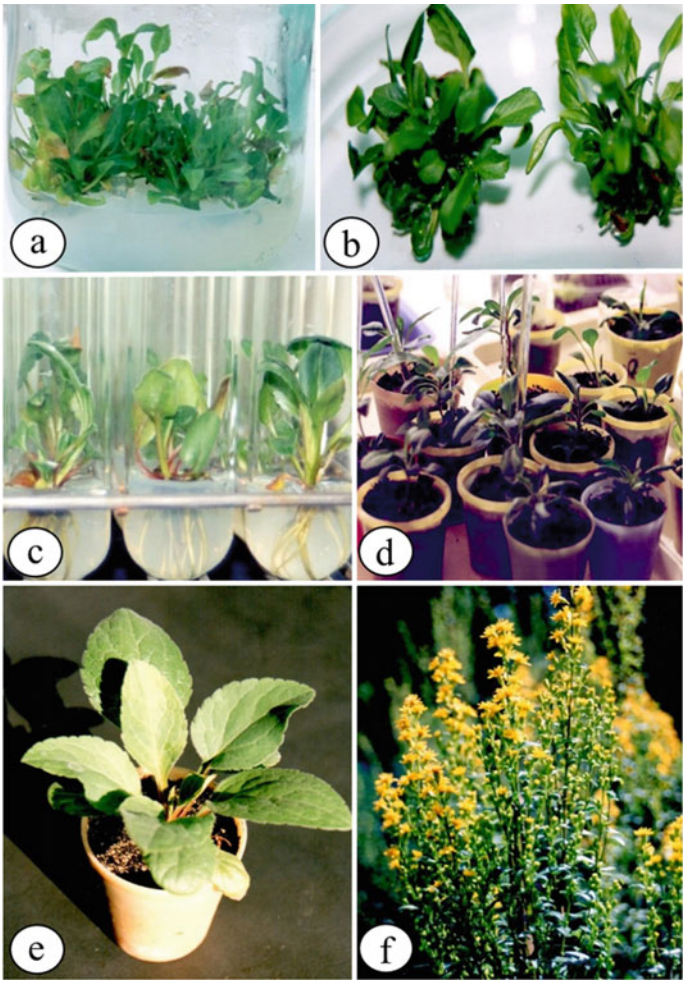


Fig. 20.2 Micropropagation of *Solidago virgaurea* L. **a**, **b** in-vitro multiplied shoots, **c** rooted shoots, **d**, **e** hardened plants in the pots, **f** acclimatized flowering plants in the experimental plot

were obviously devoted for shoot multiplication; nonetheless, the addition of an auxin (IAA) greatly improved the process (Fig. 20.2). At the next stage, micropropagated plantlets were transferred to pots and then to the field, where they bloomed and fruited (Fig. 20.2). Fragments of seedlings as well as leaves and roots were used as explants for callus induction on MS medium supplemented with 2,4-D and BAP. Such callus was characterized by yellow color with red centers and a friable structure (Fig. 20.3) (Skrzypczak et al. 1999). Although *S. virgaurea* propagation took place via axillary buds, the method applied to propagate medicinal plants with genetic stability, the number of passages and phytohormones at high concentrations could affect the appearance of somaclonal variability in cultures. Confirmation of genetic stability is of particular importance in medicinal plants in-vitro propagation. The flow cytometric analysis showed that there was no difference between the DNA content in seedlings and micropropagated plantlets. The genome size during one year of in-vitro culture was stable (Sliwinska and Thiem 2007).

These first pilot studies were of general nature, certainly requiring more detailed investigation, especially in the estimation of biotechnology process parameters. However, the experiments were the first records regarding the possibility of introducing *Solidago* species, including *S. virgaurea*, into in-vitro cultures. A reliable

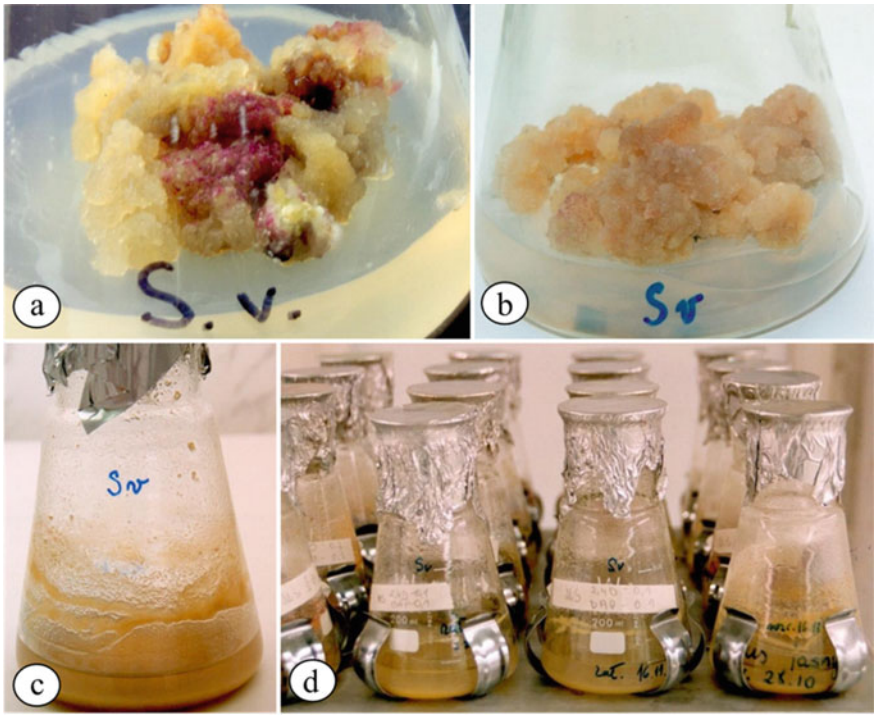


Fig. 20.3 In-vitro cell cultures of *Solidago virgaurea* L. **a**, **b** callus, **c** cell suspension, **d** cell cultures on a rotary shaker

and repeatable protocol of *S. virgaurea* micropropagation was established by the Indian team of researchers led by J. P. Paul 13 years ago. In-vitro cultures were not initiated from seeds, but from nodal explants plants growing in the botanical garden. The explant sterilization process resulted in the highest efficiency of the process (92.3%). Clonal propagation was implemented via bud initiation, while development by applying nodal segments as explants. The highest number of new shoots in in-vitro propagation was obtained on MS medium supplemented with kinetin and IAA. The highest number of roots was achieved on MS medium with IAA and BAP. In-vitro-propagated plantlets were successfully transferred to the field (Paul et al. 2012).

In-vitro cultures of *S. virgaurea* have created the possibility of producing biomass for genetic (Sliwinska and Thiem 2007), phytochemical, (the essential oils (Kalemba and Thiem 2004)), phenolic compounds (Thiem et al. 2001), and biological (antimicrobial (Thiem and Goślińska 2002)) studies of this valuable species.

20.5 Chemistry

Solidago virgaurea L. contains a wide spectrum of bioactive compounds including bisdesmosidic phenol glycosides—leiocarposide and virgaureoside A, flavonoids—mostly quercetin, kaempferol, isorhamnetin, and their glycosides, phenolic acids with caffeic acid as the main compound, depsides—chlorogenic acid and di- or tricaffeoylquinic acids, *cis*-clerodane-type diterpenes, oleanane-type triterpenoid saponins, the essential oil with the germacrene D, limonene, *α-p*inene, and myrcene as the major constituents, and anthocyanins.

20.5.1 Phenolic Compounds

The content of total flavonoids, total *o*-dihydroxyphenols and total polyphenols in the herb, flowers, leaves, and stems of *S. virgaurea* of Polish origin was determined using Christ-Müller, Arnov and Folin-Ciocalteu methods, respectively (Kalemba et al. 1992). The contents were significantly lower than those found in the related medicinal species, namely *S. canadensis* and *S. gigantea.*

20.5.1.1 Phenol glucosides-

During investigations of the saponin fraction from *S. virgaurea* L. var. *leiocarpa* (Benth) A. Grey (currently synonym for *S. virgaurea* L. (The Plant List 2020), a compound with foaming properties typical of saponins was isolated, but it appeared to be a new ester, phenol—bisdesmosidic glucoside, and it was

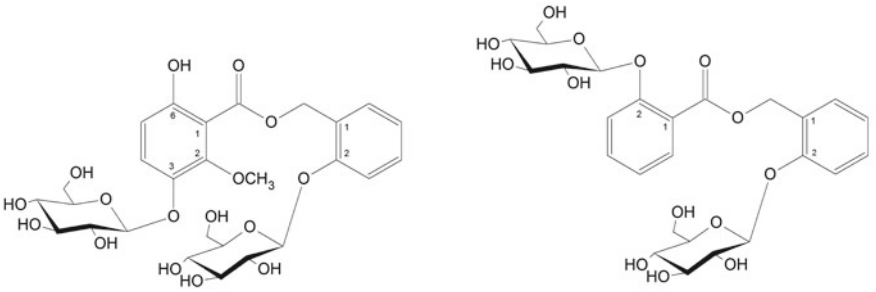


Fig. 20.4 Chemical structures of leiocarposide and virgaureoside A

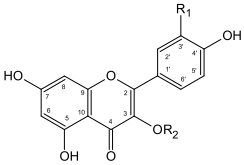
named leiocarposide (Hiller et al. 1979; Gründemann et al. 1979). The structural elucidation of ester conjugates before the era of correlation NMR spectroscopy (2D NMR) was particularly challenging, therefore, the final structure, of leiocarposide—2-(β-D-glucopyranosyloxy)benzyl 3-(β-D-glucopyranosyloxy)-6-hydroxy-2-methoxybenzoate (Fig. 20.4), was established later (Fötsch et al. 1988). It is interesting to note that a very well-known compound—salicin (2-glucopyranosyloxy-benzyl alcohol), is a part of a leiocarposide molecule. A partly characterized colorless, crystalline phenol glycoside (melting point, optical rotation), isolated during work on flavonoids in *S. virgaurea* growing wild in Poland (Skrzypczakowa 1962), was identified as leiocarposide (Skrzypczak and Ellnain-Wojtaszek 1981) by direct comparison to the sample (Hiller et al. 1979; Gründemann et al. 1979).

Yet another bisdesmosidic phenol glucoside was isolated and named virgaureoside A (Hiller et al. 1985) (Fig. 20.4). The content of leiocarposide and virgaureoside A in aerial parts varied in the ranges of 0.08–0.48% and 0.01–0.14%, respectively (Hiller and Fötsch 1986). Leiocarposide is restricted to *S. virgaurea* and definitely absent from the related species of similar medicinal uses that is *S. canadensis* and *S. gigantea* (Budzianowski et al. 1990). The compound was isolated from intact plants (inflorescences) and in-vitro cultured *S. virgaurea* plants, from the water soluble portion of the methanol extract by column chromatography on polyamide. The identity of the isolate was established by ^{1}H and ^{13}C NMR verified by 2D NMR (HH-COSY, HMQC and HMBC) spectroscopy (Skrzypczak et al. 1999). Unfortunately, leiocarposide was not synthesized in in-vitro cultured callus tissue (Thiem et al. 2001).

20.5.1.2 Flavonoids-

In all the investigations on flavonoids, *S. virgaurea* exhibited the presence of flavonols only, namely kaempferol, quercetin and isorhamnetin substituted with mono- and disaccharide moieties exclusively at the C-3 position (summarized by (Wittig and Veit 1999)) (Table 20.1). The early studies resulted in isolation and identifica-

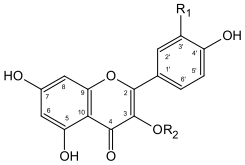
Table 20.1 Flavonoids of *Solidago virgaurea*



	Compound name	R_1	R_2	References
1	kaempferol	H	H	Budzianowski et al. (1990)
2	kaempferol 3-*O*-arabinoside	H	ara-	Budzianowski et al. (1990)
3	kaempferol 3-*O*-glucoside (astragalin)	H	glc-	Skrzypczakowa (1962), Schilcher (1964), Hiller et al. (1979), Budzianowski et al. (1990), Rosłon et al. (2014)
4	kaempferol 3-*O*-galactoside	H	gal-	Budzianowski et al. (1990)
5	kaempferol 3-*O*-rutinoside (nicotiflorin)	H	rha-(1–6)-glc-	Schilcher (1964), Hiller et al. (1979), Budzianowski et al. (1990), Pietta et al. (1991)
6	kaempferol 3-*O*-robinobioside	H	rha-(1–6)-gal-	Budzianowski et al. (1990), Pietta et al. (1991)
7	quercetin	OH	OH	Skrzypczakowa (1962), Budzianowski et al. (1990)
8	quercetin 3-*O*-arabinopyranoside	OH	arap-	Budzianowski et al. (1990)
9	quercetin 3-*O*-glucoside (isoquercitrin)	OH	glc-	Schilcher (1964), Hiller et al. (1979), Budzianowski et al. (1990)

(continued)

Table 20.1 (continued)



	Compound name	R_1	R_2	References
10	quercetin 3-*O*-galactoside (hyperoside)	OH	gal-	Budzianowski et al. (1990), Rosłon et al. (2014)
11	quercetin 3-*O*-rhamnoside (afzelin)	OH	rha-	Schilcher (1964)
12	quercetin 3-*O*-rutinoside (rutoside)	OH	rha-(1–6)-glc-	Skrzypczakowa (1961), Hiller et al. (1979), Budzianowski et al. (1990), Pietta et al. (1991), Rosłon et al. (2014)
13	quercetin 3-*O*-robinobioside	OH	rha-(1–6)-gal-	Budzianowski et al. (1990)
14	isorhamnetin	OCH_3	H	Budzianowski et al. (1990)
15	isorhamnetin 3-*O*-glucoside	OCH_3	glc-	Budzianowski et al. (1990)
16	isorhamnetin 3-*O*-galactoside	OCH_3	gal-	Budzianowski et al. (1990)
17	isorhamnetin 3-*O*-rutinoside	OCH_3	rha-(1–6)-glc-	Budzianowski et al. (1990), Pietta et al. (1991)

arap arabinopyranose, *gal* galactose, *glc* glucose, *rha* rhamnose

tion of kaempferol and quercetin 3-*O*-glucosides and quercetin 3-*O*-rutinoside (3-*O*-rhamno-1 → 6-glucoside) (Skrzypczakowa 1961). The butanol fraction of the methanol extract from inflorescences was separated by column and preparative thin-layer chromatography on polyamide to give 16 flavonoids. They were identified by ^{1}H and ^{13}C NMR and the analyses of sugar residues liberated by hydrolytic or oxidative degradation was performed (Budzianowski et al. 1990). Flavonoids are well-known valuable chemotaxonomic markers. Two-dimensional thin-layer chromatograms (2D-TLC) on polyamide of the butanol fractions proved to be an excellent tool (TLC finger-printing) for discrimination of *S. virgaurea* from the other

related species like *S. gigantea* and *S. canadensis* varieties *canadensis* and *scabra* (Budzianowski et al. 1988, 1990).

More recent investigations on the occurrence of enantiomeric flavanones in a few plant species revealed small quantities of eriodyctiol (13.1 μg/g) in *S. virgaurea* flowers, predominantly as the (S)-enantiomer (Kruk et al. 2019). Also, solid liquid extraction-solid phase extraction-ultra high performance liquid chromatography-tandem mass spectrometry (SLE-SPE-UHPLC-MS/MS) assay showed quercetin and quercetin 3-*O*-rutinoside to be abundant flavonoids in leaves, flowers and stems (Bajkacz et al. 2018).

20.5.1.3 Phenolic acids-

Caffeic acid was the first compound found in *S. virgaurea* (Björkman and Holmgren 1960; Borkowski and Skrzypczakowa 1962). 80% Methanol extract from aerial parts of *S. virgaurea* collected from natural habitats in Poland was processed to obtain the free acids fraction and the fractions of phenolic acids liberated by either acidic or alkaline hydrolysis (Kalemba 1992). Those three phenolic acid fractions were silylated and analyzed by GC. The main free acids were salicylic, ferulic, sinapic, and protocatechuic acids—9.9, 7.7, 5.1 and 4.8 mg/100 g, respectively. The minor acids were vanillic, caffeic, *p*-coumaric, syringic, and *p*-hydroxybenzoic acids. The most abundant acids, following acidic and alkaline hydrolyses, were protocatechuic acid (6.1 mg/100 g) and caffeic acid (1765.1 mg/100 g), respectively.

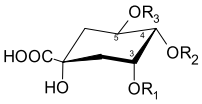
Very recent investigations of wild-growing plants employing SLE-SPE-UHPLC-MS/MS assay indicated that 4-hydroxybenzoic, 3,4-dihydroxybenzoic, caffeic, *p*-coumaric, and ferulic acids are the most abundant free phenolic acids in leaves, flowers and stems (Bajkacz et al. 2018). Their content varied in the range of 1423 ng/g of 4-hydroxybenzoic in flowers and 104.3 ng/g for *p*-coumaric acid in stems.

20.5.1.4 Depsides-

Chlorogenic acid (5-caffeoylquinic acid) was the first depside found in *S. virgaurea* (Björkman and Holmgren 1960; Borkowski and Skrzypczakowa 1962). Four caffeoyl esters of quinic acid (Table 20.2) and one ester of shikimic acid (5-caffeoylshikimic acid) were isolated from dried aerial parts of *S. virgaurea* by column chromatography on Sephadex LH20, polyamide, RP-18 silica gel and HPLC (Poetsch 1999). Their structures were established by means of NMR spectroscopy and molecular modeling as described by Pauli et al. (1998). The quantitative determination by HPLC showed that 5-caffeoylquinic acid and 3,5-dicaffeoyl-quinic acid (Fig. 20.5) were the main constituents—1.10% and 2.80% of dry weight, respectively (Poetsch 1999). The minor compounds were 3,4- and 4,5-dicaffeoyl derivatives.

The commercial dry extract prepared from aerial parts of *S. virgaurea* with 30% ethanol was partitioned between the water and organic solvents (Motaal et al. 2016). The ethyl acetate-butanol 2:1 (v/v) fraction thus obtained was separated by

Table 20.2 Caffeoylquinic acids of *Solidago virgaurea*



No	Compound name/compound name	R1	R2	R3	References
1	chlorogenic acid (5-*O*-caffeoylquinic acid)	H	H	caffeoyl	Björkman and Holmgren (1960), Borkowski and Skrzypczakowa (1962)[a], Poetsch (1999)[b], Thiem et al. (2001[c])
2	neochlorogenic acid (3-*O*-caffeoylquinic acid)	caffeoyl	H	H	Poetsch (1999)[b]
3	3,4-di-*O*-caffeoylquinic acid	caffeoyl	caffeoyl	H	Motaal et al. (2016)[d]
4	3,5-di-*O*-caffeoylquinic acid	caffeoyl	H	caffeoyl	Poetsch (1999)[b], Thiem et al. (2001)[c], Motaal et al. (2016)[d]
5	4,5-di-*O*-caffeoylquinic acid	H	caffeoyl	caffeoyl	Poetsch (1999)[b], Motaal et al. (2016)[d]
6	3,4,5-tri-*O*-caffeoylquinic acid	caffeoyl	caffeoyl	caffeoyl	Motaal et al. (2016)[d]

[a]Aerial part
[b]Aerial part
[c]In-vitro cultures (callus)
[d]Commercial extract of aerial parts

repeated reversed phase vacuum liquid chromatography (VLC) on silica gel RP-8. Four isolated caffeoylquinic acids, including 3,4,5-tri-*O*-caffeoylquinic acid—a new compound for the genus *Solidago*, were identified by ^{1}H and ^{13}C NMR (Table 20.2).

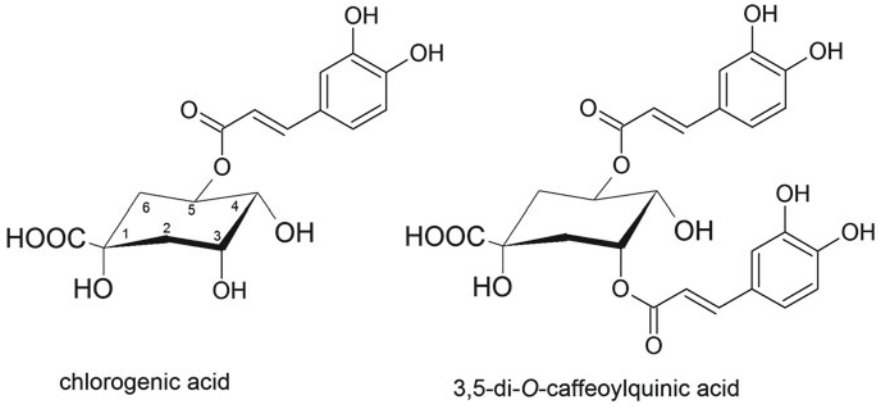


Fig. 20.5 Major caffeoylquinic acids of *Solidago virgaurea*

20.5.2 Terpenoids

20.5.2.1 Diterpenes-

The processed chloroform extract from aerial parts of *S. virgaurea* collected in Northeast India yielded 12 diterpenes after column and thin-layer chromatography on silica gel (Goswami et al. 1984). The structures, established mainly by NMR spectroscopy, showed they are all *cis*-clerodane lactones, for example, compounds 1 and 2 in Fig. 20.6. Nine clerodanes containing carboxylic groups were isolated from the ethanol-ethyl acetate extract of *S. virgaurea* of Bulgarian origin by means of silica gel, RP-18 and HPLC chromatography (Starks et al. 2010). These compounds were structurally elucidated by means of 1D and 2D-NMR and HR-ESI-MS—the example compounds—3 and 4, are shown in Fig. 20.6.

20.5.2.2 Saponins-

A number of saponins were isolated from *S. virgaurea.* Those compounds were glycosides of an oleanane-type triterpene—polygalacic acid, being 3- or 16-monodesmosides, 3,28- or 16,28-bidesmosides and 3,16,28-tridesmosides, mostly with glycosyl residues esterified with monomeric, dimeric and trimeric β-hydroxybutyric acid or acetic acid (Fig. 20.7).

The *n*-butanol fraction was prepared from 80% methanol extract of aerial parts of *S. virgaurea* cultivated in Europe. It was dissolved in methanol and the saponin fraction was precipitated with ethyl ether and purified by column chromatography on Sephadex LH-20. The products of hydrolysis were sugars—glucose, xylose and rhamnose, and a triterpene—polygalacic acid as the most abundant sapogenin (Hiller et al. 1975). In the following investigations, it was established that saponins were

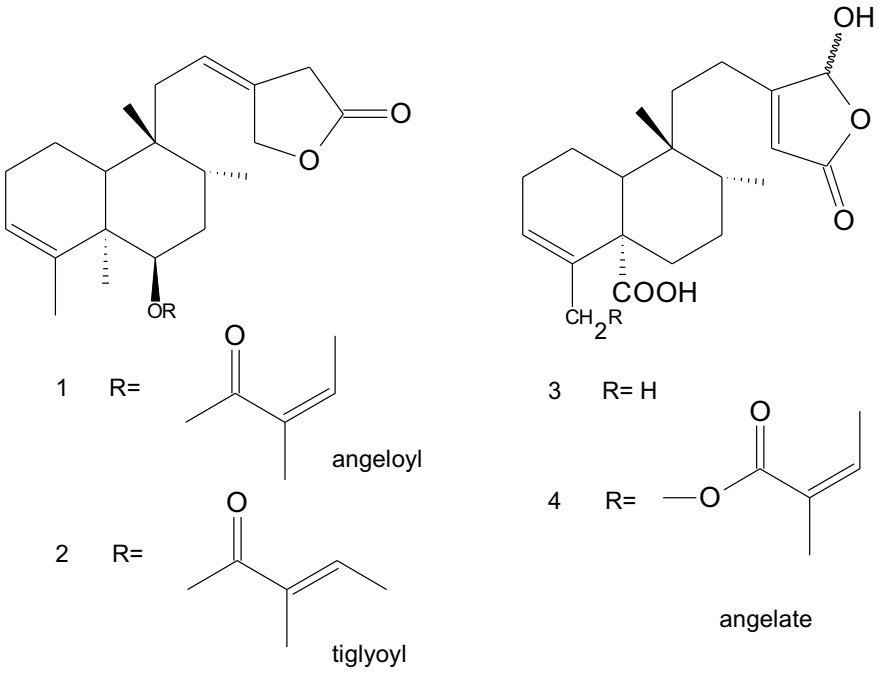


Fig. 20.6 Examples of clerodane lactones isolated from *Solidago virgaurea*

esterified with aliphatic carboxylic acids. Thus, the saponin fraction was deacylated by treatment with 1% potassium hydroxide and then new deacylated bidesmosidic saponins, named virgaureasaponins 1, 2 and 3, were isolated and their chemical structures were elucidated (Hiller et al. 1987a, b; Bader et al. 1992) (Table 20.3).

Later, the same four main saponins were isolated separately from aerial parts and roots of *S. virgaurea* subsp. *virgauera* cultivated in the botanical gardens in Europe, and structurally elucidated as solidagosaponins XIV (VSB) and XVIII (VSC) and virgaureasaponins D (VSD) and E (VSE) (Bader et al. 1995) (Table 20.4). All those saponins were acylated with either dimeric or trimeric β-hydroxybutyric acid. Upon deacylation they produced virgaureasaponin 1 (from VSB, VSC) and virgaureasaponin 2 (from VSD, VSE).

Whole plants of *S. virgaurea* of Japanese origin were extracted with hot water. The extract was passed through column of the synthetic polymer gel—Diaion HP-20 and the adsorbed material was eluted sequentially with water, 50% methanol and 100% methanol. The latter eluate was separated by combination of repeated column chromatography on various adsorbents to give about 30 saponins (Inose et al. 1991, 1992; Miyase et al. 1994). Those were mostly undescribed compounds named solidagosaponins I-XXIX, with exception of virgaureasaponin 1 (Hiller et al. 1987a), virgaureasaponin 2 (Hiller et al. 1987b) and bellisaponin BA_2 (Schöpke et al. 1991) (Table 20.3).

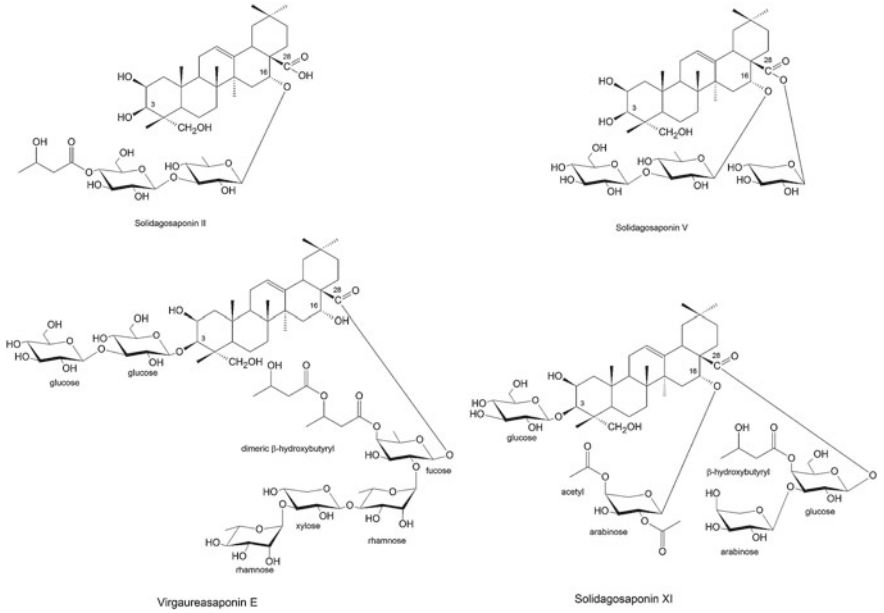


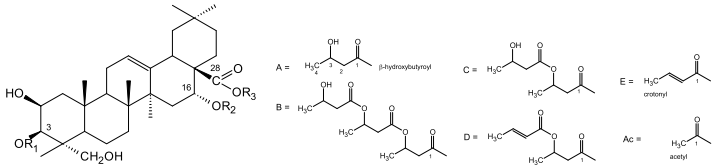
Fig. 20.7 Structural diversity of saponins occurring in *Solidago virgaurea*: examples of 16-monodesmosidic (Solidagosaponin II), 16,28-bidesmosidic (Solidagosaponin V), 3,28-bidesmosidic (Virgaureasaponin E), and 3,16,28-tridesmosidic (Solidagosaponin XI) saponins

20.5.2.3 Volatile Oil-

Early investigations of the volatile oil of *S. virgaurea* grown in Japan revealed the presence of germacrene D, limonene, α-pinene, and myrcene, (Fig. 20.8) as main constituents, and also β-elemene, β-caryophyllene and δ-cadinene (Fujita 1990). Dried aerial parts of wild plants from three localities in Poland, collected at different stages of development, yielded similar amounts of the volatile oil—0.32–0.37%, by hydrodistillation (Kalemba 1998). The bulked sample of the volatile oil was separated by multistep preparative flash chromatography to give components which were identified by GC, GC–MS and ^{1}H NMR. Sixty compounds were identified in total, the major of which were α-pinene, myrcene, β-pinene, limonene, germacrene D, and sabinene (Fig. 20.8), and also β-caryophyllene, α-humulene and α-muurolene. The volatile oil samples from different sites of the harvest had the same components, but they differed significantly in the content of the major components.

Similar studies of aerial flowering parts of *S. virgaurea*, micropropagated through axillary shoot development and transferred to soil cultivation, showed the same content (0.32%) of the volatile oil as in wild-growing plants (Thiem and Kalemba 2004). The oil was separated by preparative flash chromatography, and the fractions obtained were analyzed by GC-FID and GC–MS, while compounds were identified by retention indices and mass spectra (MS). GC-MS analysis, which took into

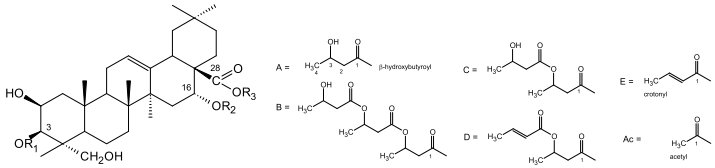
Table 20.3 Saponins isolated from whole plants of *Solidago virgaurea*



No.	Compound name	R_1	R_2	R_3	References
1	Solidagosaponin I	H	ara-(1 → 2)-glc-	H	Inose et al. (1991)
2	Solidagosaponin II	H	A-(1 → 4)-ara-(1 → 2)-glc-	H	Inose et al. (1991)
3	Solidagosaponin III	H	ara-(1 → 2)-glc-	rha	Inose et al. (1991)
4	Solidagosaponin IV	H	A-(1 → 4)-ara-(1 → 2)-glc-	rha	Inose et al. (1991)
5	Solidagosaponin V	H	ara-(1 → 2)-glc-	xyl	Inose et al. (1991)
6	Solidagosaponin VI	H	A-(1 → 4)-ara-(1 → 2)-glc-	xyl	Inose et al. (1991)
7	Solidagosaponin VII	H	ara-(1 → 2)-glc-	ara	Inose et al. (1991)
8	Solidagosaponin VIII	H	A-(1 → 4)-ara-(1 → 2)-glc-	ara	Inose et al. (1991)
9	Solidagosaponin IX	glc-(1 → 4)-glc-	H	H	Inose et al. (1991)

(continued)

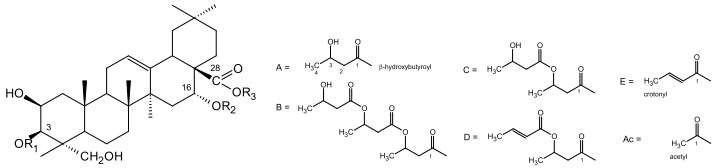
Table 20.3 (continued)



No.	Compound name	R_1	R_2	R_3	References
10	Solidagosaponin X	glc-	A-(1 → 4)-ara-(1 → 2)-glc-	3,4-diAc-ara-	Inose et al. (1992)
11	Solidagosaponin XI	glc-	A-(1 → 4)-ara-(1 → 2)-glc-	2,4-diAc-ara-	Inose et al. (1992)
12	Solidagosaponin XII	glc-	ara-(1 → 2)-glc-	3,4-diAc-ara-	Inose et al. (1992)
13	Solidagosaponin XIII	glc-	ara-(1 → 2)-glc-	2,4-diAc-ara-	Inose et al. (1992)
14	Solidagosaponin XIV (Virgaureasaponin B)	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[B (1 → 4)]-fuc-	Inose et al. (1992)
15	Solidagosaponin XV	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[Ac (1 → 4)]-fuc-	Inose et al. (1992)
16	Solidagosaponin XVI	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[A (1 → 4)]-fuc-	Inose et al. (1992)
17	Solidagosaponin XVII	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[3-Ac-A (1 → 4)]-fuc-	Inose et al. (1992)
18	Solidagosaponin XVIII	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[B (1 → 4)]-fuc-	Inose et al. (1992)

(continued)

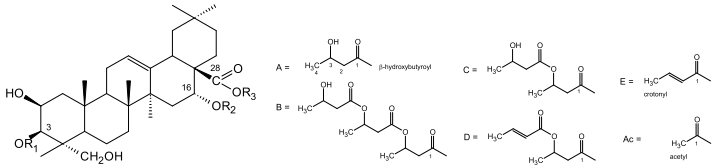
Table 20.3 (continued)



No.	Compound name	R_1	R_2	R_3	References
19	Solidagosaponin XIX (Virgaureasaponin C)	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[D (1 → 4)]-fuc-	Inose et al. (1992)
20	Solidagosaponin XX	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[5-Ac-Api (1 → 3)] [Ac-(1 → 4)]-fuc-	Inose et al. (1992)
21	Bellisaponin BA_2	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[E-(1 → 4)]-fuc-	Hiller et al. (1987), Inose et al. (1992)
22	Virgaureasaponin1	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-fuc-	Inose et al. (1992)
23	Solidagosaponin XXI	xyl-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[B-(1 → 4)]-fuc-	Miyase et al. (1994)
24	Solidagosaponin XXII	xyl-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[B-(1 → 3)]-fuc-	Miyase et al. (1994)
25	Solidagosaponin XXIII	xyl-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[5-Ac-api (1 → 3)] [B-(1 → 4)]-fuc-	Miyase et al. (1994)
26	Solidagosaponin XXIV	xyl-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[C-(1 → 4)]-fuc-	Miyase et al. (1994)

(continued)

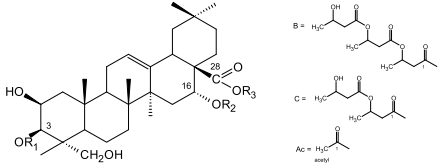
Table 20.3 (continued)



No.	Compound name	R_1	R_2	R_3	References
27	Solidagosaponin XXV	xyl-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-fuc-	Miyase et al. (1994)
28	Solidagosaponin XXVI	glc-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[B-(1 → 4)]-fuc-	Miyase et al. (1994)
29	Solidagosaponin XXVII	glc-(1 → 4)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-fuc-	Miyase et al. 1994
30	Solidagosaponin XXVIII	glc-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[4-Ac-api -(1 → 3)] [Ac-(1 → 4)]-fuc-	Miyase et al. (1994)
31	Solidagosaponin XXIX	glc-(1 → 4)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[4-Ac-Api -(1 → 3)] [Ac-(1 → 4)]-fuc-	Miyase et al. (1994)
32	Virgaureasaponin 2	glc-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-fuc-	Miyase et al. (1994)

Ac-acetyl, api-apiose, ara-arabinose, fuc-fucose, glc-glucose, rha-rhamnose, xyl-xylose

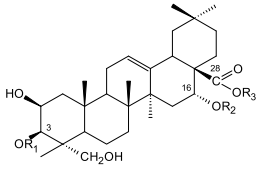
Table 20.4 Saponins isolated from the root and the herb of *Solidago virgaurea* subsp. *virgaurea*

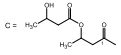


No.	Compound name	R_1	R_2	R_3	References
1	Solidagosaponin XIV	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[B-(1 → 4)]-fuc-	Bader et al. (1995)
2	Solidagosaponin XVIII	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[C-(1 → 4)]-fuc-	Bader et al. (1995)
3	Virgaureasaponin D	glc-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[B-(1 → 4)]-fuc-	Bader et al. (1995)
4	Virgaureasaponin E	glc-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[C-(1 → 4)]-fuc-	Bader et al. (1995)
5	Virgaureasaponin 1	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-fuc-	Bader et al. (1995)
6	Virgaureasaponin 2	glc-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-fuc-	Bader et al. (1995)

Fuc-fucose, glc-glucose, rha-rhamnose, xyl-xylose

Table 20.5 Saponins isolated from aerial parts of *Solidago virgaurea* subsp. *alpestris*





No.	Compound name	R_1	R_2	R_3	References
1	Virgaureasaponin 1	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-fuc-	Laurençon et al. (2013)
2	Virgaureasaponin 2	glc-(1 → 3)-glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-fuc-	Laurençon et al. (2013)
3	Virgaureasaponin C	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-[C (1 → 4)]-fuc-	Laurençon et al. (2013)
4	Virgaureasaponin 4	glc-(1 → 3)-glc-	H	xyl-(1 → 2)-rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-fuc-	Laurençon et al. (2013)
5	Virgaureasaponin 5	glc-	H	xyl-(1 → 2)-rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-fuc-	Laurençon et al. (2013)
6	Virgaureasaponin 6	glc-	H	rha-(1 → 3)-xyl-(1 → 4)-rha-(1 → 2)-)-[5-Ac-api -(1 → 3)] [C-(1 → 4)]-fuc-	Laurençon et al. (2013)

Ac-acetyl, api-apiose, fuc-fucose, rha- rhamnose, xyl-xylose

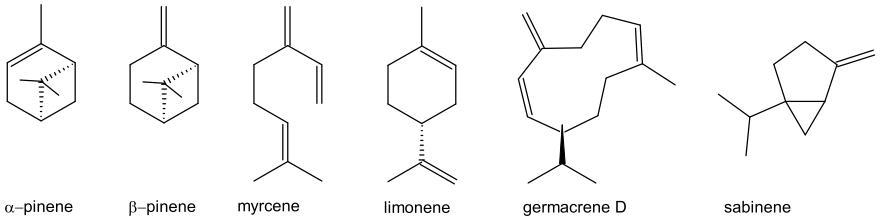


Fig. 20.8 The main constituents of the volatile oil from *Solidago virgaurea*

account 78 compounds, showed the presence of 54 constituens involving α-pinene, limonene, myrcene, and β-pinene as the major components. In general, it was found that the volatile oil of *S. virgaurea* consisted primarily of monoterpene hydrocarbons.

20.5.3 Acetylenes

Acetylenes were found in roots, but not in aerial parts of *S. virgaurea* (European origin), and identified by ^{1}H NMR spectroscopy as 2*E*,8*E*-matricaria ester, two matricaria-γ-lactones and lachnophyllum lactone (Lam 1971).

Bioassay-guided isolation of antibacterial compounds from *S. virgaurea* root extracts, using flash chromatography and semi-preparative HPLC, led to isolation and identification by NMR of two polyacetylenes, namely 2*Z*,8*Z*-matricaria ester and 2*E*,8*Z*-matricaria ester (Móricz et al. 2016) (Fig. 20.9). Those compounds appeared soon as important chemical markers allowing for ready discrimination of *S. virgaurea* from the related species by HPTLC of the root extracts. Such analysis is applicable in the wintertime, when only roots can be collected (Móricz et al. 2020).

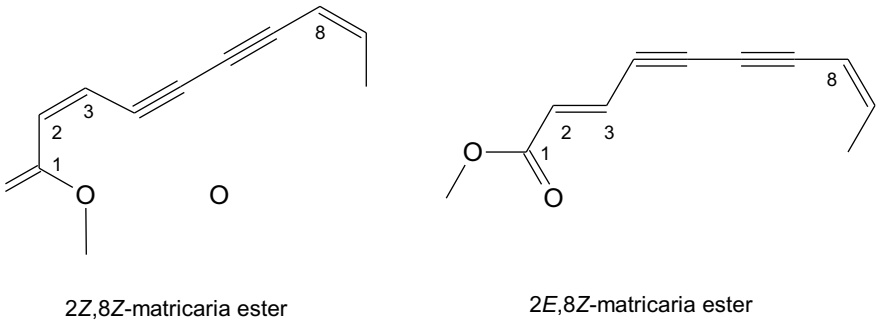


Fig. 20.9 Polyacetylenes in roots of *Solidago virgaurea*

20.5.4 Other Compounds

Anthocyanins, mainly cyanidin 3-gentiobioside, were found in the extracts of leaves, the content of which differed between lowland and mountain populations of *S. virgaurea* in Sweden (Björkman and Holmgren 1958).

20.6 Domestic Cultivation

For medicinal plants with limited distribution, such as goldenrod, destructive harvesting may result in resource exhaustion. Therefore, the sustainable use of Solidago virgaureae herb should be considered, including good harvesting practices. Despite the large demand for the raw material in Europe, goldenrod is not currently cultivated with the exception of Germany and Finland. *Solidaginis virgaureae* herb is obtained from natural sites in Bulgaria, Hungary, the former Yugoslavia, and Poland. The raw material is heterogeneous and often falsified with the related species—*S. gigantea* and *S. canadensis* due to misidentification of botanical origin. In Europe, *Solidago virgaurea* as the native species, grows all over the countries, but its occurrence is relatively rare and its populations are of low abundance, therefore valuable wild resources could be over-exploited. That is why, goldenrod could not be collected economically from their natural sites. Natural habitats in Hungary and Poland are not suitable for economical collection of *Solidago virgaurea* herb (Sztefanov et al. 2002; Kołodziej and Winiarska 2005). Therefore, the rare wild species need to be brought under cultivation. Harvesting the raw plant material from a plantation can preserve the wild population, supply the homogenous plant material and maintain uniformity in production. Some strategies and methodologies of agrotechnology and cultivation of *Solidago virgaurea* have been developed.

For pharmaceutical purposes, goldenrod herb quality is determined by the high amount of bioactive compounds, especially leiocarposide and flavonoids, which is often affected by the environmental conditions. Several studies have shown that the content of active compounds in *S. virgaurea* depends on the location and time of the raw material harvest (Kalemba et al. 1993; Rosłon et al. 2014). Leiocarposide content ranged from 0.47 to 1.60%, depending on the plant location and the time of the raw material harvest (Lück et al. 2000; Gruszczyk and Kiełtyka 2005; Kołodziej 2008). The wild-harvested raw material is not homogeneous and contains different level of secondary metabolites, therefore the production technology of this species has been effective and trial field plantations of *Solidago virgaurea* have been established. Cultivation under the controlled growth conditions, the so- called “controlled plantation” can, improve the yields of bioactive compounds and guarantee production stability (Chen et al. 2016). Moreover, good agricultural practices (GAP) should be realized to regulate the production and ensure high quality of the raw material. In order to secure goldenrod herb for the production in the future, some agricultural studies have been developed. Several methods for *Solidago virgaurea* plantation

establishment were carried out in Finland (Jokela and Galambosi 1998), Germany (Bohr and Plescher 1997, 1999; Lück et al. 2000), Hungary (Sztefanov et al. 2002), and in Poland (Gruszczyk and Kiełtyka 2005; Kołodziej 2007, 2008a, b, 2009).

In Poland, some agrotechnical studies were performed at the University of Life Sciences in Lublin between 2000 and 2010. The experiments on establishment, the yields and raw material quality cultivated in a trial field plantation were also described. The factors which determine the yields and raw material quality of goldenrod include the methods of plantation establishment, the kind of fertilization, the soil conditions and the time of the harvest. As a result of several field experiments, the authors stated that the plantation should be established from *Solidago virgaurea* ssp. *virgaurea*, a taxon rich in leiocarposide. The best methods of commercial plantation establishment are direct autumn diaspores sowing or spring seedlings planting, in a 3–4-year cycle, on heavy loam sand. A commercial plantation of goldenrod can be exploited for three years (Kołodziej 2008a, b). The effect of the soil material and nitrogen fertilization on growth and development of goldenrod was also described (Kołodziej 2002; Kucharski and Mordalski 2006). Quality of *Solidago virgaurea.* ssp. *virgaurea* herb, for example, growth, yielding, and leiocarposide content depending on a different macro-elements (N, P, K) fertilization level, was analyzed and compared by Kołodziej (2007). Fertilization with the use of all NPK (especially in double or triple doses) led to the highest level of the yields of the raw material. It was stated that phosphorous played an important role in goldenrod plant growth as well as in active substances accumulation, while nitrogen influenced the yield of aboveground parts (Kołodziej 2007, 2009). The field experiment with foliar fertilization showed the positive effect on morphological parameters, the yield and the chemical composition (Kołodziej 2008c). The optimal time for harvesting the raw material is the second and the third year of plant vegetation, when the herb yield is the highest (Kołodziej 2008b). The raw material should be harvested in the initial period of flowering and only upper stems should be collected approximately 25 cm long.

20.7 Conclusions

Solidago virgaurea, a valuable medicinal plant, has an established position in folk medicine and currently some applications have been confirmed by the scientific studies. Domestic cultivation provides the opportunity to use new techniques for solving some problems in the production of *S. virgaurea* herb—the low content of active compounds and misidentification of botanical origin. Biotechnological approaches could supply homogenous plant materials for domestic plantation establishment. Cultivation under the controlled growth conditions can improve quality and the yields of *Solidago virgaurea* herb and ensure production stability. *Solidago virgaurea* differs from other species found in Europe, for example, *S. canadensis* and *S. gigantea*, in terms of the presence of leiocarposide, which makes it the important raw material to use in urinary tract diseases.

References

Bader G, Binder K, Hiller K, Ziegler-Bohme H (1987) Zur antimyzetischen Wirksamkeit von Triterpensaponinen aus *Solidago virgaurea* L. Pharmazie 42:140

Bader G, Wray V, Hiller K (1992) Virgaurea-saponin 3, a 3, 28-bisdesmosidic triterpenoid saponin from *Solidago virgaurea.* Phytochemistry 31:621–623

Bader G, Wray V, Hiller K (1995) The main saponins from the aerial parts and the roots of *Solidago virgaurea* subsp. *virgaurea.* Planta Med 61:158–161

Bajkacz S, Baranowska I, Buszewski B, Kowalski B, Ligor M (2018) Determination of flavonoids and phenolic acids in plant materials using SLE-SPE-UHPLC-MS/MS method. Food Anal Methods 11:3563–3575

Björkman O, Holmgren P (1958) Preliminary observations on anthocyanins and other flavonoid compounds and respiration rates in different ecotypes of *Solidago virgaurea.* Physiol Plant 11:54–157

Björkman O, Holmgren P (1960) Polyphenols and polyphenol oxidases in leaves of *Solidago virgaurea.* Physiol Plant 13:582–594

Bohr C, Plescher A (1997) Empfehlungen für den Anbau von Echler Goldrute (*Solidago virgaurea* L.) in Thüringen. Drogenreport 10(18):24–27

Bohr Ch, Plescher A (1999) Optimation of the cultivation parameters of golden rod (*Solidago virgaurea* L.) relating to the yield of active compounds. Drogenreport 9(14):28

Borchert VE, Czyborra P, Fetscher C, Goepel M, Michel MC (2004) Extracts from *Rhois aromatic* and *Solidaginis virgaurea* inhibit rat and human bladder contraction. Naunyn Schmiedeberg's Arch Pharmacol 369:281–186

Borkowski B, Skrzypczakowa L (1962) Związki wielofenolowe w krajowych gatunkach nawłoci *Solidago* L. Acta Pol Pharm 19:491–495

Brantner A (1999) Die antimikrobielle Wirkung von *Solidago*-haltigen Phytourologica. Drogenreport 12:27–28

Brantner AH. Antibakterielle Wirkunf von Solidagoextrakt-hältigen Urologika. http://www.agrar.hu-berlin.de/pflanzenbau/pflanz/themal

Budzianowski J, Skrzypczak L, Wesołowska M (1988) Studies on the genus *Solidago* L II. Phenolic compounds in taxonomy of *Solidago canadensis* L. Acta Biol Cracov Ser Bot 30:33–37

Budzianowski J, Skrzypczak L, Wesołowska M (1990) Flavonoids and leiocarposide in four *Solidago* taxa. Sci Pharm (wien) 58:15–23

Budzianowski J (1999) Die urologische Wirkung des Leiocarposids. Drogenreport 12:20–21

Chen SL, Yu H, Luo HM, Wu Q, Li ChF, Steinmetz A (2016) Conservation and sustainable use of medicinal plants. Chin Med 11:1–10

Chodera A, Dabrowska K, Sećzuk M, Wasik-Olejnik A, Skrzypczak L, Budzianowski J, Ellnein-Wojtaszek M (1985) Badania nad działaniem diuretycznym estrowego glukozydu z rodzaju Solidago L. Acta Polon Pharm 42:199–204

Chodera A, Dąbrowska K, Skrzypczak L, Budzianowski J (1986) Dalsze badania nad działaniem diuretycznym lejokarpozydu. Acta Polon Pharm 43:499–503

Chodera A, Dąbrowska K, Bobkiewicz-Kozłowska T, Tkaczyk J, Skrzypczak L, Budzianowski J (1988) Wpływ lejokarpozydu na doświadczalną kamicę moczową u szczurów. Acta Polon Pharm 45:181–185

Chodera A, Dąbrowska K, Śloderbach A, Skrzypczak L, Budzianowski J (1991) Wpływ frakcji flawonoidowych z rodzaju Solidago L. na diurezę i poziom elektrolitów. Acta Polon Pharm-Drug Res 48:35–37

Debnath M, Malik CP, Bisen PS (2006) Micropropagation: a tool for the production of high quality plant-based medicines. Curr Pharm Biotechnol 7:33–49

Demir H, Açik L, Bali EB, Koç LY, Kaynak G (2009) Antioxidant and antimicrobial activities of *Solidago virgaurea* extracts. Afr J Biotechnol 8(2):274–279

Derda M, Hadaś E, Thiem B (2009) Plant extracts as natural amoebicidal agents. Parasitol Res 104:705–708

El-Ghazaly M, Khayyal MT, Okpanyi SN, Arens-Corell M (1992) Study of the anti-inflammatory activity of *Populus tremula*, *Solidago virgaurea* and *Fraxinus excelsior*. Arzneim-Forsch/Drug Res 42:333–336

EMEA. European Medicines Agency. Evaluation of Medicines for Human Use. Assessment report on *Solidago virgaurea* L., herba (2008) London, 4 September 2008 Doc. Ref. EMEA/HMPC/285759/2007

European Pharmacopoeia 9.0 (2017) Council of Europe Strasbourg. The monograph European Goldenrod, *Solidaginis virgaureae herba* 1376–1378

Fötsch G, Gründemann E, Pfeifer S, Hiller K, Salzwedel D (1988) Zur struktur von Leiocarposid. Pharmazie 43:278–280

Fötsch G, Pfeifer S, Bartoszek M, Franke P, Hiller K (1989) Biotransformation der Phenolglycoside Leiocarposid und Salicin. Pharmazie 44:555–558

Fujita S (1990) Miscellaneous contributions to the essential oils of plants from various territories. Components of the essential oils of *Solidago virgaurea* Linn. ssp. Nippon Nogei Kagaku Kaishi 64:1729–1732 (Jpn). Chem Abstr 1991, 114:98252d

Goswami A, Barua RN, Sharma RP, Baruah JN, Kulanthaivel P, Herz W (1984) Clerodanes from *Solidago virgaurea.* Phytochemistry 23:837–841

Gruszczyk M, Kiełtyka A (2005) Yields and raw material quality of *Hypericum perforatum* L. and *Solidago virga aurea* L. from one-year and two-year plantations. Herba Pol 51:5–9

Gründemann E, Gil-Rjong K, Hiller K (1979) ^{1}H-und ^{13}C-NMR-spektroskopische Untersuchungen an Leiocarposid, einem neuen Phenologlykosid. Pharmazie 34:430–431

Hiller K, Bader G, Dube G (1987) Zur struktur von Virgaureasaponin 2. Pharmazie 42:744–745

Hiller K, Bader G, Schulten H-R (1987a) Virgaureasaponin 1, ein neues bisdesmosidisches Triterpensaponin aus *Solidago virgaurea* L. Pharmazie 42:541–543

Hiller K, Dube G, Zeigan D (1985) Virgaureosid A-ein neues, bisdesmosidisches Phenolglycosid aus *Solidago virgaurea* L. Pharmazie 40:795–796

Hiller K, Fötsch G (1986) Zur Guantitativen Verteilung der Phenolglycoside Virgaureosid A und Leiocarposid in *Solidago virgaurea* L. Pharmazie 41:415–416

Hiller K, Genzel S, Murach M, Franke P (1975) Zur Kenntnis der Saponine der Gattung *Solidago.* 1 Mitteilung: Über die Saponine von *Solidago virgaurea* L. Pharmazie 30:188–190

Hiller, K., Gil-Rjong, R., Otto, A (1979) Über Die Flavonoide von *Solidago virgaurea* L. var. *leiocarpa* (Benth.) A. Gray. Pharmazie 34:571

Hirano M, Sakaguchi S, Takahashi K (2017) Phenotypic differentiation of the *Solidago virgaurea* complex along an elevational gradient: Insights from a common garden experiment and population genetics. Ecol Evol 7:6949–6962

Inose Y, Miyase T, Ueno A (1991) Studies on the constituents of *Solidago virga-aurea* L. I. Structural elucidation of saponins in the herb. Chem Pharm Bull 39:2037–2042

Inose Y, Miyase T, Ueno A (1992) Studies on the constituents of *Solidago virga-aurea* L. II. Structures of solidagosaponins X-XX. Chem Pharm Bull 40:946–953

Jacker HJ, Voight G, Hiller K (1981) Zum antiexsudativen verhalten einiger triterpensaponine. Pharmazie 37:380–382

Jokela K, Galambosi B (1998) Effect of ridge cultivation and plant density on growth and yield of some medicinal plants in Finland. Z Arzn Gew Pfl 3:139–145

Kalemba D (1992) Phenolic acids in four *Solidago* species. Pharmazie 47:471–472

Kalemba D (1998) Constituents of the essential oil of *Solidago virgaurea* L. Flavour Fragr J 13:373–376

Kalemba D, Góra J, Kurowska A, Lis A, Lasocka I (1993) Polyphenolic compounds in four *Solidago* species. Acta Pol Pharm- Drug Res 50:441–442

Kalemba D, Thiem B (2004) Constituents of the essentials oils of four micropropagated *Solidago* species. Flavour Fragr J 19(1):40–43

Kiełtyk P, Mirek Z (2014) Taxonomy of the *Solidago virgaurea* group (Asteraceae) in Poland, with special reference to variability along an altitudinal gradient. Flora Geobot 49:259–282

Kołodziej B (2007) The effect of NPK fertilisation on golden rod (*Solidago virgaurea* L., ssp. *virgaurea*) fields and quality parameters. Herba Pol 53:129–134
Kołodziej B (2008a) Effect of agrotechnical factors on the field of golden rod (*Solidago virgaurea* L. ssp. *virgaurea*). Herba Pol 54(4):28–34
Kołodziej B (2008b) Goldenrod (*Solidago virgaurea* L. ssp. *virgaurea*) reaction to foliar fertilization. Agro Sci 63(4):1–9
Kołodziej B (2008c) The effect of plantation establisment method and Atonik application in goldenrod (Solidago virgaurea L. spp. virgaurea) cultivation. Acta Sci Pol Hortorum Cultus 7(3):33–39
Kołodziej B (2009) Optimalisation of mineral fertilization in goldenrod (*Solidago virgaurea* L. ssp. *virgaurea*) cultivated for pharmaceutical purposes. Acta Sci Pol, Hortorum Cultus 8(1):27–36
Kołodziej B, Kowalski R, Kędzia B (2011) Antibacterial and antimutagenic activity of extracts aboveground parts of three *Solidago* species: *Solidago virgaurea* L., *Solidago canadensis* L., and *Solidago gigantea* Ait. J Med Plants Res 5(31):6770–6779
Kołodziej B, Winiarska S (2005) Nawłoć pospolita (*Solidago virgaurea* L.)—cenna roślina lecznicza pozyskiwana z upraw polowych. Herba Pol 51:Supplement 1
Kołodziej B (2002) The effect of soil material and nitro gen fertilization on growth and development of golden rod (Solidago virga-aurea L.) Folia Hortic 14(1): 187–193
Kruk J, Baranowska I, Buszewski B, Bajkacz S, Kowalski B, Ligor M (2019) Flavonoids enantiomer distribution in different parts of goldenrod (*Solidago virgaurea* L.), lucerne (*Medicago sativa* L.) and phacelia (*Phacelia tanacetifolia* Benth.) Chirality 31:138–149
Kucharski W, Mordalski R (2006) Optimalisation of nitrogen fertilization in goldenrod (*Solidago virgaurea* L.) cultivation for raw material. Herba Pol 52:119
Lam J (1971) Polyacetylenes of *Solidago virgaurea*: Their seasonal variation and NMR long-range spin coupling constants. Phytochemistry 10:647–653
Laurençon L, Sarrazin E, Chevalier M, Prêcheur I, Herbette G, Fernandez X (2013) Triterpenoid saponins from the aerial parts of Solidago virgaurea alpestris with inhibiting activity of Candida albicans yeast-hyphal conversion. Phytochemistry 86:103–111
Lück L, Schenk R, Weber K, Bader G, Abel G (2000) Wildherkünfte von *Solidago virgaurea* L. –Erträge und Variabilität morphologischer und phytochemische Merkmale. Z Arzn Gew Pfl 1:20–27
Madaus G (1938) *Solidago Virga Aurea*. In: Madaus Gerhard, Lehrbuch der Biologischen Heilmittel. Abteilung I: Heilpflanzen, Band III. Georg Thieme Verlag, Leipzig 2571–2576
Melzig MF (2004) Echtes Goldrutenkraut- ein Klassiker in der urologischen Phytotherapie. Wien Med Wochenschr 154:523–527
Metzner J, Hirschelmann R, Hiller K (1984) Antiphlogistische und analgetische Wirkungen von Leiocarposid einem phenolischen Bisglucosid aus *Solidago virgaurea* L. Pharmazie 39:869–870
Miyase T, Inose Y, Ueno A (1994) Studies on the constituents of *Solidago virga-aurea* L. III. Structures of solidagosaponins XXI–XXIX. Chem Pharm Bull 42:617–624
Motaal AA, Ezzat SM, Tadros MG, El-Askary H (2016) In vivo anti-inflammatory ectivity of caffeoylquinic acid derivatives from *Solidago virgaurea* in rats. Pharm Biol 54(12):2864–2870
Móricz ÁM, Jamshidi-Aidji M, Krüzselyi D, Darcsi A, Böszörményi A, Csontos P, Sz B, Ott PG, Morlock GE (2020) Distinction and valorization of 30 root extracts of 5 five goldenrod (*Solidago*) species. J Chromatogr A 1611:460602
Móricz ÁM, Ott PG, Häbe TT, Darcsi A, Böszörményi A, Alberti Á, Krüzselyi D, Csontos P, Béni S, Morlock GE (2016) Effect-directed discovery of bioactive compounds followed by highly targeted characterization, isolation and identification, exemplarily shown for *Solidago virgaurea*. Anal Chem 88:8202–8209
Okpanyi SN, Schirpke-von Panczensky R, Dickson D (1989) Antiphlogistische, analgetische und antipyretische Wirkung unterschiedlicher Pflanzenextrakte und deren Kombination im Tiermodell. Arzneim-Forsch/ Drug Res 39:698–703

Paul JP, Revathy I, Johnson M (2012) In vitro propagation of *Solidago virgaurea* L. through nodal culture. Res Plant Biol 2(4):8–15; PDR. Physican Desk Reference for Herbal Medicinaes (2000) European Goldenrod. Med Econ 2890291

Pepeljnjak S, Kustrak D, Vukusic I (1998) Investigation of the antimycotic activity of *Solidago virgaurea* and *Solidago gigantea* extracts. Pharm Pharmacol Lett 8:85–86

Pietta P, Gardana C, Mauri P, Zecca L (1991) High-performance liquid chromatographic analysis of flavonol glycosides of *Solidago virgaurea*. J Chromatogr A 558:296–301

Poetsch F (1999) Caffeic acid derivatives from *Solidago* species. Drogenreport 21:15–21

Polish Pharmacopoeia 8.0 (2008) Office for Registration of Medicinal Products, Medical Devices and Biocidal Products, Warsaw, Poland

Polish Pharmacopoeia 11 (2017) Office for Registration of Medicinal Products, Medical Devices and Biocidal Products, Warsaw, Poland

Precheur I, Rolland Y, Hasseine L, Orange F, Morisot A, Landreau A (2020) *Solidago virgaurea* L. Plant extract targeted against *Candida albicans* to reduce oral microbial biomass: a double blind randomized trial on healthy adults. Antibiotics (Basel). 25;9(4):137

Rola J, Rola H (2010) *Solidago* spp. biowskaźnikiem występowania odłogów na gruntach rolnych. Fragm Agron 27(3):122–131

Rosłon W, Osińska E, Mazur K, Geszprych A (2014) Chemical characteristics of European goldenrod (*Solidago virgaurea* L. subsp. *virgaurea*) from natural sites in central and Eastern Poland [Charakterystyka chemiczna nawłoci Pospolitej (*Solidago virgaurea* L. subsp. *virgaurea*) ze stanowisk naturalnych w środkowej i Wschodniej Polsce] Acta Scient Pol Hortorum Cultus 13:55–65

Różański H, Bobak Ł, Trziszka T (2016) Znaczenie nawłoci (*Solidago*) w fitoterapii. Meaning of goldenrod (*Solidago*) in phytotherapy. Herbalism 1(2):160–173

Schaser J, Bonaterra GA, Kelber O, Weiser D, Kinscherf R, Metz J (2006) Untersuchung antiinflammatorischer effekte des phytopharmakons Phytodolor® und seiner einzelkomponenten in einem monozyten-testmodel. Persusion 19:76–77

Schilcher H (1964) Über das Vorkommen von Flavonoiden und Hydroxyzimtsäuren in *Solidago virgaurea* L. und *Solidago serotina* Ait. Die Naturwissenschaften 51:636

Schöpke Th, Wray V, Rzazewska B, Hiller K (1991) Bellissaponins BA1 and BA2, acylated saponins from *Bellis perennis*. Phytochemistry 30:627–631. https://doi.org/10.1016/0031-9422(91)83740-C

Skidmor-Roth L (2010) Herbs & natural supplements. Mosby Elsevier, St. Louis, Missouri, pp 306–308

Skrzypczak L, Ellnain-Wojtaszek M (1981) 2-β-D-glucopyronosyloxybenzyl-4-β-D-glucopyronosyloxy-2-hydroxy-3-methoxybenzoate of *Solidago* species (Asteraceae). Pol J Chem 55:683–685

Skrzypczak L, Wesołowska M, Thiem B, Budzianowski J (1999) XXIII *Solidago* L. species (goldenrod): in vitro regeneration and biologically active secondary metabolites. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 43. Medicinal and aromatic plants XI. Springer, Berlin, Heidelberg

Skrzypczakowa L (1961) Flawonoidy w zielu nawłoci pospolitej - *Solidago virgaurea* L. I Wyodrębnienie i identyfikacja 3-rutynozydu kwercetyny (rutyny). Acta Pol Pharm 18:39–48

Skrzypczakowa L (1962) Flawonoidy w zielu nawłoci pospolitej - *Solidago virgaurea* L. II Wyodrębnienie i identyfikacja dalszych pochodnych flawonolowych. Acta Pol Pharm 19:481–490

Sliwinska E, Thiem B (2007) Genome size stability in six medicinal plant species propagated in vitro. Biol Plantarum 51(3):556–558

Starks CM, Williams RB, Goering MG, O'Neil-Johnson M, Norman VL, Hu J-F, Garo E, Hough GW, Rice SM, Eldridge GR (2010) Antibacterial clerodane diterpenes from Goldenrod (*Solidago virgaurea*). Phytochemistry 71:104–109

Sztefanov A, Petheő F, Bernáth J, Sárdi É (2002) Comparative Analysis of Hungarian *Solidago virga-aurea* Populations. Acta Hort 576:53–55

The Plant List (2020) Version 1.1. Published on the Internet. http://www.theplantlist.org/. Accessed Sept 12

Thiem B, Goślińska O (2002) Antimicrobial activity of *Solidago virgaurea* L. fom in vitro cultures. Fitoterapia 73:514–516

Thiem B, Wesołowska M, Skrzypczak L, Budzianowski J (2001) Phenolic compounds in two *Solidago* L. species from in vitro culture. J Acta Pol Pharm 58:277–281

Wang Z, Kim JH, Jang YS, Kim CH, Lee J-Y, Lim SS (2017) Anti-obesity effect of *Solidago virgaurea* var. *gigantea* extract through regulation of adipogenesis and lipogenesis pathways in high-fat diet-induced obese mice (C57BL/6N). Food Nut Res 61(1):1273479

Westendorf J, Vahlensieck W (1981) Spasmolytische und kontraktile Einflüsse eines pflanzlichen Kombinations-präparates auf die glatte Muskular des isolierten Meerschweinchendarms. Arzneim_Forsch/ Drug Res 31:40–43

Wittig J, Veit M (1999) Analyse der Flavonolglycoside von "*Solidago*"-spezies in einem zusammengesetzten Pflanzenextrakt. Drogenreport 12:18–20

Chapter 21
Biology and Biotechnological Strategies for Conservation Management of *Pueraria tuberosa*, a Traditionally Established Medicinal Liana

Bhanupriya Kanthaliya, Abhishek Joshi, Supriya Meena, and Jaya Arora

Abstract *Pueraria tuberosa* (Roxb. ex Willd.) DC, "Vidarikand" is a medicinal liana generally known as Indian Kudzu belongs to the family Fabaceae. This plant's tuber is widely used in ethnomedicine as well as in traditional systems of medicine, particularly in Ayurveda. This is an ironic source of bioactive constituents with many pharmacological properties such as restorative tonic, antiageing, spermatogenic, immune booster, demulcent, galactagogue, purgative, cholagogue, refrigerant, emollient, laxative, aphrodisiac, diuretic, emetic, cardiotonic, and expectorant. The tuber is a rich source of different classes of phytoconstituents including alkaloids, steroids, glycosides, tannins, terpenoids, flavonoids, coumarins, and anthocyanidins. There are many reports available on the improvement of the production of isoflavonoids and their mechanism of action from *P. tuberosa* but still needs research and innovation to protect this medicinally important plant as well as enhancement of important phytochemicals under in vivo and in vitro conditions. In this review, distribution of plant, biology, phytochemical constituents, biotechnological approaches to conserve and enhance the active principle, pharmacological properties, and therapeutic uses are presented with discussion on existing cultivation practises on *P. tuberosa*. Strategies to meet our domestic and commercial demands by conservation planning of this valuable plant species.

Keywords *Pueraria tuberosa* · *Medicinal liana* · Bioactive constituents · Isoflavonoids · Puerarin · Cultivation

21.1 Introduction

The genus *Pueraria* (Family: Fabaceae) is a small group of perennial lianas distributed throughout Eastern to South-East Asia to America, and some parts of the European Union. Amongst the twenty-six known species of *Pueraria*, only a few species (i.e. *P. lobata*, *P. thomsonii*, *P. candollei* var. *mirifica* and *P. tuberosa* etc.)

B. Kanthaliya · A. Joshi · S. Meena · J. Arora (✉)
Laboratory of Biomolecular Technology, Department of Botany, Mohanlal Sukhadia University, Udaipur 313001, Rajasthan, India

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_21

have cosmopolitan distribution (Van der Maesen 2002; Pan et al. 2015). The Kudzu is the most popular herb in traditional Chinese medicine and also received attention in the recent years due to their immense pharmaceutical and nutraceutical properties (Miao et al. 2019; Wang et al. 2020). The Indian Kudzu (*P. tuberosa*), commonly known as "Vidarikanda", is the most exploited species in the traditional Indian system of medicine, the Ayurveda (Sharma and Ramawat 2013; Goyal et al. 2015). It has also received attention from modern pharmacopoeia and many other research fields due to their broad spectrum of phytochemicals and bioactivities. The tubers are the most widely used plant part due to localization of generous amount of isoflavones and other constituents (Pandey et al. 2007; Satpathy et al. 2017; Mocan et al. 2018). A large number of findings is available on *P. tuberosa* phytochemicals, especially polyphenols, to validate their traditional claims and identify the molecular mechanism and pharmacokinetic profile (Srivastava et al. 2017; Hsueh et al. 2017; Ahmad et al. 2020). Recent research interests mainly focussing their beneficial interaction with other herbs and drugs to appraise efficacy in humans and other animals (Gulizia and Downs 2019; Tungmunnithum et al. 2020; Zhang et al. 2020). The commercial cultivation of *P. tuberosa* is usually associated with two major glitches, firstly, the long periods between planting and harvesting of tubers, and secondly, the variation in the isoflavonoids quantity and yield (Rathore and Shekhawat 2009; Sharma et al. 2018). On the other hand, increasing market demand of the plant material and anthropogenic activities causes overexploitation from natural populations. The propagative failure due to seed dormancy and seed predation by the insects, are also major bottlenecks in this regard (Kanthaliya et al. 2019). The previous reviews on *P. tuberosa* described only the phytochemical and therapeutic potential (Maji et al. 2014; Wang et al. 2020). Therefore, the aim of this review is to provide insights on distribution of plant, biology, phytochemical constituents; biotechnological approaches to conserve and enhance the active principle, pharmacological properties, and therapeutic uses of *P. tuberosa* with discussion on existing cultivation practises.

21.2 Scientific Classification

Kingdom	Plantae
Subkingdom	Trachebionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Pueraria*
Species	*tuberosa*

21.3 Geographic Distribution and Biology of Plant

P. tuberosa is a woody climber or liana found wild in tropical and temperate zones of the South-East Asia. It is native to India, widespread up to an altitude of 1200 meters in hilly tracts of Aravalli range to the deciduous forest of Western and Eastern Ghats (Van der Maesen 2002; Sharma et al. 2018). Being a woody climber, stems sometimes extending 20 m in height, with many tuberous roots penetrating deep in the soil. Leaves are also often large with three entire lobed leaflets arranged pinnately (Fig. 21.1). Flowering time is February to April, with bisexual, blue, or purplish-blue flowers arranged on long racemes (Kanthaliya et al. 2019). Leguminous fruits are typical pods with outer bristly brown hairy texture constricted densely between the seeds. Each pod contains 3–6 seeds; fruiting time is May–June. Seeds are bean-shaped and different types of texture are present on their upper surface. A detailed account of distribution pattern and biology of *Pueraria* species in India is presented in Table 21.1.

21.4 Phytochemical Constituents

The phytochemistry of *Pueraria* species has been studied extensively and numerous groups of phytochemicals such as polyphenols, alkaloids, phyto-sterols have been isolated and characterised being an established medicinal plant (Maji et al. 2014; Mocan et al. 2018; Wang et al. 2020). Isoflavonoids and triterpenoids glycosides are the major constituents of *Pueraria* plants and their concentration usually ranges from 0.01 to 1% dry weight (Satpathy et al. 2017; Kanthaliya et al. 2019). However, changes of the environmental dynamics or engaging a plant into tissue culture practises produce new and sometime different phytochemical profile. To date, more than 103 compounds, consisting of 49 isoflavones, 12 triterpenoid saponins, 7 sterols, 6 flavone and flavonols, 6 coumestrols, 2 xanthones, 2 puerariafurans and some other miscellaneous compounds (i.e. tuberosin, puetuberosanol, puerol B etc.) have been isolated from different species of *Pueraria.* The chemical structures of the predominant phytochemicals isolated from *P. tuberosa* are shown in Fig. 21.2.

21.5 Therapeutic Uses

Since ancient time, *P. tuberosa* is being used by Asian people for the purposes of food, decoration, and herbal medicine (Maji et al. 2014). Tubers of this plant carry immense therapeutic promises and are part of many Ayurvedic health supplements such as Chawanprash, Indrokta Rasayan, and Vidaryadi Ghrita. It is meant to reserve the strength, stamina, vitality and to increase the immunity, whilst stalling the course of ageing (Sharma et al. 2018, 2019). Studies also confirmed their beneficial effects

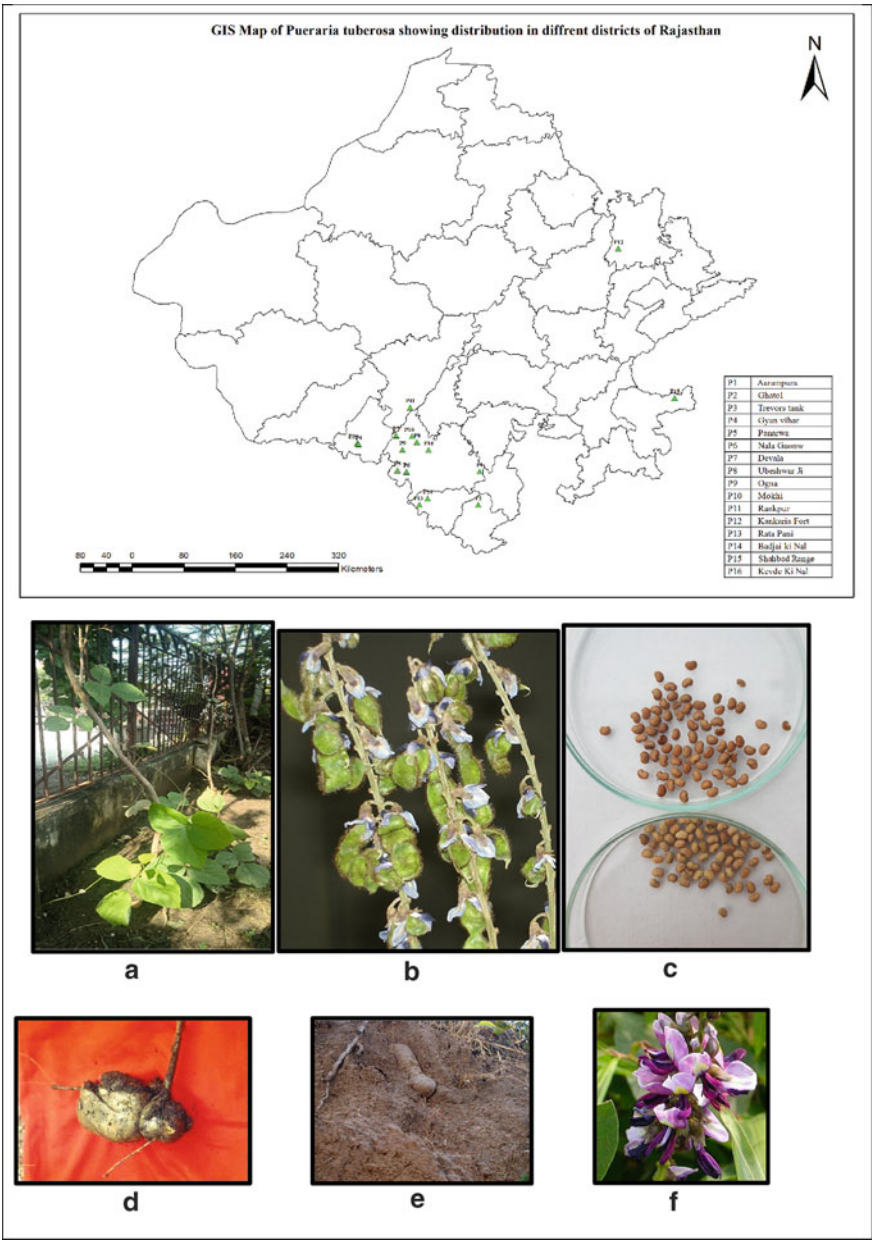


Fig. 21.1 Distribution of *P.tuberosa* in Rajasthan state and different stages of *P. tuberosa* in habitat (A—Plant, B—Pods, C—Seeds, D, E—tuber, F—Inflorescence)

Table 21.1 Geographical distribution and morphology of Indian species of *Pueraria*

Name of species	Geographical locations in India	Habitat	Nature of climbing	Leaflets	Inflorescence	Flowering and fruiting time
P. bella	Arunachal Pradesh	In hills, sprawling over boulders in river bed, 200–1000 m	Woody climber with glabrous branches	Leaflets long-elliptic, not lobed	Inflorescences 35 cm long	August–December
*P. candollei*var. *Candollei*	Assam, Andamans	Climbing on limestone rocks, lake banks, 0–1300 m	Strong woody climber, sparsely hairy or glabrous	Large leaflets	Inflorescence often more than 30 cm long	February–April
P. edulis	Manipur, Sikkim	Climbing over dwarf bushes or oak trees, on hill slopes, in forests, near streams, on sandy and rocky soils, 1300–3300 m	Woody climber	Stipellae four near petioles of side leaflets; leaflets always prominently lobed	Inflorescence 30–75 cm long	Flowering between July and October, fruiting up to November
P. peduncularis	Eastern Himalaya, Khasi Hills	Climbing or pendant on shrubs and trees, medium wet forest, hill slopes, along jungle edges, bamboo forests, 1200–3600 m	Woody climber	Ovate to Rhomboid leaflets	Inflorescence single or paired, up to 40 cm long	Flowering April to October, fruiting April to November
*P. phaseoloides*var. *Subspicata*	North-East India	Mixed deciduous forest, scrub vegetation, along roads and irrigation tanks, 0–1300 m,	The large-flowered variety with long-hairy bracts and calyces	Leaflets large, entire to usually deeply lobed	–	–

(continued)

Table 21.1 (continued)

Name of species	Geographical locations in India	Habitat	Nature of climbing	Leaflets	Inflorescence	Flowering and fruiting time
P. tuberosa	Aravalli hills, North-East India,	In hill forest and deciduous vegetation, in exposed and eroded areas, covering Ground, bushes and trees (0–1300 m)	Woody climber	Large rounded–ovate leaflets up to 32 cm long, conspicuously yellow in dry season before shedding	Inflorescence long and lax, yellow–brown pubescent or glabrous	Flowering after leaf fall from December to February
P. sikkimensis	Sikkim, W. Bengal	In deciduous forests or scrub, in plains, river valleys, from 330–1600 m	Woody climber with rusty pubescence	_	Inflorescence crowded, rusty pubescent	Flowering in March and fruiting in April–May
P. wallichii	Eastern Himalaya	In or near dry evergreen forests, open grassy vegetation, on slopes and along rivers	Shrub, sometimes straggling, sparsely pubescent and glabrous with age	Rhomboidal-liptic leaflets	_	Flowering October–January, fruiting into February
*P. montana*var. *Chinensis*	North East India	_	_	Leaflets trilobed, sometimes entire, about as wide as long	_	_

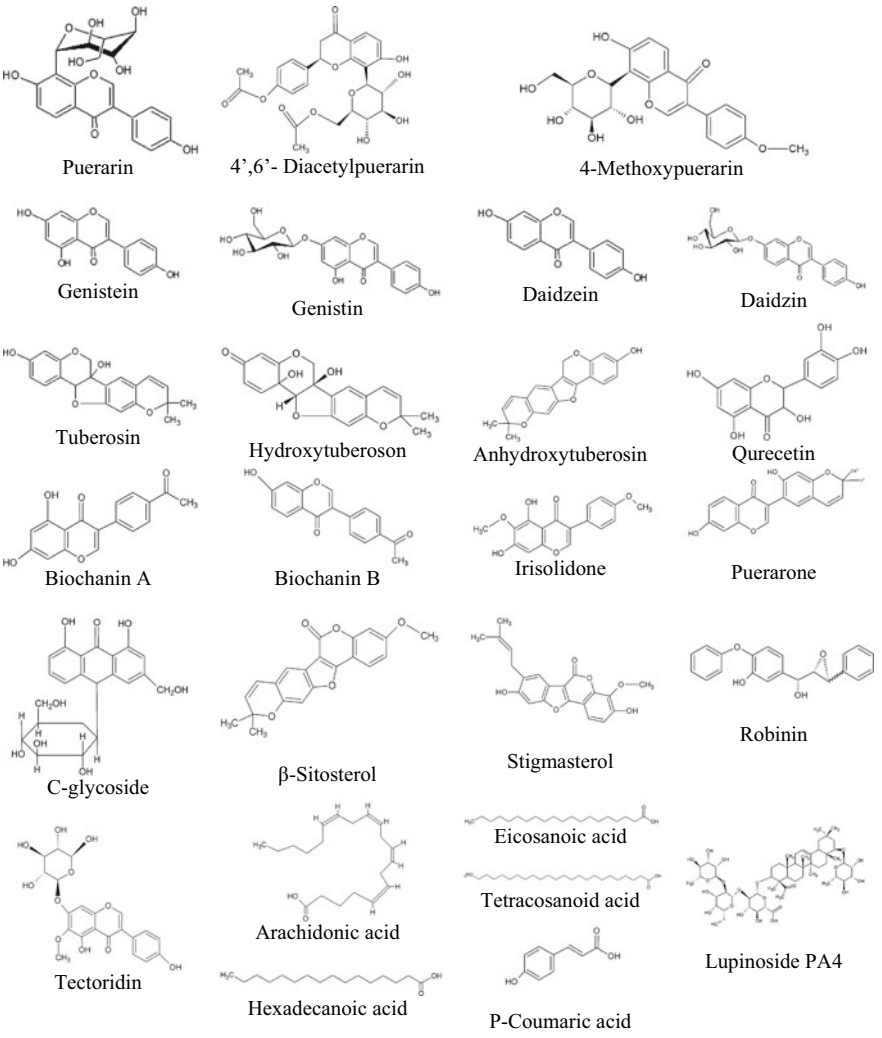


Fig. 21.2 2D structures of bioactive constituent reported in *P. tuberosa*

against the vitiated disorders such as arthritis, cardiac debility, hepatosplenomegaly, asthma, pharyngitis, and cough (Venkatasubramanian et al. 2009; Anilkumar et al. 2017). It has also been reported for skin and hair care and improve complexion. It promotes hair follicle growth and reduces the chance of alopecia condition, used in the treatment for hypertension and angina pectoris (Likhitkar et al. 2016; Rawtal et al. 2019). It is also possess numerous other activities like anti-inflammatory, antioxidant, anti-diabetic, nootropic, and antifertility (Rehman et al. 2019; Li et al. 2020; Ojo et al. 2021). Anti-tumour and anti-apoptotic activity have also been reported against ovarian, breast, brain, and multidrug resistant cancer cell lines (Hu et al. 2018; Hua

Table 21.2 Nutrient compositions of *Pueraria* species

Nutritive substances	*P. tuberosa*	*P. montana* var. *lobata*			*P. phaseoloides*	*P. lobata*
	Tuber	Raw tuber	Fresh leaves	Tuber starch	Fresh leaves and stems	Root
Moisture content (%)	82.35	68.6	76.9	16.5	80.9	*
Total carbohydrates (%)	52.26	27.8	10.2	83.1	7.9	85.21–88.94
Crude protein (%)	9.99	2.1	4.0	0.2	3.8	0.14–0.92
Fat/ oil (%)	6.16	0.1	0.6	0.1	0.4	*
Fibre (%)	18.9	0.7	6.8	*	5.5	*
Ash (%)	*	1.4	1.5	0.1	1.5	*
References	Singh (2011)	Bodner and Hymowitz (2002)				Wang et al. (2016)

*Not determined

et al. 2018; Lee et al., 2019). Singh et al. (2020) recommended its use in the treatment of Covid-19 due to its potent role in the prevention of diabetic nephropathy and cardiovascular diseases, in combination with other plants (i.e. *Pterocarpus*, *Sida*, *Asparagus*, *Boerhavia, Desmodium*, *Terminalia* etc.). Also, the presence of the nutritive substance such as carbohydrates, proteins and fibres (Table 21.2) make it a desirable food for human and animal consumption (Gulizia and Downs 2019).

21.6 Modern Scientific Validation

The authenticity of herbal drugs and formulation relies upon scientific validation methods involving taxonomical characterization of collected plant material, standardization of extraction protocols, purity, toxicity testing, various clinical trials (Bhosle and Banerjee, 2020). Figure 21.3 shows various steps involved in the process of validation of herbal drugs (Sen and Chakraborty 2017). The safety data including dosage intervals, age group, pre- and post-effects must be evaluated before launch of any herbal drug in the market. Here various validation methods come in scene.

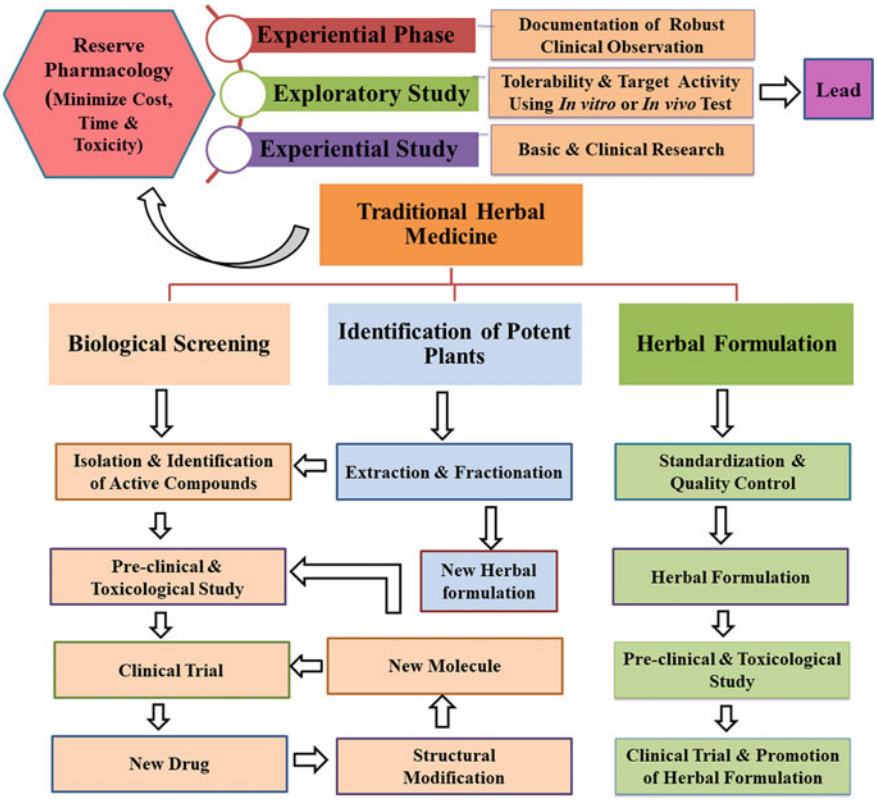


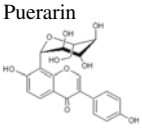
Fig. 21.3 Schematic presentation of various approaches to obtain lead drugs and their validation methods

21.7 Mechanism of Action

The active constituents of the *Pueraria,* mainly isoflavones (i.e. puerarin, genistein, daidzein etc.), have been extensively used in the treatment of various physiological and metabolic disorders related to brain, heart, and liver and other organs (Wang et al. 2020). These constituents have significant protective influence on specific disease conditions of particular target organs by modulating the different signalling pathways and factors. For example, chemo-preventive or anticancer mechanism of both the puerarin and genistein include mitigation of DNA damage, induction of apoptosis (via both the extrinsic and intrinsic pathways), regulation of cell cycle and oxidative stress, as well as the positive immunomodulation (Ahmad et al. 2020; Lee et al. 2019). Similarly, the anti-diabetic mechanisms include improvement of insulin resistance and glucose tolerance, inhibition of Maillard reaction, and induction of glycation end products (AGEs) formation (Weng et al. 2019; Li et al. 2014). Interestingly, daidzein and their metabolite equols have been reported to affect cardiovascular protection

through regulation of blood lipid metabolism and endothelial dysfunction attenuation (Das et al. 2018; Wei et al. 2019). The hepato-protective mechanism of genistin involves the suppression of oxidative stress-mediated pro-inflammatory cytokines (tumour necrosis factor-α, Interleukin-1β, and InterleukinL-12) and ROS-related enzymes (glutathione reductase, glutathione peroxidase and superoxide dismutase) levels (Islam et al., 2020; Wang et al. 2020). Table 21.3 summarizes pharmacological activity and mechanism of action of active constituents from *P. tuberosa.*

Table 21.3 Pharmacological activity and mechanism of action of active constituents from *P. tuberosa*

Active constituents	Pharmacological activity	Mode of action mechanism	References
Puerarin 	Anti–coronavirus disease 2019 (COVID-19)	Suppression of oxidative stress and inflammatory cascades, Interleukin (IL)-17 signalling, mitogen-activated protein kinase (MAPK) signalling and TNF (tumour necrosis factor) signalling	Qin et al. (2020)
	Anti-diabetic	Antagonist binding sites of peroxisome proliferator activated receptor - gamma (PPARγ), 11-β hydroxysteroid dehydrogenase type 1 (11-β HSD1), glutamine fructose-6-phosphate amido transferase (GFAT), protein-tyrosine phosphatase 1B (PTP1B) and mono-ADP-ribosyltransferase sirtuin-6 (SIRT6)	Ojo et al. (2021)
		Increased the expression levels of GLUT4(glucose transporter type 4) and insulin resistance by inhibiting NO (nitric oxide) production	Lertpatipanpong et al. (2020)
		Upregulation of uridine diphosphate (UDP)-glucuronosyltransferase 1a1 and 1a7	Dong et al. (2018)
		Enhanced insulin sensitivity and inhibition of sodium dependent glucose transport	Carlson et al. (2014)
		Increased pancreatic β-cell mass via β-cell apoptosis inhibition, increased serum insulin and decreased blood glucose levels	Li et al. (2014)
	Antioxidant	Attenuated 1-methyl-4-phenylpyridinium (MPP +)-induced oxidative stress through elevating biosynthetic capacity of Nrf2-dependent glutathione (GSH)	Li et al. (2020)
		Production of reactive oxygen species induced by t-BHP	Chang et al. (2016)

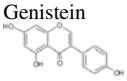
(continued)

Table 21.3 (continued)

Active constituents	Pharmacological activity	Mode of action mechanism	References
		Down regulation of reactive oxygen species (ROS)	Xu et al. (2016)
	Hepto-protective and hypo-lipidemic	Increased expressions of BHMT (betaine homocysteine methyltransferase) CBS (cystathionine β-synthase) and CTH (cystathionine γ-lyase)	Chen et al. 2020
		Reduced the hepatotoxicity via lowered the plasma levels of alanine aminotransferase and aspartate aminotransferase	Chang et al. 2016
	Anti-osteoporosis	Improves graft bone defect through decreasing the levels of pro-inflammatory cytokines [tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-17A, IL-6 and transforming growth factor (TGF)-β1] and increasing the levels of anti-inflammatory cytokines (IL-2 and IL-10)	Zhou et al. (2020)
		Enhance expression of alkaline phosphatase and type I collagen	Tiyasatkulkovit et al. 2014
		Stimulates osteoprotegerin (OPG) and inhibits receptor activator of nuclear factor-κB ligand (RANKL) and Interleukin-6 (IL-6) produced by human osteoblastic MG-63 cells	Wang et al. (2014)
	Anti-inflammatory	Suppressed phosphorylated IkBα, p65, p38, extracellular signal-regulated kinase 1 and 2 (ERK), and c-Jun N-terminal kinase (JNK)	Wu et al. (2016)
		Inhibition of expression of iNOS(inducible nitric oxide synthase) CRP(C-reactive protein) and COX-2(cyclooxygenase-2)	Mahdy et al. (2014)
	Anti-cancer	Promoted cell apoptosis and cell autophagy through phosphoinositide-3-kinas (PI3K)/Akt and mitogen-activated protein kinases (MAPK)/ extracellular signal-regulated kinases (ERK) 1/2 signalling pathways	Hu et al. (2018)
		Block the cell cycle in the G0/G1 phase and induce apoptosis	Jiang et al. (2018)
		Increased levels of caspase-3, caspase-7, caspase-9 and Bax, and reduced levels Bcl-2	Chen et al. (2016)

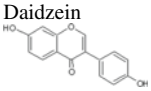
(continued)

Table 21.3 (continued)

Active constituents	Pharmacological activity	Mode of action mechanism	References
	Neuro-protective and brain stock protective	Induces GCLc(glutamate cysteine ligase) through glycogen synthase kinase (GSK-3β)/Fyn pathway	Li et al. (2020)
		Activated extracellular signal-regulated kinase (ERK)1/2 and cyclic AMP response element binding protein (CREB), and subsequently induced brain-derived neurotrophic factor (BDNF)	Zhao (2015)
		The number of Nissl body, cleaved caspase-3 and GFAP positive cells increased	Wang et al. (2014)
	Cardio protective	Activated TRPV4 (transient receptor potential vanilloid 4) channels, enhanced endothelium-dependent vasodilation and decreased the SBP (systolic blood pressure) and MAP (mean arterial pressure)	Zhou et al. (2020)
		Increased cell viability, decreased lactate dehydrogenase activity and upregulated microRNA(miR)-21 expression	Xu et al. (2019)
		Prolonged APD via its inhibitory effect upon Kv 7.1 and IKs	Xu et al. (2016)
	Nephro-protective	Attenuating SIRT1(silent information regulator 1) /FOXO1(forkhead box protein O1) pathway cc	Xu et al. (2016)
Genistein 	Anti-diabetic	Improving brain insulin signalling	Li et al. (2020)
		Modulation of gene expression in the hypothalamus through circadian entrainment pathway	Zhou et al. (2019)
		Direct consequences for β-cell expansion and glucose-triggered insulin discharge	Weng et al. (2019)
		Act as survival factor for β-cells via G protein receptor (GPR)30-initiated and Gαs-mediated activation of cAMP-response element binding protein (CREB)	Luo et al. (2018)
	Antioxidant	Achieving an adequate antioxidant defence system and scavenging free radicals	Rajaei et al. (2019)
		Altered Maillard reaction pathway by trapping the advanced glycation end products (AGEs)	Mazumder and Hongsprabhas (2016)

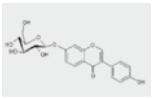
(continued)

Table 21.3 (continued)

Active constituents	Pharmacological activity	Mode of action mechanism	References
	Cardio protective	Reduced serum cardiac troponin and redox markers (ROS), LPO, 4-hydroxynonenalprotein adducts [HNE] levels	Bai and Wang (2019)
		Reduction of serum creatine kinase MB isozyme (CK-MB) and lactate dehydrogenase (LDH) leakage	Yang et al. 201(8)
	Anti-osteoclastic	Regulating autophagy induction, inhibiting osteoclast and activation the production of inflammation mediators	Bhattarai et al. (2017)
	Anti-inflammatory	Decreasing the levels of interleuin-6, tumour necrosis factor (TNF)-α and C-reactive protein and increasing level of glucagon-like peptide-1 (GLP-1)	Rehman et al. (2019)
		Ameliorated neuro-inflammatory condition by varying TNF-α, IL-1β, and nitrite levels	Rajput and Sarkar (2017)
	Neuro-protective and brain stock protective	Increased expression levels of the nerve growth factor (NGF) and brain-derived neurotrophic factors (BDNF), and reduced Aβ deposition and the level of hyper-phosphorylated Tau protein	Li et al. (2020)
		Upregulate the expression levels of p-extracellular signal-regulated kinase (ERK), p-cyclic AMP response element binding protein (CREB) and BDNF proteins	Lu et al. (2018)
	Anti-cancer	Increases hepatocyte apoptosis through energy-dependent caspase pathways and inhibits the initiation as well as progression of hepatocellular carcinoma (HCC)	Lee et al. (2019)
		Increased cell cycle arrest in the G0/G1 and G2/M phase and decreased p125FAK activity	Li et al. (2017)
		Induces cell apoptosis and promotes caspase-3/9 activation of A549 cells through miR-27a-mediated MET signalling	Yang et al. (2016)
Daidzein 	Anti-influenza	Regulates virus replication via signal transduction through 5-lipoxygenase products	Horio et al. (2020)
	Anti-diabetic	Impaired glucose level, lipid metabolism and adenosine monophosphate activated protein kinase (AMPK) phosphorylation	Das et al. (2018)

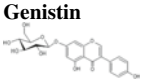
(continued)

Table 21.3 (continued)

Active constituents	Pharmacological activity	Mode of action mechanism	References
		Inhibit protein-tyrosine phosphatase (PTP) 1B and α-glucosidase	Seong et al. (2016)
	Anticancer	Induce apoptosis of choriocarcinoma cells via mitochondrial apoptotic pathway	Zheng et al. (2018)
		Regulation of PI3K/Akt/mTORpathway	Zhu et al. (2018)
		Upregulation of B-cell lymphoma 2-associated X protein, cytochrome c, cleaved caspase-3 and -9, and cleaved poly (ADP-ribose) polymerase, triggered G2/M cell arrest through down regulation of pCdc25c, Cdc25c, pCdc2, Cdc2 and cyclin B1	Hua et al. (2018)
		Suppressing signal-regulated kinases (ERK) pathway and afterwards arresting cell cycle at G1 phase	Zheng et al. (2017)
	Antioxidant	Increased superoxide dismutase (SOD) activity by downregulating Keap-1 and upregulating Nrf2 expression	Yu et al. (2020)
		Up regulation of FOXO3/SOD2 signalling pathway	Lee and Park (2018)
	Neuroprotective	Improving cognitive dysfunction	Wei et al. (2019)
		Improving locomotor function through PI3K/Akt signalling	Johnson et al. (2020)
		Promoting the expression of the α7 nicotinic acetylcholine receptor	Li et al. (2018)
	Reproductive performance	Modulation of serum hormones and expression levels of reproductive-related genes	Zhang et al. (2018)
Daidzin 	Anti-epileptic	Increased the expression of brain-derived neurotrophic factor (BDNF) /HO-1 and reduced the expression of vascular endothelial growth factor (VEGF)	Kazmi et al. (2020)
	Anti-diabetic	Reducing blood glucose, serum HbA1c, and serum insulin	Zang et al. (2015)
	Anti-inflammatory	Inhibiting the expression of lipoxygenase (LOX), cyclooxygenase-2 (COX-2), interleukin (IL)-1β and IL-6	Wu et al. (2019)

(continued)

Table 21.3 (continued)

Active constituents	Pharmacological activity	Mode of action mechanism	References
	Anti-cancer	Attenuating signal transducer and activator of transcription 3(STAT3) signalling cascade	Yang et al. (2020)
	Bladder dysfunction	Restoring phase 2 activity and inhibiting the expressions of purinergic (P)2 × 2, P2 × 3, and muscarinic (M) 3 receptors	Wu et al. (2018)
	Anti-atherosclerotic	Inhibiting monocyte-endothelial adhesion through expression of vascular cell adhesion molecule-1, monocyte chemotactic protein-1 and phosphorylation of IκB kinase and IκBα	Lee et al. (2018)
	Anti-viral	Inactivate virus by binding with Japanese encephalitis virus (JEV) fsRNA	Zhang et al. (2012)
Genistin 	Cardio-protective	Suppressing P2X7/NF-κB pathways	Gu et al. (2016)
	Anti-cancer	Suppressing estrogen receptor alpha (ERα) signalling	Hwang et al. (2020)
		Regulation of the phosphatidylinositol3kinase/mammalian target of rapamycin (PI3K/Akt/mTOR) pathways	Zhu et al. (2018)
	Hepato-protective	Regulating ROS-related enzymes	Kim et al. (2015)
Tectorigenin	Neuro and brain protective	Suppressed caspase-3 activity and cytochrome c expression	Gong et al. (2017)
		Suppressing NF-κB andextracellular signal-regulated kinase(ERK) /c-Jun N-terminal kinase (JNK) related pathways	Lim et al. (2018)
	Anti-diabetic	Enhance pancreas/duodenum homeobox protein 1 (PDX1)	Yao et al. (2020)
	Antioxidant	Abolished the downregulation of superoxide dismutase, catalase and glutathione peroxidase	Gong et al. (2017)
	Nephro-protective	Modulate expression of adiponectin receptor 1/2 (AdipoR1/2), pi- liver kinase(LK) B1, pi-AMPKα and peroxisome proliferator activated receptor alpha (PPARα)	Yang et al. (2020)
		Down-regulated expression of crystal modulator genes and pro-fibrotic genes	Divya et al. (2019)

(continued)

Table 21.3 (continued)

Active constituents	Pharmacological activity	Mode of action mechanism	References
	Anti-osteoarthritis	Suppression of NF-κB signalling	Ma et al. (2018)
		Inhibiting articular cartilage degeneration and chondrocyte apoptosis via the NF-κB P65 pathway	Wang et al. (2017)
Kaikasaponin III	Androgenic alopecia	Inhibit testosterone 5a-reductase activity	Murata et al. (2012)
	Antioxidant	Up-regulating or down-regulating antioxidant mechanisms via the changes in Phase I and II enzyme activities	Choi et al. (2004)
Lupeol and lupenone	Neuro-protective	Inhibition of β-site amyloid precursor protein cleaving enzyme 1 (BACE1) and amyloid beta (Aβ) production	Koirala et al. (2017)

21.8 Biotechnological Approaches

Plant tissue and cell culture is highly acclaimed technique not only for conservation of rare and endangered species, but also large-scale production of valuable phytochemicals and their biosynthesis (Joshi et al. 2019; Ramawat 2019). The *P. tuberosa* has broad applications in pharmaceutical sector; therefore, most of the tissue culture studies with this plant have been focussed on biologically active constituents and their bio-transformation. A number of research investigations has been reported for secondary metabolite production, mainly isoflavonoids, using various methods such as callus culture, shoot culture and cell suspension culture using callus cultures, cell cultures in shake flasks and bioreactor (Fig. 21.4, Table 21.4). Being a liana of Fabaceae, known for their recalcitrant nature to regenerate, it was difficult to obtain a fairly fast-growing callus from explants and several variations and combinations of salts, plant growth regulators and nutrients were tested (Goyal and Ramawat 2007, 2008). The *Agrobacterium* mediated hairy root transformation strategy has also been used by the researchers to increase the isoflavonoid quantity and yield. The elicitation using both the biotic (*Cuscuta*, yeast extract, etc.) and abiotic elicitors (methyl jasmonate, chitosan, etc.) have also been applied to enhance the product yield as well as viability of the production process. In an attempt to develop regenerative protocols, a high number of plantlets was obtained through enhanced axillary branching from nodal segments obtained through in-vitro raised seedlings (Fig. 21.5). Such a regenerative systems were grown in growtek bioreactor also (see Table 21.4 for references).

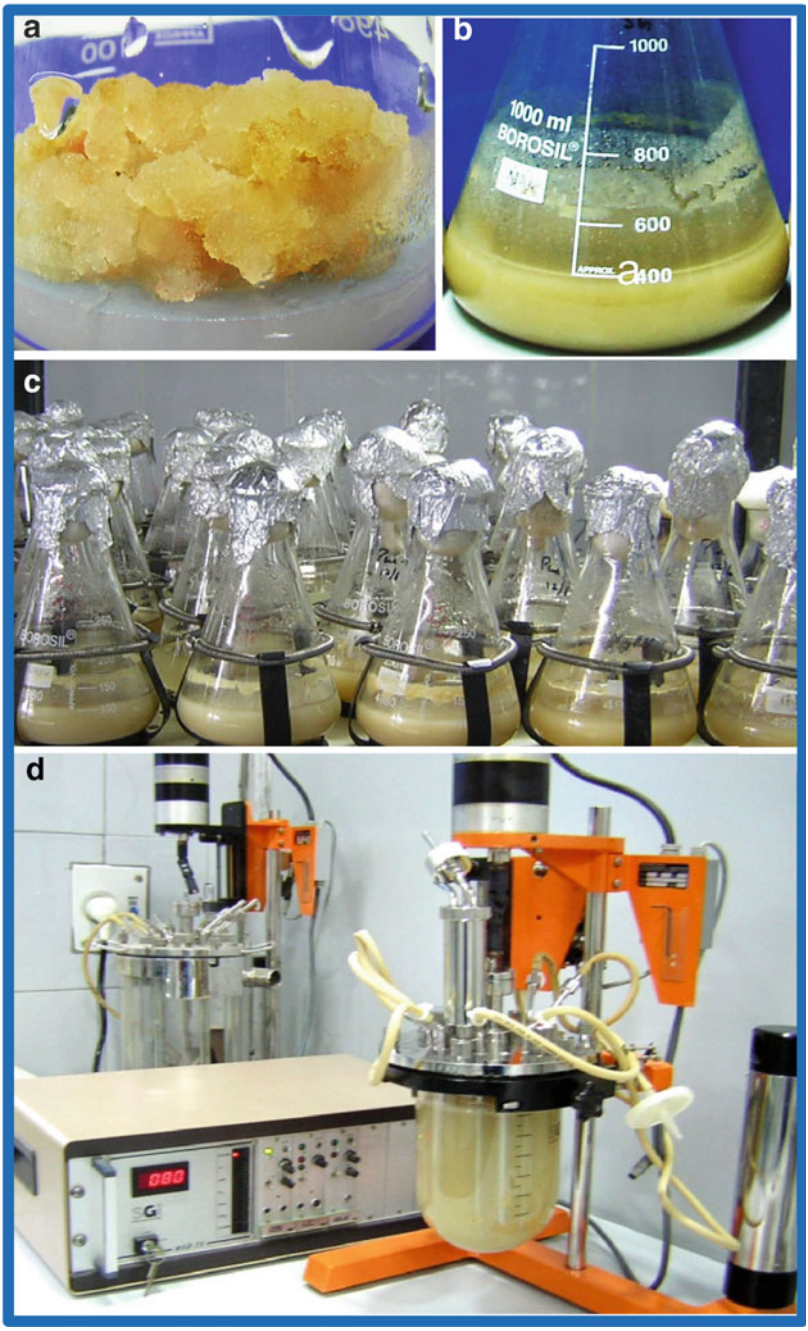


Fig. 21.4 A fairly fast-growing callus used for cell cultures and subsequently used in scale-up studies using the production medium and elicitation. Callus (A) and cell suspension cultures on large-scale (B, C) and cell cultures in stirred tank bioreactor (D) of *P. tuberosa*

Table 21.4 Biotechnological advancement and work of tissue culture about *Pueraria* species

Name of species	Culture conditions	Significance	References
P. tuberosa	CSC-MS containing 1 mg/l 2,4-D, 0.5 mg/l NAA, 1 mg/l kinetic	Enhanced production of puerarin (up to 216.41 μg/g DW)	Karwasara, and Dixit (2012)
	Shoot culture-MS containing 1.13 μM TDZ and 0.25 μM IBA + aeration treatment (20%v/v) in Growtek	Increased isolflavonoids with Puerarin (1484 μg/g DW)	Sharma et al. (2011)
	CSC- MS containing morphactin 0.1 mg/l and 2iP 5 mg/l + elicitation with *Cuscuta reflexa*	Isoflavonoid accumulation (7,006 μg/g DW)	Goyal et al. (2011)
	Shoot culture, MS containing 4.44 μM BA and 0.57 μM IAA and additives	Accumulation of puerarin (leaf, 421.35 μg/g DW)	Rathor and Shekhawat (2009)
	CSC- MS medium with morphactin (0.1 mg/l) and 2iP (5.0 mg/l) and 20% inoculum	Increased isoflavonoid content up to 6351 μg/g DW	Sharma et al. (2009)
	CSC- MS medium (KNO3 475 mg/l, thiamine 1 mg/l, biotin 1 mg/l, calcium pantothenate 1 mg/l) containing 0.1 mg/l 2,4,5-trichloroacetic acid and 0.1 mg/l kinetin + Ethrel (20–400 μM)	Total isoflavonoid accumulation up to 981 μg/g DW	Goyal and Ramawat (2008)
	CSC- MS medium (KNO3 475 mg/l, thiamine 1 mg/l, biotin 1 mg/l, calcium pantothenate 1 mg/l) containing 0.1 mg/l 2, 4, 5-trichloroacetic acid and 0.1 mg/l kinetin + sucrose (6%)	Isoflavonoid content up to 598 μg/g DW	Goyal and Ramawat (2007)
	Callus culture, MS medium containing BA (4.1 μM) and sucrose (20-60 g/l)	Isoflavonoid content (up to 2.97 μg/g DW)	Vaishnav et al. (2006)

(continued)

Table 21.4 (continued)

Name of species	Culture conditions	Significance	References
P. candollei var. *mirifica*	CSC- MS containing TDZ (0.1 mg/l), NAA (1 mg/l) and BA (0.5 mg/l) + elicitation with methyl jasmonate, yeast extract and chitosan	Enhanced production of isoflavonoid (up to 548 μg/g DW) and deoxymiroestrol(up to 976 μg/g DW)	Udomsin et al. (2020)
	HRC, 1/2MS medium containing cefotaxime (100–400 mg/l) + elicitation with methyl jasmonate	Production of deoxymiroestrol (up to 113 μg/g DW) and isoflavonoid (up to 9.94 mg/g DW)	Udomsin et al. (2019)
P. candollei	HRC-MS medium containing cefotaxime(100-300 mg/l) + elicitation with methyl jasmonate, chitosan, salicylic acid, and yeast extract	Total isoflavonoid up to 60 mg/g DW	Udomsuk et al. (2011)
P. lobata	Shoot culture, MS containing 4.6 μM kinetin and 5.7 μM IAA + elicitation with methyl jasmonate	Increased accumulation of isoflavonoid (up to 10.44 mg/g DW)	Thiem and Krawczyk (2010)
	HRC- ½ MS medium with 500 mg/l cefotaxime (supplemented with or without 0.5 mg/l IBA	Production of puerarin (0.382 mg/g DW)	Kim et al. (2012)

*2,4,5-T = 2,4,5- Trichlorophenoxy acetic acid, 2,4-D = 2,4- Dichlorophenoxy acetic acid, 2iP = N- isopentylamino purine, B5 = Pantothenic acid, BAP = N6- Benzyl adenine, IAA = Indole-3-acetic acid, IBA = Indole-3-butyric acid, Kn = Kinetin; 6-furfuryl amino purine, MS = Medium (Murashige and Skoog, 1962), NAA = 1 Naphthaleneacetic acid, TDZ = Thidiazuron, DW = Dry weight, CSC- Cell Suspension Culture, HRC- Hairy root culture

21.9 Agro-Technology for Cultivation

Forests are always been a major source of all medicinal plants including trees and herbs since time immemorial. Several species have become vulnerable to extinction due to lack of cultivation and unsustainable collection of raw material required for herbal formulations directly from the forests. The domestication of these medicinal crops includes exploration of wild medicinal plants; improvement of the desirable traits in promising species by traditional and biotechnological approaches, standardization of various agronomic methods for their large-scale exploitation, and popularization of unpopular medicinal crops amongst farmers, policymakers, and other stakeholders. *P. tuberosa* prefers sub-tropical climate, shade, and moist conditions. It

grows well in rough-textured dirt soil with high moisture contents and rich in organic matter. Partial shady areas are appropriate for its cultivation (Tropical Plant Database 2020). *Pueraria* plantation is a more successful and cost-effective by nursery rising. The mechanically scarified seeds can be sown in the nursery in May, and further seedlings can be transferred in the field in the mid of August- September (Fig. 21.5). The seedlings or cuttings can be planted in pits with a 1:1:1 blend of soil, sand, and manure. Around 18,000 plants can be planted in one-hectare land. Intercropping systems including planting of erect plants like *Desmodium and Plumbago* can be opted to develop half shade conditions for the development and advancement of this liana. Tuber collection can be done in April–May from a 5–6 years old plant. The yield of tubers through such nursery management has been reported about 5–7.5 tons per hectare (Sharma et al. 2018. The cultivation cost of *P. tuberosa* for 2016–17 has been estimated to be approximately 200 US$/ha. According to the National Ayush Mission list of prioritized plants for cultivation under the scheme of the National medicinal plant board (NMPB), *P. tuberosa*, is eligible for a 50% subsidy. Supervision of NMPB cultivation status 2016–17, cultivation of Vidarikand is being done on 5-hectare area in Gujrat ((https://www.nmpb.nic.in/content/prioritised-list-medicinal-plants-cultivation).

Fig. 21.5 *P. tuberosa* plantlets grown in Nursery

21.10 Conclusions

P. tuberosa, one of the important medicinal lianas, is owing to a tremendous international market value due to its tuber. Approximately 1400 lb dried powder/ extract of tubers was exported to various US countries in 2016 (https://www.seair.co.in/vidarikand-export-data.aspx). Tuber is a rich source of isoflavonoids with aphrodisiac and rejuvenating activities as major pharmacological properties. Direct use of tuber from wild, lack of proper cultivation has made this plant endangered. Various biotechnological approaches including callus, cell suspension culture, hairy roots culture, and micropropagation have been attempted by various researchers, and increased isoflavonoids production has been reported. Still due to lack of technology transfer from lab to land, cultivation and domestication of this medicinal crop is required. Its cultivation requires initial proper collection and sowing practises of seeds. The development of proper agro-technology will help in the proper management of this important medicinal plant and also reduce the pressure on the wild resources.

References

Ahmad B, Khan S, Liu Y, Xue M, Nabi G, Kumar S, Qarluq AW (2020) Molecular mechanisms of anticancer activities of puerarin. Cancer Manag Res 2020(12):79–90

Anilkumar K, Reddy GV, Azad R, Yarl NS, Dharmapuri G, Srivastava A, Kamal MA, Pallu R (2017) Evaluation of anti-inflammatory properties of isoorientin isolated from tubers of *Pueraria tuberosa.* Oxid Med Cell Longev 2017:5498054. https://doi.org/10.1155/2017/5498054

Anon (2016) Agro-techniques of Selected Medicinal Plants: Volume – III. Published by National Medicinal Plants Board, Ministry of AYUSH, Government of India, pp 56–58

Bai Z, Wang Z (2019) Genistein protects against doxorubicin-induced cardiotoxicity through Nrf-2/HO-1 signaling in mice model. Environ Toxicol 34(5):645–651

Bhattarai G, Poudel SB, Kook SH, Lee JC (2017) Anti-inflammatory, anti-osteoclastic, and antioxidant activities of genistein protect against alveolar bone loss and periodontal tissue degradation in a mouse model of periodontitis. J Biomed Mater Res A 105(9):2510–2521

Bhosale V, Banerjee D (2020). Scientific Validation of Herbal Medicine. https://doi.org/10.1007/978-981-13-7248-3_34

Bodner CC, Hymowitz T (2002) Ethnobotany of *Pueraria* species. In: Keung WM (ed) *Pueraria*: the genus *Pueraria* CRC Press, Taylor and Francis, pp 29–58

Carlson S, Prasain JK, Peng N, Dai Y, Wyss JM (2014) Acute and chronic kudzu improves plasma glucose tolerance in non-diabetic CD-1 mice. J Endocrinol Diabetes Mellit 2:70–77

Chang BY, Lee DS, Lee JK, Kim YC, Cho HK, Kim SY (2015) Protective activity of kudzu (*Pueraria thunbergiana*) vine on chemically-induced hepatotoxicity: in vitro and in vivo studies. BMC Complement Altern Med 16(1):1–8

Chang BY, Lee DS, Lee JK, Kim YC, Cho HK, Kim SY (2016) Protective activity of kudzu (*Pueraria thunbergiana*) vine on chemically-induced hepatotoxicity: in vitro and in vivo studies. BMC Complement Altern Med 16:39

Chen T, Chen H, Wang Y, Zhang J (2016) In vitro and in vivo antitumour activities of puerarin 6″-O-xyloside on human lung carcinoma A549 cell line via the induction of the mitochondria-mediated apoptosis pathway. Pharm Biol 54(9):1793–1799

Chen M, Zhou C, Xu H, Zhang T, Wu B (2020) Chronopharmacological targeting of rev-erbα by puerarin alleviates hyperhomocysteinemia in mice. Biomed Pharmacother 125:109936

Choi J, Shin MH, Park KY, Lee KT, Jung HJ, Lee MS, Park HJ (2004) Effect of kaikasaponin III obtained from *Pueraria thunbergiana* flowers on serum and hepatic lipid peroxides and tissue factor activity in the streptozotocin-induced diabetic rat. J Med Food 7(1):31–37

Das D, Sarkar S, Bordoloi J, Wann SB, Kalita J, Manna P (2018) Daidzein, its effects on impaired glucose and lipid metabolism and vascular inflammation associated with type 2 diabetes. BioFactors 44(5):407–417

Divya G, Albert A, Singab ANB, Ayoub IM et al (2019) Renoprotective effect of tectorigenin glycosides isolated from Iris spuria L.(Zeal) against hyperoxaluria and hyperglycemia in NRK-49Fcells. Nat Prod Res 32(11):1–6

Dong S, Zhang M, Niu H, Jiang K et al (2018) Upregulation of UDP-glucuronosyltransferases 1a1 and 1a7 are involved in altered puerarin pharmacokinetics in type II diabetic rats. Molecules 23(6):1487. https://doi.org/10.3390/molecules23061487

Gong P, Deng F, Zhang W, Ji J, Liu J, Sun Y, Hu J (2017) Tectorigenin attenuates the MPP+-induced SH-SY5Y cell damage, indicating a potential beneficial role in Parkinson's disease by oxidative stress inhibition. Exp Ther Med 14(5):4431–4437

Goyal S, Ramawat KG (2007) Effect of chemical factors on production of isoflavonoids in *Pueraria tuberosa* (Roxb. ex Willd.) DC. Suspension Culture. Indian J Exp Biol 45:1068–1072

Goyal S, Ramawat KG (2008) Increased isoflavonoids accumulation in cell suspension cultures of Pueraria tuberosa by elicitors NOPR 7(3):378-382

Goyal S, Ramawat KG (2008) Synergistic effect of morphactin on cytokinin-induced production of isoflavonoid in cell cultures of *Pueraria tuberosa* (Roxb. ex. Willd.) DC. Plant Growth Regul 55:175–181

Goyal S, Sharma V, Ramawat KG (2011) Marked effect of Cuscuta on puerarin accumulation in cell cultures of *Pueraria tuberosa* grown in shake fl asks and a bioreactor. Plant Biotechnol Rep 5(2):121–126

Goyal S, Sharma V, Ramawat KG (2015) A review of biotechnological approaches to conservation and sustainable utilization of medicinal Lianas in India. In: Parthasarathy N (ed) Biodiversity of Lianas. Sustainable development and biodiversity, vol 5. Springer, Cham, pp 179–210. https://doi.org/10.1007/978-3-319-14592-1_11

Gu M, Zheng AB, Jin J, Cui Y, Zhang N, Che ZP, Wang Y, Zhan J, Tu WJ (2016) Cardioprotective effects of genistin in rat myocardial ischemia-reperfusion injury studies by regulation of P2X7/NF-κB pathway. Evid Based Complementary Altern Med 2016:5381290. https://doi.org/10.1155/2016/5381290

Gulizia JP, Downs KM (2019) A Review of Kudzu's use and characteristics as potential feedstock. Agriculture 9(10):220. https://doi.org/10.3390/agriculture9100220

Horio Y, Sogabe R, Shichiri M, Ishida N, Morimoto R, Ohshima A, Isegawa Y (2020) Induction of a 5-lipoxygenase product by daidzein is involved in the regulation of influenza virus replication. J Clin Biochem Nutr 66(1):36–42

Hsueh TP, Lin WL, Tsai TH (2017) Pharmacokinetic interactions of herbal medicines for the treatment of chronic hepatitis. J Food Drug Anal 25(2):209–218

Hu Y, Li X, Lin L, Liang S, Yan J (2018) Puerarin inhibits non-small cell lung cancer cell growth via the induction of apoptosis. Oncol Rep 39(4):1731–1738

Hua F, Li CH, Chen XG, Liu XP (2018) Daidzein exerts anticancer activity towards SKOV3 human ovarian cancer cells by inducing apoptosis and cell cycle arrest, and inhibiting the Raf/MEK/ERK cascade. Int J Mol Med 41(6):3485–3492

Hwang ST, Yang MH, Baek SH, Um JY, Ahn KS (2020) Genistin attenuates cellular growth and promotes apoptotic cell death breast cancer cells through modulation of ERalpha signaling pathway. Life Sci 263:118594

Islam A, Islam MS, Uddin MN, Hasan MMI, Akanda MR (2020) The potential health benefits of the isoflavone glycoside genistin. Arch Pharm Res 43:395–408

Jiang K, Chen H, Tang K et al (2018) Puerarin inhibits bladder cancer cell proliferation through the mTOR/p70S6K signaling pathway. Oncol Lett 15(1):167–174

Johnson SL, Park HY, Vattem DA et al (2020) Equol, a blood-brain barrier permeable gut microbial metabolite of dietary isoflavone daidzein, exhibits neuroprotective effects against neurotoxins induced toxicity in human neuroblastoma SH-SY5Y cells and caenorhabditis elegans. Plant Foods Hum Nutr 75:512–517

Joshi A, Kanthaliya B, Arora J (2019) Evaluation of growth and antioxidant activity in *Suaeda monoica* and *Suaeda nudiflora* callus cultures under sequential exposure to saline conditions. Curr Biotechnol 8(1):42–52

Kanthaliya B, Joshi A, Arora J (2019) Evaluation of isoflavonoid content in context to tuber size and seed biology study of *Pueraria tuberosa* (Roxb. ex. Willd.) DC: a vulnerable medicinal plant Vegetos 32(3):247–253

Karwasara VS, Dixit VK (2012) Culture medium optimization for improved puerarin production by cell suspension cultures of *Pueraria tuberosa* (Roxb. ex Willd.) DC. Vitro Cell Dev Biol -Plant 48:189–199. https://doi.org/10.1007/s11627-011-9415-y

Kazmi Z, Zeeshan S, Khan A, Malik S, Shehzad A, Seo EK, Khan S (2020) Anti-epileptic activity of daidzin in PTZ-induced mice model by targeting oxidative stress and BDNF/VEGF signaling. Neurotoxicology 79:150–163

Kim SJ, Cha MS, Lee EJ, Kim IH, Kwon JE, Kang SC, Park TH (2012) *I*n vitro induction of hairy root from isoflavones-producing Korean wild arrowroot *Pueraria lobata*. J Plant Biotechnol 39(3):205–211

Kim EY, Hong KB, Suh HJ, Choi HS (2015) Protective effects of germinated and fermented soybean extract against tert-butyl hydroperoxide-induced hepatotoxicity in HepG2 cells and in rats. Food Funct 6(11):3512–3521

Koirala P, Seong SH, Jung AH, Choi JS (2017) Comparative molecular docking studies of lupeol and lupenone isolated from Puerarialobata that inhibits BACE1: probable remedies for Alzheimer's disease. Asian Pac J Trop Med 10(12):1117–1122

Lee J, Park SH (2018) Daidzein has anti-oxidant activity in normal human kidney tubular HK-2 cells via FOXO3/SOD2 Pathway. J Food Nutr Res 6(9):557–560

Lee CC, Dudonné S, Kim JH, Kim JS, Dubé P, Kim JE, Desjardins Y, Park JHY, Lee KW, Lee CY (2018) A major daidzin metabolite 7, 8, 4′-trihydroxyisoflavone found in the plasma of soybean extract-fed rats attenuates monocyte-endothelial cell adhesion. Food Chem 240:607–614

Lee SR, Kwon SW, Lee YH, Kaya P, Kim JM, Ahn C, Jung EM, Lee GS, An BS, Jeung EB et al (2019) Dietary intake of genistein suppresses hepatocellular carcinoma through AMPK-mediated apoptosis and anti-inflammation. BMC Cancer 19:6

Lertpatipanpong P, Janpaijit S, Park EY, Kim CT, Baek SJ (2020) Potential anti-diabetic activity of *Pueraria lobata* Flower (Flos Puerariae) extracts. Molecules 25(17):3970. https://doi.org/10.3390/molecules25173970

Li Z, Shangguan Z, Liu Y, Wang J, Li X, Yang S, Liu S (2014) Puerarin protects pancreatic β-cell survival via PI3K/Aktsignaling pathway. J Mol Endocrinol 53(1):71–79

Li S, Li J, Dai W, Zhang Q, Feng J, Wu L, Liu T, Yu Q, Xu S, Wang W et al (2017) Genistein suppresses aerobic glycolysis and induces hepatocellular carcinoma cell death. Br J Cancer 117:1518–1528

Li X, Huang Z, Liu S, Zeng X, Xie J, Liu C, Xiao H, Liu R, Li L, Zeng J (2018) 3'-Daidzein sulfonate sodium provides neuroprotection by promoting the expression of the α7 nicotinic acetylcholine receptor and suppressing inflammatory responses in a rat model of focal cerebral ischemia. Am J Transl Res 10(11):3455

Li RZ, Ding XW, Geetha T, Al-Nakkash L, Broderick TL, Babu JR (2020) Beneficial effect of genistein on diabetes-induced brain damage in the ob/ob mouse model. Drug Des Devel Ther 14:3325

Li X, Zhang J, Zhang X, Dong M (2020) Puerarin suppresses MPP+/MPTP-induced oxidative stress through an Nrf2-dependent mechanism. Food Chem Toxicol 144:111644

Likhitkar M, Pande M, Singh SN (2016) Evaluation of hair growth activity of *Pueraria tuberosa* (Kudzu) by using chemotherapy induced alopecia. IJIET 7(2):354–359

Lim HS, Kim YJ, Kim BY, Park G, Jeong SJ (2018) The anti-neuroinflammatory activity of tectorigenin pretreatment via downregulated NF-κB and ERK/JNK pathways in BV-2 microglial and microglia inactivation in mice with lipopolysaccharide. Front Pharmacol 9:462

Lu C, Wang Y, Xu T, Li Q, Wang D, Zhang L, Fan B, Wang F, Liu X (2018) Genistein ameliorates scopolamine-induced amnesia in mice through the regulation of the cholinergic neurotransmission, antioxidant system and the ERK/CREB/BDNF signaling. Front Pharmacol 9:1153

Luo T, Miranda-Garcia O, Sasaki G, Wang J, Shay NF (2018) Genistein and daidzein decrease food intake and body weight gain in mice, and alter LXR signaling in vivo and in vitro. Food Funct 9(12):6257–6267

Ma C, Xu K, Meng J, Ran J, Adel Abdo Moqbe S, Liu A, Yan S, Wu L (2018) Tectorigenin inhibits RANKL-induced osteoclastogenesis via suppression of NF-κB signalling and decreases bone loss in ovariectomized C57 BL/6. J Cell Mol Med 22(10):5121–5131

Mahdy HM, Mohamed MR, Emam MA, Karim AM, Abdel-Naim A, Khalifa AE (2014) The anti-apoptotic and anti-inflammatory effects of Puerarin attenuate 3-nitropropionic acid-induced neurotoxicity in rats. Can J Physiol Pharmacol 92:252–258

Maji AK, Mahapatra S, Banerjee D (2014) In-vivo immunomodulatory potential of standardized *Pueraria tuberosa* extract and its isoflavonoids. Int J Pharm Pharm Sci 6:861–867

Maji AK, Pandit S, Banerji P, Banerjee D (2014) *Pueraria tuberosa*: a review on its phytochemical and therapeutic potential. Nat Prod Res 28(23):2111–2127. https://doi.org/10.1080/14786419.2014.928291

Mazumder MAR, Hongsprabhas P (2016) Genistein as antioxidant and antibrowning agents in in vivo and in vitro: a review. Biomed Pharmacother 82:379–392

Miao XR, Niu JQ, Wang AQ, Wang DB, Fan J (2019) Complete chloroplast genome sequence of *Pueraria thomsonii*, an important traditional Chinese medicine plant. Mitochondrial DNA Part B 4(2):4163–4165

Mocan A, Carradori S, Locatelli M, Secci D, Cesa S, Mollica A, Riga S, Angeli A, Supuran CT, Celia C, Di Marzio L (2018) Bioactive isoflavones from *Pueraria lobata* root and starch: different extraction techniques and carbonic anhydrase inhibition. Food Chem Toxicol 112:441–447

Murata K, Noguchi K, Kondo M, Onishi M, Watanabe N, Okamura K, Matsuda H (2012) Inhibitory activities of *Puerariae Flos* against testosterone 5α-reductase and its hair growth promotion activities. J Nat Med 66(1):158–165

Ojo OA (2021) Puerarin as a potential drug candidate for the management of type-2 diabetes: molecular docking and pharmacophore modeling studies. Biointerface Res Appl Chem 11(2):8751–8759. https://doi.org/10.33263/BRIAC112.87518759

Pan B, Liu B, Yu ZX, Yang YQ (2015) *Pueraria grandiflora* (Fabaceae), a new species from Southwest China. Phytotaxa 203(3):287–291

Pandey N, Chaurasia JK, Tiwari OP, Tripathi YB (2007) Antioxidant properties of different fractions of tubers from *Pueraria tuberosa* Linn. Food Chem 105:219–222

Qin X, Huang C, Wu K, Li Y, Liang X, Su M, Li R (2020) Anti–coronavirus disease 2019 (COVID-19) targets and mechanisms of puerarin. J Cell Mol Med 00:1–9. https://doi.org/10.1111/jcmm.16117

Rajaei S, Alihemmati A, Abedelahi A (2019) Antioxidant effect of genistein on ovarian tissue morphology, oxidant and antioxidant activity in rats with induced polycystic ovary syndrome. Int J Reprod Biomed 17(1):11–22

Rajput MS, Sarkar PD (2017) Modulation of neuro-inflammatory condition, acetylcholinesterase and antioxidant levels by genistein attenuates diabetes associated cognitive decline in mice. Chem Biol Interact 268:93–102

Ramawat KG (2019) An introduction to the process of cell, tissue, and organ differentiation, and production of secondary metabolites. In: Ramawat KG, Ekiert HM, Goyal S (eds) Plant cell and tissue differentiation and secondary metabolites. Reference series in phytochemistry. Springer, Cham. https://doi.org/10.1007/978-3-030-11253-0_35-1

Rathore MS, Shekhawat NS (2009) Micropropagation of *Pueraria tuberosa* (Roxb. Ex Willd.) and determination of puerarin content in different tissues. PCTOC 99(3):327–334

Rawtal B, Sahatpure N, Sakharwade S (2019) *Pueraria tuberosa* (Vidarikanda): an emerging cosmeceutical herb. IJSDR 4(7):130–137

Rehman K, Ali MB, Akash MSH (2019) Genistein enhances the secretion of glucagon-like peptide-1 (GLP-1) via downregulation of inflammatory responses. Biomed Pharmacother 112:108670

Satpathy S, Patra A, Hussain MD, Ahirwar B (2017) Simultaneous estimation of genistein and daidzein in *Pueraria tuberosa* (Willd.) DC by validated high-performance thin-layer chromatography (HPTLC) densitometry method. J Liq Chrom Relat Tech 40(10):499–505

Sen S, Chakraborty R (2017) Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. J Tradit Complement Med 7(2):234–244

Seong SH, Roy A, Jung HA, Jung HJ, Choi JS (2016) Protein tyrosine phosphatase 1B and α-glucosidase inhibitory activities of *Pueraria lobata* root and its constituents. J Ethnopharmacol 194:706–716

Sharma V, Ramawat KG (2013) Tuberous medicinal plants of India: Biology and biotechnology. In: Ramawat KG, Merillon JM (eds) Bulbous plants: biotechnology. CRC Press, Taylor and Francis, pp 311–337

Sharma V, Goyal S, Ramawat KG (2009) Scale up production of isoflavonoids in cell suspension cultures of *Pueraria tuberosa* grown in shake flasks and bioreactor. Eng Life Sci 9(3):267–271

Sharma V, Goyal S, Ramawat KG (2011) Increased puerarin biosynthesis during in vitro shoot formation in *Pueraria tuberosa* grown in growtek bioreactor with aeration. Physiol Mol Biol Plants 17(1):87–92

Sharma S, Agrawal M, Lal M (2018) Cultivation of "Vidarikand" (*Pueraria tuberosa* Dc): a drug of potential importance. IJIRR 05(05):5460–5462

Sharma R, Martins N, Kuca K, Chaudhary A, Kabra A, Rao MM, Prajapati PK (2019) Chyawanprash: a traditional Indian bioactive health supplement. Biomolecules 9(5):161

Singh N (2011) Wild edible plants: a potential source of nutraceuticals. Int J Pharm Sci Res 2(12):216–225

Srivastava S, Shree P, Tripathi YB (2017) Active phytochemicals of Pueraria tuberosa for DPP-IV inhibition: in silico and experimental approach. J Diabetes Metab Disord 16(1):46

Thiem B, Krawczyk A (2010) Enhanced isoflavones accumulation in methyl jasmonate-treated in vitro cultures of kudzu [*Pueraria lobata Ohwi*]. Herb a Polonica 56(1):48–56

Tiyasatkulkovit W, Malaivijitnond S, Charoenphandhu N, Havill LM, Ford AL, VandeBerg JL (2014) *Pueraria mirifica* extract and puerarin enhance proliferation and expression of alkaline phosphatase and type I collagen in primary baboon osteoblasts. Phytomedicine 21(12):1498–1503

Tropical Plants Database, Ken Fern. tropical. the ferns.info. 2020-09-17. tropical.theferns.info/viewtropical.php?id=Pueraria+tuberosa

Tungmunnithum D, Intharuksa A, Sasaki Y (2020) A promising view of kudzu plant, *Pueraria montana var. lobata* (Willd.) Sanjappa & Pradeep: flavonoid phytochemical compounds, taxonomic data, traditional uses and potential biological activities for future cosmetic application. Cosmetics 7(1):12. https://doi.org/10.3390/cosmetics7010012

Uddin MS, Hossain MF, Al Mamun A et al (2020) Exploring the multimodal role of phytochemicals by modulating cellular signal pathways to combat age-related neurodegeneration. Sci Total Environ 138313

Udomsin O, Yusakul G, Kraithong W, Udomsuk L, Kitisripanya T, Juengwatanatrakul T, Putalun, W (2019) Enhanced accumulation of high-value deoxymiroestrol and isoflavonoids using hairy root as a sustainable source of *Pueraria candollei var. mirifica.* PCTOC 136(1): 141–151

Udomsin O, Yusakul G, Kitisripanya T et al (2020) The deoxymiroestrol and isoflavonoid production and their elicitation of cell suspension cultures of *Pueraria candollei* var. *mirifica*: from shake flask to bioreactor. Appl Biochem Biotechnol 190:57–72. https://doi.org/10.1007/s12010-019-03094-y

Udomsuk L, Jarukamjorn K, Tanaka H, Putalun W (2011) Improved isoflavonoid production in Pueraria candollei hairy root cultures using elicitation. Biotech Lett 33(2):369–374
Vaishnav K, Goyal S, Ramawat KG (2006) Isoflavonoids production in callus culture of Pueraria tuberosa, the Indian kudzu. Indian J Exp Biol 44(12):1012–1017
Van der Maesen LJG (2002) Pueraria: botanical characteristics. In: Keung WM (ed) *Pueraria:* the genus Pueraria. Taylor and Francis, New York, pp 1–28
Venkatasubramanian P, Kumar KS, Venugopal SN (2009) Use of as a substitute for as per Ayurvedic descriptions. Indian J Tradit Knowl (IJTK) 8(3):310–318
Wang Y, Yang C, Xie WL, Zhao YW, Li ZM, Sun WJ, Li LZ (2014) Puerarin concurrently stimulates osteoprotegerin and inhibits receptor activator of NF-κB ligand (RANKL) and interleukin-6 production in human osteoblastic MG-63 cells. Phytomedicine 21(8–9):1032–1036
Wang N, Zhang Y, Wu L et al (2014) Puerarin protected the brain from cerebral ischemia injury via astrocyte apoptosis inhibition. Neuropharmacology 79:282–289
Wang ZB, Chen BB, Luo L, Yan JK (2016) Fractionation, physicochemical characteristics and biological activities of polysaccharides from *Pueraria lobata* roots. J Taiwan Inst Chem Eng 67:54–60
Wang CL, Wang CD, Xiao F, Zhu JF, Shen C, Zuo B, Cui YM, Wang H, Gao Y, Hu GL, Zhang XL (2017) Anti-inflammatory and anti-osteoarthritis effects of tectorigenin. Biol J Open 6(8):1130–1136
Wang S, Zhang S, Wang S, Gao P, Dai L (2020) A comprehensive review on Pueraria: insights on its chemistry and medicinal value. Biomed Pharmacother 131:110734
Wei J, Yang F, Gong C, Shi X, Wang G (2019) Protective effect of daidzein against streptozotocin-induced Alzheimer's disease via improving cognitive dysfunction and oxidative stress in rat model. J Biochem Mol Toxicol 33(6):e22319
Weng L, Zhang F, Wang R, Ma W, Song Y (2019) A review on protective role of genistein against oxidative stress in diabetes and related complications. Chem Biol Interact 310:108665
Wu H, Zhao G, Jiang K, Chen X, Zhu Z, Qiu C, Deng G (2016) Puerarin exerts an antiinflammatory effect by inhibiting nf-kb and mapk activation in staphylococcus aureus-induced mastitis. Phytother Res 30(10):1658–1664
Wu KC, Chiang BJ, Tsai WH, Chung SD, Chien CT (2018) I-Tiao-Gung extract through its active component daidzin improves cyclophosphamide-induced bladder dysfunction in rat model. Neurourol Urodyn 37(8):2560–2570
Wu KC, Lin WY, Sung YT, Wu WY, Cheng YH, Chen TS, Chiang BJ, Chien CT (2019) Glycine tomentella hayata extract and its ingredient daidzin ameliorate cyclophosphamide-induced hemorrhagic cystitis and oxidative stress through the action of antioxidation, anti-fibrosis, and anti-inflammation. Chinese J Physiol 62(5):188–195
Xu X, Zheng N, Chen Z, Huang W, Liang T, Kuang H (2016) Puerarin, isolated from *Pueraria lobata* (Willd.), protects against diabetic nephropathy by attenuating oxidative stress. Gene 591(2):411–416
Xu H, Zhao M, Liang S et al (2016) The effects of puerarin on rat ventricular myocytes and the potential mechanism. Sci Rep 6:35475
Xu HX, Pan W, Qian JF, Liu F, Dong HQ, Liu QJ (2019) MicroRNA-21 contributes to the puerarin-induced cardioprotection via suppression of apoptosis and oxidative stress in a cell model of ischemia/reperfusion injury. Mol Med Rep 20(1):719–727
Yang Y, Zang A, Jia Y, Shang Y, Zhang Z, Ge K, Zhang J, Fan W, Wang B (2016) Genistein inhibits A549 human lung cancer cell proliferation via miR-27a and MET signaling. Oncol Lett 12:2189–2193
Yang R, Jia Q, Liu XF, Ma SF (2018) Effect of genistein on myocardial fibrosis in diabetic rats and its mechanism. Mol Med Rep 17(2):2929–2936
Yang S, Ma C, Wu H, Yuan F, Yang G, Yang Q, Jia L, Liang Z, Kang L (2020) Tectorigenin attenuates diabetic nephropathy by improving vascular endothelium dysfunction through activating AdipoR1/2 pathway. Pharmacol Res 153:104678

Yang MH, Jung SH, Chinnathambi A, Alahmadi TA, Alharbi SA, Sethi G, Ahn KS (2020) Attenuation of STAT3 signaling Cascade by Daidzin can enhance the apoptotic potential of Bortezomib against multiple myeloma. Biomolecules 10(1):23. https://doi.org/10.3390/biom10010023

Yao X, Li K, Liang C, Zhou Z, Wang J, Wang S, Liu L, Yu CL, Song ZB, Bao YL, Zheng LH (2020) Tectorigenin enhances PDX1 expression and protects pancreatic β-cells by activating ERK and reducing ER stress. J Biol Chem 295(37):12975–12992

Yu Z, Yang L, Deng S, Liang M (2020) Daidzein ameliorates LPS-induced hepatocyte injury by inhibiting inflammation and oxidative stress. Eur J Pharmacol 885:173399

Zang Y, Igarashi K, Yu C (2015) Anti-obese and anti-diabetic effects of a mixture of daidzin and glycitin on C57BL/6J mice fed with a high-fat diet. Biosci Biotechnol Biochem 79(1):117–123

Zhang T, Wu Z, Du J, Hu Y, Liu L, Yang F, Jin Q (2012) Anti-Japanese-encephalitis-viral effects of kaempferol and daidzin and their RNA-binding characteristics. PLoS ONE 7(1):e30259

Zhang Q, Chen D, Yu B, Mao X, Huang Z, Yu J, Luo J, Zheng P, Luo Y, He J (2018) Effects of dietary daidzein supplementation on reproductive performance, serum hormones, and reproductive-related genes in rats. Nutrients 10(6):766

Zhang Y, Yang M, Ho NJ, Mok RY, Zhang Z, Ge B, Leung PC, Fung KP, San Lau CB, Lee VHL, Lin Z (2020) Is it safe to take Radix Salvia Miltiorrhiza-Radix *Pueraria lobate* product with warfarin and aspirin? A pilot study in healthy human subjects. J Ethnopharmacol 262:113151. https://doi.org/10.1016/j.jep.2020.113151

Zheng W, Sun R, Yang L, Zeng X, Xue Y, An R (2017) Daidzein inhibits choriocarcinoma proliferation by arresting cell cycle at G1 phase through suppressing ERK pathway in vitro and in vivo. Oncol Rep 38(4):2518–2524

Zheng W, Liu T, Sun R, Yang L, An R, Xue Y (2018) Daidzein induces choriocarcinoma cell apoptosis in a dose-dependent manner via the mitochondrial apoptotic pathway. Mol Med Rep 17(4):6093–6099

Zhou L, Xiao X, Zhang Q, Zheng J, Li M, Yu M, Wang X, Deng M, Zhai X, Li R, Liu J (2019) Dietary genistein could modulate hypothalamic circadian entrainment, reduce body weight, and improve glucose and lipid metabolism in female mice. Int J Endocrinol 2019:2163838. https://doi.org/10.1155/2019/2163838

Zhou T, Wang Z, Guo M, Zhang K, Geng L, Mao A, Yang Y, Yu F (2020) Puerarin induces mouse mesenteric vasodilation and ameliorates hypertension involving endothelial TRPV4 channels. Food Funct 11:10137–10148

Zhou T, Wang Z, Guo M et al (2020) Puerarin induces mouse mesenteric vasodilation and ameliorates hypertension involving endothelial TRPV4 channels. Food Funct 11:10137–10148

Zhu Y, Yao Y, Shi Z, Everaert N, Ren G (2018) Synergistic effect of bioactive anticarcinogens from soybean on anti-proliferative activity in MDA-MB-231 and MCF-7 human breast cancer cells in vitro. Molecules 23(7):1557.ssss https://www.mdpi.com/1420-3049/23/7/1557

Chapter 22
Integrated Approach for the Quality Assurance of Commercially Important Himalayan Medicinal Plants

Prateek Singh Bora, Patil Shivprasad Suresh, Surekha Kumari, Anmol, Shivani Puri, and Upendra Sharma

Abstract The gradual growth of the herbal drug industries has necessitated the need for the quality control measures for the quality assessment of herbal preparations. This has resulted in the employment of a comprehensive set of analytical methods that helps to give a complete picture of the quality of herbal material in terms of its phytochemical composition and chemo taxonomical origin. Hence, this chapter discusses the issue of adulteration and also elaborates on the integrated analytical approach which has come out as a comprehensive tool in the assessment of botanicals authenticity. Twelve commercially important Himalayan medicinal plants whose trade volume exceeds 100 metric tons annually has been discussed. Although, various efforts have already been made towards the quality assessment of these commercially important Himalayan medicinal plants, the integrated set of analytical methods discussed in this chapter can be applied for the complete assessment of their authenticity to further enhance their trade volume in the international market.

Keywords Quality control · Himalayan medicinal plants · Adulteration · Phytochemistry · Chemometrics

22.1 Introduction

In the last decade, the methods for the quality assessment of herbals (drugs, preparations, and medicinal products) have developed significantly. Initially, quality control methodologies focused basically on plant source (wild/cultivated) and the parts of the plant which were identified based on microscopic and macroscopic botanical features. However, the gradual development of various analytical techniques including wet-chemical tests, hyphenated chromatographic methods coupled with

P. S. Bora · P. S. Suresh · S. Kumari · Anmol · S. Puri · U. Sharma (✉)
Chemical Technology Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur 176061, Himachal Pradesh, India
e-mail: upendraihbt@gmail.com; upendra@ihbt.res.in

Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201002, Uttar Pradesh, India

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_22

various detection systems such as UV–Vis, ELSD, and MS, etc. and tests for various impurities have led to a notable improvement in the quality control methodology. Any herbal material is said to be authentic if it is exactly as claimed, certified, and most importantly conforms to the criteria of originality. The herbal sample must be identical, or it must have as many alike features as possible on comparison with the original sample. Hence the identity is an inherent concept of the quality of herbal material. Identification of particular herbal material relies on genetic and morphological characteristics, and the distinct phytochemical composition of plant material prepared under optimized conditions of extraction that represents its distinct metabolomic profile (Kroes 2014; Pferschy-Wenzig and Bauer 2015) (The United States Pharmacopoeia Convention (USP) 2013). Factors that affect the phytochemical composition of genetically identical herbal material include soil composition, conditions of cultivation and post cultivation processing, time of harvest, and process of extraction. These parameters come under the concept of traceability information which is a primary step and major factor in the process of quality control of medicinal plants.

The concept of "*pure, unadulterated herbal material*" is indispensable to quality control of medicinal plants. The absence of adulteration is a fundamental condition of quality control. Hence the definition of adulterant is indispensable to the concept of quality control of herbal material.

World Health Organization (WHO) guidelines for quality control of medicinal plant material defines an adulterant as "*herbal constituent or other substance that is either deliberately or non-intentionally added to a herbal material or a finished herbal product*" (WHO expert committee 2017). Based on the way it manifests itself, adulteration can be of two types such as unintentional and intentional adulteration.

An unintentional adulteration can be environmental contamination, resulting from cultivation on polluted land, improper harvesting technique, and misidentification of the plant material that results in the inclusion of unwanted materials. Quality of herbal material can be lost even due to poor storage and handling conditions, and also due to phytochemical degradation during processing. Hence poor quality control processes in the supply and manufacturing chains can also result in unintentional adulterations (European Medicines Agency/Herbal Medicinal Products Committee 2006; Kroes 2014).

Intentional adulterations are motivated by economic gains and therefore has a higher rate of incidence throughout the world. USP defines the economically motivated adulteration in the context of food deception as: "*the fraudulent addition of non-authentic substances or removal or replacement of authentic substances without the purchaser's knowledge for the economic gain of the seller*" (Johnson 2014; Spink and Moyer 2013). Intentional adulteration can be done in two ways:

(a) Replacement (complete/partial) of the herbal material by some similar species, or by a different part of the plant which works as a cheaper alternative. For example, different leaves of *Abies spectabilis* and *Rhododendran anthopogan* are sold in the markets under the name of *Taxus wallichiana* (Calahan et al. 2016).

(b) Addition of un-authorized chemical compounds in various proportions to increase the bioactivity and to improve the overall efficacy of the product (Ahmed and Hasan 2015; Johnson 2014; Spink and Moyer 2013; Dhami and Mishra 2015). For example, flavonols (kaempferol and rutin) and inexpensive plant materials rich in flavonoids have been used as the most common adulterants in *Ginkgo biloba* based marketed products (Mosihuzzaman and Choudhary 2008; Simmler et al. 2018).

Quality control is a stepwise procedure of validation and certification that employs both validated methodologies and reference materials. All steps for the quality control should be achieved in accordance with the different official monographs and agency guidelines such as the Food and Drug Administration (FDA), Pharmacopoeias, World Health Organization (WHO). Hence, after defining the notion of identity and adulteration, the quality assessment of botanicals can be done by a three-step procedure to assure the authenticity of herbal material. This procedure also conforms to the U.S. Current Good Manufacturing Practices (cGMP) guidelines for quality control of dietary supplements (Brown 2017; Whitsitt et al. 2013; U.S. Food and Drug Administration 2007). The three steps include:

(a) Assessment of information regarding traceability which includes data of geographic origin, cultivation/collection, and preparation conditions.
(b) Validation of morphological and taxonomical identity.
(c) Assessment of phytochemical composition representing chemotaxonomic identity, supporting its efficacy and meanwhile also confirming the absence of any impurities and adulterations.

22.2 Need for the Quality Control of the Himalayan Medicinal Plants

The global herbal drugs market is presently estimated at about US $90 billion with an annual growth rate of 10–15% and it is expected to reach US $5 trillion by 2030. Although the global herbal drug market is growing at a very fast pace, India accounts for only 2% share. It is estimated that the Indian herbal industry uses approximately 960 plant species out of which 70% of export (Rs. 10 billion per annum) consists largely of raw materials. Only 30% of the export consists of finished herbal products, including herbal extracts (Sahoo and Manchikanti 2013). Over one thousand companies (with revenues approx. US$60 billion) are procuring either the raw material or the bulk of the herbal products from Asian, African, and South American countries with rich biodiversity (S. 2002).

The major reason for the low share of the Indian herbal drug industry is the lack of proper quality, safety, and assurance of the efficacy of herbal drugs and raw materials despite having in-depth knowledge in terms of Ayurveda. Indian herbal drug industry is unable to capitalize at the global level due to the mammoth problem of adulteration and substitution due to the use of unauthenticated raw material in the

production and direct export of plant material. Developing countries like India with a traditional knowledgebase can convert their rich bio-resources to valuable products for the generation of economic wealth contributing to the prosperity of the nation.

There are 8000 medicinal plant species in India out of which only 960 plant species are actively traded in the national and international markets, with 178 plant species being traded in trade volume exceeding 100 metric tons (Srirama et al. 2017). The Indian Himalayas are biodiversity hotspots (angiosperms species: 8000, gymnosperms species: 44 and of pteridophytes species: 600) with 1748 species of medicinal plants (Samant et al. 1998). The trans-Himalayan region sustains comparatively less number of medicinal plants species (about 337) (Kala 2003), due to the distinct geographic and ecological conditions (Kala and Mathur 2002).

Currently, increasing awareness towards untapped traditional medicines has also resulted in an immense focus on Himalayan medicinal resources especially in the high-altitude region for the exploration of medicinally important species. According to the national medicinal plant board report, 21 species of Himalayan medicinal plants have huge commercial importance as their trade volume exceeds 100 metric tons annually (Table 22.1). These commercially important Himalayan medicinal plants include *Aconitum heterophyllum* (Atis), *Berberis aristata* (Daruhaldi), *Fritillaria roylei* (Kakoli), *Jurinea macrocephala* (Dhoop), *Nardostachys jatamansi* (Jatamansi), *Onosma hispidum* (Ratanjot), *Picrorhiza kurroa* (Kutaki), *Rheum austral* (Revandchini), *Swertia chirayita* (Chirata), *Trillium govanianum* (Nag Chattri), *Valeriana jatamansi* (Mushakbala) and *Viola pilosa* (Banafasha) (Ved and Goraya 2007). These are marketed either as raw materials or are constituent of various preparations according to their final usages such as herbal/botanical drugs, phytopharmaceuticals, herbal medicines or products, and nutraceuticals. Exploration of chemistry and the therapeutic potential of these medicinally important plants has led to an increase in the commercialization of these plants at the global level. The commercialization of the rare and endangered plants has given birth to the acute problem of adulteration due to their high value and demand by the industry worldwide. Most of these species are in the endangered category due to over-exploitation from wild and lack of cultivation.

There are many reports regarding adulteration as well as substitution of these commercially important Himalayan medicinal plants. *Aconitum heterophyllum* is substituted with the common weed *Cyperus rotundus* (Venkatasubramanian et al. 2010). *Fritillaria pallidiflora* raw material being adulterated with 8 species of *Fritillaria* genus and its raw bulbs are also often substituted with *Withania somnifera* (Wang et al. 2005). *Nardostachys jatamansi* is often being substituted with *Selinum vaginatum* although these two plant species have distinct phytochemical profiles as *N. jatamansi* consisted of phenolics whereas *S. vaginatum* consisted of only hydroxycinnamic acid derivatives (Srirama et al. 2017).

There are presently no uniform global standard practices/protocols to identify the different types of adulterant and/or substituent present in herbal products sourced from these commercially important Himalayan medicinal plants. In the very recent past, people started to realize the adverse effects of adulteration on health and safety. Hence, if this issue is not addressed with immediate effect, adulteration could

Table 22.1 Commercially important Himalayan medicinal plants with their major supply source price in Kg and annual trade in terms of metric tons (HF = Himalayan Forest), (echarak—https://echarak.in/echarak/marketprice.do and National Medicinal Plant Board report on demand and supply of medicinal plants in India)

Botanical name	Trade name	Major supply source	Annual trade (MT)	Price per kg
Aconitum heterophyllum	Atis	HF	100–200	3500–10,500
Berberis aristata	Daruhaldi	HF	500–1000	15–35
Fritillaria roylei	Kakoli	HF	50–100	3500–10,500
Jurinea macrocephala	Dhoop	HF	1000–2000	60–150
Nardostachys grandiflora	Jatamansi	HF	200–500	110–150
Onosma hispidum	Ratanjot	HF	500–1000	50–60
Picrorhiza kurroa	Kutaki	HF	200–500	220–230
Swertia chirayita	Chirata	HF	500–1000	200–225
Rheum australe	Revandchini	HF	500–1000	25–30
Trillium govanianum	Nag Chhatri	HF	200–500	2000–2500
Valeriana jatamansi	Mushakbala	HF	100–200	95–100
Viola pilosa	Banafasha	HF	200–500	300–350

adversely impact consumer health as well as trade to the global market (Techen et al. 2014).

As we know that Himalayan medicinal plants are the precursors of many commercialized herbal products, so assessment of quality is necessary to support the purported health effects and efficacy claims to assure the safety of any commercialized product. Hence in this chapter, we have reviewed the concept of quality control and its different parameters in the context of adulteration and have also attempted to summarize various quality control methods for the twelve commercially important Himalayan medicinal plants. We have also emphasized on the fact that the assessment of the quality of an herbal product can only be achieved by the employment of a multi-layered set of analytical techniques which will help to identify the sample chemically and taxonomically while assuring that it is devoid of any adulteration.

22.3 Guidelines of Different Agencies for Quality Control

Herbal formulation-based medicines are not only easily, economically, and widely accessible, but also provided a chemical diversity for the designing and development of potent therapeutic candidates. The majority of the global population can't afford Western medicines due to high costs, specifically most Third World nations, thus they utilized traditional medicines (Kumar 2012). Moreover, it's estimated that approximately 30,000–70,000 medicinal plant samples have not been systematically characterized, consequently since the ancient eras; the traditional herbal formulations are frequently utilized without standardization. The authorized procedure and guidelines for the standardization and quality control (QC) of botanical medicines vary from nation to nation due to ethnic and cultural diversity. Therefore, only limited herbal formulations have been scientifically validated. The World Health Organization (WHO) has provided globally accepted guidelines for the evaluation of quality, safety, and efficiency of herbal formulations (Akerele 1992). Additionally, the WHO has designed a pharmacopeia database on medicinal plants and herbal formulations, and the foundation of methods for the evaluation of herbal medicines (WHO 1993; Zhang 1998). Various regional quality control regulatory organizations for herbal formulation and medicines have been working on several aspects, including herbal drug prescriptions, over-the-counter drugs, folk medicines, and nutritional supplements. In the USA, the WHO published globally accepted guidelines for quality assurance and control (QA & C) of herbal medicines and since 1994, the quality of herbal formulations has been standardized under the "Dietary Supplement Health and Education Act". This act established that herbal formulations are not certified by the FDA and these formulations have not ever purported for diagnosis and treatment of diseases. WHO provided QA & C guidelines for medicinal plant and herbal formulations from 1998, which WHO associated nations can utilize for the standardization of the regional medicinal plant's based herbal formulations (WHO Expert Committee 2011). WHO mostly evaluated the following features of the herbal formulations such as QC of raw plant samples/herbal formulations, shelf life and stability valuation, toxicological and safety evaluation and evaluation of the scientific efficacy of traditional herbal formulations. According to the WHO association with FDA, International Atomic Energy (IAE) herbal formulation should be scientifically validated based on bioactive or major phytomolecules with the chromatographic or spectroscopic profiling. The various references of herbal formulation standardization established by the WHO guidelines involved botanical evaluation, foreign organic and inorganic residues, histochemical and analytical profiling, physicochemical, pharmacological, and toxicological parameters (WHO Expert Committee 2011). The following parameters of herbal samples are analyzed for the quality control assessments:

(a) Authentication and collection: (taxonomy, geographical locations, vegetative stage, portions of the plant, ethnobotanical status, histological examination, etc.)

(b) Foreign matter: (collection should be clean, with no traces of soil, pests, and excreta, etc.)
(c) Organoleptic evaluation: (appearance, odor, taste, etc.)
(d) Tissues of investigative significance present in the drug.
(e) Ash and extractive values.
(f) Volatile material.
(g) Moisture content.
(h) Chromatographic and spectroscopic fingerprinting.
(i) Analysis of heavy and toxic metals.
(j) Pesticide residue.
(k) Microbial screening.
(l) Radioactive screening.

Standardization of herbal medicines in European Union is done by the several institutions including the European Directorate for the Quality of Medicines and Health Care (EDQM), Strasbourg, France); the European Medicines Agency (EMA), London(U.K.) and Amsterdam (Netherlands); the Federal Institute for Drugs and Medical Devices in Germany (BfArM) (Knoess and Wiesner 2019). The EDQM and European Pharmacopoeia are key institutions involved in the quality standardization of herbal medicinal products (Uerpmann-Wittzack 2017). The European Pharmacopoeia has published general and specific monographs regarding the Quality assurance and control guidelines for the herbal medicines or preparation of herbal formulations.

22.4 Sampling Methods in Quality Assessment of Medicinal Plants

WHO recommends the use of a series of tests to determine the quality of plant material including sampling methods, determination of toxic heavy metals, pesticide residue, microorganism, and aflatoxins. During the initial inspection following factors must be taken care:

(a) The solubility of plant material must be determined in terms of a millilitre of solvent required to dissolve one gram of plant material.
(b) The container used for the storage of plant material must be chosen in such a way that it should not interact with plant material neither physically nor chemically.
(c) Plant material should be protected from light and should be placed in a dark place.
(d) The humidity level and temperature used for the storage of plant material must be specified.
(e) During the powdering of plant material fineness of powder and sieve size should also be taken into consideration.

All these factors must be properly noted down as the variability in these factors can alter the quality of herbal medicines (World Health Organization 2011). Before implementing quality control tools, the selection of the desired sample is also taken into account as the sample represents the quality of the complete batch (Indrayanto 2018).

Sampling of Material in Bulk: Herbal material usually lacks homogeneity *i.e.* it does not have uniform composition throughout. So special handling tools should be used to get uniformity of sample. WHO recommended the following method for selection of containers from a batch of plant material (Table 24.2):

(a) If the number of containers or packaging units containing plant material is less than 6 then samples should be taken from all the units.
(b) If packaging units are between 6 and 50, samples should be taken from any 5 units systematically.
(c) If packaging units or containers are above 50, then take samples from 10% of containers. Proper rounding off should be applied to the nearest multiple of 10 i.e. 76 containers should be rounded off to 80, so samples should be taken from 8 containers.

From the selected container, three samples should be taken from the top, middle, and bottom layers of containers. Selected samples should properly undergo inspection for noting factors like color, odor, texture, insects, sand, or glass particles should be done and noted. Samples should be taken in such a way that there is minimum fragmentation of material (World Health Organization 2011).

United States Pharmacopeia (USP) which was established to provide standards of quality, purity, identity, and packaging for drug substance has the recommended sampling method to ensure the quality of drugs based on botanical origin. The first step is to divide the batch of plant material into sub-batches (so that it can be as homogenous as possible). Then samples should be taken from the top, middle, and bottom of each sub-batches or containers (Table 22.2).

While taking the samples from sub-batches the size of components of plant material is also taken into account. If the component part is <1 cm or completely powdered, the sampling device is used in such a way that it removes a core from top to bottom. However, if the component part is >1 cm, samples should be taken by hands in such a way that there is no moisture content left. While taking the sample, quantity of plant material is also considered. If the quantity of plant material is <1 kg in the container (or sub-batch), take less quantity required to do quality tests. If the

Table 22.2 Selection of samples from sub-batches (Indrayanto 2018)

Number of sub-batches (N_1), (round off N_1 to next highest whole number)	Number of sub-batches to be sampled (N_2)
1–10	All
11–19	11
≥20	$N_2 = 10 + (N_1/10)$

quantity is (1–5 kg), withdraw sample from the top, middle, and bottom layer of a container containing plant material. However, if quantity is >5 kg, take samples weighing ≥250 g from the top, middle, and bottom layer of a container containing the plant material (World Health Organization 2011).

22.5 Different Parameters for Assessing the Quality of Medicinal Plants

22.5.1 Documentation of Traceability Information

The traceability information is very vital to the concept of quality control as it helps to ensure transparency in the supply chain and helps in the identification of risks related to any adulterations. Microorganisms, pesticides, herbicides, heavy metals, and mycotoxins are some of the well-known examples of the primary category of adulterants which mainly occurs due to the use of pesticides. Hence, lack of traceability data can result in the procurement of poor quality of plant material having environmental contaminants (The United States Pharmacopeia Committee 2016; Govindaraghavan and Sucher 2015; Manning and Soon 2014). Good Agricultural and Collection Practices (GACP) guidelines are the one which helps to minimize such contamination during the production and supply chains (Kroes 2014; European Medicines Agency/Herbal Medicinal Products Committee 2006; World Health Organization 2003; American Herbal Products Association 2006). Essential aspects of GACP include a record of cultivation location, harvest time, and growth promoters, fumigants, fertilizers, pesticides, and herbicides used during production (Kroes 2014). WHO (WHO Expert Committee 2011) and other quality control agencies guidance documents such as the herbal medicine compendium of USP (The United States Pharmacopeia 2013), HPA (American Herbal Products Association 2012; American Herbal Products Association 2014), and EMA (Kroes 2014) also listed standard methods to be used for the control of environmental contamination. Any lacunae in documenting the traceability information can be exploited for economic benefits, therefore employing different analytical techniques for the identification of the elemental composition of herbal material for the tracing the mode of cultivation and geographical origin is essential. For this purpose, mainly Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) has also been applied. Other techniques used for heavy metals detection in plant material include Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and Flame Atomic Absorption (AA) (United States Pharmacopeia 2017; Avula et al. 2010).

22.5.2 Determination of Non-specific Parameters

Some non-specific parameters including ash content, extractive values, volatile oils, water and volatile matter, bitterness value, haemolytic activity, foaming index, swelling index and total tannins must be predetermined in addition to traceability to assure the overall safety of the herbal product.

Ash Content: Ash comprises of inorganic radicals such as carbonates, silicates, and phosphates of Na, K, Mg, Ca, etc. Ash content is determined by igniting the plant material and the material left after ignition is considered as total ash. This total ash is then boiled with dilute HCl and the remaining insoluble matter is ignited which is measured as acid-insoluble ash (Ahmad et al. 2014; WHO 1998; Pradhan et al. 2015).

Extractive Values: Extractive values are the measures of the weights of extractable active components present in a given amount of herbal material by using different solvents. It can be achieved by the following methods: hot extraction, cold maceration with ethanol, water, or combination of ethanol and water. Extractive values are helpful for the determination of adulterated drugs, and for the purity and quality of the crude drug (WHO 1998; Pradhan et al. 2015; Chaudhari and Girase 2015; Nafiu et al. 2017).

Water and Volatile Matter: The presence of moisture leads to the decomposition of herbal material as moisture content promotes the fungi or insects, microbial growth, and thus deteriorates the whole material. The moisture content can be determined by an azeotropic method that involves the distillation of the sample with an immiscible solvent and the water content gets absorbed by the solvent. Both moisture and volatile matter can be determined by *loss on drying* method in which the sample is dried by heating to 100–105 °C or can be dried at room temperature, under reduced pressure or atmospheric pressure in a desiccator above phosphorus pentoxide R (WHO 1998; Pradhan et al. 2015).

Volatile Oils: These are easily recognized by their characteristic fragrance, oil-like appearance, and can vaporize at room temperature. For determination of the volume of volatile oil, the distillation of plant material is done with water and then the distillate is stored in a graduated test tube. The oil content is calculated in ml/100 g of the plant material (WHO 1998).

Bitterness Value: Bitter taste medicinal plant materials are used as appetizing agents to stimulates the gastric juice which secretes in the gastrointestinal tract. The bitterness is determined by the comparison of the threshold bitter concentration of plant extract with a dilute solution of quinine hydrochloride (WHO 1998; Surekha et al. 2016).

Hemolytic Activity: Herbal materials derived from families *Araliaceae, Caryophyllaceae, Dioscoreaceae, Primulaceae*, and *Sapindaceae* contain saponins which can cause hemolysis on adding to a suspension of blood and cause hemoglobin to disperse

into the surrounding medium. Therefore, the hemolytic activity of medicinal plant materials or a formulation containing saponins is measured by comparing with saponin R, a reference material with the hemolytic activity of 1000 units/g (WHO 1998; Surekha et al. 2016).

Foaming Index: Aqueous decoction of saponins bearing plant materials can produce persistent foam on shaking and this is expressed in terms of foaming index. For this, 1 g of powdered plant material is diluted with boiling water, cooled, and then filtered. This decoction is then transferred into different test tubes in sequential portions of 1, 2, 3 ml, etc. up to 10 ml then the volume of each test tube is made 10 ml with water, shaken for 15 s and allowed to stand for 15 min so that foam height can be measured. If the foam height in each test tube is <1 cm then the foaming index is <100. If the foam height is 1 cm then the volume of the decoction in a test tube is used to measure the foaming index. If the foam height in each test tube is >1 cm then the foaming index is over 1000 (WHO 1998; Surekha et al. 2016).

Swelling Index: Most of the medicinal plants have their pharmaceutical or therapeutic benefits due to their swelling properties. The swelling index is the volume taken in ml, under specified conditions, by swelling of 1 g medicinal plant material. For this, the plant material is shaken repeatedly with water or swelling agent for one hour and then allowed to stand for about 2–3 h. Then the volume covered by the mixture is measured and the mean value of the independent analysis related to 1g plant material is calculated (WHO 1998).

Astringent Property: Tannins are astringent biomolecules that turn the animal hides into leather by binding to proteins thus forming water-insoluble substances that cause resistance to proteolytic enzymes. When this process is considered in the case of living tissues then it is known as "astringent" action and is responsible for the use of tannins in therapeutics (WHO 1998; Surekha et al. 2016).

Foreign Matter: Medicinal plant material used to prepare herbal formulations should have to be pure and completely free from contamination caused by molds or insects, chemical residues, animal excreta, and visible contamination like soil, sand, dust, stones and poisonous foreign matter. No sign of deterioration, abnormal odor, and stain should be detected. The products should be stored in a clean and hygienic place to avoid contamination. In cases where foreign matter closely resembles the medicinal plant material itself then it becomes necessary to take the sample of the plant material to apply either chemical or physical test. TLC is frequently employed to detect foreign matter when it closely resembles to chemical residue (Ahmad et al. 2014; Kunle 2012; WHO 1998). An example of a medicinal plant i.e. *Tinospora cordifolia* for which all such determinations have been carried out for assessing its quality. Quality control parameters for *Tinospora cordifolia* has been determined and compared with the parameters set by WHO and FDA. The average value of non-specific parameters is expressed in percentage, of dried material, which includes total ash (7.5%), acid insoluble ash (1.16%), water-soluble extractive value (12.05%), alcohol soluble extractive value (7.27%) and loss on drying (2.31%). They have also analyzed for the presence of heavy metals and pesticide residues. Their results

Table 22.3 Determination of heavy metals in *Tinospora cordifolia* sample

Heavy metals	As per WHO (ppm)	As per FDA (ppm)	Results
Lead	10.0	10.0	<5 ppm
Arsenic	10.0	10.0	Nil
Mercury	1.00	1.00	Nil
Cadmium	0.30	0.30	Nil

showed that only lead was present in the sample whereas mercury, arsenic, and cadmium were not present as shown in Table 22.3 (Nasreen et al. 2010).

22.6 Morphological and Genetic Identity Tests

These tests help to distinguish different species of the same plant and different parts of the plant from potential adulterants (Simmler et al. 2014). Anatomical and DNA-based identity tests serve as a very helpful tool in the identification of different types of plant material from their possible adulterants (Techen et al. 2014; Chen et al. 2014).

22.6.1 Morphological Identification Methods

As we know that the phenotype of the plant is best described by its morphologic description of the plant which further helps in taxonomic identification of the herbal material. Macroscopic, microscopic, and some sensory analyses are of great utility in the identification of the herbal material which is already well known for a certain therapeutic effect (Pferschy-Wenzig and Bauer 2015; Smillie and Khan 2010). The relevance of vouchered herbal samples and morphologic identification procedures has been highlighted in various publications and documents related to GACP (European Medicines Agency/Herbal Medicinal Products Committee 2006; World Health Organization 2003; American Herbal Products Association 2006; Culley 2013). Technical methods and taxonomic keys for the identification of different herbal material can be found in monographs of different agencies (EP, WHO, AHP, and Herbal Medicines Compendium of USP) (World Health Organization 1999). Macroscopic examination of a plant material enables the detection of foreign substances such molds, insects, soil etc. while microscopic analyses also allows the identification and discrimination of the selected plant organ from other parts. Hence morphological identification is an important tool that act as a preliminary test for the detection of contaminations.

22.6.2 *DNA Profiling as an Identification Method for Authentication of Medicinal Plants*

The institutions like pharmacopeias and quality control regulatory agencies recommend different approaches like histochemical analysis and chromatographic and spectroscopic chemo-profiling for quality standardization of herbal medicine (Balammal et al. 2012; Nikam et al. 2012). But such approaches have limitations like cell types, morphological features, environments, and geographical locations, which can be overcome by the analysis of the molecular markers. Molecular markers like DNA or RNA are advanced, more informative, and reliable over the typical chemical and phenotypical markers. Molecular markers of every species are unique and polymorphic, which are not affected by the environmental conditions, morphological, and anatomical factors (Chan 2003). Moreover, genomic samples can be extracted and isolated from the living as well as non-living tissues (Warude et al. 2003). The accurate identification and authentications of medicinal plants are very crucial (Zhao et al. 2006). The several DNA-based methodologies including AFLP, ARMS, CAPS, DAF, ISSR, RAPD, RFLP, SSR, DNA hybridization, and microarrays techniques are systematically applied for the identification of botanical samples (Heubl 2010). A recently developed technique for medicinal plant authentication includes DNA barcoding, which specifically recognizes the internal transcribed spacer (ITS), (Pennisi 2007). DNA barcoding is sensitive technique for the authentication of plants, and is significantly suitable for the quality assurance and taxonomy studies of medicinal plants (Zhu et al. 2008; Yao et al. 2010; Cimino 2010). Various DNA fingerprinting, PCR and hybridization-based techniques have been used for the quality assessment of Himalayan medicinal plants. The authentication of *Saussurea lappa* C. B Clarke (Kushta) was characterized by the ITS DNA and 5S rRNA sequencing method (Chen et al. 2008). RAPD marker-based DNA fingerprinting technique used for the profiling of *Tinospora cordifolia* (Guduchi) by the Op A-16, Op C-7, Op C-13, and Op G-5 primers to detect the occurrences of drugs in herbal formulations (Shinde and Dhalwal 2010). RAPD markers amplification can also distinguish the two different populations of *Picrorhiza kurrooa,* which have similar morphological features. DNA fingerprinting of *Withania somnifera* (Ashwagandha) is analysed and authenticated by using Inter Simple Sequence Repeats (ISSR) Markers (Bamhania et al. 2013).

22.7 Phytochemical Composition Analysis

Phytochemical composition analysis helps to determine the type of secondary metabolites present in the herbal material. There are two general approaches for the quality control of herbal material which are known as phytochemical profiling/fingerprinting. Phytochemical profiling helps to determine only a limited set of chemical compounds with the help of targeted analysis. It employs chemical

markers for the simultaneous qualitative and quantitative identification whereas in phytochemical fingerprinting the overall chemical composition of herbal material is investigated. A phytochemical fingerprint enables the determination of both known as well as unknown compounds by the use of untargeted metabolomics (Hall 2006; Simmler et al. 2016b). Both of these approaches are complementary for the quality assurance of herbal materials.

22.7.1 Targeted Phytochemical Analysis

22.7.1.1 Application of Chemical Markers as the Quality Control Tool

A targeted analysis helps to determine the specified limit of the selected chemical markers in a given herbal material. The term targeted is used for the identification and quantification of specific markers known as chemical markers of the selected herbal material. It helps to gain a selective overview of sample composition in terms of some distinct chemical entities which are present in the herbal sample. Chromatographic techniques hyphenated with different detection systems are the most widely used analytical techniques for the targeted identification of phytochemicals in herbal material as well as for the detection/quantification of known adulterants. High performance thin layer chromatography, liquid chromatography, ultra-high pressure systems coupled with ultra-violet, mass spectrometry, as well as other detectors, and gas chromatography, coupled with MS detection system are the major analytical technique used for the quality control of medicinal plants (Smillie and Khan 2010; Simmler et al. 2016a; Khan and Smillie 2012). Calibrants and certified reference chemical standards are usually employed for the analysis which helps in the identification and quantification of multiple markers. The overall advantage of this approach is to reveal the uniqueness of the sample under investigation. The official monographs of different pharmacopeia (e.g., USP, EP) and Association of Official Analytical Communities (AOAC), serve as a guidance document that comprehensively mentions the limits of the amount of different chemical markers for different herbal material.

Targeted LC/GC-based analyses have also been widely applied for the identification of potential known adulterants in different herbal material which are added to enhance their overall efficacy. For instance, there are various reports regarding the addition of antihypertensive, anti-inflammatory agents, anabolic steroids to different herbal products in order to improve overall efficacy (Calahan et al. 2016). MS-based methods are also very useful for the identification/quantification of adulterants added to different herbal products (Vaclavik et al. 2014). Various already published reviews articles have highlighted the detection of different synthetic compounds as adulterants in different Indian herbal medicines especially in sexual enhancers and slimming products (Savaliya et al. 2010; Skalicka-Woźniak et al. 2017). Techniques such as NMR, Capillary Electrophoresis, and HPTLC techniques (Brown 2017; Calahan

et al. 2016; Ordoudi et al. 2017) also have wide applicability for the identification of chemical adulterants in herbal samples.

The only limitation of the targeted LC/GC-based approach is the non-identification of unknown compounds as these methods are designed for specific chemical markers and known adulterants. Hence, there is a need to extend the targeted analysis approach with the untargeted metabolomics for the identification of known as well as unknown adulterants.

22.7.1.2 Analysis of Toxic Compounds

Analysis of toxic compounds is essential for the overall safety of any herbal formulation. The concentration of such compounds should be below the maximum residual limit which means that the sample detection limit of the method used must be below its maximum residual limit (Indrayanto 2018).

Chemical Evaluation: Chemical evaluation is associated with the screening of crude drug to the purification of chemical constituents from that drug. This procedure involves isolation, identification, and purification of chemical constituents using qualitative chemical tests. Chemical tests are performed to check the purity or to identify certain drugs. Therefore, qualitative chemical tests help to establish the identity of the drug material and also useful in the detection of possible adulteration (Ahmad et al. 2014; Kunle 2012).

Heavy Metals: Contamination of herbal materials with toxic metals could be ascribed to many causes such as pesticide residues and environmental pollution and and can pose severe health hazards. Arsenic, mercury, and lead are the most frequently detected toxic along with copper, cadmium, and thallium. The limits for heavy metals like mercury, arsenic, lead, and cadmium should be minimum as set by WHO and FDA. Color reaction using special reagents and instrumental methods form the basis for the identification of heavy metals. The instrumental methods employed for metal detection include atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP), and neutron activation analysis (NAA) (Shukla 2009; Sahoo et al. 2010; Ahmad et al. 2014; Zhang et al. 2012; Kunle 2012; WHO 1998; Sekhon 2011).

Pesticide Residues: Medicinal plants are susceptible to traces of pesticides accumulated from spraying, soil treatment during cultivation, and management of fumigants in storage. Therefore, it is advised that every country associated with the production of medicinal plant material should have an established control laboratory that could be capable to perform the determination of pesticide residues. However, limited reports are there on toxicity due to existence of pesticides and fumigants, it is necessary to make herbal plant material and products either free from all these chemicals or should be in the permitted limit (Ahmad et al. 2014; Zhang et al. 2012; Kunle 2012; WHO 1998).

Mycotoxins: Mycotoxins include aflatoxins, fusarial toxin, ochratoxin, penicillic acid, citreoviridin, etc., and are produced by different fungi species including *Aspergillus, Penicillium*, and *Fusarium.* Aflatoxins are the most commonly found toxins and are highly poisonous and may prove hazardous to health even if absorbed in minute quantities. Aflatoxins contamination in herbal material is determined by the procedure recommended by the WHO (Ahmad et al. 2014; Zhang et al. 2012; Kunle 2012).

Radioactive Contamination: Exposure to ionizing radiation is not avoidable as there are radionuclides that occur naturally in the ground as well as in the atmosphere or may occur due to nuclear accidents. The WHO together with other organizations has recommended some guidelines if any case of global contamination by radionuclides occurs. There is no generalized method available so far for the measurement of radioactive contamination. However, such contamination should be of serious concern and the suspected sample should be examined by an appropriate laboratory (Kunle 2012; WHO 1998).

Toxicity Data: Toxicological evaluation of herbal drugs is required to reveal its contribution to toxicity in quality control. In toxicity analysis, the techniques used are cell line assay, microarray, in vitro, in vivo, and modern standardization techniques. A comprehensive phytochemical and pharmacological study is needed to evaluate the toxic effects of phytoconstituents of herbal formulation. Misidentification and substitution of herbal material, monitoring and surveillance system development, toxicity assessment, risk assessment approaches, and regulatory approaches are utilized in the toxicity assessment of herbal drugs (Ahmad et al. 2014; Sekhon 2011; Bandaranayake 2006).

22.7.2 Untargeted Chemical Profiling Method as QC Tool for the Quality Assessment of Medicinal Plants and Their Derivatives

The overall efficacy of an herbal material/preparation is the synergistic effect of different types of chemical entities present in it. The claimed health effects are not due to only a few bioactive markers but they are due to poly-pharmacological effects of different chemical compounds. Hence, the quality control assessment must take into account the overall phytochemical complexity in addition to the targeted evaluation of fixed chemical markers. Small-molecule metabolomics with the employment of untargeted chemical profiling is one of the best suited method for the phytochemical investigation of the herbal material. Different types of spectroscopic/spectrometric techniques can be employed to develop a complete plant profile by performing simultaneous identification and quantification of plant metabolites (Hall 2006). HPTLC, HPLC–UV/MS and IR/NMR fingerprinting methods are widely applied for the untargeted chemical profiling. Flow injection MS (FIA-MS) techniques help in producing

complex mass spectrometric fingerprints of herbal extracts (Huang et al. 2015; Sun and Chen 2011; Harnly et al. 2015). Direct spectroscopic fingerprinting can be developed by either vibrational spectroscopy techniques such as FTIR and Raman spectroscopy (Wang and Yu 2015; Rooney et al. 2015) or by NMR spectroscopy (Simmler et al. 2014; Monakhova et al. 2018). To achieve a comprehensive picture of phytochemicals of a sample requires an appropriate amount of diversity of the herbal samples which are genetically and morphologically identical. The sample diversity is important as it helps to rule out different influences such as species, chemotypes, geographic variation, cultivation conditions, etc. (Hall 2006; Simmler et al. 2016b; Commisso et al. 2013; Oms-Oliu et al. 2013). Hence, an adequate amount of sample diversity will help to identify unknown adulterants from the sample herbal material. Direct spectroscopic/ metric techniques such as FIA-MS, IR and NMR generate complex data set as they do not involve physiochemical separation of different phytometabolites (Simmler et al. 2016b; Wang and Yu 2015; Dondorp et al. 2017; Simmler et al. 2014; Monakhova et al. 2018). Hence, to handle this complex data, chemometrics ie. multivariate statistical analysis is employed for the objective comparison with the reference data set and to identify unknown adulterants.

22.8 Chemometrics as an Innovative Tool Set for the Determination of Authenticity of Medicinal Plants and Their Derivatives

Modern analytical and spectroscopic tools play a vital role during the authenticity, efficiency, safety, and quality assurance of medicinal plants/herbal products. These methods provide floods of data. In the case of chromatographic techniques, this data can be of the form of peak area, retention time while in the case of spectroscopic techniques data can be of the form of factors like absorbance. Different forms of data are regarded as variables which are recorded for different samples. So here chemometrics tools are used for data handling and for extracting useful information from the data. According to the journal "Chemometrics *and Intelligent Laboratory Systems*"—Chemometrics is a branch in which mathematical and statistical tools are used to design or select ideal procedures and experiments and to extract maximum useful information from chemical data. It is often termed as multivariate data analysis tools due to the existence of multiple variables. Mathematical and statistical concepts such as covariance, regression, orthogonality, normalization are used in this type of data mining. Development in computer science and availability of software like Unscrambler, SIMCA, MaTlab has made possible the use of chemometrics tools easily. Generally, in multivariate analysis, a matrix is formed whose rows represent the sample while columns represent the variables. Firstly, preprocessing methods such as peak alignment are done to reduce unwanted sources of variation. Pattern recognition is done using tools such as Principle Component Analysis (PCA) (Bansal et al. 2014). This method aims to find patterns i.e. similarity

and difference between sample and variables. Pattern recognition is also termed as Exploratory Data Analysis. The main aim behind PCA is to reduce dimensionality by undergoing a linear transformation of data i.e. whole data set containing information of samples and variables is converted into principal components that cover maximum information from data. Along with PCA, several supervised pattern recognition methods are also used nowadays. It includes soft independent modeling of class analogy (SIMCA), partial least squares–discriminant analysis (PLS-DA), orthogonal projections to latent structures–discriminant analysis (OPLS-DA) (Gad et al. 2013). For visualization and interpretation of multiple variables, tools like S-plot is used. For example, Jarouche M. et al. carried out the quality control of "Qi Ju Di Huang Wan" (QJDHW), an eight-herb formulation which is used to relieve hypertension using Herb MaRS and chemometrics tools. Herbal Chemical Marker Ranking System (Herb MaRS) was used to select the maker compounds for testing the quality. Factors chosen for ranking systems were bioactivity, availability of standards, physiological activity. Among different analytes present in the herbs collected from eleven different sources, seven analytes were selected for quantification by HPLC–ESI–MS. PCA was used for understanding variation among different samples. PCA plot differentiate the samples, showing that some of the samples have a low concentration of selected analytes. Hence the quality of herbal formulation from different sources was tested using Herb-MaRS for marker compound selection, HPLC-ES-MS for standardization and PCA as chemometrics tool showing chemical variability (Jarouche et al. 2019).

22.9 Selected Commercially Important Himalayan Medicinal Plants

22.9.1 Aconitum heterophyllum

Aconitum heterophyllum which is known as *Atish* in Hindi and *Ativisha* in Sanskrit is widely used in Ayurvedic system of medicine. It belongs to Ranunculaceae family and Plantae kingdom. Dried and tuberous roots of Atish are known to have various therapeutic effects and are used in different Ayurvedic formulations as well as in the traditional Chinese medicinal system.

Geographic Distribution: This indigenous medicinal plant is found in the alpine regions of Himalayas. Aconitum consist of 300 species which are present all over the world among them 24 species are found in the Himalayan sub-alpine and alpine region in altitude ranging from 2400 to 3600 m. Generally ideal condition for its growth include moist soil with rainfall ranging between 664.2 and 1485.7 mm (Paramanick et al. 2017).

Medicinal Properties and Ethnomedicinal Usage: As per *Charaka Samhitha* (Sutra Sthana Chapter—25), Atish has been recommended for digestion, carminative action,

absorbing *Tridosha balancing properties*. In *Sushrutha Samhitha* it was suggested as a remedy against diarrhea. Ayurveda has also mentioned its antimalarial activity. It is prescribed against cough irritation, bronchitis and is effective against blood pressure. Aqueous extract of the roots of this plant is prescribed in chronic fever and diarrhea. Heart and Nerve sedative properties of this herb are also shown (Prasad et al. 2014). Pharmacological evaluations on the plant include antipyretic, antimicrobial, analgesic, antifungal, insecticidal, antiviral, antidiabetic activity and is used to treat diseases of the nervous system, digestive system and fever (Ukani et al. 1996).

Phytochemistry: Preliminary phytochemical studies revealed that leaf, roots, and stem contain alkaloids, carbohydrates, proteins, amino acids, flavonoids, saponins, quinones, and terpenoids (Paramanick et al. 2017). *Aconitum heterophyllum* generally possess diterpenoid alkaloids. Structurally complex phytomolecules of this plant include hetisine, atisenol, heteratisine, heterophyllinine-A, heterophyllinine-B, as shown in Fig. 22.1, (Jacobs and Craig 1942; Nisar et al. 2009).

Quality Control Approaches: To authenticate *A. heterophyllum*, Seethapathy et al., developed SCAR (Sequence characterized Amplified Region) markers of this plant using nuclear ribosomal DNA based Internal Transcribed Spacer sequence (nrDNA ITS). Using this system, they check the authenticity of some of the herbal products where *A. heterophyllum* is used as one of the ingredient. Genomic DNA from the plant tubers was extracted and then ITS amplification using universal primers was done. Species-specific SCAR primers were designed and then compared with the DNA of commercial products containing *A.heterophyllum* (Seethapathy et al. 2014). It is essential to include all the quality control tools for quality assurance of Aconitum species which are traded in a larger amount and price being high so that quality can be checked easily.

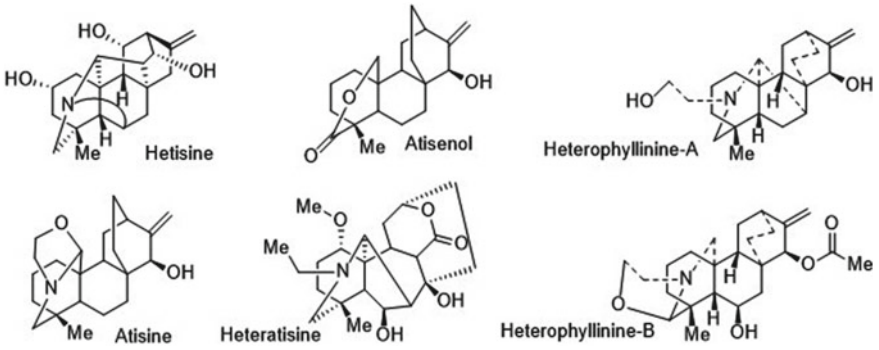


Fig. 22.1 Phytochemical constituents of *Aconitum heterophyllum*

22.9.2 *Berberis aristata*

Berberis aristata belongs to the family Berberidaceae and locally known as Daruharidra means 'the wood having yellow color'. *B. aristata* is one of the high-value medicinal shrubs which are indigenous to the Indian subcontinent. *B. aristata* is a spiny and woody shrub with the height ranging from 2 to 3 m. The bark of *B. aristata* has externally appeared brownish or yellowish while internally it appears as dark yellow. Due to medicinal applications of *B. aristata* it has high trade value and especially supplied from Indian state Himachal Pradesh (Ved and Goraya 2008).

Geographic Distribution: It is indigenous to the Himalayan region at an altitude of 6000 to 10,000 ft. specifically in India, Nepal, and Sri Lanka (Kirtikar and Basu 1995).

Medicinal Properties and Ethnomedicinal Usage: *B. aristata* has been used in the Indian Ayurvedic system of medicine since from 900 to 1000 BC (Sack and Froehlich 1982). *B. aristata* based Ayurvedic formulation has used for the management of wounds, rheumatism, bacillary dysentery, diabetes, eye care, jaundice, and skin diseases. Moreover, pharmacological potentials of *B. aristata* has been evaluated experimentally including antidiarrheal (Joshi et al. 2011), immunomodulatory, anti-inflammatory (Sohni and Bhatt 1996), hepatoprotective (Sohni and Bhatt 1996), anti-platelet activating factor (Tripathi and Shukla 1996), cardiotonic (Gilani et al. 1999), cytotoxic (Das et al. 2009), antioxidant (Andola et al. 2008), and anti-microbial (Singh et al. 2007).

Phytochemistry: Phytochemical investigations of *B. aristata* revealed the presence of glycosides, alkaloids, amino acids, saponins, tannins, flavonoids, carbohydrates, and proteins (Patel et al. 2012). The major group of phytoconstituents in *B. aristata* is alkaloids including karachine, pseudopalmatine chloride, aromoline, pseudoberberine chloride, oxyberberine, oxyacanthine, berbamine, berberine, berberine chloride, pakistanine, tetrahydropalmatine, palmatine chloride, and taxilamine as shown in Fig. 22.2 (Blasko et al. 1982; Potdar et al. 2012). Besides that, the flower contains polyphenolic flavonoids such as quercetin, rutin, and chlorogenic acid, etc. (Sivakumar and Kamachandran Nair 1991). The aliphatic hydrocarbon like *n*-docosane was isolated from the ethanolic extract of *B. aristata* (Katiyar et al. 2011).

Quality Control Approaches: Balasubramani et al., has described ITS-based DNA markers for the reorganization and differentiation of *B. aristata* from the other similar species (Balasubramani et al. 2011). Kreuzer et al., also employed a phylogenomic approach to analyze the plastid sequence to developing DNA barcodes for *B. aristata* (Kreuzer et al. 2019). Majority of chemical analysis was performed by using chromatographic analysis i.e. HPLC or HPTLC and their major focus on berberine alone, rather than other Phyto molecules of *B. aristata* (Joshi et al. 2011; Pasrija et al. 2010; Pant and Rajasekaran 2011; Patel et al. 2012).The mass spectroscopic technique was applied for phytochemical fingerprinting of *B aristata* to identify 11

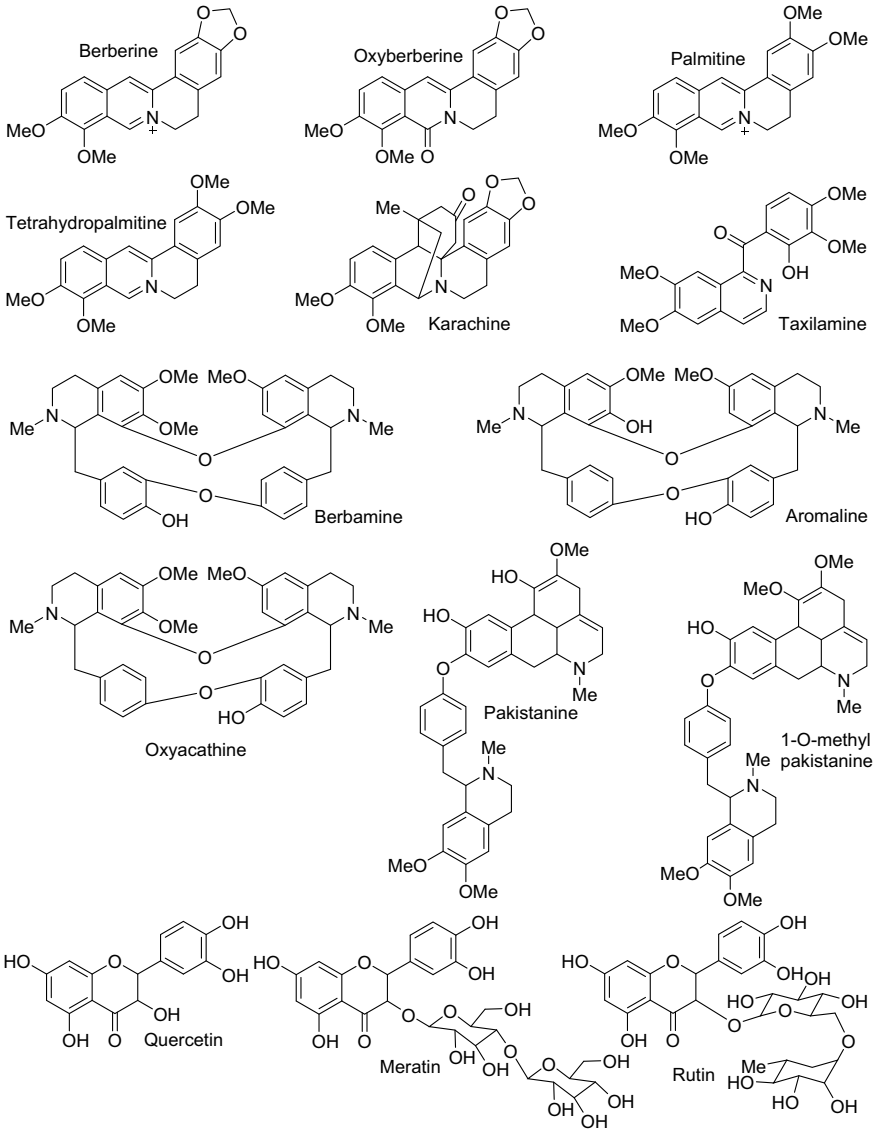


Fig. 22.2 Phytochemical constituents of *Berberis aristate*

marker peaks (Bajpai et al. 2015). Toxicity analysis of ethanol and aqueous fractions of *B. aristata* was evaluated by the acute oral toxicity method with a dose limit is 2000 mg/kg body weight. The results of the toxicity analysis indicated that of orally administrated samples of *B. aristata* did not show toxicity or fatality in animals up to 5000 mg/kg body weight (Joshi et al. 2011). Chemometric analysis of *B. aristata*

was performed for the authentication of herbal samples combination with PCA and DART-MS data (Bajpai et al. 2015).

22.9.3 Fritillaria roylei

The genus Fritillaria belongs to the family Liliaceae and has about 130–165 species. It is indegineous to temperate region of the Northern Hemisphere and is represented by six species in India (Sastri 1950). Different species of Fritillaria has been used in Traditional Chinese Medicine for thousands of years. In India, it is known by the Hindi name "Kakoli" and is an important ayurvedic herbal medicine.

Geographic Distribution: It is found in the temperate and alpine pastures at the height of 2400–3900 m above sea level in Jammu and Kashmir, Himachal Pradesh and Uttarakhand in India, Afghanistan and Pakistan. This belongs to the category of critically endangered plant species of western Himalayas (Shafi et al. 2018).

Phytochemistry: The main class of phytoconstituents reported from this plant are steroidal alkaloids. Steroidal alkaloids like peimine, peiminine and peimisine, and sepeimine shown in Fig. 22.3, has been reported to be present in the bulbs of *F. roylei.* (Yun-Hsi 1944; Chou 1947; Jiang et al. 2001).

Medicinal Properties and Ethnomedicinal Usage: *F. roylei* is an important part of ayurvedic system of medicine where it one of the eight important ingredients of Ashtavarga (Dhyani et al. 2010). According to Chinese traditional medicine, it is used to treat cold and bronchitis as it helps to clear heat and moisten dryness. Anti-inflammatory, antitussive, and expectorant activities of *F. roylei* are also reported in the literature (Hao et al. 2015).

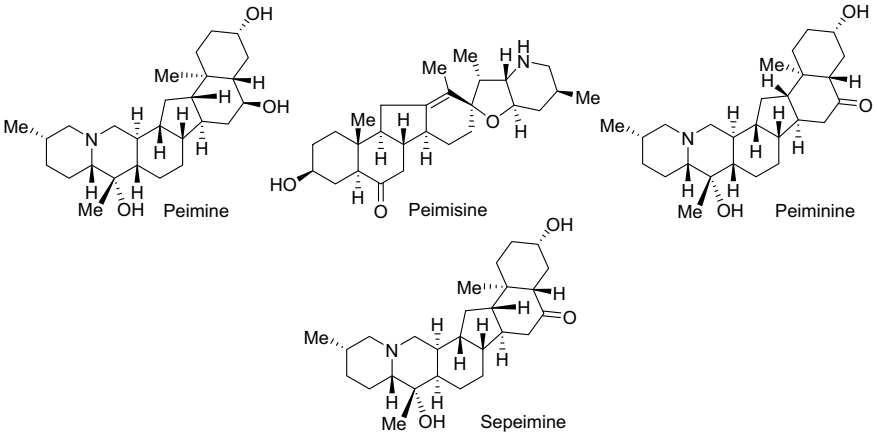


Fig. 22.3 Phytochemical constituents of *Fritillaria roylei*

Quality Control Approaches: Kanwaljeet et al. studied the morphological, anatomical, and palynological characters of *F. roylei* to develop DNA barcodes for its molecular authentication. Genomic DNAs were extracted from the dried bulbs samples and high-quality marker sequences were obtained for one nuclear and one cytoplasmic molecular marker (Singh et al. 2020a). Kumar et al. have reported a selective, sensitive, and rapid liquid chromatography-electrospray ionization mass spectrometry (LC–ESI–MS) analytical method for the qualification of sipeimine and peimine (Kumar et al. 2020) in wild and in vitro cultures samples of *Fritillaria roylei*. Although there are numerous reports regarding the chemical marker development and chemical profiling of other species of *Fritillaria* genus no detailed work has been found in the context of *F. roylei.*

22.9.4 Jurinea macrocephala

Jurinea macrocephala, member of family Asteraceae, is popularly known as Himalayan Dolomiaea. It is found in the morainic alpine regions of Himalayas up to 3400–5000 m. In India, this plant has reached on verge of extinction due to exploitation for its valuable tuberous roots which are provided as raw material to incense industry (Dogan et al. 2009; Charak et al. 2018). The roots are applied on eruptions as poultice, used as antiseptic and root decoction is considered to be effective in colic. The alcoholic extract of plant has shown anti-bacterial and anti-malarial properties (Charak et al. 2018; Singh et al. 2015).

Geographic Distribution: It is distributed in Irano-Turanian phytogeographic region and in India, at an altitude of 3400–5000 m (Dogan et al. 2009; Charak et al. 2018).

Phytochemistry: Six molecules have been isolated from this plant which include β-sitosterol, physcion, lupenone, ptiloepoxide, 20, 21α-epoxytaraxastan-3β-ol and chlorogenic acid (Kumar and Agnihotri 2020) as shown in Fig. 22.4.

Medicinal Properties and Ethnomedicinal Usage: The roots of *J. macrocephala* are utilized for making gums, applied as poultice on rashes, used as antiseptic and its decoction is helpful in colic. The roots are effective in puerperal fever and fever after childbirth because of their cordial and stimulant effects. The aromatic oil obtained from roots of *J. macrocephala* is effective in eye infection, gout and rheumatism, diarrhoea and stomachache (Kumar and Agnihotri 2020; Kumar et al. 2009; Kunwar et al. 2006).

Quality Control Approaches: Although, a HPLC–DAD analysis of antioxidant active fraction has been reported, there is no particular method for the proper authentication of *J. macrocephala* (Shah et al. 2014).

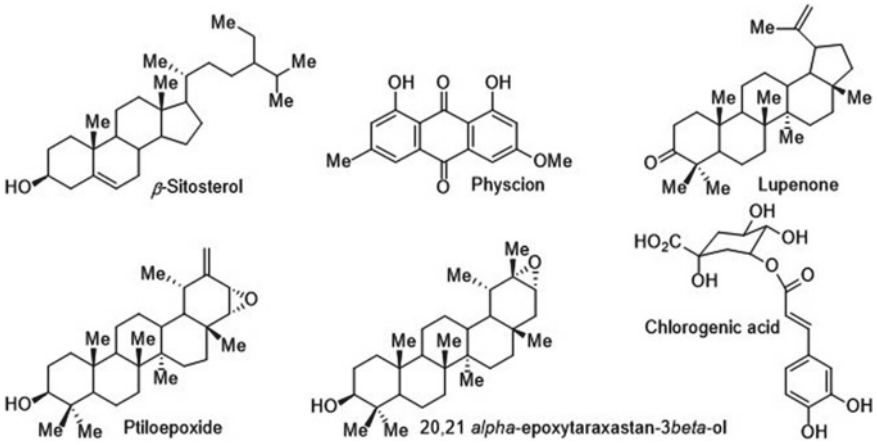


Fig. 22.4 Phytochemical constituents of *Jurinea macrocephala*

22.9.5 Nardostachys jatamansi

Nardostachys jatamansi, a perennial herb, belonging to family Velarianacae found only in the alpine regions of Himalayas. *N. jatamansi* is a well-known Ayurvedic herb and has been used for a long period to treat various disorders because of its various pharmacological activities such as anti-parkinson's (Ahmad et al. 2006), anti-convulsant (Rao et al. 2005), anti-diabetic (Nelson Kumar et al. 2011), tranquilizing (Arora et al. 1962), hypotensive (Ashfaque et al. 2017), neuroprotective (Khan et al. 2012), hepatoprotective (Ali et al. 2000) etc.

Geographic Distribution: *N. jatamansi* is found only in the alpine regions of Himalayas ascending up to an altitude of 3000–5000 m from Pakistan to India (Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim) (Purnima and Kothiyal 2015).

Phytochemistry: *N. jatamansi* contains volatile essential oil and other pharmacologically active compounds. Jatamansone is the major compound obtained from the rhizomes of this plant. The principal active compounds of *Nardostachys jatamansi* are sesquiterpenes and coumarins. Sesquiterpenes include the major compound jatamansone, jatamansic acid, nardostachone, dihydrojatamansin, nardal, nardostachysin, jatamol A and B, nardosinone, calarene, seychellene and coumarins such as xanthogalin (Gottumukkala et al. 2011), (Rahman et al. 2011), (Sahu et al. 2016). Other chemical constituents reported from this plant include, nardostachone, patchouli alcohol, alpha-patchoulene, beta-patchoulene, calarene, nardin, resin, sugar, starch, gum, ketone, etc. An alkaloid actinidine is also reported from this plant (Rahman et al. 2011), (Sahu et al. 2016). Other phytochemicals reported from this plant include neolignan, lignans, coumarins, and terpenoids (Gottumukkala et al.

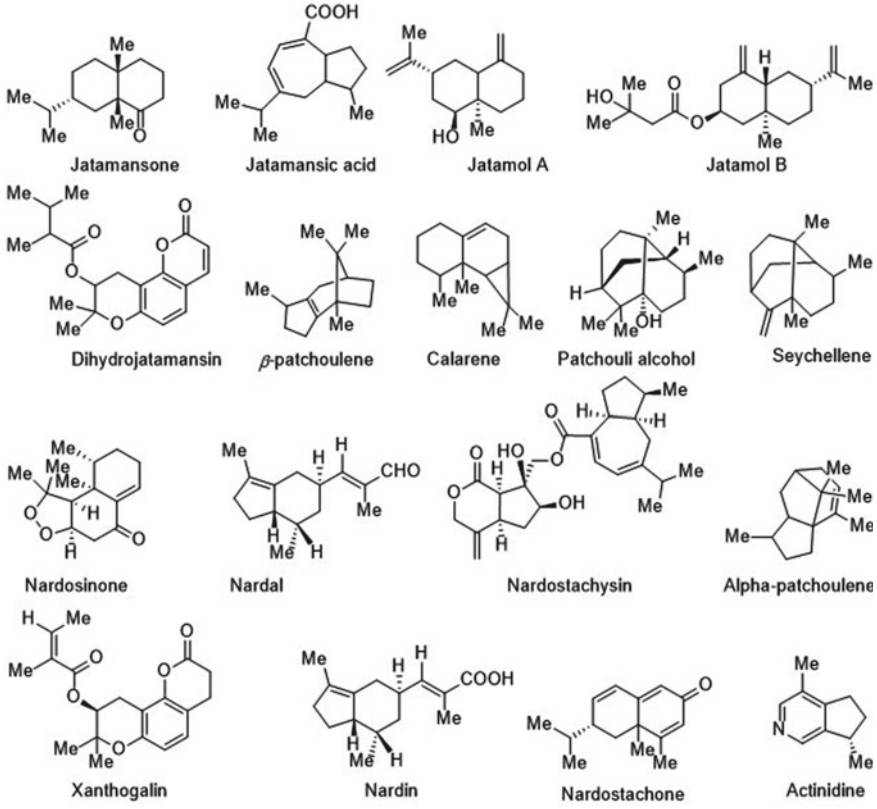


Fig. 22.5 Phytochemical constituents of *Nardostachys jatamansi*

2011). Chemical structures of different phytoconstituents found in *N. jatamansi* are shown in Fig. 22.5.

Medicinal Properties and Ethnomedicinal Usage: *N. jatamansi* is one of the most popular medicinal and aromatic herbs mentioned in Indian traditional medicinal system and is used in a wide range of ailments such as cholera, palpitation, hysteria, epilepsy, etc. (Rahman et al. 2011; Gupta et al. 2012). Roots and rhizomes of *N. jatamansi* have a bitter taste, aromatic, diuretic, antispasmodic, nerve stimulant, nerve sedative, carminative, promotes appetite and digestion hepatoprotective (Ali et al. 2000; Rahman et al. 2011). In Ayurveda *N. jatamansi* is mentioned to promote sleep, alleviate mental diseases, increase the digestion, stop skin disorders, syncope, stop burning sensations and promote hair growth (Bagchi et al. 1991; Mude et al. 2020). In India, the roots and rhizomes of *N. jatamansi* are marketed as Ayush56 which is an anticonvulsant Ayurvedic drug (Chatterjee et al. 2005; Mude et al. 2020). It also exhibits significant tranquilizing activity (Arora et al. 1962), anti-oxidant (Sharma and Singh 2012), hepatoprotective (Ali et al. 2000), neuroprotective (Khan

et al. 2012), hypotensive (Ashfaque et al. 2017), anticonvulsant (Rao et al. 2005) and antiarrhythmic activities (Arora and Madan 1956).

Quality Control Approaches: The microscopic and macroscopic characteristics of *N. jatamansi* were studied and the physicochemical parameters were established for assessing the quality. The average values of non-specific parameters are expressed in the percentage of dried material which includes total ash (20.53%), acid insoluble ash (0.76%), water-soluble ash (1.005%), alcohol soluble extractive (2.88%), water-soluble extractive (17.1%). They have also performed HPTLC fingerprinting analysis for proper authentication of *N. jatamansi* (Jha et al. 2012). A UPLC-PDA method had been developed for the quality control of *N. jatamansi*, for chemical investigation and simultaneous quantification of six serotonin transporter modulatory constituents. For chromatographic fingerprinting analysis, samples of *N. jatamansi* were collected from different retail sources and 24 common peaks were considered as characteristic peaks for assessing the consistency of all the samples. Out of 24 peaks, six peaks were identified by phytochemical investigation as buddleoside, desoxonarchinol A, nardosinone, isonardosinone, kanshone H and (-)-aristolone (Zhang et al. 2018).

22.9.6 Onosma hispidum

There are 150 species of the genus *Onosma* in Asia and 29 more species are known which are indigenous to China. The name Ratanjot (Ratan means gem and jot means luster) is associated with 15 species of genus *Onosma. Onosma hispidum* belongs to family Boraginaceae which is known for roots due to the production of a red/maroon dye used as a natural coloring agent in spices, foods, oils and medicines.

Geographic Distribution: *O. hispidum* is distributed up to 2000–4500 m in the western Himalayas and it is a perennial herb that can reach up to 70 cm in height (Kumar et al. 2013).

Phytochemistry: The main chemical constituents present in the plant are onosmin A and B, hispidone, benzoic acid, and 4-hydroxy benzoic acid. Ethanolic extract of root's bark is reported to contain vanillic acid and ferulic acid. Other compounds reported from this species include apigenin, apigenin 7-O-β-D-glucoside and (2S)-5,2'-dihydroxy-7,5'-dimethoxy-flavanone (Kumar et al. 2013; Naz et al. 2006) (Fig. 22.6).

Medicinal Properties and Ethnomedical Usage: The roots of *O. hispidum* are mainly used as flavoring and coloring agent mainly in medicines and food stuffs due to production of red dye in roots (Asghar et al. 2018). This plant recommended for the treatment of fever, abdominal pains, infectious diseases, and as a laxative, anthelmintic. Ratanjot is also used in the treatment of blood disorders, bronchitis, abdominal pain, eye diseases. Flowers of *O. hispidum* flowers are used as cardiac tonic and stimulant. Bruised roots of the plant are applied over the cutaneous layer eruptions and can also be used in the treatment of diabetis (Hussain et al. 2017; Kumar

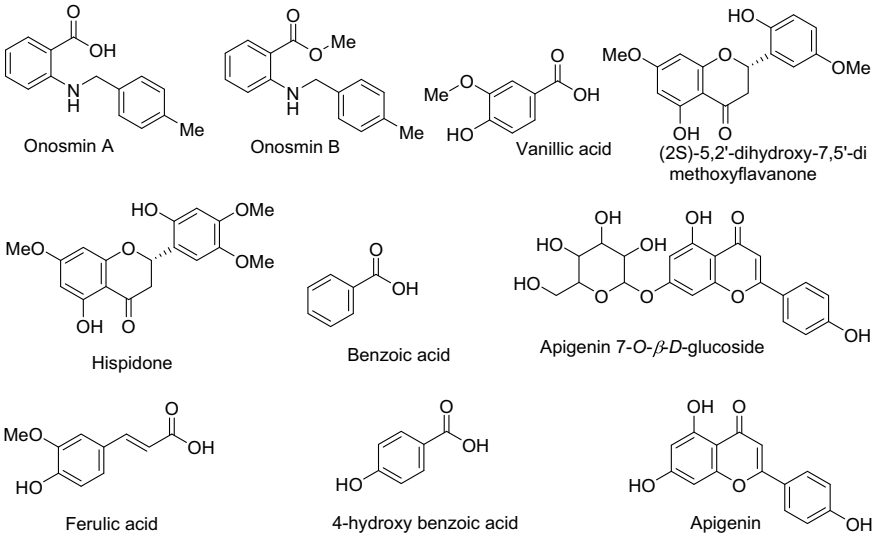


Fig. 22.6 Phytochemical constituents of *Onosma hispidum*

et al. 2013). Various other traditional uses have also been proved experimentally (Kumar and Gupta 2009). The chemical constituent isolated from the plant such as ferulic acid and hispidone possess antibacterial activity and cholinesterase inhibition property, respectively. The methanolic extract of roots are also reported to possesses hypoglycaemic activity (Kumar et al. 2010).

Quality Control Approaches: Chemical profiling of other *Onosma* species such as *O. sericea, O. stenoloba, O. ambigens, O. bracteatum, O. aucheriana, O. frutescens*, and *O. tauricum* has been reported but there is no report on chemical profiling of *O. hispidum* (Katanić Stanković et al. 2020; Kirkan et al. 2018; Sarikurkcu et al. 2020). DNA profiling of *Onosma* species has not been reported till date.

22.9.7 Picrorhiza kurroa

Picrorhiza kurroa Royle is a renowned medicinal herb belonging to foxglove family Scrophulariaceae. It has been used in Ayurveda in the traditional system of medicine for treatment of liver diseases. It is being classified as bitter drugs due to its intense bitter taste (Debnath et al. 2020). It has well established ethnopharmacological role in the various ailments ranging from indigestion, spleen disorders to liver complications (Singh 2004). The medicinal properties of the plant are mainly attributed to its main bioactive components picrosides, phenolic compounds, and cucurbitacins. Active phytoconstituents are mainly present in the roots and rhizomes of the herb. There is a lot more demand for this herb which is approximately 500 tons per year but

indiscriminate utilization of herbal plants has added this plant in the endangered list of medicinal plants (Verma et al. 2009).

Geographic Distribution: *P. kurroa* is a perennial herb found in the alpine Himalayan region in India, Nepal, and China at an elevation of 2700–5000 m above mean sea level. It is also named White Christmas Rose due to the arrival of its flowers in winter at the time of Christmas.

Phytochemistry: The main active constituents of *P. kurroa* are iridoids glycosides which includes picrosides (I, II and III), kutkosides, cucurbitacins (Fig. 22.7). It also

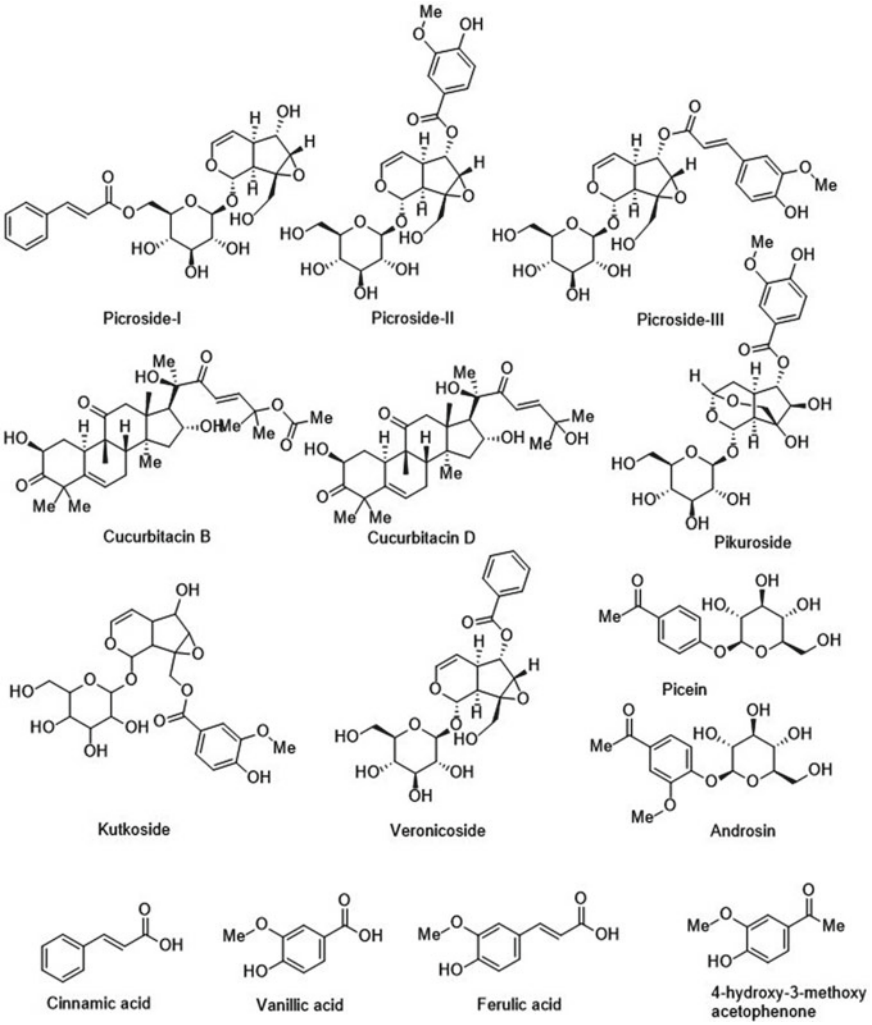


Fig. 22.7 Phytochemical constituents of *Picrorhiza kurroa*

contains 4-hydroxy-3-methoxy acetophenone, veronicoside, pikuroside, minecoside, phenol glycosides i.e. picein and other bioactive phenolic compounds (Masood 2015). Other phytoconstituents in this plant are androsin and apocynin which possess anti-inflammatory, antioxidant activity. Among cucurbitacins, it contains cucurbitacin B, D and R. Carbohydrate such as *D*-mannitol and aromatic acids like ferulic acid, vanillic acid, and cinnamic acid are also present in it (Debnath et al. 2020).

Medicinal Properties and Ethnomedical Usage: *P. kurroa* roots possess its main ethnopharmacological use as a hepatoprotective agent. Its roots and rhizomes are used in Unani medicine as anthelmintic, stomachic, carminative and laxative and purgative in the higher doses. It is used to kill intestinal worms, to strengthen stomach function in case of indigestion, in ascites and the several brain ailments. In higher doses, it is used for the treatment of paralysis, epilepsy, and as cathartic, emetic, abortifacient, as the emmenagogue to maintain menstrual flow. It is also used as antidote in the dog bite (Akbar 2020). Apocynin present in the roots is mainly used as a potent NADPH oxidase inhibitor and also has significant antioxidant and anti-inflammatory properties. Androsin present in *P. kurroa* possesses anti-asthmatic activity (Singh 2004). *P. kurroa* is used in the treatment of several ailments such as jaundice, chronic dysentery, asthma, and as potent herbal ingredient in the preparation of polyherbal medicines for their antihepatotoxic effect in liver disorders (Akbar 2020).

Quality Control Approaches: The phytochemical profiling of *P. kurroa* has been performed in which quantification of picroside-I and picroside-II has been done using high performance thin layer chromatography (HPTLC) (Singh et al. 2005). LC–ESI–MS/MS analysis of hydroalcoholic extract (70%) of *P. kurroa* was done where iridoid glucosides such aspicroside-I, picroside-II, picroside-III, pikuroside, kutkoside and some flavonoids such as apocynin and vanillic acid were determined (Krupashree et al. 2014). ISSR and RAPD markers have been reported to estimate the genetic stability of in-vitro developed plants of *P. kurroa* (Rawat et al. 2013). In an another report, 20 novel genic SSR markers were found which can be utilized in the diversity characterization in broad geographical regions for the identification and selection of quality genotypes. This study could be utilized for the conservation of this endangered species and commercial cultivation (Singh and Sharma 2020).

22.9.8 Rheum australe

Rheum australe is commonly known as Himalayan or Indian rhubarb is a medicinal herb belonging to polygonaceae family. There are approximately 60 species of *Rheum* have been identified all over the world (Pandith et al. 2018). It has been mentioned in the ancient texts for the treatment of liver disorders, gastritis, blood purification, stomach, and mensural disorders. Himalayan rhubarb has been actively utilized in the Ayurveda, Chinese, Homeopathy, Tibetan, and Unani medicinal system (Rokaya et al. 2012).

Geographic Distribution: *R. australe* is an endemic, robust, perennial herb with stout rhizomes and is found in the temperate and subtropical regions of the North-western Himalayas. It covers the areas of India, China, Bhutan, Pakistan, Nepal, and Myanmar. It grows at an altitude of 3500 m in the grassy and rocky slopes.

Photochemistry: *R. australe* contains different categories of phytoconstituents as stilbenes, anthrones, anthraquinones, phenols, sterols, flavonoids, chromones, oxantrone ethers, and esters. The most commonly found secondary metabolites are anthraquinone derivatives viz. emodin, aloe-emodin, chrysophanol, rhein, physcion, and their glycosides and stilbenes such as piceatannol, resveratrol and its glycoside derivatives as shown in Fig. 22.8.

Medicinal Properties and Ethnomedical Usage: *R. australe* is the oldest known medicinal herb used in traditional as well as modern medicine system. Its root, bark and leaves extract are used as laxative, purgative, astringent tonic and stomachic since ancient times. In the north west Himalayas, its leaves and stalks are consumed as a vegetable source. According to USFDA nutrient database, Indian rhubarb is a good source of vitamins such as vitamin K and C and minerals like calcium and potassium. *R. australe* roots are used in the treatment of muscular swelling, mumps, cuts, tonsillitis, and also as expectorant, appetizer, etc. (Pandith et al. 2018). Indian rhubarb is also of great importance in veterinary science as a general panacea for livestock. It is recommended that rhizomes of *R. austral* should not be taken by people who tend to develop epilepsy, gout, rheumatism, and particularly for people who have a history of uric acid disease or renal or gall bladder stones. Rhizomes of rhubarb produce a yellow dye which is employed in cosmetics and textiles, hairs, and wooden material for coloration purposes. It is used as an environmentally friendly and natural source of dye in the woolen yarn samples.

Quality Control Approaches: Phytochemical investigation of anthraquinone derivatives in the root extract of *R. australe* was performed using HPLC–UV analysis (Kumar and Spandana 2013). They have identified four different anthraquinones, aloe-emodin, emodin, chrysophanol, and physcion in this analysis. There are several reports on phytochemical screening of other species of genus *Rheum* viz., *R. palmatum, R. cordatum. R. spiciforme,* and *R. webbianum* (Tabin et al. 2016)

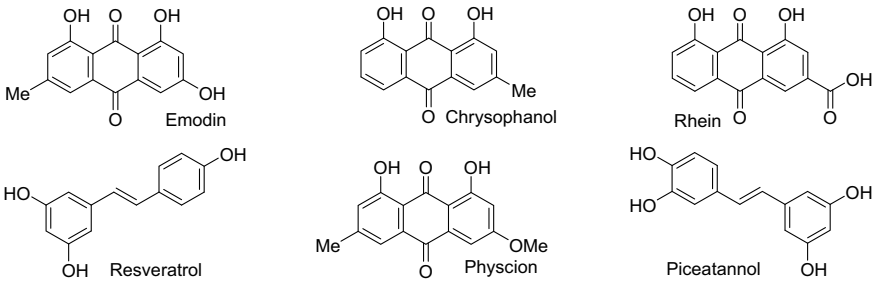


Fig. 22.8 Phytochemical constituents of *Rheum australe*

(Zhumashova et al. 2019; Liang et al. 2013). Aloe-emodin, emodin, rhein, chrysophanol, and physcion are the chemical markers used in the phytochemical profiling of *R. australe.* No reports are available on the DNA profiling of *R. australe.*

22.9.9 Swertia chirayita

Swertia chirayita belonging to family Gentianaceae is a well-known medicinal herb used in traditional medicinal system. Genus *Swertia* having 170 species are distributed in Asia, America, Africa, and Europe. In India, almost 40 species of Swertia are found among which *S. chirayita* (Chiratitka in Hindi, Kairata in Sanskrit) is most efficient due to its medicinal properties. Secondary metabolites present in this herb have shown numerous biological activities hence making this endangered medicinal herb critically important (Joshi and Dhawan 2005).

Geographical Distribution: *S. chirayita* is erect, annual, perennial herbal, and is 2–4 cm to 1.5 m high. It is a flowering plant species and shows a wide range of colors and floral outlines. Acidic soil conditions are suitable for its growth. Generally, this herb grows in temperate regions of altitude ranging from 1200 to 2100 m above the sea level. This plant is distributed throughout the Himalayan region, Nepal being dominant in its distribution and trade (Khanal et al. 2014).

Medicinal Properties and Ethnomedicinal Usage: Gentianaceae family is known for having glycosides which is bitter flavoured compound known for curing digestive disorders. Use of this plant as a whole is mentioned in Ayurveda, Siddha, and Unani system (Kumar and Van Staden 2016). Medicinal use of this plant against malaria, inflammation, asthma, anaemia, hepatotoxic disorders, weakness, joint pains, headache, skin diseases, scorpion bite, and vaginal discharge is well known. Extracts of this plant possess activity against the hepatitis B virus. Extracts of *S. chirayita* are used in different herbal formulations like Ayush-64 and possess antipyretic, antifungal, antiviral, and antibacterial activities (Gopalkrishna et al. 2008; Kumar and Van Staden 2016).

Phytochemistry: *S. chirayita* is known to have different classes of compounds such as alkaloids, flavonoids, lignans, xanthones, terpenoids, etc. Compound chiratanin is present in different parts of this plant. Amarogentin is known to show anti-cancer and anti-diabetic activity (Pal et al. 2012). Swertiamarin shows anti-hepatitis and anti-arthritic activity (Wang et al. 2001). Different classes of secondary metabolites present in this herb possesses significant pharmacological activities. The chemical structure of some important secondary metabolites found in different parts of *S. chirayita* are shown in Fig. 22.9.

Quality Control Approaches: Latif et al., performed physicochemical and phytochemical standardization of *S. chirayita* to confirm its identity, safety, quality assurance. Different plant species were regarded as substitutes and adulterants for *S. chirayita,* thereby misguiding in the selection or utilization of genuine medicinal

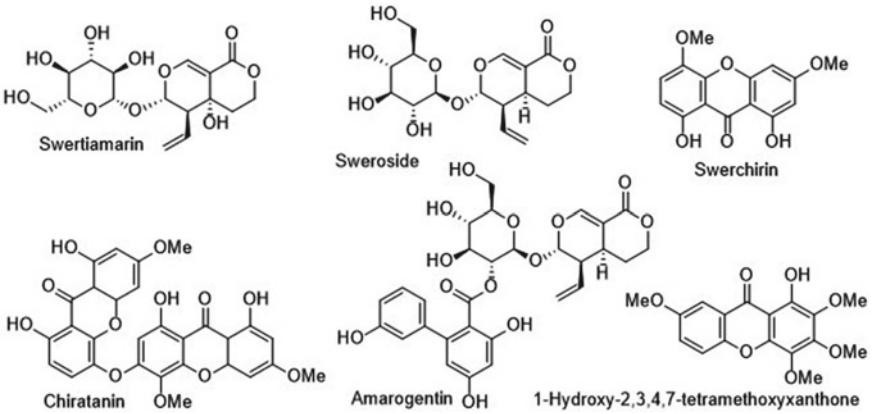


Fig. 22.9 Phytochemical constituents of *Swertia chirayita*

herb. They studied parameters like ash content, moisture content, loss on drying, melting range, solubility, IR studies, Fluorescence analysis. This study was aimed to set up pharmacopoeial standards in determining the quality of *S. chirayita* (Latif and Rehman 2014). However, there is a lack of quality control methods implemented in this herb. Being a commercially important plant traded in huge amounts, there is a need of implementing modern quality control parameters for its quality assurance. It is necessary to build complete quality parameters that should be implemented easily to check its efficiency.

22.9.10 Trillium govanianum

Trillium govanianum Wall.ex D. Don also known as Nagchhatri (Family: Melanthiaceae) is a perennial high-value medicinal herb that endemic to the Himalayas. It's one of two species of genus *Trillium* which grown in India along with *T. tschonoskii* (Chauhan et al. 2019). Species of the genus *Trillium* are slow-growing with prolonged regeneration periods from seeds which takes 5–15 years (Case Jr and Case 1997). The size of *T. govanianum* is about 15–30 cm long with tree leaves connected at the apex of the green stem and a single, reddish-tri-petals, purple flower in the middle of leaves stalk. *T. govanianum* has currently endangered and overexploited due to its illegal trading to fulfilled demand in pharmaceutical industries.

Geographic Distribution: *T. govanianum* has grown under natural habitat in the all over Himalaya from temperate and sub-alpine ranges in Afghanistan, Pakistan, India, Tibet, and Nepal at an altitude range of 2500–4000 m (Vidyarthi et al. 2013). Jammu and Kashmir, Himachal Pradesh, and Uttarakhand are the Indian Himalayan region where *T. govanianum* has grown, while its population is rare in the North-Eastern Indian Himalaya (Chauhan et al. 2019).

Phytochemistry: The principle phytochemicals of *T. govanianum* are steroidal saponins. To date, approximately 13 phytomolecules was isolated from *T. govanianum,* including steroidal saponins, phytoecdysteroids, and trihydrylate fatty acids (Ismail et al. 2015), (Rahman et al. 2017; Singh et al. 2020b). Chemical structures of different compounds isolated from *T. govanianum* are shown in Fig. 22.10.

Medicinal Properties and Ethnomedical Usage: In Ayurvedic medicine, the rhizome part of *T. govanianum* recommended for therapeutic ailments associated with inflammation, pain, and sexual disorders (Rani et al. 2013). It has partially studied for its traditionally well-known therapeutic applications. Beside this some researchers have also carried out preliminary in vitro and in vivo bio-activities of *T. govanianum* which show that it has anti-fungal (Ismail et al. 2015), analgesic and anti-inflammatory (Ur Rahman et al. 2016), anti-fertility (Sharma et al. 2018), cytotoxicity (Khan et al. 2016; Sharma et al. 2016) and anti-oxidative activities (Rahman et al. 2015; Singh et al. 2020b).

Quality Control Approaches: Recently approximately, 24 steroidal saponins from parent extract (Singh et al. 2020c) and nine hydrophilic compounds from *n*-hexane fraction (Maqbool et al. 2018), were tentatively identified in *T. govanianum* by UPLC-ESI–MS/MS and GC/MS respectively. The quantitative analysis of steroidal saponins from *T. govanianum* indicated that the occurrences of borassoside E, protodioscin, and govanoside B in rhizome part were abundant therefore they can be considered as marker compounds for quality control. Sharma et al., was developed approximately 21 novel microsatellite markers of the authentication of *T. govanianum* (Sharma et al. 2017).

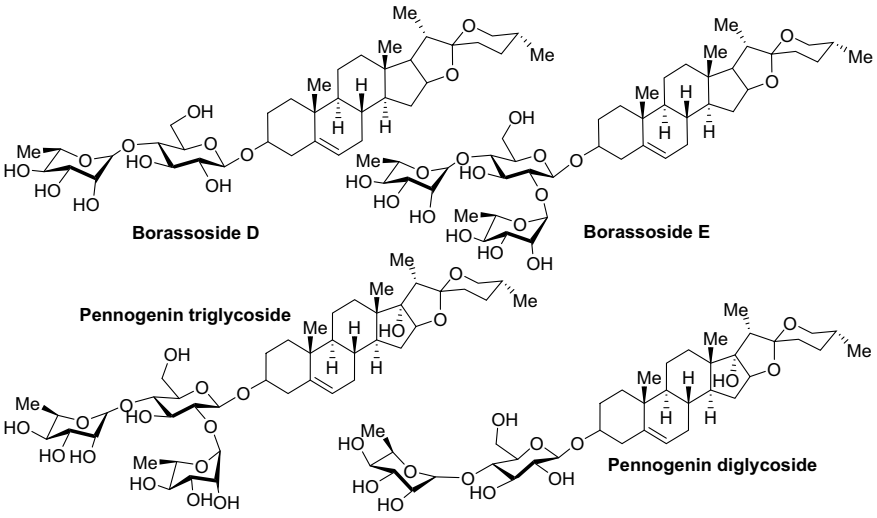


Fig. 22.10 Phytochemical constituents of *Trillium govanianum*

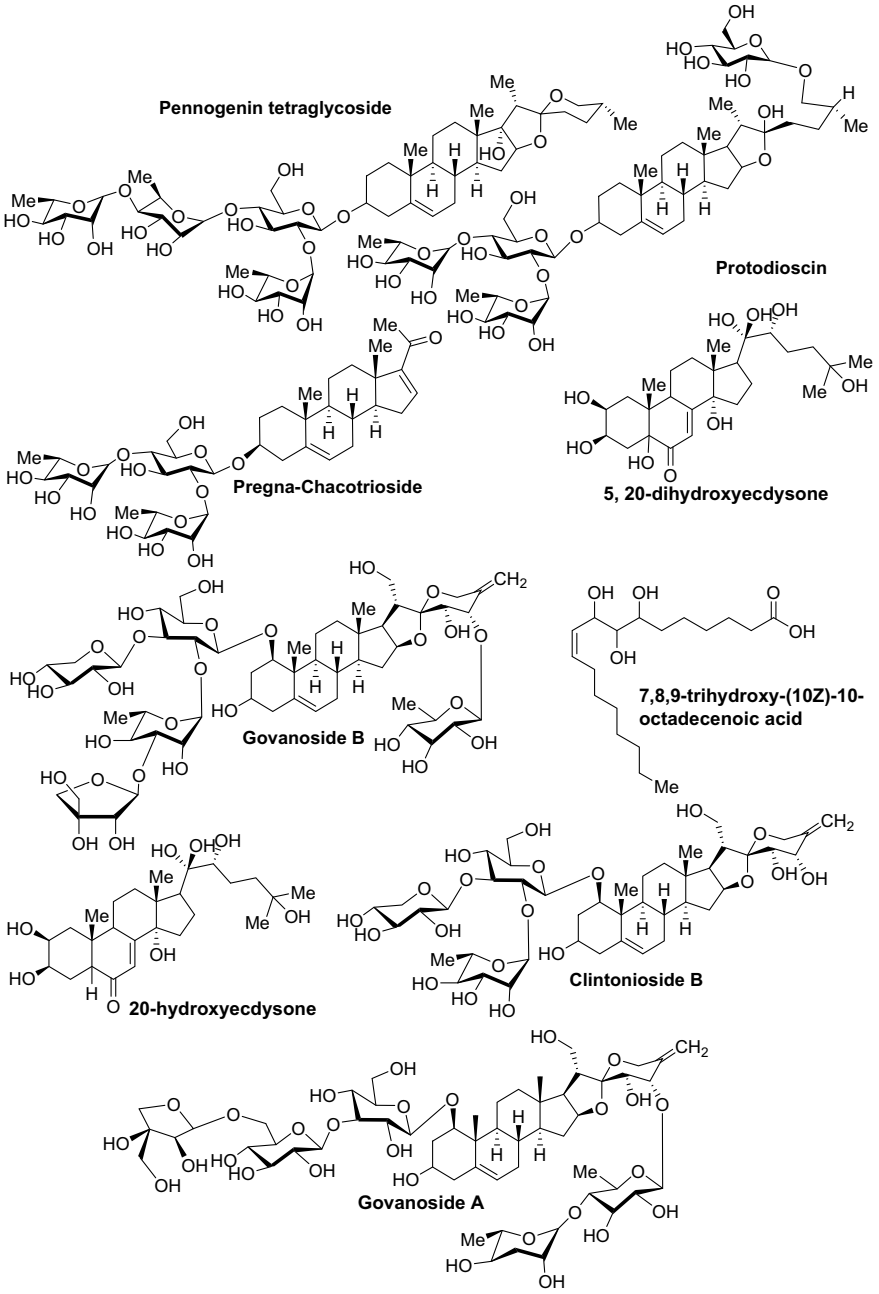


Fig. 22.10 (continued)

22.9.11 Valeriana jatamansi

Valeriana jatamansi Jones (Indian valerian) is a perennial herb belonging to family Valerianaceae. Valeriana is an important aromatic and medicinal plant of the temperate Himalayan region. Indian valerian (English) is also popularly known as Mushkbala (Kashmiri/Hindi) and Sugandhbala or Tagar (Sanskrit). *V. jatamansi* is the most primitive species of plants belonging to family Valerianeaceae (Sundaresan and Ilango 2018). The roots and rhizomes of valerian possess high value essential oil which is used in perfumery industries. The different traditional medicine system such as Siddha, Homeopathy, Ayurveda Ethnomedicine, and Indian system of Medicine have shown the significant usage of Indian valerian of the treatment of various ailments (Rather et al. 2012).

Geographic Distribution: *V. jatamansi* is mainly distributed in all temperate regions of the Himalayas. It is collected from the wild in Europe, Japan, Eastern Europe, France, Belgium and Netherlands (Jugran et al. 2019). It is a perennial dwarf herb with rhizomatous roots covered with hairs and fibres. *V. Jatamansi* resides up to an altitude of 3000 m. It mainly grows in the rocky, moist, grassy slopes, steep areas, and on stones with sandy loam soil.

Phytochemistry: The main chemical constituents present in roots and rhizomes of *V. Jatamansi* are flavonoid, flavone glycosides, lignans, phenolics, sesquiterpenes, essential oils, iridoids/valproates, and other phytoconstituents. The major sesquiterpenes present in indian valeriana are jatamansone or valeranone. Some other sesquiterpenes such as α-patchoulene, angelicin, β-patchoulene, calarene, β-sitosterol, jatamansin, jatamansinol, patchouli alcohol, seychelane are also present. Among valproates it contains valtrate, acevaltrate, didrovaltrate, isovaltrate and others like valerenic acid, valerinone are also present in *V. Jatamansi*. Several other compounds such as jatamansic acid, terpenic, jatamansone semicarbazone, etc. are also reported for the roots and rhizomes of valeriana (Sundaresan and Ilango 2018). Chemical structures of different compounds reported from are shown in Fig. 22.11.

Medicinal Properties and Ethnomedical Usage: *V. jatamansi* roots and rhizomes are mainly used due to presence of high-quality essential oil which is mainly employed in perfumery. Iridoid compounds are reported to produce sedative effects and also used in the treatment of leprosy and dementia. Valeric acid and valerinone are used in the development of top-selling herbal drug valerian. Valepotriates or iridoids are known to be the most active phytoconstituents of the species. They possess various activities like neuroprotective, in Alzheimer's disease, anticoagulant, hepatoprotective, antiprotozoal, anticancer, antifungal, antibacterial, and anti-inflammatory. Indian valeriana is used since ancient times in the Indian system of medicine such as in Rigveda and Charka Samhita. It was used mainly for its aromatic, carminative, stimulant and antispasmodic effects. It is also used as a curative measure in case of obesity, anxiety, tranquilizer, skin diseases, insanity, sciatica, failing reflexes, and in treatment of snake poisoning.

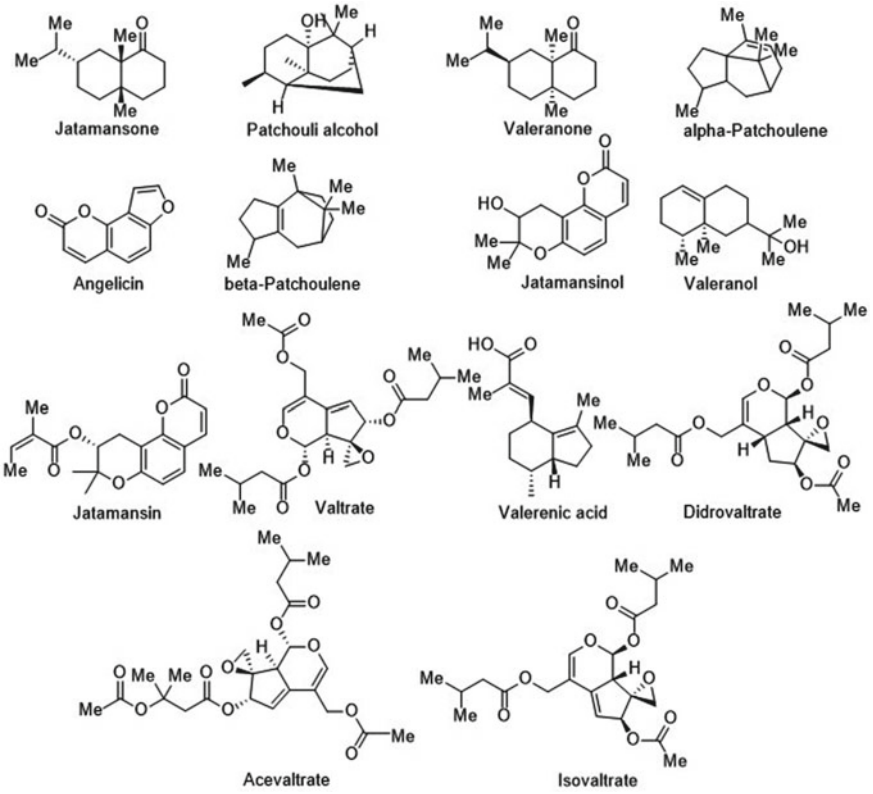


Fig. 22.11 Phytochemical constituents of *Valeriana jatamansi*

Quality Control Approaches: HPLC–PDA–MS and TLC analysis of chlorinated valproates was reported from the whole plant of *V. Jatamansi*. In this, 15 chlorinated valproates were isolated and their structure was elucidated by NMR spectroscopic method (Lin et al. 2013). Head space GC–MS and GC-MS analysis of *V. Jatamansi* has also been reported for the profiling of the volatile constituents present in the plant (He et al. 2018). Flavonoids, valerenic acid, and phenylpropanoids are also determined using liquid chromatography with UV detection (Navarrete et al. 2006). DNA profiling studies showed that ARMS based on the 5S rDNA sequence can be used to distinguish *Valeriana jatamansi* from four other *Valeriana* species namely *V. pseudofficinalis, V. officinalis* var. *latifolia, V. hardwickii* and *V. flaccidissima* (Diao et al. 2010). The genetic diversity of *V. Jatamansi* can also be evaluated using randomly amplified polymorphic DNA markers (Kumar et al. 2012). Jatamansone (valerenone), valerianannoids A–C, kaempferol, quercetin, hesperidine, and patchouli alcohol are some chemical markers used in chemical profiling. And among valepotriates, jatamanvaltrate B, chlorovaltrate F, jatamanvaltrate A, jatamanvaltrate D, jatamanvaltrate C, volvaltrate B, didrovaltrate, jatamanvaltrate H, chlorovaltrate

J, AHD-valtrate, 5-hydroxydidrovaltrate, 10-acetoxy valtrathydrin, IVHD valtrate, chlorovaltrate L-N, and acetoxyhydrin are also utilized for phytochemical profiling (Lin et al. 2015).

22.9.12 Viola pilosa

Viola pilosa is belonging to the family Violaceae and it has commonly known as 'Banafsha' which is characterized as a small perennial herb with smooth leaves and white violet flowers. *V. pilosa* are utilized for the preparation of several ailments at all over the country. Approximately 400 species of *Viola* reported all over the world, out of that 30 species are growing in India. *Viola* species hybridize easily thus, they are naturally accessible in multiple cytotypes with 2n = 20, 37, or 54, etc. (Anonymous 1976; Canne 1987). Around 5–7 long-stalked leaves are originated from the base of herb and approximately 5 heterogeneous petals are composed of flowers. The flowers are developed between March–May which has generated a pleasant smell.

Geographic Distribution: *V. Pilosa* is preliminarily grown in the Indian subcontinent and some parts of South Asia i.e. Afghanistan, Myanmar, Thailand, and Indonesia. *V. Pilosa* is predominantly dispersed in the temperature Zones and restricted to the high altitude of 1200–3000 m in the Himalayas from Afghanistan to South-East Asia (Anonymous 1965, 1976).

Phytochemistry: The phytochemical analysis of the extracts and fractions of *V. Pilosa* was analysed that indicated that methanolic extract consists of diverse groups of molecules such as alkaloids, carbohydrates, fats and oils, flavonoids, saponins, sterols, tannins, saponins, and proteins. Low polarity fractions like *n*-hexane and ethyl acetate do not have alkaloids present in them (Panni and Bakht 2018).

Medicinal Properties and Ethnomedical Usage: The leaves and flowers of *V. Pilosa* have used to treat several illnesses and it has shown diuretic, demulcent, expectorant diaphoretic, astringent, emollient, refrigerant, and purgative, purgative activity (Shinwari and Khan 2011). It has also been reported that *V. Pilosa* shows antimicrobial and is used for the treatment of eczema and inflammation (Adhikary et al. 2011).

Quality Control Approaches: A PCR-based methodology for the authentication of different *Viola* species by utilizing a species-specific DNA sequence.

22.10 Conclusions

Over the recent decade, the gradual development of various analytical techniques has led to a significant advancement in the standards of quality for herbals products. The employment of various hyphenated chromatographic techniques with the

DNA barcoding technique has revolutionized the quality control approach for medicinal plant material. Quality control of botanicals has become a stepwise procedure of validation and certification that employs both validated methodologies and reference materials established according to official monographs and guidelines of agencies such as the WHO, FDA, and Pharmacopoeias, etc. The three-step procedure includes the assessment of traceability information and other non-specific parameters such as ash content, extractive values, volatile oils, water and volatile matter, bitterness value, hemolytic activity, foaming index, swelling index, and total tannins. The second step involves the validation of morphological and taxonomical identity by the macro and microscopic examination of anatomical features and by the help of the modern DNA barcoding technique. The final step involves the assessment of its phytochemical composition (targeted as well as untargeted approaches) by the application of spectroscopic/spectrometric techniques so as generate the complete chemical profile of the herbal product to support its efficacy and also to confirm the absence of any toxic compound.

In this chapter, various aspect related to quality assessment of commercially important Himalayan medicinal plants which are in active trade in the international markets has been reviewed. In through survey of literature, it was found that there was a clear lack of uniform approach in the context of quality assessment of raw material for these Himalayan medicinal plants and the integrated approach has not been applied to access the authenticity of these plant species. Although, significant advancement has been made in the development of chemical markers and complete phytochemical fingerprinting, DNA barcoding and chemometric techniques have not been explored in the context of these commercially important Himalayan medicinal plants. If this issue is not addressed with immediate effect, adulteration could adversely impact the trade of the raw material in the global market. Hence, to enable the Indian herbal drug industry to capitalize the rich bioresource in the international market, there is a need to address the issue of adulteration in the context of the commercially important Himalayan medicinal plants by the adoption of uniform global standard practices for the assessment of herbal products.

Acknowledgements The authors are grateful to the Director, CSIR-IHBT for his regular support. Mr. Patil and Ms. Surekha thank CSIR, New Delhi for senior research fellowship. Mr. Anmol thank DST for DST-INSPIRE Fellowship. Financial support from CSIR, New Delhi [MLP0154 (Health Mission), and MLP0159 (Agri Nutri Biotech Mission)] is also acknowledged. CSIR-IHBT Communication No. for this chapter is 4679.

References

Adhikary P, Roshan KC, Kayastha D, Thapa D, Shrestha R (2011) Phytochemical screening and anti-microbial properties of medicinal plants. Int J Pharm Biol Sci Arch 2(6):1663–1667

Ahmad I, Ahmad Khan MS, Cameotra SS (2014) Quality assessment of herbal drugs and medicinal plant products. Encycl Anal Chem 2:1–17

Ahmed S, Hasan MM (2015) Crude drug adulteration: a concise review. World J Pharm Pharm Sci 4(10):274–283
Ahmad M, Yousuf S, Badruzzaman Khan MNH, Ahmad AS, Mubeen Ahmad Ansari TI, Ashok Kumar Agrawal FI (2006) A review on *Nardostachys jatamansi* DC a flowering plant with therapeutic and pharmacognostic profiles 9(4):870–873
Akbar S (2020) *Picrorhiza kurroa* Royle ex Benth. (Plantaginaceae). In: Handbook of 200 medicinal plants. Springer, Cham, pp 1409–1417
Akerele O (1992) WHO guidelines for the assessment of herbal medicines. Fitoterapia 63:99–110
Ali S, Ansari KA, Jafry MA, Kabeer H, Diwakar G (2000) *Nardostachys jatamansi* protects against liver damage induced by thioacetamide in rats. J Ethnopharmacol 71(3):359–363
American Herbal Products Association (2006) Good agricultural and collection practice for herbal raw materials. Am Herb Pharmacopoeia
Andola HC, Rawal RS, Bhatt ID (2008) Antioxidants in fruits and roots of *Berberis asiatica* Rox. ex. DC: a highly valued Himalayan plant. Natl Acad Sci Lett 31(11):337–340
Anonymous (1965) Indian pharmacopoeia. Edn Govt of India. https://doi.org/10.1080/14786419.2020.1762184
Anonymous (1976) Wealth of India. PID, CSIR, New Delhi 10:514–517. https://doi.org/10.1080/14786419.2019.1700245
Arora RB, Madan BR (1956) Antiarrhythmics. III. Antiarrhythmic activity of Nardostachys jatamansi (an Indian indigenous drug). Indian J Med Res 44(2):259
Arora MS, Kanta C (1962) Tranquilizing activity of jatamansone—a sesquiterpene from *Nardostacys jatamansi*. Life Sci 1(6):225–228
Asghar M, Islam M, Saeed H, Imtiaz F, Saleem B, Saleem Z, Iqtedar M (2018) Investigations on *Onosma Hispidum* wall root extracts for in-vitro antidiabetic, proliferative and cytotoxic effects. J Anim Plant Sci 28(5):1339–1347
Ashfaque M, Ahmad N, Begum Z, Nasreen F (2017) Evaluation of antihypertensive activity of Sumbul-ut-Tib (*Nardostachys jatamansi*) in adrenaline induced dog's blood pressure. J Pharmacogn Phytochem 6(1):93–95
Avula B, Wang YH, Smillie TJ, Duzgoren-Aydin NS, Khan IA (2010) Quantitative determination of multiple elements in botanicals and dietary supplements using ICP-MS. J Agric Food Chem 58:8887–8894
Bagchi A, Oshima Y, Hikino H (1991) Neolignans and lignans of *Nardostachys jatamansi* roots. Planta Med 57(01):96–97
Bajpai V, Singh A, Arya KR, Srivastava M, Kumar B (2015) Rapid screening for the adulterants of *Berberis aristata* using direct analysis in real-time mass spectrometry and principal component analysis for discrimination. Food Addit Contam Part A 32:799–807
Balammal G, Sekar Babu M, Reddy PJ (2012) Analysis of herbal medicines by modern chromatographic techniques. Int J Preclin Pharm Res 3(1):50–63
Balasubramani SP, Goraya GS, Venkatasubramanian P (2011) Development of ITS sequence-based markers to distinguish *Berberis aristata* DC. from B. lycium Royle and *B. asiatica* Roxb. Biotech 1(1):11–19
Bamhania K, Khatakar S, Punia A, Yadav OP (2013) Genetic variability analysis using ISSR markers in *withania somnifera* L. Dunal genotypes from different regions. J Herbs Spices Med Plants 19:22–32
Bandaranayake, WM (2006) Quality control, screening, toxicity, and regulation of herbal drugs. Modern Phytomed 25–57
Bansal A, Chhabra V, Rawal RK, Sharma S (2014) Chemometrics: a new scenario in herbal drug standardization. J Pharm Anal 4(4):223–233
Blasko G, Murugesan N, Freyer AJ, Shamma M, Ansari AA (1982) Karachine: an unusual protoberberine alkaloid. J Am Chem Soc 104(7):2039–2041
Brown AC (2017) An overview of herb and dietary supplement efficacy, safety and government regulations in the United States with suggested improvements. Food Chem Toxicol 107(A):449–471

Calahan J, Howard D, Almalki AJ, Gupta MP (2016) Chemical adulterants in herbal medicinal products: a review. Planta Med 82(6):505–515
Canne JM (1987) Determinations of chromosome numbers in *Viola* (Violaceae). Can J Bot 65(4):653–655
Case FW Jr, Case RB (1997) Trilliums. Timber Press 1:285
Chan K (2003) Some aspects of toxic contaminants in herbal medicines. Chemosphere 52(9):1361–1371
Charak SS, Pratti P, Pandey BN, Chandra S (2018) Evaluation of ecological attributes of *Jurinea macrocephala* (royle) C.B. Clarke, A threatened medicinal herb of western Himalayas. Eco Chronicle 13(3):83–88
Chatterjee A, Basak B, Datta U, Banerji A, Neuman PT (2005) Studies on the chemical constituents of *Nardostachys jatamansi* DC (Valerianaceae). Cheminform 44B(02):430–433
Chaudhari RK, Girase NO (2015) Determination of soluble extractives and physico-chemical studies of bark of *Sesbania sesban* (L) Merr. J Chem Pharm 7(8):657–660
Chauhan HK, Bisht AK, Bhatt ID, Bhatt A, Gallacher D (2019) Trillium–toward sustainable utilization of a biologically distinct genus valued for traditional medicine. Bot Rev 85(3):252–272
Chen S, Pang X, Song J, Shi L, Yao H, Han J, Leon C (2014) A renaissance in herbal medicine identification: from morphology to DNA. Biotechnol Adv 32(7):1237–1244
Chen F, Chan HYE, Wong KL, Wang J, Yu MT, But PPH, Shaw PC (2008) Authentication of *Saussurea lappa,* an endangered medicinal material, by ITS DNA and 5S rRNA sequencing. Planta Med 74(08):889–892
Chou T (1947) Some minor alkaloids of Pei-Mu, *Fritillaria roylei.* J Am Pharm Assoc Am Pharm Assoc (Baltim) 36(7):215
Cimino MT (2010) Successful isolation and PCR amplification of DNA from national institute of standards and technology herbal dietary supplement standard reference material powders and extracts. Planta Med 76(5):495
Commisso M, Strazzer P, Toffali K, Stocchero M, Guzzo F (2013) Untargeted metabolomics: an emerging approach to determine the composition of herbal products. Comput Struct Biotechnol J 4(5):1–7
Culley TM (2013) Why vouchers matter in botanical research. Appl Plant Sci 1(11):1–5
Das S, Das MK, Mazumder PM, Das S, Basu SP (2009) Cytotoxic activity of methanolic extract of *Berberis aristata* DC on colon cancer. Glob J Pharmacol 3(3):137–140
Debnath P, Rathore S, Walia S, Kumar M, Devi R, Kumar R (2020) *Picrorhiza kurroa*: a promising traditional therapeutic herb from higher altitude of western Himalayas. J Herb Med 23:100358
Dhami N, Mishra AD (2015) Phytochemical variation: how to resolve the quality controversies of herbal medicinal products? J Herb Med 5(2):118–127
Dhyani A, Nautiyal BP, Nautiyal MC (2010) Importance of Astavarga plants in traditional systems of medicine in Garhwal, Indian Himalaya. Int J Biodivers Sci Ecosyst Serv Manag 6(1–2):13–19
Diao Y, Duan WT, Lin XM, Liao C, Hu Z, Wang Y (2010) Molecular authentication of *Valeriana jatamansi* by ARMS based on 5S rDNA sequence. Acta Pharm Sin 45(8):1067–1070
Dogan B, Duran A, Hakki E (2009) Numerical analyses of wild *Jurinea* spp. (asteraceae) in turkey. Bangladesh J Bot 38(1):47–53
Dondorp AM, Smithuis FM, Woodrow C, von Seidlein L (2017) How to contain artemisinin- and multidrug-resistant falciparum malaria. Trends Parasitol 33(5):353–363
European Medicines Agency/Herbal Medicinal Products Committee (2006) Guideline on good agricultural and collection practice (GACP) for starting materials of herbal origin
Gad HA, El-Ahmady SH, Abou-Shoer MI, Al-Azizi MM (2013) Application of chemometrics in authentication of herbal medicines: a review. Phytochem Anal 24(1):1–24
Gilani AH, Janbaz KH, Aziz N, Herzig MJU, Kazmi MM, Choudhary MI, Herzig JW (1999) Possible mechanism of selective inotropic activity of the n-butanolic fraction from *Berberis aristata* fruit. Gen Pharmacol Vasc Syst 33(5):407–414

Gopalkrishna V, Verma H, Patil P, Kolhapure R (2008) Antiviral activity of the Indian medicinal plant extract, *Swertia chirata* against herpes simplex viruses: a study by in-vitro and molecular approach. Indian J Med Microbiol 26(4):322

Gottumukkala VR, Annamalai T, Mukhopadhyay T (2011) Phytochemical investigation and hair growth studies on the rhizomes of *Nardostachys jatamansi* DC. Pharmacogn Mag 7(26):146–150

Govindaraghavan S, Sucher NJ (2015) Quality assessment of medicinal herbs and their extracts: criteria and prerequisites for consistent safety and efficacy of herbal medicines. Epilepsy Behav 52:363–371

Gupta RK, Disket J, Mann S (2012) A review on spikenard (*Nardostachys jatamansi* DC.) An 'endangered' essential herb of India. Int J Pharm Chem 2:52–60

Hall RD (2006) Plant metabolomics: from holistic hope, to hype, to hot topic. New Phytol 169(3):453–468

Hao DC, Gu XJ, Xiao PG (2015) Medicinal plants: Chemistry, Biology and Omics. Woodhead Publishing

Harnly J, Chen P, Sun J, Huang H, Colson KL, Yuk J, McCoy JAH, Reynaud DTH, Harrington PB, Fletcher EJ (2015) Comparison of flow injection MS, NMR, and DNA sequencing: methods for identification and authentication of black cohosh (*Actaea racemosa*). Planta Med 82(3):250

He X, Wang S, Shi J, Sun Z, Lei Z, Yin Z, Xie H (2018) Genotypic and environmental effects on the volatile chemotype of *valeriana jatamansi* jones. Front Plant Sci 9:1003

Heubl G (2010) New aspects of DNA-based authentication of Chinese medicinal plants by molecular biological techniques. Planta Med 76(17):1963–1974

Huang H, Sun J, McCoy JA, Zhong H, Fletcher EJ, Harnly J, Chen P (2015) Use of flow injection mass spectrometric fingerprinting and chemometrics for differentiation of three black cohosh species. Spectrochim Acta Part B Spectrosc 105:121–129

Hussain F, Ali A, Shahid M (2017) Antidiabetic potential of *Onosma hispidum* roots. West Indian Med J

Indrayanto G (2018) Recent development of quality control methods for herbal derived drug preparations. Nat Prod Commun 13(12):1599–1606

Ismail M, Shah MR, Adhikari A, Anis I, Ahmad MS, Khurram M (2015) Govanoside A, a new steroidal saponin from rhizomes of *Trillium govanianum*. Steroids 104:270–275

Jacobs A, Craig C (1942) The aconite alkaloids Ix. The isolation of two new alkaloids from *Aconitum heterophyllum*, heteratisine and hetisine. J Biol Chem 143(3):605–609

Jarouche M, Suresh H, Low M, Lee S, Xu C, Khoo C (2019) Quality control and variability assessment of an eight-herb formulation for hypertension using method validation and statistical analysis. Molecules 24(8):1520

Jha SV, Bhagwat AM, Pandita NS (2012) Pharmacognostic and Phytochemical studies on the rhizome of *Nardostachys jatamansi* DC using different extracts. Pharmacogn J 4(33):16–22.

Jiang RW, Ma SC, But PPH, Dong H, Mak TCW (2001) Sipeimine, a steroidal alkaloid from *Fritillaria roylei* Hooker. Acta Crystallogr Sect C Cryst Struct Commun 57(2):170–171

Johnson R (2014) Food fraud and 'economically motivated adulteration' of food and food ingredients. Congr Res Serv Rep 40

Joshi P, Dhawan V (2005) *Swertia chirayita*—an overview. Curr Sci 89(4):635–640

Joshi PV, Shirkhedkar AA, Prakash K, Maheshwari VL (2011) Antidiarrheal activity, chemical and toxicity profile of *Berberis aristata*. Pharma Bio 49(1):94–100

Jugran AK, Rawat S, Bhatt ID, Rawal RS (2019) *Valeriana jatamansi*: an herbaceous plant with multiple medicinal uses. Phyther Res 33(3):482–503

Kala CP (2003) Medicinal plants of Indian trans-Himalaya: focus on Tibetan use of medicinal resources. Bishen Singh Mahendra Pal Singh, Dehra Dun

Kala CP, Mathur VB (2002) Patterns of plant species distribution in the Trans-Himalayan region of Ladakh India. J Veg Sci 13(6):751–754

Katanić Stanković JS, Ceylan R, Zengin G, Matić S, Jurić T, Diuzheva A, Aktumsek (2020) Multiple biological activities of two Onosma species (*O. sericea* and O. *stenoloba*) and HPLC-MS/MS characterization of their phytochemical composition. Ind Crops Prod 144:112053

Katiyar D, Singh RK, Singh S, Singh D, Singh V (2011) Isolation and characterization of n-docosane from heartwood of *Berberis aristata*. Intl J Pharm Sci Res 2(2):331

Khan IA, Smillie T (2012) Implementing a 'quality by design' approach to assure the safety and integrity of botanical dietary supplements. J Nat Prod 75(9):1665–1673

Khan Kashif M, Nahar L, Al-Groshi A, Zavoianu AG, Evans A, Dempster NM, Wansi JD, Ismail FMD, Mannan A, Sarker SD (2016) Cytotoxicity of the roots of *Trillium govanianum* against breast (MCF7), liver (HepG2), lung (A549) and urinary bladder (EJ138) carcinoma cells. Phytother Res 30(10):1716–1720

Khan MB, Hoda MN, Ishrat T, Ahmad S, Khan MM, Ahmad A, Yusuf S, Islam F (2012) Neuroprotective efficacy of *Nardostachys jatamansi* and crocetin in conjunction with selenium in cognitive impairment. Neurol Sci 33(5):1011–1020

Khanal S, Shakya N, Nepal N, Pant D (2014) *Swertia chirayita*: the Himalayan herb. Int J Appl Sci Biotechnol 2(4):389–392

Kirkan B, Sarikurkcu C, Ozer MS, Cengiz M, Atılgan N, Ceylan O, Tepe B (2018) Phenolic profile, antioxidant and enzyme inhibitory potential of *Onosma tauricum* var. tauricum. Ind Crops Prod 125:549–555

Kirtikar KR, Basu BD (1995) Indian medicinal plants International book distributors, pp 1–456

Knoess W, Wiesner J (2019) The globalization of traditional medicines: perspectives related to the European union regulatory environment. Engineering 5(1):22–31

Kreuzer M, Howard C, Adhikari B, Pendry CA, Hawkins JA (2019) Phylogenomic approaches to DNA barcoding of herbal medicines: developing clade-specific diagnostic characters for *Berberis*. Front Plant Sci 10:586

Kroes BH (2014) The legal framework governing the quality of (traditional) herbal medicinal products in the European Union. J Ethnopharmacol 158(2):449–453

Krupashree K, Hemanth Kumar K, Rachitha P, Jayashree GV, Khanum F (2014) Chemical composition, antioxidant and macromolecule damage protective effects of *Picrorhiza kurroa* Royle ex Benth. South African J Bot 94:249–254

Kumar A, Agnihotri VK (2020) Phytochemical studies of Jurinea macrocephala roots from Western Himalaya phytochemical studies of *Jurinea macrocephala* roots from Western Himalaya. Nat Prod Res 34(3):421–424

Kumar A, Shirkot P, Thakur AK, Raina R (2012) Assessment of genetic diversity in *Valeriana jatamansi* Jones. Germplasm using RAPD markers. Natl Acad Sci Lett 35(3):221–226

Kumar H, Spandana M (2013) Qualitative and quantitative study of anthraquinone derivatives in the root extract of *Rheum australe* of Nepal (syn. *Rheum emodi*) by HPLC. J Pharmacogn Phytochem 2(4):40–42

Kumar N, Gupta AK (2009) Wound-healing activity of *onosma hispidum* (ratanjot) in normal and diabetic rats. J Herbs Spices Med Plants 15(4):342–351

Kumar N, Gupta AK, Prakash D, Kumar P (2010) Hypoglycemic activity of *Onosma hispidum* (Ratanjot). Int J Diabetes Dev Ctries 30(4):213–216

Kumar N, Kumar R, Kishore K (2013) Onosma L.: a review of phytochemistry and ethnopharmacology. Pharmacogn Rev 7(14):140

Kumar P, Partap M, Ashrita RD, Kumar P, Warghat AR (2020) Metabolite and expression profiling of steroidal alkaloids in wild tissues compared to bulb derived in vitro cultures of Fritillaria roylei—High value critically endangered Himalayan medicinal herb. Ind Crops Prod 145:1–11

Kumar V, Van Staden J (2016) A review of *Swertia chirayita* (Gentianaceae) as a traditional medicinal plant. Front Pharmacol 6(308):1–14

Kunle OF, Egharevba HO, Ahmadu PO (2012) Standardization of herbal medicines—a review. Int J Biodiv Conserv 4(3):101–112

Kunwar RM, Kshhetri HB, Rai SK, Bussmann RW (2006) Ethnomedicine in Himalaya: a case study from Dolpa, Humla, Jumla and Mustang districts of Nepal. J Ethnobiol Ethnomed 2(1):27

Latif A, Rehman S (2014) Original research paper standardization of a herbal medicine-*swertia chirayita* linn. Pharmacophore 5(1):98–108

Liang Z, Sham T, Yang G, Yi L, Chen H, Zhao Z (2013) Profiling of secondary metabolites in tissues from *Rheum palmatum* L. using laser microdissection and liquid chromatography mass spectrometry. Anal Bioanal Chem 405(12):4199–4212

Lin S, Ye J, Liang X, Zhang X, Su J, Fu P, Yang XW (2015) Mass spectrometric profiling of valepotriates possessing various acyloxy groups from *Valeriana jatamansi*. J Mass Spectrom 50(11):1294–1304

Lin S, Zhang ZX, Chen T, Ye J, Dai WX, Shan L, Xu XK (2013) Characterization of chlorinated valepotriates from *Valeriana jatamansi*. Phytochemistry 85:185–193

Manning L, Soon JM (2014) Developing systems to control food adulteration. Food Policy 49:23–32

Maqbool KK, Nahar L, Mannan A, Ul-Haq I, Arfan M, Ali Khan G, Hussain I, Sarker SD (2018) Cytotoxicity, in vitro anti-leishmanial and fingerprint HPLC-photodiode array analysis of the roots of *Trillium govanianum*. Nat Prod Res 32(18):2193–2201

Masood M (2015) *Picrorhiza kurroa*: an ethnopharmacologically important plant species of Himalayan region. Pure Appl Biol 4(3):407

Monakhova YB, Holzgrabe U, Diehl BWK (2018) Current role and future perspectives of multivariate (chemometric) methods in NMR spectroscopic analysis of pharmaceutical products. J Pharm Biomed Anal 147:580–589

Mosihuzzaman M, Choudhary MI (2008) Protocols on safety, efficacy, standardization, and documentation of herbal medicine (IUPAC technical report). Pure Appl Chem 80(10):2195–2230

Mude S, Khandar AS, Gulhane S, Mude G (2020) A review on *Nardostachys jatamansi* DC a flowering plant with therapeutic and pharmacognostic profiles. J Pharmacogn Phytochem 9(4):870–873

Nafiu MO, Hamid AA, Muritala HF, Adeyemi SB (2017) Preparation, standardization, and quality control of medicinal plants in Africa. In: Kuete V Medicinal spices and vegetables from Africa: therapeutic potential against metabolic, inflammatory, infectious and systemic disease. Academic Press, pp 171–204

Nasreen S, Radha R, Jayashree N, Selvaraj B, Rajendran A (2010) Assessment of quality of *Tinospora cordifolia* (WILD) MIERS (Menispermaceae) pharmacognostical and phytophysicochemical profile. Pharmacie Globale 5(03):1–4

Navarrete A, Avula B, Choi YW, Khan IA (2006) Chemical fingerprinting of valeriana species: simultaneous determination of valerenic acids, flavonoids, and phenylpropanoids using liquid chromatography with ultraviolet detection. J AOAC Int 89(1):8–15

Nisar M, Obaidullah, Ahmad M, Wadood N, Lodhi MA, Shaheen F, Choudhary MI (2009) New diterpenoid alkaloids from Aconitum heterophyllum Wall: Selective butyrylcholinestrase inhibitors. J Enzyme Inhib Med Chem 24(1):47–51

Naz S, Ahmad S, Ajaz Rasool S, Sayeed SA, Siddiqi R (2006) Antibacterial activity directed isolation of compounds from *Onosma hispidum*. Microbiol Res 161(1):43–48

Nelson Kumar S, Ravindrareddy K, Kanhere RS, Krishna YZ, Raja K, Mahesh K (2011) Antidiabetic activity of ethanolic extract of *Nardostachys jatamansi* on alloxan induced diabetic rat. Int J Adv Pharm Res 2(6):263–268

Nikam PH, Kareparamban J, Jadhav A, Kadam V (2012) Future trends in standardization of herbal drugs. J Appl Pharm Sci 2(6):38–44

Oms-Oliu G, Odriozola-Serrano I, Martín-Belloso O (2013) Metabolomics for assessing safety and quality of plant-derived food. Food Res Int 54(1):1172–1183

Ordoudi SA, Cagliani LR, Melidou D, Tsimidou MZ, Consonni R (2017) Uncovering a challenging case of adulterated commercial saffron. Food Control 81:147–155

Pal D, Sur S, Mandal S, Das A, Roy A, Das S, Panda CK (2012) Prevention of liver carcinogenesis by amarogentin through modulation of G 1/S cell cycle check point and induction of apoptosis. Carcinogenesis 33(12):2424–2431

Pandith SA, Dar RA, Lattoo SK, Shah MA, Reshi ZA (2018) *Rheum australe*, an endangered high-value medicinal herb of North Western Himalayas: a review of its botany, ethnomedical uses, phytochemistry and pharmacology. Phytochem Rev 17(3):573–609

Panni MK, Bakht J (2018) In vitro antibacterial activity and phyto-chemistry of samples from the roots of *Viola pilosa.* Pak J Pharm Sci 31(6):2523–2527
Pant J, Rajasekaran A (2011) HPTLC fingerprinting profile of marker compound (Berberine) in roots of *Berberis aristata* DC. Pharmacogn J 3(19):41–44
Paramanick D, Panday R, Shukla SS, Sharma V (2017) Primary pharmacological and other important findings on the medicinal plant "*Aconitum Heterophyllum"* (Aruna). J Pharmacopuncture 20(2):89–92
Pasrija A, Singh R, Katiyar CK (2010) Validated HPLC-UV method for the determination of berberine in raw herb Daruharidra (*Berberis aristata* DC), its extract, and in commercially marketed ayurvedic dosage forms. Intl J Ayu Res 1(4):243
Patel DK, Patel K, Dhanabal SP (2012) Standardization of *Berberis aristata* extract through conventional and modern HPTLC techniques. Asian Pac J Trop Dis 2:S136–S140
Pennisi E (2007) Taxonomy wanted: a barcode for plants. Science 80:190–191
Pferschy-Wenzig EM, Bauer R (2015) The relevance of pharmacognosy in pharmacological research on herbal medicinal products. Epilepsy Behav 52(B):344–362
Potdar D, Hirwani RR, Dhulap S (2012) Phyto-chemical and pharmacological applications of *Berberis aristata.* Fitoterapia 83(5):817–830
Pradhan N, Gavali J, Waghmare N (2015) WHO (World Health Organization) guidelines for standardization of herbal drugs. Int Ayurvedic Med J 3(8):2238–2248
Prasad SK, Jain D, Patel DK, Sahu AN, Hemalatha S (2014) Antisecretory and antimotility activity of *Aconitum heterophyllum* and its significance in treatment of diarrhea. Indian J Pharmacol 46(1):82
Purnima MB, Kothiyal P (2015) A review article on phytochemistry and pharmacological profiles of *Nardostachys jatamansi* DC-medicinal herb. J Pharmacogn Phytochem 3(5):102–106
Rahman H, Shaik HA, Madhavi P, Eswaraiah MC (2011) A review: pharmacognostics and pharmacological profiles of *nardastachys jatamansi* dc. Elixir Pharm 39:5017–5020
Rahman SU, Adhikari A, Ismail M, Shah MR, Khurram M, Anis I, Ali F (2017) A new trihydroxylated fatty acid and phytoecdysteroids from rhizomes of *Trillium govanianum.* Antiseptic 3:5
Rani S, Rana JC, Rana PK (2013) Ethnomedicinal plants of Chamba district, Himachal Pradesh India. J Med Plant Res 7(42):3147–3157
Rao VS, Rao A, Karanth KS (2005) Anticonvulsant and neurotoxicity profile of *Nardostachys jatamansi* in rats. J Ethnopharmacol 102(3):351–356
Rather AM, Nawchoo IA, Ganie AH, Singh H, Dutt B, Wani AA (2012) Bioactive compounds & medicinal properties of *Valeriana jatamansi* Jones—a review. Life Sci J 9(2):847–850
Rawat JM, Rawat B, Mehrotra S, Chandra A, Nautiyal S (2013) ISSR and RAPD based evaluation of genetic fidelity and active ingredient analysis of regenerated plants of *Picrorhiza kurroa.* Acta Physiol Plant 35(6):1797–1805
Rokaya MB, Münzbergová Z, Timsina B, Bhattarai KR (2012) *Rheum australe* D. Don: a review of its botany, ethnobotany, phytochemistry and pharmacology. J Ethnopharmacol 141(3):761–774
Rooney JS, McDowell A, Strachan CJ, Gordon KC (2015) Evaluation of vibrational spectroscopic methods to identify and quantify multiple adulterants in herbal medicines. Talanta 138:77–85
Shukla SS (2009) Approaches towards standardization and quality assessment of herbals. J Res Educ Indian Med 15(1):25–32
Sack RB, Froehlich JL (1982) Berberine inhibits intestinal secretory response of *Vibrio cholerae* and *Escherichia coli* enterotoxins. Inf Immu 35(2):471–475
Sahoo N, Manchikanti P (2013) Herbal drug regulation and commercialization: an Indian industry perspective. J Altern Complement Med 19(12):957–963
Sahoo N, Manchikanti P, Dey S (2010) Fitoterapia herbal drugs: standards and regulation. Fitoterapia 81(6):462–471
Sahu R, Dhongade HJ, Pandey A, Sahu P (2016) Medicinal Properties of *Nardostachys jatamansi* (a review) Orient J Chem 32(2):859–866
Samant SS, Dhar U, Palni LMS (1998) Medicinal plants of Indian Himalaya. Gyanodaya Prakashan

Sarikurkcu C, Sahinler SS, Tepe B (2020) *Onosma aucheriana*, *O. frutescens*, and *O. sericea*: phytochemical profiling and biological activity. Ind Crops Prod 154:112633
Sastri B (1950) The wealth of India, a dictionary of raw material and industrial products. Publ Inf Dir (CSIR New Delhi)
Savaliya AA, Shah RP, Prasad B, Singh S (2010) Screening of Indian aphrodisiac ayurvedic/herbal healthcare products for adulteration with sildenafil, tadalafil and/or vardenafil using LC/PDA and extracted ion LC-MS/TOF. J Pharm Biomed Anal 52(3):406–409
Seethapathy GS, Balasubramani SP, Venkatasubramanian P (2014) nrDNA ITS sequence based SCAR marker to authenticate *Aconitum heterophyllum* and *Cyperus rotundus* in ayurvedic raw drug source and prepared herbal products. Food Chem 145:1015–1020
Sekhon NC, Bhupinder S (2011) An overview of advances in the standardization of herbal drugs. J Pharm Educ Res 2(2):55–70
Shafi H, Nawchoo IA, Shah SA, Kaloo ZA (2018) Ex situ conservation strategies for *Fritillaria cirrhosa* D. Don (Liliaceae): a critically endangered medicinal herb of Kashmir Himalaya. Proc Natl Acad Sci India Sect B Biol Sci 88(1):33–41
Shah NA, Khan MR, Naz K, Khan MA (2014) Analysis of neglected medicinal *Jurinea dolomiaea* Roots. BioMed Res Int 1(1):1–4
Sharma S, Mehta V, Sharma P, Jaggi K, Udayabanu M, Sood H (2018) Antifertility activity and contraceptive potential of the hydroalcoholic rhizome extract of *Trillium govanianum* in female wistar rats. Asian J Pharm Clin Res 11(11):329–332
Sharma S, Sharma A, Mehta V, Chauhan RS, Malairaman U, Sood H (2016) Efficient hydroalcoholic extraction for highest diosgenin content from *Trillium govanianum* (Nag chhatri) and it's in vitro anticancerous activity. Asian J Pharm Clin Res 9(4):386–392
Sharma SK, Singh AP (2012) In vitro antioxidant and free radical scavenging activity of *Nardostachys jatamansi* DC. J Acupunct Meridian Stud 5(3):112–118
Sharma V, Wani MS, Singh V, Kaur K, Gupta RC (2017) Development and characterization of novel microsatellite markers in *Trillium govanianum*: a threatened plant species from North-Western Himalaya. Biotech 7(3):190
Shinde VM, Dhalwal K (2010) DNA fingerprinting of *Tinospora cordifolia* using RAPD analysis. J Glob Pharma Technol 2:38–42
Shinwari MI, Khan MA (2011) Folk use of medicinal herbs of Margalla hills national park Islamabad. Am Eurasian J Sustain Agric 69(1):45–56
Simmler C, Chen SN, Anderson J, Lankin DC, Krause E, Dietz B, Bolton JL, Nikolic D, van Breemen RB, Pauli GF (2016) Botanical integrity: part 2. Traditional and holistic modern analytical approaches. HerbalEGram 109:60–64
Simmler C, Graham JG, Chen SN, Pauli GF (2018) Integrated analytical assets aid botanical authenticity and adulteration management. Fitoterapia 129:401–414
Simmler C, Kulakowski D, Lankin DC, McAlpine JB, Chen S-N, Pauli GF (2016) Holistic analysis enhances the description of metabolic complexity in dietary natural products. Adv Nutr 7(1):179–189
Simmler C, Napolitano JG, McAlpine JB, Chen SN, Pauli GF (2014) Universal quantitative NMR analysis of complex natural samples. Curr Opin Biotechnol 25:51–59
Singh AP (2004) Kutkins—a review of Chemistry and pharmacology. Ethnobot Leafl 2004(1):9
Singh M, Srivastava S, Rawat AKS (2007) Antimicrobial activities of Indian Berberis species. Fitoterapia 78(7–8):574–576
Singh N, Gupta AP, Singh B, Kaul VK (2005) Quantification of picroside-I and picroside-II in Picrorhiza kurroa by HPTLC. J Liq Chromatogr Relat Technol 28(11):1679–1691
Singh P, Sharma RK (2020) Development of informative genic SSR markers for genetic diversity analysis of *Picrorhiza kurroa*. J Plant Biochem Biotechnol 29(1):144–148
Singh K, Kumar P, Kumar P, Lone JF Gairola PRS (2020a) Morpho-anatomical and palynological standardization and DNA barcoding of *Fritillaria cirrhosa* D Don. (*Fritillaria roylei* Hook). Plant Arch 20(2):1304–1313

Singh P, Singh R, Sati N, Sati OP, Kumar N (2015) Antioxidant and antibacterial activity of *Jurinea dolomiaea* boiss extracts. Int J Life Sci Sci Res 1:74–78

Singh PP, Bora PS, Suresh PS, Bhatt V, Sharma U (2020b) Qualitative and quantitative determination of steroidal saponins in *Trillium govanianum* by UHPLC-QTOF-MS/MS and UHPLC-ELSD. Phytochem Anal 31 (6): 861–873

Singh PP, Suresh PS, Bora PS, Bhatt V, Sharma U (2020c) Govanoside B, a new steroidal saponin from rhizomes of *Trillium govanianum.* Nat Prod Res https://doi.org/10.1080/14786419.2020.1761360

Sivakumar R, Kamachandran Nair AG (1991) Polyphenolic constituents of the flowers of *Berberis aristata.* J Indian Chem Soc 68(9):531–532

Skalicka-Woźniak K, Georgiev MI, Orhan IE (2017) Adulteration of herbal sexual enhancers and slimmers: the wish for better sexual well-being and perfect body can be risky. Food Chem Toxicol 108:355–364

Smillie TJ, Khan IA (2010) A comprehensive approach to identifying and authenticating botanical products. Clin Pharmacol Ther 87(2):175–186

Sohni YR, Bhatt RM (1996) Activity of a crude extract formulation in experimental hepatic amoebiasis and in immunomodulation studies. J Ethnopharmacol 54(2–3):119–124

Spink J, Moyer DC (2013) Understanding and combating food fraud. Food Technol 67(1):1

Srirama R, Santhosh Kumar JU, Seethapathy GS, Newmaster SG, Ragupathy S, Ganeshaiah KN, Uma Shanker R, Ravikanth G (2017) Species adulteration in the herbal trade: causes, consequences and mitigation. Drug Saf 40(8):651–661

Sun J, Chen P (2011) A flow-injection mass spectrometry fingerprinting method for authentication and quality assessment of *Scutellaria lateriflora*-based dietary supplements. Anal Bioanal Chem 401(5):1577

Sundaresan N, Ilango K (2018) Review on valeriana species-*valeriana wallichii* and *valeriana jatamansi.* J Pharm Sci Res 10(11):2697–2701

Surekha D, Rajan VST, Kumar NA, Parameswari SA, Chetty CM (2016) A review on role of quality control and quality assurance system in regulation of herbal drugs. Int J Res Phytochem Pharmacol 6(2):32–40

Tabin S, Gupta RC, Kamili AN, Bansal G (2016) Phytochemical analysis of wild and in vitro raised plants of rheum species using HPLC. Biochem Pharmacol Open Access 5(4):1–7

Techen N, Parveen I, Pan Z, Khan IA (2014) DNA barcoding of medicinal plant material for identification. Curr Opin Biotechnol 25:103–110

The United States Pharmacopeia (2013) Monographs in the herbal medicines compendium guidelines documents version 1.0. The United States Pharmacopeia

The United States Pharmacopeia Committe (2016) Ensuring the quality of dietary supplements, The United States Pharmacopeia

Tripathi YB, Shukla SD (1996) *Berberis aristata* inhibits PAF induced aggregation of rabbit platelets. Phytother Res 10(7):628–630

U.S. Food and Drug Administration (2007) Current good manufacturing practice in manufacturing, packaging, labeling, or holding operations for dietary supplements. In: Federal register. U.S. Food and Drug Administration, pp 34751–34958

Uerpmann-Wittzack R (2017) European Directorate for the quality of medicines and healthcare (EDQM). In: The Council of Europe : Its law and policies, pp 394–406

Ukani MD, Mehta NK, Nanavati DD (1996) *Aconitum heterophyllum* (ativisha) in ayurveda. Anc Sci Life 16(2):166–171

United States Pharmacopeia (2017) Elemental impurities-procedures/chemical tests. In: USP40-NF Gen. Chapters, pp 8066–8069

Ur Rahman S, Adhikari A, Ismail M, Raza Shah M, Khurram M, Shahid M, Ali F, Haseeb A, Akbar F, Iriti M (2016) Beneficial effects of *Trillium govanianum* rhizomes in pain and inflammation. Molecules 21(8):1095

Ur Rahman S, Ismail M, Shah MR, Iriti M, Shahid M (2015) GC/MS analysis, free radical scavenging, anticancer and β-glucuronidase inhibitory activities of *Trillium govanianum* rhizome. Bangladesh J Pharmacol 10(3):577–583

Vaclavik L, Krynitsky AJ, Rader JI (2014) Mass spectrometric analysis of pharmaceutical adulterants in products labeled as botanical dietary supplements or herbal remedies: a review. Anal Bioanal Chem 406(27):6767–6790

Ved DK, Goraya GS (2008) Demand and supply of medicinal plants. Medplant-ENVIS Newslett Med Plants 1(1):2–4

Ved DK, Goraya GS (2007) Demand and supply of medicinal plants in India. NMPB, New Delhi & FRLHT, Bangalore, India

Venkatasubramanian P, Kumar KS, Nair VSN (2010) *Cyperus rotundus*, a substitute for *Aconitum heterophyllum*: Studies on the ayurvedic concept of Abhava Pratinidhi Dravya (drug substitution). J Ayurveda Integr Med 1(1):33

Verma PC, Basu V, Gupta V, Saxena G, Ur Rahman L (2009) Pharmacology and Chemistry of a potent hepatoprotective compound picroliv isolated from the roots and rhizomes of *Picrorhiza kurroa* Royle ex Benth. (Kutki). Curr Pharm Biotechnol 10(6):641–649

Vidyarthi S, Samant SS, Sharma P (2013) Dwindling status of *Trillium govanianum* Wall. ex D. Don-A case study from Kullu district of Himachal Pradesh, India. J Med Plant Res 7(8):392–397

Wang CZ, Li P, Ding JY, Jin GQ, Yuan CS (2005) Identification of *Fritillaria pallidiflora* using diagnostic PCR and PCR-RFLP based on nuclear ribosomal DNA internal transcribed spacer sequences. Planta Med 71(4):384–386

Wang CZ, Maier UH, Eisenreich W, Adam P, Obersteiner I, Keil M, Zenk MH (2001) Unexpected biosynthetic precursors of amarogentin—a retrobiosynthetic 13C NMR study. European J Org Chem 2001(8):1459–1465

Wang P, Yu Z (2015) Species authentication and geographical origin discrimination of herbal medicines by near infrared spectroscopy: a review. J Pharm Anal 5(5):277–284

Warude D, Chavan P, Joshi K, Patwardhan B (2003) DNA isolation from fresh and dry plant samples with highly acidic tissue extracts. Plant Mol Biol Report 21(4):467–467

Whitsitt V, Beehner C, Welch C (2013) The role of good manufacturing practices for preventing dietary supplement adulteration. Anal Bioanal Chem 405(13):4353–4358

WHO (1993) Research guidelines for evaluating the safety and efficacy of herbal medicines. Res Guidel Eval Saf Effic Herb Med

WHO expert committe (2017) WHO guidelines for selecting marker substances of herbal medicines

WHO Expert Committee (2011) Quality control methods for herbal materials. World Health Organisation

World Health Organization & ŚOZ (2003) WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants. World Health Organization

World Health Organization (1998). Quality control methods for medicinal plants materials. Geneva

World Health Organization (1999) WHO monographs on selected medicinal plants. Essent Med Heal Prod Inf Portal 1. World Health Organization, pp 183–194

Yao H, Song J, Liu CH, Luo K, Han J, Li Y, Pang X, Xu H, Yingjie Zhu Y, Xia P, Chen S (2010) Use of ITS2 region as the universal DNA barcode for plants and animals. PLoS One 5:e13102

Yun-Hsi W (1944) The constituents of *Fritillaria roylei*. J Am Chem Soc 66(10):1778–1780

Zhang J, Wider B, Shang H, Li X, Ernst E (2012) Quality of herbal medicines: challenges and solutions. Complement Ther Medicine 20(1–2):100–106

Zhang W, Nan G, Wu HH et al (2018) A simple and rapid UPLC-PDA method for quality control of *Nardostachys jatamansi*. Planta Med 50(08):536–543

Zhang X (1998) Regulatory situation of herbal medicines: a worldwide review. World Heal Organ 26:223–228

Zhao Z, Hu Y, Liang Z, Yuen JPS, Jiang Z, Leung KSY (2006) Authentication is fundamental for standardization of Chinese medicines. Planta Med 72(10):865–874

Zhu S, Fushimi H, Komatsu K (2008) Development of a DNA microarray for authentication of ginseng drugs based on 18S rRNA gene sequence. J Agric Food Chem 56(11):3953–3959

Zhumashova G, Kukula-Koch W, Koch W, Baj T, Sayakova G, Shukirbekova A, Sakipova Z (2019) Phytochemical and antioxidant studies on a rare *Rheum cordatum* Losinsk. Species from Kazakhstan. Oxid Med Cell Longev (2):1–9

Part III
Regional Importance of Medicinal Plants

Chapter 23
Blessings of Medicinal Plants—History and Prospects

Maiko Inoue and Shinichiro Hayashi

Abstract Medicinal and aromatic plants have sustained the life of human and animals, alleviated ailments, improved quality of life. The history of medicinal and aromatic plants as medicines was unthinkably long and the use based on vast empirical achievement. While the traditional and ethnobotanical remedies have been passed down orally and books, modern science showed rapid growth. Especially second compounds of medicinal and aromatic plants have been powerful and magical natural medicines for human, which was eventually proven due to isolation and practical application in last 200 years and yet a part of plants. Simple and inexpensive remedies using familiar medicinal and aromatic plants are useful for human and animals, which has not changed in all this time. Perhaps a hint of discovery of new medicine might be in traditional plants, ethnobotany, or rain forest.

Keywords Preventive medicine · Integrative medicine · Traditional medicine · Medical marijuana

23.1 Introduction

People think that medicinal and aromatic plants being in our life usually. However, this is a result that human repeated trial and error in long history. The relationship between human and medicinal and aromatic plants is inseparable when we talk about human's history. For example, medicinal plants are continually used as medicines when human suffered from ailments, as flavoring and masking for unpleasant tastes, as cosmetics, formerly used for ritual and myth, and at times used as a currency. Thus, the use of medicinal and aromatic plants ranges widely (Table 23.1). Native medicinal and aromatic plants spread together with human's action to the whole world, many of them were cultivated successfully at the drifted place, and various plant-derived products are easy to get at reasonable prices and for all year-round in present. The

M. Inoue (✉)
Specified Nonprofit Corporation of Horticulture Therapy and Zen, Hamamatsu, Japan

S. Hayashi
GREEN FLASK Inc., Tokyo, Japan

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_23

Table 23.1 Example use of medicinal and aromatic plants

Medicine, preservative, offering, rite, luxuries, tonic, supplement, prize, deadly poison, antidote, dye, scent, talisman, philter, spice, food, anesthetic, relaxation, literature, culture, garden, art, esthetics, phytotherapy

herbal medicine is becoming a big market, in 2012 the whole sales of Chinese herbal medicines reached more than US$83 billion which was 20% more than the market in 2011. It has estimated that the market will reach more than US$115 billion by 2020 (Jamshidi-Kia et al. 2018). Herbal medicines effective for a maintenance of health and presymptomatic state which is difficult to diagnose in modern Western medicines, anybody could handle, generally side effects are insignificant in familiar herbs. In Japan, government offered to aim preventive health care for future welfare, which means Japanese try to build a body and mind of difficult to get ill, to prevent diseases, and to maintenance healthy body individually. It is better than to treat body after get sick (Ministry of Health, Labour and Welfare 2015). The government encourages to adopt of simple remedies for presymptomatic state and some exercise in daily life and to be interested in a wide variety of things about health, medicine, and your body condition. In present, various remedies-related medicinal and aromatic plants are known, for example, aromatherapy, phytotherapy, horticulture therapy, *kampo*, traditional Chinese medicines, and Ayurveda, and part of them could use readily by anybody besides medical doctor. In recent years, cannabis, especially medical marijuana, is remarked in the world. Many countries started to legalize for medical use of cannabis, by today 30 countries have legalized. Medical marijuana has lots of capability for treatment suffering people due to serious illness. Cannabidiol (CBD) derived from cannabis was reported the effectiveness against numerous diseases, such as pediatric epilepsy, Parkinson's disease, Alzheimer's disease, multiple sclerosis, Huntington's disease, hypoxia–ischemia injury, pain, psychosis, anxiety, depression, cancer, nausea, inflammatory diseases, rheumatoid arthritis, infection, inflammatory bowel and Crohn's diseases, cardiovascular diseases, and diabetic complications (Pisanti et al. 2017; WHO 2017). In humans, CBD exhibits no effects indicative of any abuse or dependence potential (WHO 2017). Though cannabis used to be familiar from ancient times for human, the new potential is revealing in recent years. Thus, many medicinal and aromatic plants which well known, still potentials is expanding.

23.2 Historical Progress Related to Medicinal and Aromatic Plants

Present use of medicinal and aromatic plants was established by huge knowledge from experience which started in prehistory. Development of as medicine owed not only interaction between plants and human but also among science, art, literature, culture, environment, and others.

Although only small occurrences might be became clear compare with enormous facts which occurred in long history, which is still important clue to satisfy our curiosity. The history related with medicinal plants is indicated in Table 23.2 included recorded plant name. Probably the start was a recognition of certain plants which significantly affect human body. The certain plants cause dramatic change of human body even taking little dose, for example, hallucination, floating, paralysis, death. The comprehensible actions were impressed, the information of the plants and experiences of the use were accumulated, and the plants were classified to toxicogenic, medicinal, and innocuous. Each tribe instructed information of indigenous plants orally customarily, thereafter someone who could manipulate the special plants became healers or shamans. Ephedra, opium, henbane, mandrake, hellebore, and jimsonweed were shown in the history from early times, 7000 BC, some of them use as powerful drug in present too.

Amazingly each original remedy using medicinal plants were occurred in China, Egypt, and India from 2500 BC to 1500 BC. A lot of kind of indigenous medicinal plants were used to alleviate human ailments, the knowledge was improved, and remedies became reliable. The old remedies were splendid and founded on traditional medicine such as traditional Chinese medicine, Ayurveda, Unani, and Western medicine, and the traditional medicines help even modern people. Circa 1500 BC in Egypt, royalty member used perfumes and cosmetics made from many aromatic plants. Since this time, aromatic plants were used to enrich their life.

Spices were very attractive for ancient people as same as medicinal plants. Indian spices such as cardamom and turmeric were cultivated in the gardens of Babylon around 800–700 BC. Thus, eastern spices started to spread to Middle East and Europe little by little. Later ancient Greeks imported Eastern spices to the Mediterranean area, such as pepper, cassia, cinnamon, and ginger about 400 BC (McCormick Science Institute 2020), conceivably at that time due to Hippocrates (460–377 BC) was well versed in medicinal plants and his great prescience might have an influence upon the import of eastern spices. Hippocrates prescribed not only powerful such as opium and deadly nightshade but also mild active plants such as pomegranate and mint to heal patients. Ancient Greeks and Romans may have used the juice of silphium (*Ferula* sp.) as a contraceptive, but silphium was extinct by the third or fourth century A.D., probably due to overcollection (Sumner 2000).

Around the first century, ancient Roman Empire treading herbs and spices largely from Arabia. All eastern spices were through Arab because not any other route exist by that time. And the place of origin of eastern spices had kept secret by Arabian caravan for their monopoly, which leaded the price of eastern spices extremely high and continued until adventurers find sea routes in thirteenth century. When Roman Empire expanded their lands to northern Alps, several herbs and spices were introduced such as pepper, caraway, rosemary, and thyme (McCormick Science Institute 2020). In Greece, Pedanius Dioscorides wrote a five-volume book, *De Materia Medica* which was the first pharmacopeia, described treatment of the properties, uses, cultivation, and selection of 657 medicinal plants in 77 AD (Fig. 23.1).

Except several herbs and spices of the New World, various medicinal herbs and spices were spread from East Asia to Europe by twelfth century. Avicenna also

Fig. 23.1 Title page of Thomas Johnson's expended edition of *Gerard's Herball* in 1633 (Wikipedia)

known as Ibn Sina was a Persian polymath, significant physician, wrote *The Canon of Medicine* which was a medical encyclopedia described the contemporary medical knowledge of the Islamic world. It is an important text in Unani medicine and was used as a standard medical textbook through the eighteenth century in Europe. Avicenna used steam distillation to produce essential oils derived from aromatic plants.

After thirteenth century, European adventurers started to find Spice Island using sea route because imported spices passed through Arab were extremely high price by Arabian monopoly. During the sea voyage, the New World was discovered, thus several spices which is native to the Americas, such as vanilla, red pepper, and allspice were introduced into Europe. Around 1500, the stream of spices was taken the leaded by Arab caravan ended, then another competition for spice monopoly was started in Western Europe. The spice war among Portugal, Spain, England, France, and the Netherlands was significant point of world spice history. Eventually, Frenchman stole spices from the Netherland territory and began to cultivate on French-controlled islands in the Indian Ocean, which was the trigger of end of war. In 1444, Johannes Gutenberg invented a technique of movable type printing, which leaded the Printing Revolution to Europe. Therefore, publication of herbal book increased rapidly in Europe from sixteenth to eighteenth century, and the movable type printing contributed to the development of the Renaissance, Reformation, the Age of Enlightenment, and the scientific revolution. In 1753, Carl Linnaeus developed new system of binomial nomenclature, it have been adopted to the present (Petrovska 2012).

In 1803, alkaloid, morphine was isolated for the first time from Opium poppy (*Papaver somniferum*) by German pharmacist, since organic chemistry developed, various new medicines were started to synthesize and used in modern Western medicine, and became conventional medicine.

In USA, the Shakers begun wholesale medicinal herb business, native New England plants were cultivated and processed under the strict control around 1820. Shaker physician made free use of medicinal plant for treatment of their patients in Shaker villages (Sumner 2000; Post 2010). In twentieth century, many Western medicines were synthesized based on plant extracts, and development of vaccines and antibiotics discouraged use of medicinal plants in advanced countries, however traditional medicine has been used by 70–80% of world population for the primary treatment. Recently, traditional and natural medicine revived as complementary alternative medicine (CAM), integrative medicine which combine with both benefits of modern Western medicine and CAM are introduced to remedy of disease and health care (Table 23.2).

23.3 Significant Discoveries and Isolations in Nineteen Century

Proportionate progress of science, biologist, and chemist found various components and the molecular structure in scientific level and indicated comprehensive explanation what occurred in human body when taken traditional medicinal plant remedies. The medicinal affections and effects of medicinal plants were caused by phytochemicals which produced as secondary compounds in plants. Secondary compounds are produced by biosynthetic pathways in plant, for example, phenols, tannins, aromatic alkaloids are produced by shikimic acid pathway, terpenes, steroids, alkaloids are produced by mevalonate pathway, and phenols and alkaloids are produced by acetate-malonate pathway. Once secondary compound is taken into human body, it works as a great natural medicine and occasionally is toxically for the regardless dosage. Historically, remarkable discoveries and the isolation of chemical compounds from traditional medicinal plants become a foundation to synthesize a new medicine and proof of the validity were indicated the uses to today (Table 23.3).

The first isolation of medicinal component was morphine from Opium poppy by German pharmacist, Friedrich Sertümer in 1805, which leaded discoveries of alkaloids, such as emetine, piperine, caffeine, and quinine in next 15 years. Alkaloids have been used as cures and poisons since ancient times, generally conspicuously affect the central nervous system of human body therefore very little dosage are used in any cases.

Glycosides consist of a sugar and another non-sugar compound (aglycon) in molecular level, and aglycon has medical potential. Glycosides are abundant in

plants; however, specific aglycons are shown in certain families, for example, glucosinolates exist in Brassicaceae, Cyanogenic Glucosides are in Rosaceae, and phenylpropanoid, iridoid, and cardiac glycosides are in Scrophulariaceae. Digoxin is cardiac glycoside derived from *Digitalis purpurea*, (foxglove, Scrophulariaceae family) and treats for atrial fibrillation, atrial flutter, and heart failure.

Table 23.2 History of medicinal plants

Time line	Historical events	Recorded plants
60000 BC	Plants have been cultivated as drugs	
58000 BC	Early knowledge of plant medicines passed from healer to healer	
7000–6500 BC	*Avesta*, book of Zoroastrianism, indicated Iranian used medicinal plants	Ephedra
4000 BC	300 medicinal plant remedies were listed on Sumerian clay tablets	Opium, myrrh, henbane, mandrake, thyme
3000 BC	Egyptian schools of herbalists have existed	Borage, rosemary
2500 BC	The *Pen Tsao* described 365 medicinal plants in China	Cassia, opium, ephedra, hemp, chaulmoogra, cinnamon bark, jimson weed, ginseng, camphor, *Podophyllum*, *Rhei rhisoma*
1500 BC	850 plant medicines and remedies were listed in *Ebers papyrus* in Egypt, slaves were fed garlic and royalty used perfumes and cosmetics	Mandrake, aloe, castor bean, opium, garlic, juniper berries, onions, pomegranate, senna, fig, willow, coriander, common centaury, fennel, cumin, thyme
	Rig Veda includes 1,500 plants-derived medicines in India	Snakeroot, nutmeg, pepper, clove
800 BC	Homer's epics, *The Iliad and The Odysseys* included 63 plant species	Elecampane, *Artemisia*, castor, hellebore, garlic, sea onion, mustard, cabbage
800–700 BC	King of Babylonia grew 64 different species of plants in his royal garden	Cardamom, coriander, garlic, thyme, saffron, turmeric
	Spices indigenous to India were cultivated in the gardens of Babylon	Cardamom, turmeric
668–633 BC	A scroll of cuneiform writing of Assyria records a long list of aromatic plants	Thyme, sesame, cardamom, turmeric, saffron, poppy, garlic, cumin, anise, coriander, silphium, dill, myrrh
~600 BC	Aromatic plants became popular condiments in Persia. Persia produced essential oils	Onion, garlic, shallot, rose, lily, coriander, saffron
~400 BC	Ancient Greeks imported Eastern spices to the Mediterranean area	Pepper, cassia, cinnamon, ginger

(continued)

Table 23.2 (continued)

Time line	Historical events	Recorded plants
460–377 BC	Hippocrates relied upon 300 plant species to heal patients	Wormwood, common centaury, garlic, opium, henbane, deadly nightshade, mandrake, hellebore, sea onion, celery, parsley, asparagus, oak, pomegranate, Queen Anne's lace, silphium, saffron, Cinnamon, thyme, coriander, mint, marjoram
371–287 BC	*Historia Plantarum* covered the collection and preparation of plant medicines, spices, and perfumes written by Theophrastus in Greece	Mandrake, peony, hellebore, cinnamon, iris rhizome, mint, pomegranate, cardamom, monkshood
300–200 BC	Chinese courtiers carried cloves in their mouths for fresh breath	Cloves
	Ancient surgeon used aromatic plants for its antiseptic action	White mustard, sesame
~100	Ancient Roman Empire did trading spices came from Arabia largely	Cassia, cinnamon
23–79	Pliny wrote about 1,000 medicinal plants in his book *Historia naturalis*	
77	*De Materia Medica* described the properties, uses, cultivation, and selected 657 medicinal plants written by Pedanius Dioscorides	Opium, white willow, hellebore, buttercup, jimson weed, henbane, deadly nightshade, chamomile, garlic, onion, marshmallow, ivy, nettle, sage, common centaury, coriander, parsley, sea onion, oak bark
131–200	Galen compiled the first list of drugs, *De succedanus*	*Uvae ursi folium*
	Medical writing of *Charaka* and *Susruta II* referenced spices and herbs	Cinnamon, cardamom, ginger, turmeric, pepper, cumin, mustard seed, cloves
~400	Medical school and library existed in Egypt	
400–500	Ginger pots were used to provide fresh food for long sea voyages	Ginger
570–632	Mohammed co-owned a shop that stocked spices	Myrrh, frankincense, Asian spices
~700	Slavic people used medicinal plants for remedy, cosmetics, and hunting	Rosemary, basil, garlic, hellebore, cucumber, nettle, yarrow, monkshood

(continued)

Table 23.2 (continued)

Time line	Historical events	Recorded plants
742–814	King of France, Charlemagne encouraged to develop and grow local herbs	Sage, sea onion, iris, mint, common centaury, anise, fennel, fenugreek, thyme, parsley, coriander
850	John Mesue wrote *De Re Medica*	Aloe, deadly nightshade, henbane, coffee, ginger, strychnos, saffron, curcuma, pepper, cinnamon, rheum, senna
980–1037	Avicenna wrote *Canon of Medicine* and Razi wrote *al-Hawi*	
1197–1248	Ibn Baitar wrote *Liber Magnae Collections Simplicum Alimentorum Et Medicamentorum* describing 1,400 plants	
1175–1230	Michael Scott developed anesthetic for surgery using botanical mixture	Henbane, opium, mandrake
1180	King Henry II founded a pepper's guild of wholesale merchants	Cinnamon, clove, sage, saffron, garlic, juniper
1254–1498	Marco Polo's and Vasco De Gama's journeys brought many medicinal plants into Europe	Cacao, lobelia, vanilla, mate, tobacco, red pepper
~1400	An epidemic of plague happened. Physicians wore hoods and face cones filled with aromatic herbs	Wormwood
1493	Christopher Columbus discovered the new spices on his second voyage	Red pepper, allspice
1501	Portugal had large quantities of Indian spices	Cinnamon, cassia, ginger, pepper, nutmeg, mace, clove
1525	Richard Bancke wrote *An Herbal*	Rosemary
1530–1536	*Herbarum Vivae Eicones* was written by Otto Brunfels with accurate illustrations, however the text was fraught with discrepancies	
1539	Hieronymus Bock wrote *New Kreuterbuch* describing the life histories of herbaceous plants	
1542	Leonhard Fuchs authored *De Historia Stirpium* which including 500 plant species	Corn, pumpkins
1546	*Dispensatorium* was the first pharmacopeia, an official listing of medicines describing their preparation and use	

(continued)

Table 23.2 (continued)

Time line	Historical events	Recorded plants
1588	Giambattista della Porta wrote *Phytognomonica*	Pine cones, thistles, catkins, lily bulbs, moss, pomegranate, walnuts
1597	John Gerard compiled *The Herball or Generall Historie of Plants*	Solomon's seal
1629	*Paradisi in Sole Paradisis Terrestris* is a gardening text written by John Parkinson	
1633	Thomas Johnson published a revised edition of *The Herball* which described 2,850 medicinal plants	
~1700	Quinine bark overwhelmed England, France, and Germany	Quinine bark
1749	Carl Linnaeus published Materia Medica included information on illnesses, specific medicines, pharmaceutical effects, dosage, and origin of medicinal plants	
1753	Carl Linnaeus developed new system of binomial nomenclature in *Species Plantarum*	Mandrake, feverbark, digitalis, twinflower
~1800	USA entered the world spice trade	Pepper, cassia, clove, cinnamon, ginger
1803–1878	Discovery, substantiation, and isolation of alkaloids	Poppy, ipecacuanha, strychnos, quinine, pomegranate
1820	The first edition of the *United States Pharmacopoeia* listed 650 drugs, of which 455 were from plants	
1826	Wholesale medicinal herb business begun by the Shakers in USA	Mayflower, butterfly weed, liverwort, black henbane, Jimson weed, thorn apple
1828	Organic chemistry was studied and urea was made in the laboratory by Friedrich Wöhler	
1820–1850	The Harvard Shakers cultivated and processed native New England plants and 212 herbs were sold	Culver's root, purple angelica
1846	An overproduction of spices brought a decline in its economic importance	Pepper
1848	Asa Gray wrote prescription and *the Manual of the Botany of the Northern United States*	Mustard, black pepper

(continued)

Table 23.2 (continued)

Time line	Historical events	Recorded plants
1936	The eleventh edition of the *United States Pharmacopoeia* listed 570 drugs, of which 260 were from plants	
1930s	Plant-derived drugs have appeared in the pharmaceutical marketplace	Curare, sweet clover, Madagascar periwinkles, Rauvolfia species, tropical yams, monkshood, pomegranate, henbane, opium, benzoin, saffron, castor
1900–2020	Development of vaccines and antibiotics Revived interest in medicinal plants and starting integrative medicine	

(*Source* Sumner (2000), Sánchez and Kelley (2002), Petrovska (2012), Jamshidi-Kia et al. (2018), McCormick Science Institute (2020), Inoue et al. (2014))

Terpenes is the most significant compound group in plant, which is biosynthesized from acetate in mevalonate pathway. Terpenes could be classified by the number of isoprene unit (C_5H_8), such as monoterpene ($C_{10}H_{16}$), sesquiterpene ($C_{15}H_{24}$), diterpene ($C_{20}H_{32}$), and triterpene ($C_{30}H_{48}$). Monoterpene is principal compound in essential oil, for instance, cineol of *Eucalyptus* spp. and pinene of *Pinus* spp., and sesquiterpene exists mainly as sesquiterpene lactone and bitter compound. Artemisinin is one of sesquiterpene lactones, which is used for treatment of malaria for its *anthelmintic* action. Taxol is a complex terpene which was found from Pacific yew (*Taxus brevifolia*), and useful for treating several cancers by interfering with cell divisions.

Vast medicines have been synthesized based on phytochemicals including the historical significant discoveries and have been saved lives to today, interestingly Sumner (2000) explained that for plants, probably phytochemicals are for surviving from predatory animals and insects and expansion for their territory.

23.4 Useful Remedies Continue to Present from Ancient Times

Ancient time, perhaps human tried to alleviate their ailments using plants surround them and used various plants using trial and error. In the way, human chanced upon a discovery likely plant for alleviation. Since then, human have explored natural medicines, accumulated the knowledge, and handed down the use to offspring. The similar thing happened sporadically all over the world, consequently in present each region has remained an indigenous remedy. Consider the fact of remain those remedies with knowledge over enormous time, the reliability of remedies is no longer any doubt from uncountable clinical experience. Additionally plant resource has been on

Table 23.3 Medical significant discoveries from medicinal plants in human history

Isolated year	Compound	Plant material	Medicine	Medicinal actions	Component group
1805	Morphine	Opium poppy (*Papaver somniferum*)	Statex, MSContin, Oramorph, Sevredol, others	Analgesic	Alkaloid
1860	Cocaine	Coca (*Erythroxylon coca*)	Procaine, Novocain	Anesthetic	Tropane alkaloid
1935	Tubocurarine	Moonseed vine (*Chondrodendron tomentosum*)	d-tubocurarine chloride, Tubarine	Skeletal muscle relaxant	Alkaloid mixtures
1893	Acetylsalicylic acid	Willow (*Salix alba*)	Aspirin	Analgesic, antiplatelet, antipyretic, anti-inflammatory	Glycoside
1952	Reserpine	Snakeroot (*Rauvolfia serpentina*)	Reserpine	Antihypertensive, mental illness	Alkaloid
1885	Kombe, strophanthin	Vine (*Strophanthus kombe*)	Cardiac glycosides	Treatment for heart failure	Cardiac glycoside
1882	Ouabain, G-strophanthin	Vine (*Strophanthus gratus*)	Strodival	Antihypertensive, antiarrhythmic	Cardiac glycoside
1930	Digoxin	Foxglove (*Digitalis purpurea, D. lanata*)	Lanoxin, others	Treatment for atrial fibrillation, atrial flutter, heart failure	Cardiac glycoside
1961 (vincristine) 1958 (vinblastine)	Vincristine and Vinblastine	Madagascar periwinkle (*Catharanthus roseus*, syn. *Vinca rosea*)	Oncovin, Vincasar, Velban, others	Treatment for Hodgkin's disease, choriocarcinoma, childhood leukemia, breast cancer	Alkaloid

(continued)

Table 23.3 (continued)

Isolated year	Compound	Plant material	Medicine	Medicinal actions	Component group
1966	Taxol	Pacific yew (*Taxus brevifolia*), parasitic fungus on yew bark	Paclitaxel, Taxol, others	Cancer treatment for lung, ovarian, breast, head, neck, Kaposi's sarcoma	Terpene-based
1820	Quinine	Cinchona tree (*Cinchona* spp.)	Qualaquin, Quinate, Quinbisul, others	Antimalaria	Alkaloid
1972	Artemisinin	Sweet wormwood (*Artemisia annua*)	Artemisinin	Antimalaria	Terpene

(*Source* Sumner (2000))

earth, therefore, human can use practically to today. Though act that human try to treat diseases has not changed in all this time, most of people in advanced countries attach great importance to synthesized modern Western medicine rather than traditional natural medicine for treatment of disease last 200 years. Consequently, modern people in advanced countries forgot how to use, the efficacy, and even the existence of traditional natural remedies, while up to 80% of developing country populations rely on traditional medicine for their primary health care according to World Health Organization (WHO 2004). General traditional remedies were indicated in Table 23.4. Most of plant materials could be found fairly ease in shop or online also are easy to grow own garden. Table 23.4 includes information of medical actions and chemical compounds, which were revealed by various scientific researches. Science started to prove mechanism of medical action of traditional natural medicines little by little, however proving all scientifically would take long time. No scientific proof of medical actin does not mean traditional remedies do not work, just science caught up with proof of clinical experiences accumulated from ancient time.

Some herbs show same medical action, for example, more than half of herbs have anti-inflammatory (Table 23.4, Fig. 23.2). And some of them have antimicrobial, antibacterial, antiviral, or antifungal too. Thus, herbs have at least several medical actions just in one plant. Valerian, Linden, and German chamomile are well known as hypnotic herbs, while garlic, ginger, mint, rosemary, and thyme have stimulant action. Materials of remedy could be chosen from candidate herbs which have same medical action. Particular chemical compound and medical action were distinguished, for instance inulin from Echinacea (*Echinacea angustifolia, E. purpurea, E. pallida*) acts as immunomodulatory and Feverfew (*Tanacetum parthenium*) is useful for migraine treatment because of parthenolide, sesquiterpene lactone. A lot of application could be arranged according to use, for example, ginger crystallized is good for car sickness due to the antiemetic action, and a cream made from plantain leaves is help to treatment of insect bites and stings due to the anti-inflammatory, vulnerary, and demulcent actions.

23.5 Medicinal Plants for Animal Self-Medication and Veterinary Practice

Animal uses plants as medicine as well as human. The science of animal self-medication is relatively a new field and called zoopharmacognosy was coined by Eloy Rodriguez and Richard Wrangham in 1992. A behavior suggested self-medication was observed in Tanzania, which chimpanzee selected the leaves of *Aspilia* species and swallowed intact large leaves covered in dense hairs. This behavior is fairly different from usual leaf-eating. Actually, reports suggested that *Aspilia* leaves produce thiarubrine-A, which kills viruses, fungi, and parasitic worms. An alternative explanation is that the densely hairy surface may physically dislodge parasitic worms from intestines in a process of digestion (Sumner 2000). Leaf-swallowing

Table 23.4 Useful remedies continuous from ancient to present using familiar medicinal plants

Common name (scientific name)	Parts used	Applications	Medical actions	Chemical compounds
Aloe vera (*Aloe vera*)	Clear central leaf pulp, juice from leaf	Cream (including aloe vera gel) for sunburn and wounds, shampoo for dry and itchy scalps	Cell regeneration, anticancer, laxative, hepatic	Aloe-emodin, bitters
Calendula (*Calendula officinalis*)	Flower, leaf, essential oil	Gel (including infusion) for acne, balm (including infused oil) for chapped hands and sores, ointment, cream	Anti-inflammatory, antimicrobial, antiseptic, antifungal, antispasmodic, lymphatic, astringent, vulnerary, emmenagogue, hormone, and vitamin A precursors	Salicylic acid, faradiol monoester, triterpenes, flavonoids, volatile oil, chlorogenic acid, carotenoids, phytosterol, bitters, polysaccharides
Cranberry (*Vaccinium macrocarpon*)	Fruit	Fruit leather or fresh juice for cystitis	Antiadhesion of bacteria	Proanthocyanidins, fructose, vitamin C, arbutin
Dandelion (*Taraxacum officinale*)	Flower, leaf, stem, root	Decoction for water retention	Diuretics, hepatic, cholagogue, antirheumatic, laxative, tonic, anti-inflammatory, detoxify the blood	Inulin, vitamin A, C, minerals, terpenes, sterols, xanthophylls, flavonoids, polysaccharides, bitters, phenolic acid
Echinacea (*Echinacea angustifolia, E. purpurea, E. pallida*)	Leaf, stem, rhizome	Tincture and tea for colds and flu, throat spray made from tincture	Antimicrobial, immunomodulator, anti-inflammatory, anticatarrhal, vulnerary, alterative, antiviral	Inulin, isobutylamides, echinocoside, cichoric acid, alkylamides, polysaccharides, polyacetylenes, essential oil

(continued)

Table 23.4 (continued)

Common name (scientific name)	Parts used	Applications	Medical actions	Chemical compounds
Elder flower (*Sambucus nigra*)	Flower, leaf, shoot, root, fruit, bark	Tea, poultice, infused honey, lotion, cordial, jam, pie, alcoholic drink	Expectorant, diuretic, diaphoretic, emollient, vulnerary, anticatarrhal, antispasmodic, laxative, antirheumatic, antiallergic, anti-inflammatory	Sambunigrin, triterpenes, fixed oil, miscellaneous, phenolic acids, pectin, sugars, cyanogenetic glycosides, flavonoids, fatty acids, alkanes, tannins, minerals
Feverfew (*Tanacetum parthenium*)	Leaf, flower, essential oil	Tea, smoothie, food, tincture, and tablet for migraine prevention	Analgesic, anti-inflammatory, vasodilator, emmenagogue, antirheumatic, antispasmodic, antithrombotic	Parthenolide, camphor, parthenolide, sesquiterpene lactones, onoterpenes and sesquiterpenes, flavonoids, bitters, essential oil
Garlic (*Allium sativum*)	Flower, bulblets	Fresh or powder for athlete's foot, food, garlic oil capsules	Antifungal, antimicrobial, diaphoretic, hypocholesteremic, cholagogue, hypotensive, antispasmodic, stimulant, expectorant, carminative, cardiotonic, anticatarrhal, alterative, hypoglycemic, hypothyroidism	Allyl sulfides, cysteine, diallyl disulfide, alliin, miscellaneous, vitamin B, minerals, flavonoids
German chamomile, annual chamomile (*Matricaria recutita*)	Flower, essential oil	Cream for eczema, syrup including decoction for colic, tea, infused oil, essential oil, tincture, poultice, bath	Antihistamine, antiseptic, antiallergenic, carminative, digestive, nervine, antispasmodic, anti-inflammatory, antimicrobial, vulnerary, moisture-retention, hypnotic, emmenagogue	Chamazulene, bisabolol, bisabolol oxides, sesquiterpene lactones, flavonoid glycosides, apigenin, luteolin, quercetin, isorhamnetin, bitters

(continued)

Table 23.4 (continued)

Common name (scientific name)	Parts used	Applications	Medical actions	Chemical compounds
Ginger (*Zingiber officinale*)	Rhizome, essential oil, flower, leaf, shoot	Crystallized for morning and travel sickness, food, tea, tincture, cordial, steam inhalation, massage oil	Stimulant, carminative, antispasmodic, rubefacient, diaphoretic, emmenagogue, antirheumatic, anti-inflammatory, digestive, choleresis, antiemetic, analgesic	Gingerols, shogaols, sesquiterpenes zingiberene and β-bisabolene, oleoresin, lipids
Ginkgo (*Ginkgo biloba*)	Leaf, fruit, seed	Tea for memory enhancer, tablet, tincture, extract	Anti-inflammatory, vasodilator, relaxant, digestive bitter, uterinestimulant, cardiovascular tonic, antioxidation	Ginkgolides A, B, C, and J, bilobalide, flavonol glycosides, ginkgolic acid
Hawthorn (*Crataegus* spp.)	Leaf, flower, fruit, wood, seed	Fruit leather for cholesterol reducer, tea, tincture	Cardiotonic, diuretic, astringent, hypotensive, anti-inflammatory	Flavonoids, oligomeric procyanidins, triterpene acids, phenolic acids
Lavender (*Lavandula* spp.)	Flower, leaf, stem, essential oil	Steam inhalation, massage oil, ointment, cream, lotion, tea, potpourri for repellent, essential oil	Carminative, antispasmodic, relaxing nervine, antidepressant, rubefacient, emmenagogue, antimicrobial, anti-inflammatory, antiseptic	Licareol, linalool, linalyl acetate, volatile oil, coumarins, triterpenes, flavonoids, tannins
Linden (*Tilia* spp.)	Flower, leaf, stem, bark, wood	Lotion for moisturizing, tea, tincture	Nervine, antispasmodic, hypotensive, diaphoretic, diuretic, anti-inflammatory, astringent, hypnotic, emmenagogue, heart tonic	Volatile oil, flavonoids, miscellaneous, phenolic acids, tannins

(continued)

Table 23.4 (continued)

Common name (scientific name)	Parts used	Applications	Medical actions	Chemical compounds
Marshmallow (*Althaea officinale*)	Flower, leaf, root, seed	Syrup for cough and sore throat, tea, tincture, poultice	Demulcent, emollient, diuretic, anti-inflammatory, expectorant, anticatarrhal	Mucilage, miscellaneous, flavonoids, scopoletin, polyphenolic acids
Mint (*Mentha* spp.)	Flower, leaf, essential oil	Tea for Irritable Bowel Syndrome, steam inhalation, tincture, food, ointment, powder, spray, mouthwash	Carminative, anti-inflammatory, antispasmodic, aromatic, diaphoretic, antiemetic, nervine, antimicrobial, analgesic, stimulant, Emmenagogue, anticatarrhal, cholagogue	Phenolic acids, essential oil, menthol, flavonoids, tannins
Nettle (*Urtica dioica*)	Flower, leaf, shoot, root, fruit, seed	Tea for water retention, soup for restorative, hair tonic, tincture, juice, poultice	Diuretic, astringent, tonic, hypotensive, Antirheumatic, alterative	Vitamins, minerals, chlorophyll, lignans, flavonol glycosides, phytosterol, folic acid
Plantain (*Plantago major*)	Leaf, stem, seed	Cream for insect bites and stings, tea for water retention, tincture, ointment, poultice	Anti-inflammatory, anti-allergenic, diuretic, vulnerary, expectorant, demulcent, astringent, antimicrobial, mucilaginous	Iridoids, flavonoids, tannins, oleanolic acid, plant acid
Rosemary (*Rosmarinus officinalis*)	Flower, leaf, twig, essential oil	Potpourri of dried leaves for repellent, infused wine for memory booster, bath, tincture, tea, lotion	Carminative, antispasmodic, antidepressant, rubefacient, antimicrobial, emmenagogue, insect repellent, stimulant, cholagogues, astringent, antirheumatic, antioxidation, antiseptic, circulation	Camphor, borneol, volatil oil, flavonoids, rosmarinic acid, phenolic acids, diterpenes, rosmaricine, triterpenes

(continued)

Table 23.4 (continued)

Common name (scientific name)	Parts used	Applications	Medical actions	Chemical compounds
Sage (*Salvia officinalis*)	Flower, leaf, essential oil	Fresh or powder for athlete's foot, tea for hot flush and night sweat, potpourri for repellent, honey for sore throat, toothpowder for plaque remover and gum soother, tincture	Carminative, insect repellent, antiseptic, anti-inflammatory, decongestant, antispasmodic, antimicrobial, astringent, emmenagogue, cholagogue, anticatarrhal	Camphor, thujone, volatile oil, diterpene bitters, flavonoids, phenolic acids, salviatannin
St. John's wort (*Hypericum perforatum*)	Flower, leaf, stem, fruit	balm (including infused oil) for chapped hands and sores, tea, tincture	Anti-inflammatory, astringent, vulnerary, nervine, antimicrobial, antispasmodic, antidepressant, antiviral, sedative	Xantholignans, anthraquinone, hypericin, dihydrodianthrone, volatile oil, phloroglucinols, flavonoids, tannins
Thyme (*Thymus vulgaris*)	Flower, leaf, stem, essential oil	Tincture for breath spray and mouthwash, antiseptic soap using essential oil, infused oil for external application, lotion, tea	Antiseptic, carminative, antimicrobial, antispasmodic, expectorant, astringent, anthelmintic, emmenagogue, anticatarrhal, stimulant	Thymol, volatile oil, carvacrol, flavonoids, miscellaneous, tannins, saponins
Valerian (*Valeriana officinalis*)	Leaf, root, essential oil	Decoction, tea, and powder for anxiety and insomnia, tincture	Nervine, hypnotic, antispasmodic, carminative, hypotensive, emmenagogue	Isovaleric acid, valepotriate, essential oil

(*Source* Wong (2009), Hayashi (2011), Bremness (2002), Hoffmann et al. (2003), Pengelly (2004))

Echinacea
(*Echinacea purpurea*)

Elder flower
(*Sambucus nigra*)

Hawthorn
(*Crataegus* spp.)

Linden (*Tilia* spp.)

Plantain (*Plantago major*)

St. John's wort
(*Hypericum perforatum*)

Fig. 23.2 Useful medicinal plants for home remedy

occurs in other animals as well, such as Pygmy chimpanzees, eastern lowland gorillas, and African great apes (Sumner 2000). Other purposes using ethno-medicine were supposed, such as pain killer, induce labo, and cure stomach or intestinal upset by Malay elephant, African elephant, and South American wolf, respectively (Huffman 2003). Biologist Michael Huffman established widely used criteria for judgment of animal self-medication. (1) The plant eaten cannot be a regular part of the animal's diet. (2) The plant must provide little or no nutritional value to the animal. (3) The plant must be consumed during certain times of year, such as the rainy season when parasites are most likely to cause infections. (4) Other animals in the group do not participate (Shurkin 2014). Investigation of zoopharmacognosy is very tough because researchers have to keep following the target wild animals within a certain distance for weeks, collect feces, and others. Therefore, not so many studies could be performed, however to know what plants are used as a medicine for animals might be clue for finding new medicinal plant for human.

Regarding livestock, medicinal and mostly indigenous plants are used for ailments in Europe, Africa, and India (Parthiban et al. 2016; Verma 2014; Tolossa et al. 2013; Mayer et al. 2014; Luseba et al. 2007). Healers have traditional and ethnobotanical knowledge and prescribe orally or externally. Various parts of plant are used, such as bark, leaf, root, shoot, flower, seed, bulb, fruit, latex, stem, aerial part, whole plant, butt, rhizome, pod, and seed oil for several ailments, for example, wounds, parasites, arthritis, diarrhea, and retained placenta. Families which frequency used in at least Europe, Africa, and India for medical practices are *Fabaceae*, *Lamiaceae*, *Asteraceae*, *Euphorbiaceae*, and *Solanaceae*. Natural medicine is necessary for organic livestock and very economical.

23.6 Prospects

23.6.1 Future Medicine: Integrative Medicine and Preventive Medicine

Recently, a view of medicine is starting to change because personalized medicine is important due to lifestyle-related diseases and various mental diseases caused by daily stress so-called the modern disease is deeply related to personal circumstances. Integrative medicine is a person-centered care system that uses both modern Western and other CAM. The advantage of modern Western medicine is a rapid and local treatment for acute diseases, such as operation, vaccine, and radiology and the testing technique for diagnosis, while other complementary and alternative medicine advantage for chronic diseases, a presymptomatic state, maintenance of health, and vitalize. Integrative medicine has become popular among advanced countries to change the structure of disease control. For example, Dr. Andrew Weil of University of Arizona, a leading figure of integrative medicine, is a physician and herbalist and prescribes medicinal plants for treatment (Weil 2020). Before nineteenth century, the modern

Western medicine did not exist therefore earlier people was not able to use even they want to, however present is different. Integrative medicine might fit the modern people approach and lifestyle.

Preventive medicine focuses on the health of individuals and communities, and the goal is to promote health and well-being and prevent disease, disability, and death (American college of preventive medicine 2020). Disease prevention relies on anticipatory actions because disease and disability begin before individuals realize the affected. Sometimes disease and disability are caused by environmental factors, pre disposition, and lifestyle choices which are possible to control by individuals. Preventive medicine consisted of three categories, primary prevention—promotion of health, secondary prevention—early detection and treatment, and tertiary prevention—rehabilitation. For example, phytotherapy, aromatherapy, and others using medicinal plants are useful for promotion of health, periodical health examination based on modern Western medicine could avoid critical state of disease, and horticulture therapy for rehabilitation is effective means. Preventive medicine based on integrative medicine would lead to longer healthy life expectancy and reduction of medical costs in the future.

23.6.2 Possibility and Prospects of Medical Marijuana

A term of *medical marijuana* is referred an application of cannabis (*Cannabis sativa*) in the field of medicine (Fig. 23.3). While cannabis which contains a peculiar constituent Tetrahydrocannabinol (THC, Fig. 23.4) is effective against various serious illness such as cancer, multiple sclerosis, Parkinson's disease, and glaucoma,

Fig. 23.3 Cannabis (*Cannabis sativa*)

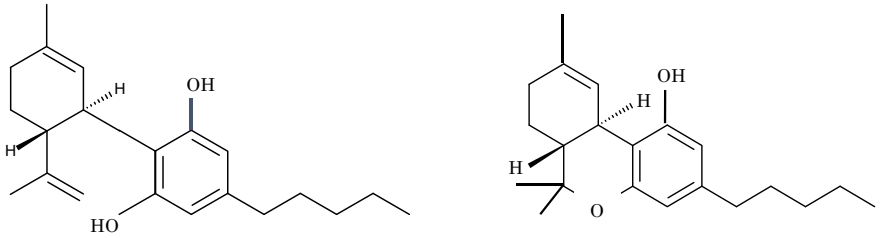


Fig. 23.4 Molecular structures of cannabidiol (CBD) and tetrahydrocannabinol (THC)

its use is limited by a law in each country. In USA, using medical marijuana is allowed in more than 30 states including Washington DC, however, it has been illegal in the US constitution. In the Netherlands, Australia, UK, and Germany, in these days Canada and Korea approve its use thus a stream of the legalization is accelerating. In Japan, Japanese government prohibits the possession, import, and uses of cannabis even if only for the purpose of medical treatments under *the Cannabis Control Law* which was enacted in 1947. However, if consider the situation of a rise in medical expenses because of the increasing of death for cancer and various serious illness, it is unavoidable topic for the necessity of medical marijuana.

Cannabis has a history of which was applied for a medical treatment from ancient times. A Chinese oldest agriculture and medicinal plant book "*Pen Tsao Ching*" which was believed written by Shen Nung described that cannabis works on rheumatic pains, malaria, gynecological disease, and constipation, and Ayurveda referred to widely uses for pain, insomnia, and digestive dysfunction. An Irish physician William Brooke O'Shaughnessy recommended cannabis for a great variety of therapeutic purposes in India, and in 1842, he returned to England where he introduced cannabis to Western medicine and ultimately an extract and tincture made by cannabis have been published in English Pharmacopoeia. In Japan, before until World War II the preparations of Cannabis called tinctures or extracts of Indian hemp had been sold at pharmacy, and had been published in until 5th Japanese Pharmacopoeia, however since 6th (in 1951 revised), the preparations of Cannabis have been deleted until now because the Cannabis Control Law was promulgated in 1948.

Israeli Dr. Raphael Mechoulam isolated THC, marijuana's main psychoactive component in 1964. Subsequently, cannabinoid (a series of constituents, the similar molecular structure as THC, called cannabinoid) receptors were discovered from human body and named cannabinoid type 1 (CB1) receptors in 1988. And an endocannabinoid, anandamide was discovered in human's brain, which could attach to cannabinoid receptors like THC in 1992. Although not so many people have an experience having marijuana, the human body possesses the endocannabinoid system surprisingly. Several years later from CB1 receptors had found, cannabinoid type 2 (CB2) receptors were discovered. CB1 receptors are found mainly in the brain and central nervous system, and CB2 receptors are found in immune system cells and leukocyte. CB1 and CB2 receptors get involved in the regulation of various

physiological functions, and the spread cannabinoid system in the body coordinates biodefense and homeostasis. Medical marijuana causes pharmacological actions in many spheres, such as pain, appetite, cognition, and lipid metabolism, which means nothing less than influences upon a cannabinoid system. Cannabidiol (CBD) is different cannabinol from THC, does not attach to CB1 and CB2 receptors, however CBD affects receptors like serotonin receptors and has variegated pharmacological properties, such as anti-inflammatory, anticonvulsive, antianxiety, and antiemetic. In Japan, CBD is out of the Cannabis Control Law, thus CBD is imported in form of supplement.

Considering large segments of people assumes that marijuana has dangerous toxicity as same as a stimulant, a very little paper indicated a certain scientific evidence about a toxicity of marijuana. The 99th Mayor of New York City, Fiorello LaGuardia appointed the LaGuardia Committee for sociological and scientific investigation of smoking marijuana, which was the first in-depth study into the effects of marijuana in the USA. In 1944, the report concluded the relative harmlessness of a long-term use of marijuana for physically, psychologically, and ethically, and use of marijuana did not induce violence, delinquency, and crime (The Laguardia Committee 1944). American Psychiatric Association indicated no report proven that use of marijuana could lead to use a hard drug, such as cocaine or heroin, though a gateway drug theory or a stepping stone theory for its hazardousness are rumored.

Medical marijuana has been used for some kind of serious diseases, in the meantime the practical use of medical marijuana against cancer which is the Japanese's 30%-leading cause of death, has been hoped as soon as possible. For example, in USA, medical marijuana is used as the clinical application in integrated medicine framework. Integrated medicine means personalized medicine which put together both of conventional medicine including pharmaceuticals, operations, and radiation treatments, and complementary and alternative medicine. In here, use of medical marijuana as a supportive care could prevent an emaciation of body and mind by a chemotherapy and keep their quality of life. Medical marijuana induces effectiveness of central analgesic and pain relief against fearful pain of cancer, recovery of appetite of patient who loses energy and strength by cancer cachexia (weight loss induced by protein degradation), and is helpful in treating nausea and vomiting from chemotherapy. Recently, the direct anticancer effects, such as an angiogenesis inhibition and apoptosis induction of medical marijuana attract attention.

In the USA, 2018, data shows that every day, 128 people die after overdosing on opioids (prescription painkillers and heroin) (National Institutes of Health 2020). This opioid crisis has caused the rise of using opioids for chronic pain other than cancer. Opioids such as morphine suppress a respiratory center, therefore large enough doses produce respiratory arrest while cannabis does not affect a respiratory center, which is probably safer. Actually, states that have legalized medical marijuana have seen drops in opioid overdoses deaths (Sifferlin 2019). In addition, a possibility of combined effects of medical marijuana and opioids was reported both of increase an analgesic effect and decrease side effect. In Japan, administration of synthetic opiates is strictly and the risk of abuse and over-does is little, therefore

doctor has no idea to use medical marijuana than opioid. However, the benefits of using medical marijuana compared with opioids should be known.

Essential oil of cannabis consists of classified terpenoids, such as *β-caryophyllene*, *myrcene*, *linalool*, and *limonene* other than cannabinoids. *β-caryophyllene,* which is a main ingredient of copaiba oil, combines with CB2 receptors in extremely low concentrations and inhibit to produce proinflammatory cytokine, consequently induces anti-inflammatory and analgesic action against neuropathic pain, was reported. It means use *β-caryophyllene* together other than only THC or CBD leads a synergistic effect, in other words entourage effect, the phrase is used especially cannabis (Fukuda 2015). In the case of cannabis, use whole plant included numerous compounds other than only THC or CBD is more effective, and it is a theory of using herbs.

When legalization of medical marijuana is following one another in the West and Asia, Japan faces a necessity of revaluation of medical marijuana not just follow an existing law but have a scientific point of view. Cannabis was necessary plant for the culture of daily life before prohibition by law. Hemp seeds were important food thus cannabis was utilized for food, clothing, and housing. In Japan, a thread made of hemp was used for cutting an umbilical cord when delivery, and the baby clothes had a design of hemp. And in a rite of *Shinto* which is one of the traditional religions of Japan, hemp had important role and was deeply related with spiritual culture. Legalization of medical marijuana even leads to take identity and ecological life back for Japanese beyond medical field.

23.7 Conclusions

Since the birth of animal and human, the blessing of medicinal and aromatic plants continuously sustains human, animals, and other creatures. An overwhelming long history and uncountable discoveries could alleviate suffering of ailments and lead a good quality of life. Huge useful remedies have been handed down from ancient, which could adapt to complex modern diseases sufficiently. Therefore, integrative medicine combined modern Western and traditional medicine is necessary for present and future medicine. Now, usual conception regarding diseases and medicine needs to be updated for twenty-first century.

One of the important missions is conservation of genetic resources of medicinal and aromatic plants. Haphazard overharvesting and environmental pollution should be reduced. Absolutely, capable medicinal plants will be in existing plants, and the hint to find it out will be in accumulated knowledge from ancient. Medicinal and aromatic plants are a great treasure for human, animals, and other creatures.

References

American college of preventive medicine (2020) About preventive medicine. Accessed 29 May 2020 https://www.acpm.org/about-acpm/what-is-preventive-medicine/

Bremness L (2002) Smithsonian handbooks, Herbs. Dorling Kindersley, Inc., New York

Fukuda K (2015) Truth of medical marijuana (in Japanese) MEISOU Publishing Inc., Tokyo

Hayashi S (2011) Basic text book of herb & essential oil. IKEDA PUBLISHING CO. LTD., Tokyo

Hoffmann D (2003) Medical herbalism, the science and practice of herbal medicine. Healing Arts Press, Rochester

Huffman M (2003) Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. Proc Nutrition Soc 62:371–381

Inoue M, Craker L (2014) Medicinal and aromatic plants-uses and functions. In: Dixon G, Aldous D (eds) Horticulture: plants for people and places, vol 2. Springer, Dordrecht, pp 645–669

Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H (2018) Medicinal plants: past history and future perspective. J Herbmed Pharmacol 7(1): 1–7

Luseba D, Elgorashi E, Ntloedibe D, Staden J (2007) Antibacterial, anti-inflammatory and mutagenic effects of some medicinal plants used in South Africa for the treatment of wounds and retained placenta in livestock. South African J Botany 73:378–383

Mayer M, Vogl C, Amorena M, Hamburger M, Walkenhorst M (2014) Treatment of organic livestock with medicinal plants: a systematic review of European ethnoveterinary research. Forsch Komplementmed 21:375–386

McCormick Science Institute (2020) History of spices. Accessed 15 March 2020 https://www.mccormickscienceinstitute.com/resources/history-of-spices

Ministry of Health, Labour and Welfare (2015) Japan vision: health care 2035. Accessed 21 May 2020 https://www.mhlw.go.jp/seisakunitsuite/bunya/hokabunya/shakaihoshou/hokeniryou2035/future/ (in Japanese)

National Institutes of Health (2020) Opioid Overdose Crisis. Accessed 27 April 2020 https://www.drugabuse.gov/drugs-abuse/opioids/opioid-overdose-crisis

Parthiban R, Vijayakumar S, Prabhu S, Yabesh J (2016) Quantitative traditional knowledge of medicinal plants used to treat livestock diseases from Kudavasal taluk of Thiruvarur district, Tamil Nadu, India. Brazilian J Pharmacogn 26:109–121

Pengelly A (2004) The constituents of medicinal plants, an introduction to the chemistry and therapeutics of herbal medicine. Fragrance J LTD. Tokyo

Petrovska B (2012) Historical review of medicinal plant's usage. Pharmacogn Rev 6(11):1–5

Pisanti S, Malfitano AM, Ciaglia E, Lamberti A, Ranieri R, CuomoG AM, Faggiana G, Proto MC, Fiore D, Laezza C, Bifulco M (2017) Cannabidiol: state of the art and new challenges for therapeutic applications. Pharmacol Ther 175:133–150

Post M (2010) Medical practice in the Harvard Shaker church family 1834–1843. Am Communal Soc Quart 4(4):218–229

Sánchez E, Kelley K (2002) Herb and spice history. Penn State College of Agricultural Sciences Department of Horticulture. file:///C:/Users/Maiko/Downloads/herb-and-spice-history%20(1).pdf

Shurkin J (2014) News feature: animals that self-medicate. PNAS 111(49):17339–17341

Sifferlin A (2019) The healing power of Cannabis. TIME Marijuana: the medical movement. Meredith Corporation, NY

Sumner J (2000) The natural history of medicinal plants. Timber Press Inc., Oregon

Tolossa K, Debela E, Athanasiadou S, Tolera A, Ganga G, Houdijk J (2013) Ethno-medicinal study of plants used for treatment of human and livestock ailments by traditional healers in South Omo, Southern Ethiopia. J Ethnobiol Ethnomed 9:32

Verma R (2014) An ethnobotanical study of plants used for the treatment of livestock diseases in Tikamgarh District of Bundelkhand, Central India. Asian Pacific J Tropical Biomed 4(Suppl 1): S460–S46

Weil A (2020) Andrew Weil center for integrative medicine. The University of Arizona. Accessed 29 May 2020 https://integrativemedicine.arizona.edu/about/index.html
Wong J (2009) Grow your own drugs. HarperCollins Publishers, London
World Health Organization (2004) New WHO guidelines to promote proper use of alternative medicines. Accessed 4 May 2020 https://www.who.int/mediacentre/news/releases/2004/pr44/en/
World Health Organization (2017) Cannabidiol (CBD) pre-review report. Accessed 24 May 2020 https://www.who.int/medicines/access/controlled-substances/5.2_CBD.pdf

Chapter 24
Barberry (*Berberis vulgaris*)—Traditional and Contemporary Use

Anna Och and Renata Nowak

Abstract *Berberis vulgaris* L. has an over 3000-year tradition in folk medicine. Genus devastated in Europe and appreciated in the Middle East, is a very valuable medicinal plant. The cultivation is carried out on a global scale in Iran and Georgia. The crop is exported to Europe, where the awareness of the benefits of its use has been growing recently. It is mainly due to the intensification of scientific reports on the health-promoting effects of berberine. This compound is currently being intensively studied, also clinically. Currently, *Berberis vulgaris* L. is a commonly investigated plant species due to its highly promising effectiveness in many therapies. In this chapter, we have compieled information collected from the latest scientific literature. We have reviewed and analyzed applications of the genus and described the current knowledge of this plant as a contemporary drug and its use in various locations in the world. Numerous papers describing this valuable plant are available at present. However, there is no comprehensive review summarizing its potential in current pharmacy applications. Its effectiveness in treatment of civilization diseases, especially due to the antioxidative and chemopreventive activity, makes barberry a promising agent in this area. With its rich chemical composition and safety of use, barberry is considered a nutraceutical agent. Modern research also discusses the anti-cancerogenic activity of the plant.

Keywords *Berberis vulgaris* · Traditional medicine · Berberine · Chemoprevention · Nutrition · Civilization diseases

A. Och · R. Nowak (✉)
Department of Pharmaceutical Botany, Medical University of Lublin, 1Chodźki St., 20-093 Lublin, Poland
e-mail: renatanowak@umlub.pl

A. Och
e-mail: anna.och@umlub.pl

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_24

24.1 Introduction

Berberis vulgaris L., commonly known as barberry, has an over 3000-year tradition in folk medicine. Once very popular in Europe and America, it was eliminated due to the threats to crops posed by the species. This resulted in forgetting this excellent resource in these parts of the world. The scientific literature describes the traditional uses of barberry in many diseases, and the latest scientific reports confirm its effectiveness or show its new potential. The species was saved in the Middle East, where it is still cultivated and highly valued, especially as a culinary plant. Currently, *Berberis vulgaris* L. is a commonly investigated plant species due to its highly promising effectiveness in many therapies. In this chapter, we have compieled information collected from the latest scientific data. We have reviewed and analyzed applications of *Berberis vulgaris.* We have described the current knowledge of this plant as a contemporary drug and its usage in various locations in the world. At present, numerous papers describing *Berberis vulgaris* are available. However, there is no comprehensive review summarizing its potential in current pharmacy applications. Its effectiveness in treatment of civilization diseases, especially due to the antioxidative and chemopreventive activity, makes the species a promising agent in this field. The growing amount of research focused on the species may restore appreciation and respect of barberry in Europe.

24.2 Botanical Characteristics

Berberis vulgaris L. belongs to the *Berberidaceae* family and is a large and dense deciduous shrub. It usually reaches 2–3 m in height and a similar diameter. However, specimens reaching 8 m height and living up to 300 years have been recorded as well. The aboveground part of the plant has long branches bending towards the ground (Fig. 24.1). They are covered with 1–2 cm-long triple thorns.

The bark of barberry (*Berberidis Cortex*) is grey-yellow. It has elongated grooves with transverse crevices. The internal surface of the bark is dark-yellow and brown. It is fibrous and striated lengthwise. Frequently, the white wood adheres to the bark. In the cross-sectional view, a thin cork and dark-brown phloem are visible. The phloem is transected with bright-yellow parenchyma (Figs. 24.2 and 24.3). The bark has a delicate scent and bitter taste. It can be mistaken with the dried stem of *Berberis aristata* DC.

The deciduous leaves are 2–5 cm long and 1–2.5 cm wide. Their shape ranges from broad elliptical to ovate. The leaves are straight, ciliated, with irregular and serrated margins. They are tapered at the base and have short petioles. The leaves grow in clusters of a few, and there is a thorn underneath each cluster (Figs. 24.4 and 24.5). They have a pleasant sour taste; hence, the French name *Epine vinette*, which denotes “sour thorn”.

Fig. 24.1 Perennial specimen of *Berberis vulgaris* reaching 3 m height (Botanical Garden of Maria Curie-Skłodowska University in Lublin, Poland)

Flowers emerge in May and June and are characterized by a strong scent. They are small and yellow. They grow in loose drooping 12–22 flower clusters of 4–6 cm length. The flowers have three petals resembling sepals and six nectaries resembling petals.

Barberry produces berries, i.e., oblong bright-red clustered fruits, which ripen in September and October. They remain on the plant after leaves fall and partly in winter. They form beautiful red clusters. However, the species is not a typical garden plant.

Fig. 24.2 Yellow-green inner side of barberry bark

Fig. 24.3 White wood of *Berberis vulgaris*

The root (*Berberidis radix*) is covered by soft yellow-grey periderm. Once broken, an intensely yellow layer structure is visible. The bark is membranous, orange-yellow in color, with a smooth inner surface. The root has bitter and unpleasant taste but no aroma. It is thick, strongly branched, and rigid (Sarraf et al. 2019; Anon 2020; Roślin 2020; Rutkowski 2013) (Figs. 24.6 and 24.7).

Fig. 24.4 Immature fruits of *Berberis vulgaris* gathered in clusters

Fig. 24.5 Alternate broad-elliptical straight leaves with irregular and serrated margins

24.3 Distribution and Occurrence

Berberis vulgaris is distributed in moderate and semitropical regions of Asia, Europe, Africa, and North and South America (Rahimi-Madiseh et al. 2017) where it grows mostly in the wild or is cultivated. *Berberis vulgaris* grows in bright coniferous forests and at the margins of woods, thickets, and slopes (Roślin 2020). In Poland, *Berberis vulgaris* is the only wild barberry species. It belongs to the group of wild-growing plants with edible fruits (Atlas Roślin 2020). In landscape and urban settings, the species is used to reinforce escarpments (Atlas Roślin 2020).

Fig. 24.6 *Berberis vulgaris* bark with elongated grooves with transverse crevices

Fig. 24.7 Three-pronged thorns in the above-ground part of a *Berberis vulgaris* specimen. The bark with elongated grooves and transverse crevices is visible

Currently, it is rarely cultivated in the gardens of Europe but used to be planted in monastery gardens throughout southern Europe in the past centuries (Roelfs 1982). The species is also cultivated in gardens in the European part of Russia, mainly in the forest-steppe zone and in the North Caucasus. It is even grown in gardens in Siberia and the Ural Mountains, where special efforts are made so that the species can survive in such difficult conditions. This provides evidence for the awareness of the inhabitants of those regions of the value of its raw material. The species is not an ornamental plant, unlike *Berberis thunbergii*, i.e., a species with an extremely wide

range of beautiful garden varieties from the genus *Berberis*. In 2019, the collection of 69 cultivars of *Berberis thunbergii* from the "Kurowscy" nursery was awarded the National Plant Collection status by the Polish Dendrology Society. The decorative value of varieties of this species is appreciated in gardens worldwide.

Berberis vulgaris was brought to North America by early settlers from Europe, where it was a very popularly plant used as medication at that time (Roelfs 1982). The distribution and occurrence of barberry was strongly limited, as it is an indirect host to wheat stem rust. Winter spores of rust emerge on the bottom surface of barberry leaves. Certain types of fungi parasitize shoots: *Puccinia graminis*, *Puccinia brachypodii*, *Puccinia striiformis*, *Erysiphe berberidis*, *Phyllosticta berberidis, Sphaerulina berberidis*. In addition, Diptera larvae *Rhagoletis meigenii* and *Dasineura berberidis* as well as Hemiptera insects *Arge berberidis* feed on barberry shoots (BioInfo UK 2020). In 1918, the eradication program started and its occurrence was substantially limited (Roelfs 1982). Currently, barberry grows in North America, i.e., in New England and from the mountains of Pennsylvania down to Virginia. The species has been eradicated in Europe as well. In Europe, it is found most frequently in the Mediterranean region and the Balkans. It ranges as far as the Caucasus to the east (Jędrzejko 1997). In the Alps, barberry grows at the altitude of up to 2500 m above sea level (Dörr and Lippert 2001).

Barberry was naturalized in Asia. It is currently much more popular there than in Europe. This is associated with the wide use of the plant in medicinal and culinary applications, especially in Iran and Asia Minor. Due to this use, barberry is widely cultivated in Asia. Iran is the world center of barberry production. Production is concentrated mainly in the southern part of the Khorasan region, where the environmental conditions are unfavorable for cultivation of other horticultural crops. Approximately 6000 hectares of barberry plantations and over 4500 tons of raw material are harvested each year in the Khorasan region alone. The plantations are mainly concentrated around the cities of Ghayen and Birjand, but the cultivation has expanded to the cities of Gonabad and Ferdos in recent years (Tehranifar 2003).

Barberry likes warm, sunlit locations and xerothermic habitats. It usually grows on sandy, rocky, and alkaline soils which are rich in calcium. It prefers soils with low humus content. The plant is resistant to drought. It grows on medium-class and infertile soils (Rośl in 2020). The climatic conditions in regions with the largest plantations of the species are typical for desert and semi-dry areas with hot summers, cold winters, low relative humidity, and high fluctuations in daily temperature maximum and minimum values. The average annual rainfall is 190.3 mm in Ghayen and 173.5 mm in Birjand. The annual minimum and maximum temperatures range from −38 to + 41 °C in Ghayen and from −15 to +44 °C in Birjand. This area is characterized by high acidity and salt concentrations in water and soil and very low content of organic matter (Tehranifar 2003).

The common barberry is very popular in Georgia, where the raw material is also produced commercially. It grows in lower mountain zones, at forest margins, and along river banks. However, the species is not commercially cultivated and its current occurrence in nature does not comply with ecological standards.

24.4 Cultivation and Harvesting of Material

The species *Berberis vulgaris* has a small number of varieties, and these are very poorly described. In Arabic scientific literature, there is information about varieties *Berberis vulgaris* "*Atropurpurea*" (Lacroixx 2020; Związek Szkółkarzy Polskich 2020), *Berberis vulgaris f. alba-variegata*, *Berberis vulgaris* "*captured*", and *Berberis vulgaris* "captured Large-Fry" (Związek Szkółkarzy Polskich 2020).

The most important variety cultivated on a large scale is the seedless barberry *Berberis vulgaris* (L.) var. *asperma*, which is grown mainly to obtain fruit for the food industry. The variety has been cultivated for many years as a houseplant in the southern parts of Iran.

The most valuable barberry cultivar *Berberis vulgaris* (L.) var. *asperma* is propagated via suckers. Annual shoots emerging from the roots of adult plants are separated in mid-autumn or late winter. When moving and planting, it is important to prevent the plant from drying out. Plant roots are usually coated with a layer of mud to prevent desiccation. Barberry shrubs are routinely planted in autumn. Spring planting is possible as well, but substantially higher plant survival rates are noted in the case of autumn planting. Pre-planting treatments consist of deep plowing, disc-harrowing, fertilizing, and making individual pits. Fertilization with manure, nitrogen, phosphorus pentoxide, and potassium oxide is recommended before planting. Post-planting fertilization includes placing manure and nitrogen, phosphorus, and potassium fertilizers next to each shrub.

In new orchards, barberry shrubs are planted at 4–7 m spacing of rows and 4–5 m spacing between plants in the row. Rooted suckers are planted singly in each pit. Pruning is not commonly applied in the region unless weak, infected, and dry stems have to be removed. Cutting off the suckers for propagation is a pruning treatment as well.

Barberry can also be propagated vegetatively by rooting above-ground branches or by sowing preferably freshly collected seeds in autumn. Layered seeds are used in spring sowing. However, propagation by sowing reduces the flavor and varietal values.

Barberry is able to survive long periods of drought. However, water scarcity can reduce the yield substantially. It is especially important to water the plant a week before harvesting. Watering is also very important for the survival and growth of young plants in the first year after planting. Young orchards are usually irrigated at 7–10-day intervals. Older orchards are sometimes irrigated every 25 days. The seedless barberry is slightly more resistant to water salinity.

Barberry fruit can be expected to be harvested after five years of cultivation. At that time, each shrub produces approximately 1 kg of fruit, with higher yields provided by older plants. Full production performance is achieved from the fourteenth harvest season. Seedless barberry fruits ripen from early October to early November, depending on weather conditions. They ripen earlier in mountainous areas and later in regions with warmer climate. Due to the thorny stems and special shape of the shrubs, barberry fruit harvesting is one of the most difficult and labor-intensive stages

of barberry production. The raw material is collected manually with the method of beating shoots or by cutting off fruiting stems. Manual harvesting of the raw material involves collection of the fruit into portable containers. This laborious and expensive method is employed in small orchards with short plants. The beating shoots method is used in large orchards with tall shrubs. The berries are harvested using a piece of wood to beat the shoots, and fruits that fall onto a cloth placed underneath are collected. This, however, can bruise the shoots and fruits, thus favoring development of diseases, and the quality of the fruit is not satisfactory. Harvesting by cutting off fruiting stems is a common practice used in the Birjand region and is called "Pofaki". Fruiting shoots are cut off and dried in the shade. Fruits are left to dry for 2–3 months, and they are regarded to have the highest quality.

Currently, only a small amount of harvested barberry is sold as fresh fruit. Most of the crops are sold dried. The traditional sun-drying method is mainly used. The climate conditions and sufficient amounts of sunlight in the cultivation region render this method the most economical production mode. The shade-drying method mentioned above requires a suitable place and a longer time. Other methods include tunnel drying and vacuum drying. The temperature of the tunnel drying process reaches 70 °C. It reduces the anthocyanin content in the fruit. In turn, the vacuum drying technique is rapid and provides excellent product quality but is more expensive than the other methods. After drying, the fruit is aired, cleaned, and packed. Up to 5 kg of fresh fruit are needed to obtain 1 kg of dried barberry, depending on the moisture content in the final product.

Barberry roots can be collected throughout the growing season. They should be harvested with caution, as any loss of thin roots and bark may substantially reduce the berberine content in the raw material. Barberry roots should not be washed in water, as berberine dissolves well in water and loss of the compound during washing may be expected. The raw material must be dried at good ventilation at 40–50 °C. The dried raw material is finally cleaned, and its shelf life is 3 years.

Barberry leaves are harvested in the flower bud formation and flowering stages and should be dried at good ventilation (Tehranifar 2003).

24.5 Chemical Composition

Berberis vulgaris is a typically alkaloidal species. At the beginning of the twentieth century, it was established to have the highest alkaloid content in the genus amounting to 15% (Orechoff 1933) while the lowest alkaloid content was noted for *Berberis thunbergii* DC., i.e. 0.97% (Madaus 1938). The alkaloids occurring in the raw material belong to the group of isoquinoline alkaloids. The first alkaloids, i.e. berberine and tertiary alkaloids, were extracted from barberry in the 1970s (Petcu and Goina 1970) (Fig. 24.8). Currently, berberine has been found in the root and shoots, root and shoot bark, and berries (Imanshahidi and Hosseinzadeh 2008). Polish researchers Domagalina and Smajkiewicz extracted magnoflorine (Fig. 24.9) and

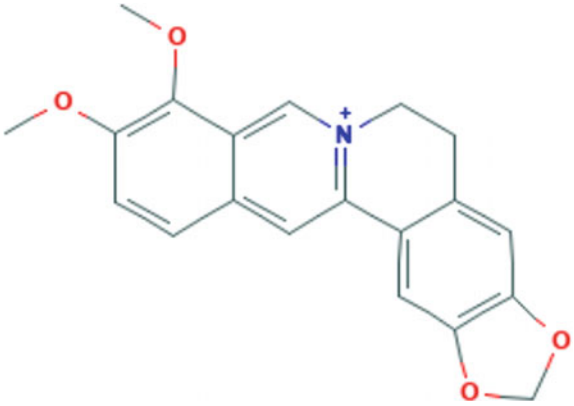


Fig. 24.8 Chemical structure of berberine

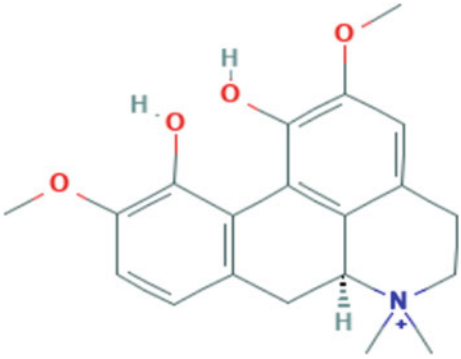


Fig. 24.9 Chemical structure of magnoflorine

columbamine (Domagalina and Smajkiewicz 1971) from barberry. In 1974, protoberberine was identified (Drost-Karbowska et al. 1974). Hošťálková et al. extracted an alkaloid named berbamine from barberry roots (Imanshahidi and Hosseinzadeh 2008; Host'álková et al. 2013).

Currently, it has been reported that barberry plant parts differ in the composition of secondary metabolites. Barberry leaves contain β-xylans and α -glucans, delphinidin 3-O-β-D-glucoside. Ascorbic acid, ursolic acid, and hyperoside are contained in leaves and fruits (Imanshahidi and Hosseinzadeh 2008).

In some fruit extracts triterpenes (lupeol, ursolic acid, oleanolic acid) and sterols (mainly stigmasterol) were determined. Moreover, barberry fruits contain caffeic acid, chlorogenic acid, quercetin, tannin, bervulcine, thaliemidine, esculetin, vitamin K, α-tocopherol, β-carotene, pectins, and sucrose (Imanshahidi and Hosseinzadeh 2008).

Pelargonin, lambertine (Fig. 24.10), magnoflorine, chrysanthemin, berbamine, berberrubine, and bargustanine have been identified in the root. Magnoflorine is also contained in the shoot and root bark. Compounds present in the bark of the aboveground and underground parts include petunidin-3-O-beta-glucoside, jatrorrhizine

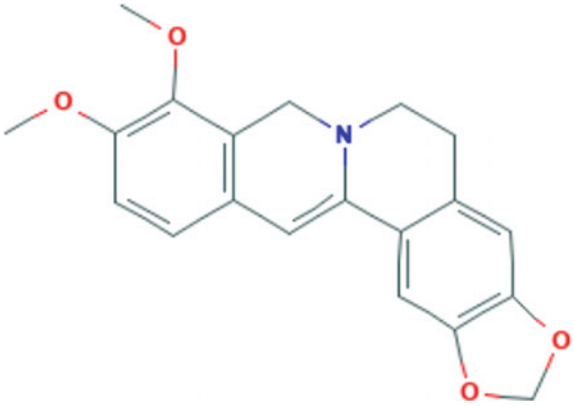


Fig. 24.10 Chemical structure of lambertine

(also contained in the root), columbamine, berberrubine, berbamine, acanthine, and berberine. The latter two compounds are more widely distributed in the plant. Acanthine has been determined in the root, bark, root bark, stem bark, shoots, and leaves. Berberine has been detected in the root, shoot, root bark, bark of aboveground parts, and fruits (Imanshahidi and Hosseinzadeh 2008).

Palmatine (Fig. 24.11) is an important alkaloid from barberry determined in the root, shoot bark and root bark (Imanshahidi and Hosseinzadeh 2008; Saied and Begum 2004). Other alkaloids are represented by oxyberberine, isocorydine, bisbenzylisoquinolines, e.g., oxycanthine (Tomosaka et al. 2008; Mokhber-Dezfuli et al.

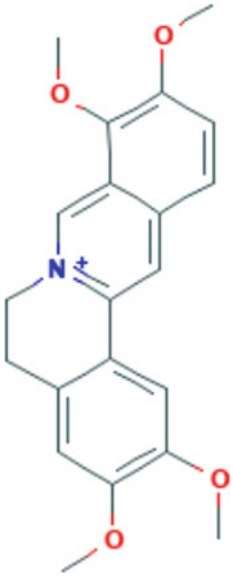


Fig. 24.11 Chemical structure of palmatine

2014), and isoquinoline alkaloids: bervulcine and thaliemidine (Imanshahidi and Hosseinzadeh 2008).

Due to the presence of cytoprotective compounds such as N-(p-trans-coumaroyl) tyramine, cannabisin G, and (±)—lyoniresinol (Tomosaka et al. 2008), *Berberis vulgaris* is highlighted as an important plant used for chemoprevention.

The whole raw material is also rich in carbohydrates, pectins, and polysaccharides (α -glucans, β-xylans). The root bark is the richest source of secondary metabolites and is therefore the most pharmacologically active part of the plant (Imanshahidi and Hosseinzadeh 2008).

A very important group of secondary metabolites in barberry are flavonoids. They are currently ascribed great importance from the point of view of nutrition and chemoprevention. In the group of flavonoids, anthocyanins are present in barberry extracts, with greater amounts in alcoholic extracts than in water extracts (Hoshyar et al. 2016). The group of anthocyanins is represented by chrysanthemin (Fig. 24.12), pelargonin (Fig. 24.13), petunidin-3-O-beta-glucoside (Fig. 24.14), and delphinidin-3-glucoside (Fig. 24.15). Sharifi and Niakousari indicate that due to the lack of an appropriate processing technology, the potential of barberry fruits in terms of extraction of anthocyanins, polyphenols, and ascorbic acid are not fully used. They also mentioned insufficient methods for obtaining hyperoside and quercetin by raw material extraction. They identified the following anthocyanins in *Berberis vulgaris* fruits: cyanidin-3,5-diglucoside, cyanidin-3-glucoside, delphinidin-3,5-diglucoside, petunidin-3-O-beta-glucoside, pelargonidin-3,5-diglucoside, and pelargonidin-3-glucoside (Sharifi et al. 2019) (Fig. 24.16).

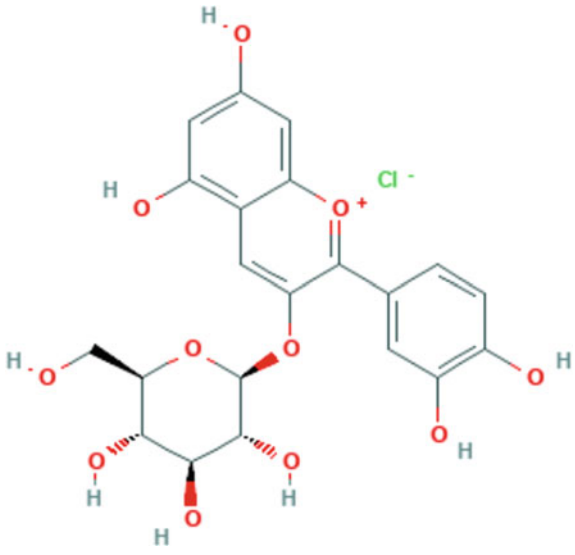


Fig. 24.12 Chemical structure of chrysanthemin

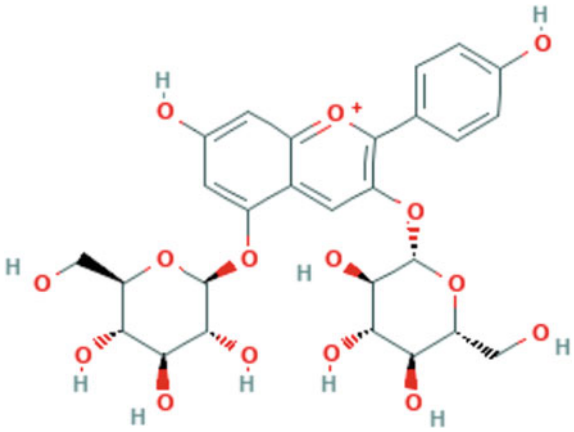


Fig. 24.13 Chemical structure of pelargonin

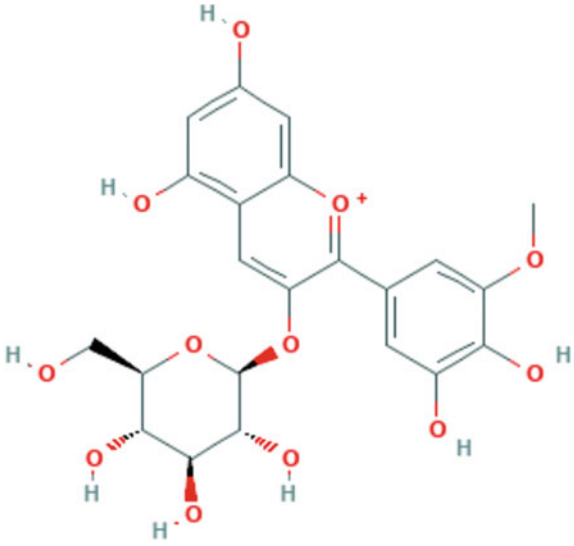


Fig. 24.14 Chemical structure of petunidin-3-O-β-D-glucoside

24.6 Barberry in Traditional Medicine and Current Position in Official Phytotherapy

Barberry has been used since antiquity in both medicine and culinary arts. The therapeutic activity of the plant was exploited in ancient Egypt, Iraq, India, and China (Rahimi-Madiseh et al. 2017). In the Middle-Ages, the species was universally employed in European medicine. The name *Berberis vulgaris* was first used in the twelfth-century literature by Arab medicine writer Averroes to describe barberry fruits. Osiander referred to the plant as a "perfect thirst-quenching and refreshment-offering" substance. Paracelsus described barberry juice as "a sour

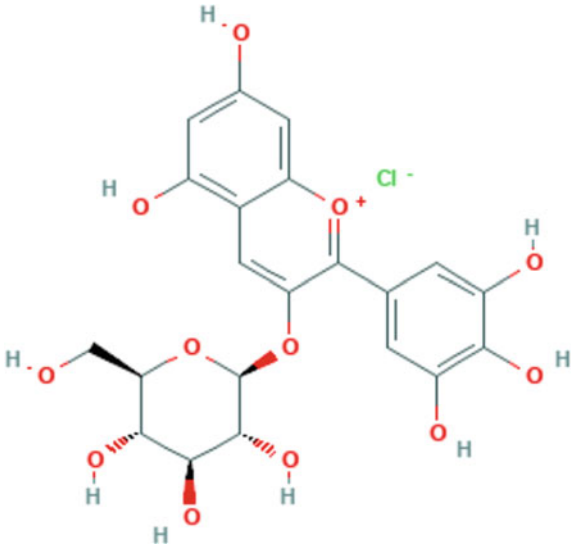


Fig. 24.15 Chemical structure of delphinidin-3-glucoside

Part of plant	**Type of chemical compound**
Root	Isoquinoline alkaloid
Bark	Isoquinoline alkaloid
Root bark	Isoquinoline alkaloid
Stem bark	Isoquinoline alkaloid
Shoots	Isoquinoline alkaloid
Leaves	Isoquinoline alkaloid
	Vitamins
	Flavonoids
	Carbohydrates
	Flavonols
	Oxygen heterocycles
Fruits	Coumarins
	Vitamins
	Phenylpropanoids
	Carotenoids
	Flavonoids
	Alkane to c4
	Isoquinoline alkaloids
	Carbohydrates
	Tannins
	Triterpenes

Fig. 24.16 Characteristics of plant parts based on the type of chemical compounds

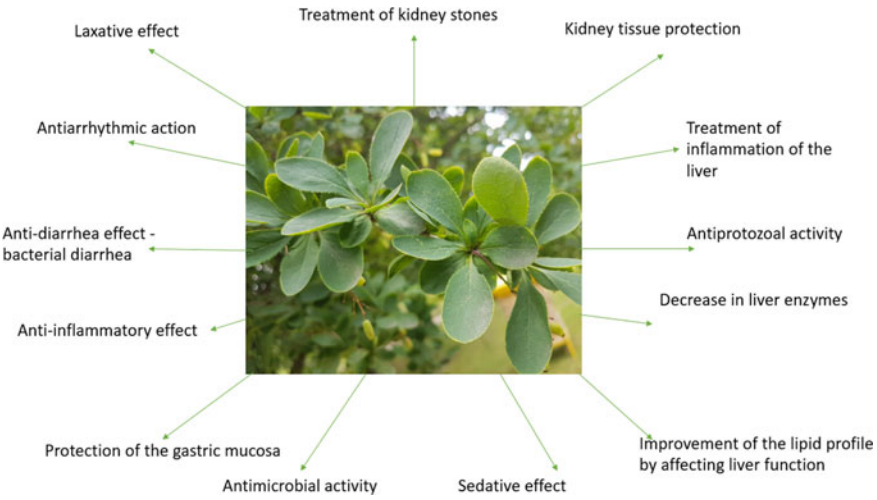


Fig. 24.17 Scientifically confirmed effectiveness of the traditional use of *Berberis vulgaris* L. and its current position in official phytotherapy

mixture". Sixteenth-century sources noted that barberry fruits were primarily used. Matthiolus and other eighteenth and nineteenth-century physicians described the application of fruits and stems (Anon 2020).

In the Near and Far East, the plant is still popular in traditional medicine, especially in Iran, which is the largest producer of *Berberis vulgaris* fruits in the world (Rahimi-Madiseh et al. 2017).

Berberis vulgaris plays a vital role in the European herbal medicine (Fig. 24.17). However, it has never been registered as a medicinal drug. A Digest of Medicinal Plants, compiled on the basis of monographs by the German E Commission of the Federal Health Office (Komission für den humanmedizinischen Bereich, phytotherapeutische Therapierichtung und Stoffgruppe) published in 1990, following the WHO recommendations concerning the definition of the term herbal medicine, features a monograph of *Berberis vulgaris*. It contains requirements for approval of the material to be used as a new medicine. However, the monograph mentions the lack of proven effectiveness of the material (Borkowski 1994). The Federal Drug Administration nor European Medicines Agency have so far not approved *Berberis vulgaris* in any therapeutic indication (FDA 2021; EMA 2021).

In consequence, it is still an herbal agent used in traditional medicine and used raw materials are *Berberidis fructus, Berberidis cortex, Berberidis radicis cortex*, and *Berberidis radix*; the term *Berberidis cortex* denotes the bark of the aboveground part of the plant. In addition to the aforementioned, Polish herbal medicine literature describes *Berberidis folium* as a medicinal material. Due to the previous eradication of the plant, collection of roots and aboveground parts of the plant in Poland has been abandoned so as not to destroy further habitats. Leaves are picked in May and June, and ripe fruits are harvested in August and September when they turn bright red.

Currently, in Poland, the material is only available as a traditional herbal product, and is not included in the list of officially registered medicinal products. Despite strong traditions of application of the plant in England, the British Pharmacopeia does not include a monograph of barberry. The French Pharmacopeia 2012 presents a monograph of *Berberidis radicis cortex.* Other barberry species are included in Chinese and British Pharmacopoeias. Chinese Pharmacopoeia 2010 comprises general information about roots of *Berberis* spp., which are traditionally named "Sankezhen" (Dan et al. 2011).

Officially, the plant is recommended as a traditional drug or a basis for homeopathic medicines. During the homeopathic boom in the nineties in Europe, *Berberis vulgaris* played a vital role in homeopathic treatment of gallstones and kidney stones and in the prophylaxis of these illnesses. Homeopathic preparations based on *Berberis vulgaris* produced by French and German companies are recommended for treatment of chronic rheumatic conditions, gout, and muscle pains.

European Commission has approved *Berberis vulgaris* as a cosmetic ingredient. The plant is found in the CosIng database. Stem, bark, and fruit extracts are registered as skin conditioning agents, while root extract is registered as antimicrobial agent (CosIng 2021).

24.6.1 Application in the Treatment of Liver Diseases

The most popular application in traditional medicine mentioned in scientific literature is associated with the treatment of liver diseases. Ayurvedic medicine mentioned *Berberis vulgaris* as an effective agent in treating liver conditions. In addition, this application has been mentioned most frequently in the contemporary European scientific literature. Traditionally, barberry root was used in the treatment of jaundice in Poland, states of New England, and Denmark (Anon 2020). In Romania, root extracts were used to treat liver inflammations (Sharifi et al. 2019). In "The great Kneipp book", Kneipp mentions barberry tea as a remedy for liver inflammations, jaundice, and gallstones (Kneipp 1935). The aforementioned german Digest of Medicinal Plants mentions Digest mentions illnesses of liver and bile ducts as a potential field of application of the plant (Anon 2020). Various investigations have examined the effect of berberine on the decrease in the activity of liver enzymes, triglycerides, and cholesterol (Majeed et al. 2015). Its effectiveness was also confirmed in the treatment of nonalcoholic fatty liver disease (Chevallier 2001).

24.6.2 Application in the Treatment of Gastrointestinal Tract Diseases

Mexicans treat stomach cancers with barberry extracts (Sarraf et al. 2019). However, such an approach has not been found in other cultures. Traditional Indian and Chinese medicine uses barberry to treat constipation and as an antiemetic agent (Anon 2020). In traditional European medicine, bark extracts (tonics or powdered bark) are used to treat constipation. The laxative effect is caused by berberine content and emerges after high dosage has been administered. On the other hand, high doses of berberine entail appetite loss. Barberry fruit juice acts as a mild laxative. Extract whose dose does not evoke the laxative effect stimulates and alleviates indigestion (Anon 2020). At present, barberry fruit is also employed as a traditional medicine in such gastrointestinal tract ailments as heartburn, stomach cramps, constipation, and appetite loss.

Traditionally, barberry is also used as an anti-diarrhea agent in bacterial diarrhea and bacterial gastrointestinal infections. This application was well-known in ancient Iran (Chevallier 2001) and was associated with the antibacterial effect of the material, which has been scientifically confirmed. The effectiveness of berberine in the treatment of severe diarrhea caused by *Escherichia coli* and *Vibrio cholerae* bacteria has been proved clinically (Rabbanim et al.1987).

It has been evidenced that barberry has a protective effect on the stomach mucous membrane. An in vivo study in mice with connective tissue proliferation, cell infiltration, and the presence of cancerous cells in esophagus induced by acetylsalicylic acid showed a reduction in the proliferation and infiltration of cancerous cells. Fruits administered in the dose of 900 mg/kg proved to have similar effectiveness to that of omeprazole (Majeed et al. 2015).

24.6.3 Application in the Treatment of Urinary System Diseases

The effectiveness of barberry in treating urinary system diseases was already known in Ayurvedic medicine (CosIng 2021). Traditional European medicine claimed that small doses were effective in treating kidney stones and urinary infections (Ullah et al. 2015). Barberry seeds are now frequently used in treating kidney diseases in Pakistan and Northern Europe. Their effectiveness is probably related to the content of flavonoids, alkaloids, and terpenes, which may have a protective effect on kidneys (Hošt'álková et al. 2013; Chevallier 2001). The effectiveness of the extract in treating kidney stones in animals has been proved recently. In an in vivo study, an extract from the bark of barberry root administered to rats impeded the emergence of calcium crystal deposits in uriniferous tubules. It also protected against associated pathological changes, including polyuria, body weight loss, and renal failure. Although animal studies validate the medical application of barberry in kidney stone disease treatment

(Bashir et al. 2010), there are no clinical data on its effect in humans. On the other hand, a clinical study indicated that berberine improved kidney functions in patients with kidney atherosclerosis (Xiaopeng et al. 2017). In a group of patients with high blood pressure and type 2 diabetes, berberine combined with hypotensive and hypoglycemic drugs prevented kidney damage and improved hemodynamic parameters (Dai et al. 2015).

24.6.4 Sedative Activity

As a sedative, *Berberis vulgaris* was used in antiquity in today's Iran (Zarei et al. 2015). The application has not been frequently mentioned in the scientific literature. However, the sedative and neuroprotective effects of *Berberis vulgaris* extracts have been described recently. In an in vivo study, the administration of the extract resulted in enhancement of the magnitude of the outward potassium current in brains of rats. This may explain the effect of barberry on brain (Fatehi et al. 2005).

24.6.5 Antibacterial Applications

Besides treatment of bacterial diarrhea, the antibacterial effect of barberry is exploited in other than intestinal infections. Traditional Indian medicine employs barberry in treating leprosy (Lomakina 1961; Kang et al. 2015). In addition, the Chinese and Japanese exploit the antibacterial effect of *Berberidis cortex*. A popular application of barberry bark or barberry berry decoction is its use as mouthwash for treating oral cavity pains accompanying aphthae or bacterial gum infections (Lomakina 1961). The antibacterial effectiveness of barberry has been confirmed. It has been documented that berberine has an antibacterial effect against various strains of bacteria, including *Actinobacillus pleuropneumoniae* (Kang et al. 2015), *Shigella dysenteriae* (Kong et al. 2010), *Streptococcus agalactiae* (Peng et al. 2015), and *Helicobacter pylori* (Zhang et al. 2014). The effectiveness of barberry extract against *Acne vulgaris* bacteria was clinically confirmed in teenagers experiencing mild to severe acne (Fouladi 2012). The extract from barberry berries also proved effective in treating bacterial vaginal infections. When applied jointly with metronidazole, it significantly enhanced the effectiveness of the treatment, which was also clinically verified. Such a combination improved the results of the treatment. Moreover, no relapse was observed (Masoudi et al.). Berberine itself enhances the activity of certain commonly administered antibiotics. It suggests its potential application in conjunction with other antibiotics in antibiotic-resistant bacterial infections (Imenshahidi and Hosseinzade 2016).

24.6.6 Antiprotozoal and Antidermatophytic Activity

In malaria-affected regions, especially in North Africa (Zarei et al.), barberry extracts are traditionally used to alleviate the course of the illness. The antiprotozoal effect is comparable to the application of quinine. However, certain authors did not report improvement of patients infected with *Plasmodium malariae*, *Plasmodium vivax*, and *Plasmodium falciparum* (Lomakina 1961).

Antifungal and antiprotozoal activity has been confirmed in vitro. It was proved that various barberry extracts and berberine exhibited high anti-dermatophytic activity against pathogenic dermatophytes *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum canis*, and *Microsporum gypseum.* Methanolic extracts of *Berberis vulgaris* had stronger anti-dermatophytic activity than aqueous extracts (Mahmoudvand et al. 2014). Other studies confirmed that barberry ethanolic extract also significantly reduced the viability of *Leishmania tropica* promastigotes, i.e. it was found to decrease the growth of amastigotes in macrophages, while failing to manifest significant cytotoxicity against mice macrophages (Mahmoudvand et al. 2014; Parvizi et al. 2020).

24.6.7 Anti-inflammatory and Antiallergenic Activity

The anti-inflammatory activity of barberry was used in Eastern traditional medicine to treat rheumatism and chronic inflammation (Zargari 1997; Ghafourian et al. 2017). Similarly, Indian traditional medicine exploited barberry in treating rheumatism (Lomakina 1961; Kang et al. 2015). As shown by Hübotter, Mongolian medicine used barberry to treat chronic ophthalmitis, arthritis, deformation of joints, rheumatism, low back pain, and spine and muscle pains (Lomakina 1961); however, treatment of liver inflammation was the most frequent application (Kneipp 1935). Currently, barberry root extracts are used in traditional Bulgarian and Eastern medicine to treat rheumatoid arthritis and other chronic inflammatory diseases (Fatehi-Hassanabad et al. 2005). Modern studies have strongly confirmed the anti-inflammatory activity of barberry extracts. Studies conducted by Kiasalari et al. concerning the effect of barberry extracts on severe and chronic inflammations in rats indicated that alcoholic berry extracts alleviated severe and chronic inflammation (Kiasalari et al. 2011). A comparative study of the effect of barberry berry extract, berberine chloride, and corticosteroids on the rat model of ulcerative colitis revealed that the prevention of colon lesions may result from the presence of anthocyanins. It was proven that it may result from the presence of anthocyanins (Minaiyan et al. 2011).

The scientific data offer few reports on the traditional use of barberry to treat allergies. However, Shamsa et al. indicated that barberry fruit extract, depending on the dose, reduced the level of histamine to an extent similar to that of dexchlorpheniramine. It was also found to decrease the level of acetylcholine with similar efficiency as that of atropine (Shamsaa et al. 1999).

Despite previous studies suggesting otherwise (Shamsaa et al. 1999), studies of the effect of barberry on inflammatory markers conducted by Ebrahimi-Mameghani et al. indicated no effect of the compound on the concentration of interleukin 6 (IL-6) and C-reactive protein (CRP) in serum (Ebrahimi-Mamaghani et al. 2009a; b).

24.6.8 Traditional Scientifically Unconfirmed Applications

Traditional Iranian, Bulgarian, and Russian medicine uses barberry to stem bleeding (Lomakina 1961; Farhadi et al. 2008; Zarei et al. 2015). As shown in Polish herbal medicine manuals, leaf extracts improve the contractility of uterine vessels and smooth muscles (Mokhber-Dezfuli et al. 2014). Traditional English medicine applies leaf extracts in cases of pregnancy complications and pathological uterine bleeding. It is due to the effect of the extracts on vessel contraction, which stems bleeding, in all organs of the abdominal cavity, including the uterus (Lomakina 1961). However, no scientific proof of their effectiveness has been provided. At present, there is no information about the safety of application during pregnancy; hence, the material is not used for treatment of bleeding in pregnant patients and the application in gynecology has changed the course of berberine use.

Traditional Iranian and Egyptian medicine used barberry in treating fevers (Lomakina 1961; Farhadi et al. 2008; Parvizi et al. 2020). In addition, barberry root extracts (bitter tonic) were used to reduce fever in Europe. It was noted that berberine itself is much more effective in this regard (Wehmer 1991). This application has been frequently raised in the scientific data. However, this effect has not been scientifically confirmed.

24.7 Barberry in Modern Therpy

Scientific data on barberry indicate that the plant is worth attention as an agent preventing the development and alleviating the course of common cardiovascular and metabolic civilization diseases (Fig. 24.18). Therefore, the search for natural remedies and preventive measures for these illnesses seems vital. At present, research on the effect of *Berberis vulgaris* indicates its considerable potential as a prophylactic against civilization diseases. The anti-oxidative properties of the material should be especially highlighted, together with the anti-cancerogenic and chemopreventive effects of barberry reported in the scientific data.

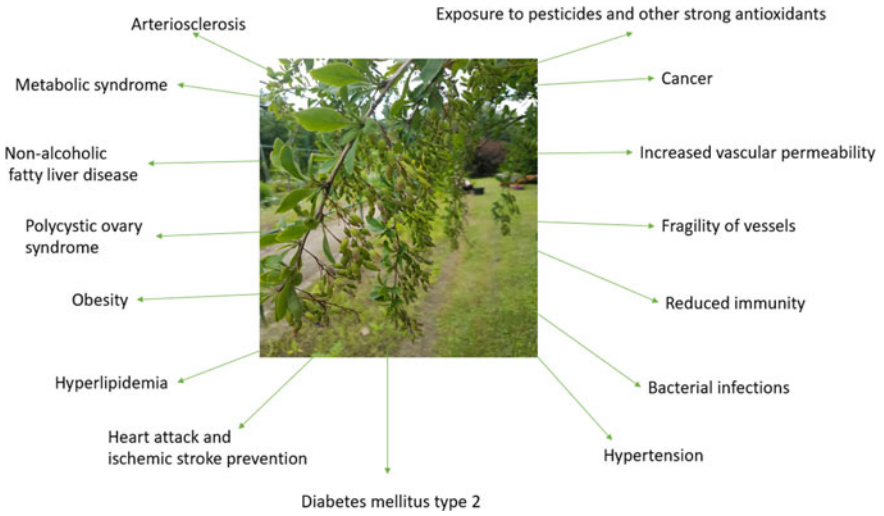


Fig. 24.18 Conditions and diseases in which barberry exhibits scientifically confirmed effectiveness according to the latest knowledge

24.7.1 Cardiovascular System

Berberis vulgaris exerts a hypotensive effect (Affuso et al. 2010; Lan et al. 2015; Abushouk et al. 2017), which results from a combined sedative, anti-arrhythmic, and anti-artherosclerotic effect and acts on the vascular endothelium (Ghafourian et al. 2017). The reduction in mean arterial pressure and heart rate in vivo is probably associated with endothelium-independent vasodilation (Imanshahidi and Hosseinzadeh 2008).

Due to the high content of berberine in the raw material, an effect of barberry extracts, similar to that of berberine, on cardiovascular diseases can be expected. Although a strong effect of barberry extracts on the reduction of liver enzyme function, triglycerides, and cholesterol have been found, hence the extracts can be used in patients with nonalcoholic fatty liver disease (Majeed et al. 2015), berberine has been subject to more comprehensive clinical studies. In patients with congestive heart failure, berberine has an antiarrhythmic effect, improves the ejection fraction, and improves left ventricular function and overall physical performance (Zeng et al. 2003). Berberine lowers blood pressure by reducing cholesterol through several mechanisms, e.g., it stimulates the hepatic uptake of serum cholesterol, intensifies LDL-C removal from the blood (Barrios et al. 2017), reduces intestinal cholesterol absorption, enhances cholesterol excretion with feces, stimulates hepatic cholesterol exchange, and formation of bile acids (Li et al. 2015). It also stimulates AMP-activated protein kinase, which may limit the synthesis of fatty acids (Brusq et al. 2006). A decrease in the concentration of TG, TC, and LDL-C has been found as well as an increase in HDL-C after three months of use (Wang et al. 2016).

Berberine also exerts anti-inflammatory and neuroprotective effects in atherosclerosis and ischemic stroke. It lowers the level of inflammatory factors in the serum (MIF and IL-6) and the volume of unstable plaque in the carotid artery (Li et al. 2016). Moreover, in patients with acute coronary syndrome, berberine improves clinical outcomes due to its anti-inflammatory effect (Meng et al. 2012).

24.7.2 Anti-diabetic Function and Effectiveness in Metabolic Syndrome

Barberry shows promising effects in the treatment of type 2 diabetes. It significantly lowers blood sugar levels and, very importantly, in such cases, it has a great ability to alleviate cardiovascular diseases resulting from complications related to type 2 diabetes and insulin resistance (Meliani et al. 2011).

This property has been the subject of extensive research and entered the clinical trial phase. It was noted that the barberry extract (3 g/d) administered for 3 months to type 2 diabetic patients reduced cardiovascular risk factors and improved blood sugar levels. It also caused reduction of triglycerides, total cholesterol, LDL fraction, apolipoprotein B, glucose, and insulin. The use of barberry extracts thus reduces the risk of coronary artery disease and myocardial infarction, which is a complication of type 2 diabetes. On the other hand, there was no difference in the levels of HDL-C, homocysteine, and hemoglobin (HbA1c) glycation. These results are consistent with in vivo studies of the function of berberine alone in rats (Safari et al. 2020).

Hyperinsulinemia and insulin resistance typical of type 2 diabetes also play an important role in the pathogenesis of polycystic ovary syndrome (Li et al. 2013). Berberine administered alone was tested with regard to polycystic syndrome, and its effects were similar to metformin in inducing ovulation in polycystic ovary syndrome with low side effects and lowered lipid parameters and BMI (An et al. 2014; Wu et al. 2015). Additionally, its effect on the lipid profile of patients with polycystic ovary syndrome ovaries was confirmed (Cicero et al. 2014). No clinical data are yet available on barberry alone in these cases; therefore, further research is expected.

The effectiveness of barberry in the treatment of metabolic syndrome was confirmed (Tabeshpour et al. 2017) in cases where 6-week supplementation with 200 mg of dried barberry significantly improved the lipid profile, lowered the level of hs-CRP (Zilaee et al. 2014), decreased the pro-oxidative-antioxidant balance (Mohammadi et al. 2014), lowered the concentration of LDL-C, and increased the level of HDL cholesterol and insulin (Ebrahimi-Mamaghani et al. 2009a, b).

24.7.3 Antioxidant and Anticancer Activity

The prevention and alleviation of the development and course of civilization diseases, such as type 2 diabetes, obesity, hypertension, and metabolic syndrome, are important premise to consider barberry as a dietary supplement for subjects at increased risk of these diseases. Barberry has a positive effect on the lipid profile, blood pressure regulation, and anti-inflammatory activity. Consequently, barberry seems to be an ideal candidate for supplementation in subjects with an increased risk of hypertension, diabetes, and related complications. Due to the high content of anthocyanins, barberry supplementation can protect against cardiovascular diseases and heart attacks. Various types of cancer are another scourge among civilization diseases. Free radicals and oxidative stress are some of the important factors in the development of cancer (Nowak et al. 2014). The antioxidant activity of barberry is a characteristic of ethanolic extracts from barberry roots, lignified stems, and leaves. Its antioxidant activity correlates well with the content of antioxidants, i.e. phenols and flavonols. This suggests an important role of these compounds in the overall antioxidant function of the tested parts of the plant. The antioxidant activity mainly varies between plant organs (Zovko Koncić 2010). Strong antioxidant efficacy of barberry has been demonstrated in vivo in rats with pulmonary fibrosis induced by paraquat, i.e. a herbicide commonly used in the US but banned in Europe. The toxicity of paraquat is mainly based on the reaction of cell membrane lipids with paraquat peroxide radicals. Most likely, the effectiveness of barberry extracts in treating paraquat-induced pulmonary fibrosis is related to its strong antioxidant and anti-inflammatory properties. In the case of paraquat-induced pulmonary fibrosis, the effect of the extract was dose-dependent (Javad-Mousavi et al. 2016). Barberry has a significant protective function also in the case of exposure to other pesticides. The level of antioxidants in the cerebellum and brain of mice exposed to the extract of diazinon and barberry was higher than that of treatment with diazinon alone. Moreover, the function of acetylcholinesterase in the brain was significantly higher after inclusion of barberry extract (Sonei et al. 2020).

Due to the proven and extensively investigated anti-cancer effects of berberine, barberry is also being increasingly studied in this area. Barberry extracts, similarly to berberine, inhibit cell proliferation in the MCF 7 breast cancer cell line, the CACO-2 colonorectal cancer cell line, and the Hep G2 liver cancer cell line (Kang et al. 2015). The most promising results in terms of anti-cancer activity have been achieved in the case of breast cancer. The above-mentioned extracts have been found to inhibit significantly the proliferation of breast cancer cells (MCF-7) without affecting normal human breast epithelial cells (MCF10-A). In the case of this cell line, the alcoholic extract has been shown to induce apoptosis (Hoshyar et al. 2016; Ghafourian et al. 2017). A clinical study in women with breast cancer showed that barberry juice administered for 8 weeks reduced the vascular endothelial growth factor (VEGF), the peroxisome proliferator-activated receptor (PPAR), and the hypoxia-induced factor (HIF), i.e. important parameters in breast cancer carcinogenesis (Pirouzpanah et al. 2019). It has also been demonstrated that barberry bark extract inhibits human

cholinesterase and butyrylcholinesterase, which play a key role in anti-cancer activity (Novák et al. 2015).

24.7.4 Other Possible Applications

The latest references to the anti-cancer effect of barberry indicate the effectiveness of the extract in the treatment of leukemia. It was confirmed that, after administration of 1 mg/ml of barberry extract, the level of p53 marker expression in the WEHI-3 leukemia cell line was lower than after exposure to doxorubicin (Saedi et al. 2015). Och et al. examined the effect of berberine on a number of leukemia cell lines (HL-60, HL-60/MX1, HL-60/MX2, CCRF/CEM, CEM/C1, J45) and found a significant reduction in p53 gene expression (Och et al. 2019).

A comparative study of the effect of berberine and barberry extract revealed that crude barberry extracts inhibited α-glycosidase to a substantially greater extent than berberine chloride. The inhibitory effect of both substances on acetylcholinesterase was the same (Pirouzpanah et al. 2019). Additionally, compared to barberry, berberine was characterized by higher cytotoxicity towards murine macrophages (Mahmoudvand et al. 2014).

The chemopreventive properties suggest that barberry should be further investigated with regard to supplementation in high-risk cancer-prone patients. It could help to avoid development of the disease and in the treatment of precancerous lesions to inhibit their transformation into cancer.

24.8 Nutrition

Barberry fruits are a natural source of easily digestible vitamins and carotenoids. They are valuable vitaminizing materials regulating metabolic processes and improving the overall resistance of the body, especially against bacterial infections, as they reduce inflammation of mucous membranes and tighten capillaries. The presence of various organic acids and their derivatives significantly increases the effectiveness of vitamin C compared to the synthetic form of the vitamin. Consequently, barberry fruits complement the treatment of bacterial infections, colds, fever, and excessive vascular permeability. They are also effective in the period of decreased immunity.

The specific sour taste of the berries, due to the high content of vitamin C, prompted the use of the fruit as an ingredient in preserves, which was popular in Antiquity. The fruit was also used as an ingredient in the food. Over time, barberries have been replaced in this respect by other species (Atlas Roślin 2020). Currently, they are used in the food industry to make jams, jellies, and wine. Unfortunately, barberry preserves have completely lost their popularity in Europe, where the risk of civilization diseases is the greatest. There are dietary supplements on the market containing *Berberis vulgaris* in the form of liquid extracts, infusions, and capsules.

In addition, capsules that contain berberine are available as well. From the point of view of supplementation, the berries are the most convenient to use. They can be eaten fresh or dried, but unripe fruit should not be eaten. Alternatively, they can be served to brew tea. The fruit is safe because barberry is not a poisonous plant. In high doses, the extracts cause diarrhea and loss of appetite; there may also be agitation or depression with hallucinations, delirium with loss of consciousness (Anon 2020). However, no serious intoxication has been reported in humans. It should be noted that berberine inhibits CYP enzymes, which in combination with other drugs may cause indirect toxicity (Zohre et al. 2017).

The European Food Safety Authority has not yet defined the standards for *Berberis vulgaris* (EFSA 2021) *Berberis vulgaris* currently occurs powdered as dietary supplements and its nutrition potential is poorly known.

24.9 Conclusions

Due to its undesirable occurrence in cereal cultivation areas, the species has a turbulent history of occurrence and cultivation. Formerly appreciated and cultivated in monastery gardens in Europe in the past centuries, today it grows rather in the wild or sporadically in European gardens. However, it is intensively cultivated in the Middle East, mainly in Iran, from where it is exported all over the world. The plant is mainly cultivated around the cities of Ghayen and Birjand, but the cultivation has expanded to the cities of Gonabad and Ferdos in recent years (Tehranifar et al. 2003), where the weather conditions facilitate large-scale production of the plant. The seedless variety is mainly cultivated and exported. Despite its long tradition, barberry has never been officially approved as a medicine. Some traditional applications have been scientifically validated. The effect of barberry in the fight against civilization diseases such as type 2 diabetes, metabolic syndrome, cardiovascular diseases, and cancer gives great hope. The rich composition of pharmacologically active and nutritional compounds in the plant indicates that barberry is an important candidate as a nutrient for subjects exposed to civilization diseases or with reduced immunity and susceptibility to bacterial infections. Despite its high medicinal potential, the genus is currently appreciated rather as a spice and only in narrow circles as a herbal medicine.

References

Abushouk AI, Salem AMA, Abdel-Daim MM (2017) Berberis vulgaris for cardiovascular disorders: a scoping literature review. Iran J Basic Med Sci 20:503–510

Affuso F, Ruvolo A, Micillo F, Saccà L, Fazio S (2010) Effects of a nutraceutical combination (berberine, red yeast rice and policosanols) on lipid levels and endothelial function randomized, double -blind, placebo-controlled study. Nutrition Metabol Cardiovasc Dis 20:656–661

An Y, Sun Z, Zhang Y et al (2014) The use of berberine for women with polycystic ovary syndrome undergoing IVF treatment. Clin Endocrinol 80:425–431
Anon (2020) Henriette's herbal homepage, Helsinki. Accessed 26 Jul 2020 https://www.henriettes-herb.com
Barrios V, Escobar C, Cicero AFG, Burke D, Fasching P, Banach M, Bruckert E (2017) A nutraceutical approach (Armolipid Plus) to reduce total and LDL cholesterol in individuals with mild to moderate dyslipidemia: review of the clinical evidence. Atherosclerosis Supplements 24:1–15
Bashir S, Gilani AH, Siddiqui AA, Pervez S et al (2010) Berberis vulgaris root bark extract prevents hyperoxaluria induced urolithiasis in rats. Phytother Res 24(8): 1250–1255
BioInfo (UK) (2020) London. Accessed 13 July 2020 www.bioinfo.org.uk
Borkowski B (1994) Rośliny lecznicze w fitoterapii : kompendium roślin leczniczych uszeregowanych według zakresów stosowania na podstawie monografii opracowanych przez Komisję E Federalnego Urzędu Zdrowia RFN. Instytut roślin i przetworów zielarskich, Poznań
Brusq JM, Ancellin N, Grondin P et al (2006) Inhibition of lipid synthesis through activation of AMP kinase: an additional mechanism for the hypolipidemic effects of berberine. J Lipid Res 47:1281–1288
Chevallier A (2001) The encyclopedia of medicinal plants. Dorling Kindersley, St Leonards
Cicero AFG, Reggi A, Parini A, Morbini M et al (2014) Berberine and monacolin effects on the cardiovascular risk profile of women with oestroprogestin-induced hypercholesterolemia. High Blood Pressure Cardiovasc Prev 21:221–226
CosIng (2021) Brussels. Accessed 10 Jan 2021 https://ec.europa.eu
Dai P, Wang J, Lin L, Zhang Y, Wang Z (2015) Renoprotective effects of berberine as adjuvant therapy for hypertensive patients with type 2 diabetes mellitus: evaluation via biochemical markers and color Doppler ultrasonography. Exp Therap Med 10:869–876
Dan Y, Liu Y, Peng Y, Qian Z, Xiao P (2011) New collection of crude drugs in Chinese pharmacopoeia 2010 II. Sankezhen (Berberis spp.). Chinese Herbal Med 3(4): 272–288
Domagalina E, Smajkiewicz A (1971) Isolation of magnophlorine and columbamine from Berberis vulgaris. Acta Pol Pharm 28(1):81–87
Dörr E, Lippert W (2001) Flora des Allgäus und seiner Umgebung. IHW-Verlag, Eching
Drost-Karbowska K, Kowalewski Z, Szaufer M (1974) Determination of protoberberine alkaloids in Berberis vulgaris L. Acta Pol Pharm 31(5):683–687
Ebrahimi-Mamaghani M, Arefhosseini SR, Golzarand M, Aliasgarzadeh A (2009a) Long-term effects of processed Berberis vulgaris on some metabolic syndrome components. Iran J Endocrinol Metabol 11
Ebrahimi-Mameghani M, Arefhosseini SR, Golzarand M, Aliasgarzadeh A (2009b) Long-term effects of processed Berberis vulgaris on some metabolic syndrome components. IJEM 11: 41–47
EFSA (European Food Safety Authority) (2021) Parma. Accessed 10 Jan 2021 https://www.efsa.europa.eu
EMA (European Medicines Agency) (2021) Amsterdam. Accessed 12 Jan 2021 https://www.ema.europa.eu
Farhadi AK, Gavadifar K, Farha A (2008) Effects of *Berberise Vulgaris* fruit extract on blood cholesterol and triglyceride in hyperlipidemic patients Koomesh. J Semnan Univ Med Sci 9:211–216
Fatehi M, Saleh TM, Fatehi-Hassanabad Z, Farrokhfal K, Jafarzadeh M, Davodi S (2005) A pharmacological study on Berberis vulgaris fruit extract. J Ethnopharmacol 102(1):46–52
Fatehi-Hassanabad Z, Jafarzadeh M, Tarhini A, Fatehi M (2005) The antihypertensive and vasodilator effects of aqueous extract from Berberis vulgaris fruit on hypertensive rats. Phytother Res 19(3):222–225
FDA (Federal Drug Administration) (2021) Silver spring. Accessed 10 Jan 2021 https://www.fda.gov
Fouladi RF (2012) Aqueous extract of dried fruit of Berberis vulgaris L. in acne vulgaris, a clinical trial. J Diet Suppl 9(4):253–261

Ghafourian E, Sadeghifard N, Pakzad I et al (2017) Ethanolic extract of Berberis Vulgaris fruits inhibits the proliferation of MCF-7 breast cancer cell line through induction of apoptosis. Infect Disord Drug Targets 17(3):192–198

Hoshyar R, Mahboob Z, Zarban A (2016) The antioxidant and chemical properties of Berberis vulgaris and its cytotoxic effect on human breast carcinoma cells. Cytotechnology 68(4):1207–1213

Host'álková A, Novák Z, Pour M, Jirosová A et al (2013) Berbanine: a new isoquinoline-isoquinolone alkaloid from Berberis vulgaris (Berberidaceae). Nat Prod Commun 8(4):441–442

Imanshahidi M, Hosseinzadeh H (2008) Pharmacological and therapeutic effects of Berberis vulgaris and its active constituent, berberine. Phytother Res 8:999–1012

Imenshahidi M, Hosseinzade H (2016) Berberis vulgaris and berberine: an update review. Phytotherapy Res 30:1745–1764

Javad-Mousavi SA, Hemmati AA, Mehrzadi S et al (2016) Protective effect of Berberis vulgaris fruit extract against Paraquat-induced pulmonary fibrosis in rats. Biomed Pharmacother 81:329–336

Jędrzejko K. (1997) Zarys wiedzy o roślinach leczniczych. Śląska Akadeima Medyczna, Katowice

Kang S, Li Z, Yin Z, Jia R, Song X, Li L (2015) The antibacterial mechanism of berberine against Actinobacillus pleuropneumoniae. Nat Prod Res 29:2203–2206

Kiasalari Z, Khalili M, Ahmadi P (2011) Effect of alcoholic extract of Berberis vulgaris fruit on acute and chronic inflammation in male rats. J Babol Univ Med Sci 58:28–35

Kneipp S (1935) The great Kneipp book. Verlag, Monachium

Kong W, Li Z, Xiao X, Zhao Y, Zhang P (2010) Activity of berberine on Shigella dysenteriae investigated by microcalorimetry and multivariate analysis. J Therm Anal Calorim 102:331–336

Lacroixx (2020) Teheran. Accessed 3 Nov 2020 https://www.lacroixx.com

Lan J, Zhao Y, Dong F, Yan Z et al (2015) Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipemia and hypertension. J Ethnopharmacol 161:69–81

Li XY, Zhao ZX, Huang M et al (2015) Effect of Berberine on promoting the excretion of cholesterol in high-fat diet-induced hyperlipidemic hamsters. J Transl Med 13:278

Li Y, Kuang H, Shen W et al (2013) Letrozole, berberine, or their combination for anovulatory infertility in women with polycystic ovary syndrome: study design of a doubleblind randomised controlled trial. BMJ Open 003934

Li Y, Wang P, Chai MJ et al (2016) Effects of berberine on serum inflammatory factors and carotid atherosclerotic plaques in patients with acute cerebral ischemic stroke. Zhongguo Zhongyao Zazhi 41:4066–4071

Lomakina VA (1961) Treatment of chronic cholecystitis with a tincture of Berberis vulgaris leaves. Sov Med 25:139–144

Madaus G (1938) Lehrbuch der Biologischen Heilmittel. Georg Thieme Verlag, Leipzig

Mahmoudvand H, Sharififar F, Rahmat MS et al (2014) Evaluation of antileishmanial activity and cytotoxicity of the extracts of Berberis vulgaris and Nigella sativa against Leishmania tropica. J Vector Borne Dis 51(4):294–299

Majeed W, Aslam B, Javed I et al (2015) Histopathological evaluation of gastro protective effect of Berberis vulgaris (Zereshk) seeds against aspirin induced ulcer in albino mice. Pak J Pharm Sci 28(6):1953–1958

Masoudi M, Kopaei MR, Miraj S (2016) Comparison between the efficacy of metronidazole vaginal gel and Berberis vulgaris (Berberis vulgaris) combined with metronidazole gel alone in the treatment of bacterial vaginosis. Electron Physician 8(8):2818–2827

Meliani N, Dib Mel A, Allali H, Tabti B (2011) Hypoglycaemic effect of Berberis vulgaris L. in normal and streptozotocin-induced diabetic rats. Asian Pac J Trop Biomed 1(6): 468–471

Meng S, Wang LS, Huang ZQ et al (2012) Berberine ameliorates inflammation in patients with acute coronary syndrome following percutaneous coronary intervention. Clin Exp Pharmacol Physiol 39:406–411

Minaiyan M, Ghannadi A, Mahzouni P, Jaffari-Shirazid E (2011) Comparative study of berberis vulgaris fruit extract and berberine chloride effects on acetic acid-induced colitis in rats. Iran J Pharm Res 10(1):97–104

Mohammadi A, Sahebkar A, Kermani T et al (2014) Barberry administration and pro-oxidant–antioxidant balance in patients with metabolic syndrome. Iran Red Crescent Med J 16: e16786

Mokhber-Dezfuli N, Saeidnia S, Gohari AR, Kurepaz-Mahmoodabadi M (2014) Phytochemistry and pharmacology of berberis species. Pharmacogn Rev 8(15):8–15

Novák Z, Hošt'álková A, Opletal L, Nováková L, Hrabinová M, Kuneš J, Cahlíková L (2015) (+)-chenabinol (revised NMR data) and two new alkaloids from Berberis Vulgaris and their biological activity. Nat Prod Commun 10(10):1695–1697

Nowak R, Olech M, Nowacka N (2014) Plant polyphenols as chemopreventive agents. In: Watson R, Preedy VR, Zibadi S (eds) Polyphenolsa in human health and disease. Elsevier, San Diego, pp 1289–1307

Och A, Zalewski D, Komsta Ł, Kołodziej P, Kocki J, Bogucka-Kocka A (2019) Cytotoxic and proapoptotic activity of sanguinarine, berberine, and extracts of *Chelidonium majus* L and *Berberis thunbergii* DC toward hematopoietic cancer cell; ines. Toxins 11(9):485

Orechoff A (1933) Über die Alkaloide von *Berberis heteropoda.* Arch Pharmaz 271(6):323–327

Parvizi MM, Zare F, Handjani F, Nimrouzi M, Zarshenas MM (2020) Overview of herbal and traditional remedies in the treatment of cutaneous leishmaniasis based on traditional persian medicine. Dermatol Ther 3566

Peng L, Kang S, Yin Z, Jia R, Song X, Li L, Jing B (2015) Antibacterial activity and mechanism of berberine against Streptococcus agalactiae. Inter J Clin Exp Pathol 8:5217–5223

Petcu P, Goina T (1970) New methods for extraction of alkaloids from *Berberis vulgaris.* Planta Med 18(4):372–375

Pirouzpanah S, Asemani S, Shayanfar A, Baradaran B, Montazeri V (2019) The effects of *Berberis Vulgaris* consumption on plasma levels of IGF-1, IGFBPs, PPAR-γ and the expression of angiogenic genes in women with benign breast disease: a randomized controlled clinical trial. BMC Complement Altern Med 19(1):324

Polskich ZS (2020) Warszawa. Accessed 3 Nov 2020 https://www.e-katalogroslin.pl

Rabbanim GH, Butler T, Knight J, Sanyal SC, Alam K (1987) Randomized controlled trial of berberine sulfate therapy for diarrhea due to Enterotoxigenic Escherichia coli and Vibrio cholerae. J Infect Dis 155:979–984

Rahimi-Madiseh M, Lorigoini Z, Zamani-gharaghoshi H, Rafieian-kopaei M (2017) Berberis vulgaris: specifications and traditional uses. Iran J Basic Med Sci 20(5):569–587

Roelfs AP (1982) Effect of barberry eradication on stem rust in the United States. Plant Disease Feb 177–181

Roślin A (2020) Warszawa. Accessed 24 July 2020 https://atlas.roslin.pl

Rutkowski L (2013) Klucz do oznaczania roślin Polski niżowej. Wydawnictwo Naukowe PWN, Warszawa

Saedi TA, Ghafourian S, Jafarlou M et al (2015) Berberis vulgaris fruit crude extract as a novel anti-leukaemic agent. J Biol Regul Homeost Agents 29(2):395–399

Safari Z, Farrokhzad A, Ghavami A et al (2020) The Effect of Barberry (*Berberis Vulgaris* L.) on glycemic indices: a systematic review and meta-analysis of randomized controlled trials. Complement Ther Med 51: 102414

Saied S, Begum S (2004) Phytochemical studies of Berberis vulgaris. Chem Nat Compd 40:137–140

Sarraf M, Babaei AB, Naji-Tabasi S (2019) Investigating functional properties of barberry species: an overview. J Sci Food Agric 99:5255–5269

Shamsaa F, Ahmadianib A, Khosrokhavara R (1999) Antihistaminic and anticholinergic activity of barberry fruit (Berberis vulgaris) in the guinea-pig ileum. J Ethnopharmacol 64(2):161–166

Sharifi A, Niakousari M, Mortazavi SA, Elhamirad AH (2019) High-pressure CO2 extraction of bioactive compounds of barberry fruit (Berberis vulgaris): process optimization and compounds characterization. J Food Meas Character 13:1139–1146

Sonei A, Fazelipour S, Kanaani L, Hadipour JM (2020) Protective effects of berberis vulgaris on diazinon-induced brain damage in young male mice. Prev Nutr Food Sci 25(1):65–70

Tabeshpour J, Imenshahidi M, Hosseinzadeh H (2017) A review of the effects of berberis vulgaris and its major component, berberine, in metabolic syndrome. Iran J Basic Med Sci 20:557–568

Tehranifar A (2003) Barberry Growing in Iran. Acta Hort 620:193–195

Tomosaka H, Chin YW, Salim AA, Keller WJ, Chai H, Kinghorn AD (2008) Antioxidant and cytoprotective compounds from *Berberis vulgaris* (barberry). Phytother Res 22(7):979–981

Ullah N, Khan S, Khan A, Ahmad W, Shah Y, Ahmad L, Ullah I (2015) A prospective pharmacological review of medicinal herbs, *Cucumis melo* and *Berberis vulgaris*, commonly used in the treatment of renal diseases in Pakistan. Acta Pol Pharm 72(4):651–654

Wang L, Peng L, Wei G, Ge H (2016) Therapeutic effects of berberine capsule on patients with mild hyperlipidemia. Zhongguo Zhong xi yijie he za zhi Zhongguo Zhongxiyi jiehe zazhi. Chinese J Integr Traditional Western Med 36:681–684

Wehmer E (1991) Die Pflanzenstoffe. Verlag von Gustav Fischer, Jena

Wu XK, Wang YY, Liu JP, Hou JP, Gao YQ, Du SM (2015) Letrozole, berberine, or a combination for infertility in Chinese women with polycystic ovary syndrome: a multicentre, randomised, double-blind, placebo-controlled trial. Lancet 386:70–70

Xiaopeng Z, Jizong X, Xusheng W, Fang C (2017) Therapeutic effect of berberine on renal atherosclerosis. Biomed Res 28:8815–8817

Zarei A, Changizi-Ashtiyani S, Taheri S, Ramezani M (2015) A quick overview on some aspects of endocrinological and therapeutic effects of *Berberis vulgaris* L. Avicenna J Phytomed 5(6):485–497

Zargari A (1997) Medicinal plants. Tehran University of Medical Sciences, Tehran

Zeng XH, Zeng XJ, Li YY (2003) Efficacy and safety of berberine for congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 92:173–176

Zhang XM, Yang YS, Gang S, Yang C, Lu M, Zhi J (2014) Berberine, Allicin- or Clarithromycin-based triple therapy for the first-line treatment of helicobacter pylori infection: an open-label, randomized trial. Gastroenterology 146:398

Zilaee M, Kermany T, Tavalaee S, Salehi M, Ghayour-Mobarhan M, Ferns GAA (2014) Barberry treatment reduces serum antiheat shock protein 27 and 60 antibody titres and high-sensitivity C-reactive protein in patients with metabolic syndrome: a double-blind, randomized placebo-controlled trial. Phytotherapy Res 28:1211–1215

Zohre S, Rad K, Rameshrad M, Hosseinzadeh H (2017) Toxicology effects of Berberis vulgaris (barberry) and its active constituent, berberine: a review. Iran J Basic Med Sci 20(5):516–529

Zovko Koncić M, Kremer D, Karlović K, Kosalec I (2010) Evaluation of antioxidant activities and phenolic content of *Berberis vulgaris* L. and *Berberis croatica* Horvat. Food Chem Toxicol 48(8–9): 2176–2180

Chapter 25
How Can Medicinal and Aromatic Plants Be Evaluated as Alternative Livelihoods for the Rural People? A Normative Assessment of the Ways to Be Addressed

Muhittin Kulak, Mehmet Zeki Kocak, Ahmet Metin Kumlay, Nagihan Kilic, Ferdi Celikcan, and Mehmet Hakki Alma

Abstract The poverty is of the most challenging and multidimensional problems for humanity. The majority of the people suffering from the poverty live in the rural areas of the developing countries. All governments with their policymakers have to produce or propose strategically relevant and adequate investments for the rural people in order to reduce the poverty with its multidimensions. In order to minimize that vexing problem of poverty, there are many alternative recipes in addition to conventional agricultural activities. For that context, collecting plants species from wild, which have been evaluated for multiple purposes, might be of the alternative and complementary way of the sustainable life of the rural people. Herewith the chapter, we herein concerned with medicinal and aromatic plants and then focused on the socio-economic of the plants, recommendations to be followed, sine-quo-non roles of woman, factors in conversion of raw materials into the functional materials, considerable roles of traditional knowledge, non-timber forest products, and agricultural policies in rural development.

Keywords Rural development · Poverty · Sustainable development · Agricultural policies · Legislation · Bibliometric analysis

Muhittin Kulak and Ahmet Metin Kumlay are equally contributed to the chapter.

M. Kulak (✉) · M. Z. Kocak · N. Kilic
Department of Herbal and Animal Production, Vocational School of Technical Sciences, Igdir University, 76000 Igdir, Turkey

A. M. Kumlay
Department of Field Crops, Faculty of Agriculture, Igdir University, 76000 Igdir, Turkey

F. Celikcan
Department of Organic Farming, College of Applied Science, Igdir University, 76000 Igdir, Turkey

M. H. Alma
Department of Forest Industry Engineering, Faculty of Forestry, Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_25

25.1 Introduction

25.1.1 How to Define Rural Development

To simply define the non-farm rural economy, it is of the strategy including activities that are not agricultural but are performed in rural areas with wage work or self-employment (Csáki and Lerman 2001). It has been reported that the 75% of the 1.2 billion poor people in the world live in rural areas, in which those people are mostly dependent on agricultural, forestry, fisheries, and related activities for their livelihood (Anriquez and Stamoulis 2007). The main aim of the rural development has been oriented around poverty reduction by diversifying their activities and making the activities non-homogenous (Csáki andf Lerman 2001).

25.1.2 Powerful Strategy for Visualization of the Relevant Studies

For the topics to be addressed through the chapter, we used VOS viewer for visualization of the most common hot topics or core subjects investigated/reported by the researchers hitherto. In that context, VOS viewer is of the powerful tools in construction and visualization of the relevant literatures, being more popular recently in almost all study areas, viz. agriculture, business, economy, geography, medicine (Kulak et al. 2019; Guney et al. 2020; Çelik 2020; Raparelli and Lolletti 2020; Szabó et al. 2020; Lou et al. 2020; Peng and Dai 2020; Donthu et al. 2020; Luo et al. 2020; Xie et al. 2020).

After a search at SCOPUS with *TITLE-ABS-KEY (rural AND development*), 89,428 document results were found without subject area limitation but "*Social Sciences*" were including the majority of the documents with a number of 35,170 (August 28, 2020). Afterward, for the scope of the present chapter, the relevant words such as "medicinal and aromatic plants" were of the criteria for limitations of documents. We, herein, obtained 187 documents with inclusion criteria "(*TITLE-ABS-KEY (rural AND development)) AND (medicinal AND aromatic AND plants*)." Now, majority of the studies corresponded to the "*Agricultural and Biological Sciences*" subject areas with a total number of 101 documents. Kulak (2018) profiled the rural area development medicinal and aromatic plants, as bibliometric analysis with 113 relevant documents out of 70,745 documents. Within about two years, the rural development term including studies increased by 26.41% (from 70,745 to 89,428), reporting that medicinal plants, Himalaya, conservation, Ethnobotany, Nepal, traditional knowledge, rural development, sustainable development, domestication, Ethno-medicine, livelihood, non-timber forest products, value chain, and marketing were the major proposed keywords, which are deemed as core of the studies, by the researchers. Furthermore, the bibliometric analysis revealed that most of the documents were from India, USA, Italy, Nepal, and South Africa. Herewith

the study, we are in target of extending the researches relevant, available and peer-reviewed by adding new publications (between 2018 and 2020). With the extension of the study, we also aimed to determine whether there were changes regarding the trends of main topics, publication numbers, or countries etc. (Figs. 25.1 and 25.2).

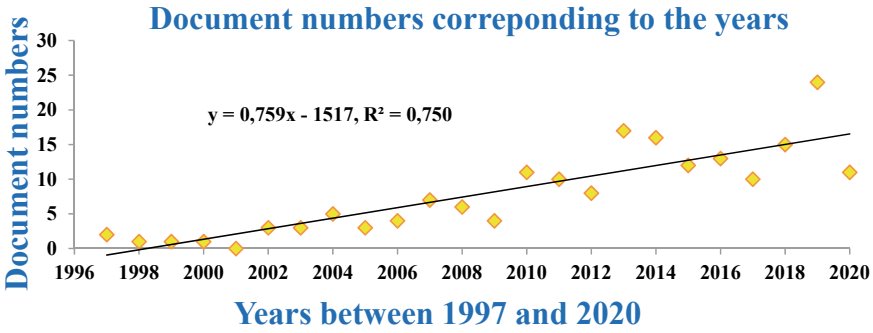


Fig. 25.1 Document numbers in accordance with the years

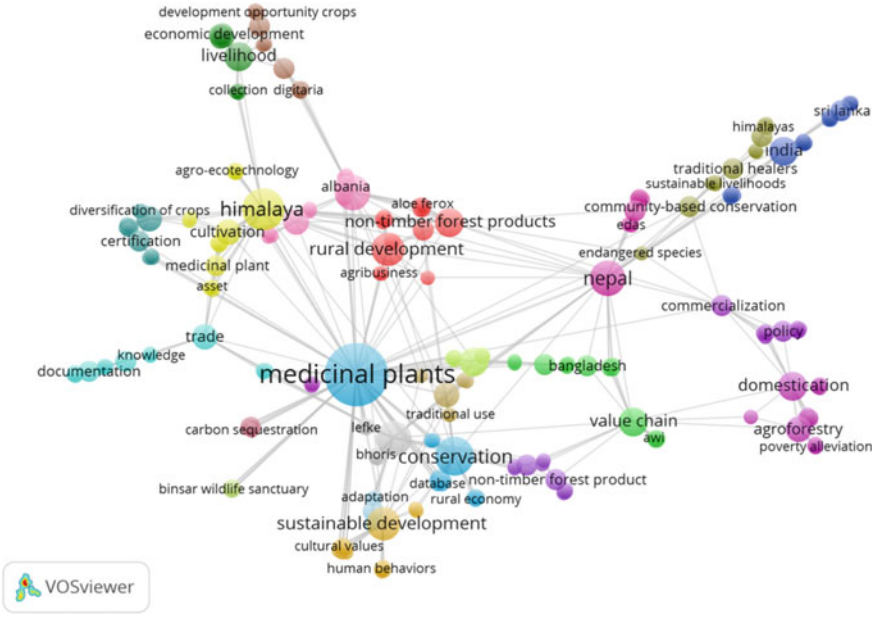


Fig. 25.2 Keywords of the rural development coupled with medicinal and aromatic plants (Kulak 2018)

25.1.3 Visualization of the Findings Along with the Keywords: A Core Content Aspect

Herewith the analyses, 674 keywords without limitation of the minimum occurrences number were used for the assessment and further visualization. Then the keyword analysis was performed in comparison with the study by Kulak (2018) in order to reveal the trends (Table 25.1; Figs. 25.2 and 25.3). As reported previously, *medicinal plants, Himalaya, conservation, Ethno-botany, Nepal, traditional knowledge, rural development, sustainable development, domestication, Ethno-medicine, livelihood, non-timber forest products, value chain, and marketing* were of the keywords (Kulak 2018). Along with the present study, we noted the major keywords such as *medicinal plant(s), ethno-botany, rural development, Himalaya, traditional knowledge, conservation, India, sustainable development, Nepal, biodiversity, domestification, ethno-medicine, livelihood, value chain, Albania, marketing, agroforestry, non-timber forest products, rural woman, traditional knowledge, and indigenous knowledge* (Table 25.1).

25.1.4 Plant Species for the Possible Rural Development: Which Is Crucial Diversity or Number?

Of the living organism, the number of terrestrial plant species including angiosperms, gymnosperms, ferns, lycophytes, and bryophytes has been estimated to be about 500,000, out of which were mostly localized in humid tropics (Corlett 2016). Of those plants, an estimation number of 50,000–80,000 flowering plants has been deemed as medicinal and aromatic plants, which are preferred for therapeutic purposes by approximately 80% of the world populations, especially in developing countries (Hendawy et al. 2010; Naguib 2011; Kulak et al. 2019). According to the bibliometric analysis by Kulak (2018), the highest number of disseminated reports was from India, which is clearly the richest source of medicinal and aromatic plants. Bibliometric analysis revealed that India topped with 51 documents, out of 18,664 total plant species but followed by Italy with 17, out of 5599 total plant species. However, interestingly, Brazil with 56,215 total plant species published only five documents regarding rural development. Similarly, China with 32,200 plant species reported only seven documents (Fig. 25.4). In that context, we cannot deduce a linear trend between total plant species and reports number. Also, it is worthy to note that all plant species cannot be considered as medicinal and aromatic plants, which in turn cannot be alternative livelihoods or income for the rurals. Moreover, the biodiversity does not equally mean the total spread of the plant species in an environment, meaning that the plant species might have limited distribution in the region. In the case of lower number species in countries, plant species might have a large quantity and wide distribution, which in turn might contribute to rural development, if the species is relevant for that purpose. Accordingly, for the assessment of a plant species for possible alternative

Table 25.1 Keywords used for visualization and assessment of the present study

Keyword	Occurrences	TLS	Keyword	Occurrences	TLS
Medicinal plants	30	137	Culture	2	15
Ethno-botany	13	58	Documentation	2	8
Rural development	12	58	Economic contribution	2	12
Himalaya	11	54	Economic development	2	8
Traditional knowledge	10	57	Economy	2	11
Conservation	9	38	Ethno-botany	2	8
India	8	36	Ethno-medicine	2	8
Sustainable development	7	38	Ethno-pharmacology	2	6
Medicinal and aromatic plants	6	28	Fidelity level	2	10
Nepal	6	31	Frankincense	2	12
Biodiversity	5	22	Fruit plants	2	8
Domestication	5	24	Geography	2	17
Ethno-medicine	5	21	Gum Talha	2	12
Livelihood	5	20	Harvesting	2	8
Value chain	5	22	Himalayas	2	8
Albania	4	17	Land-use change	2	12
Aromatic plants	4	18	Lebanon	2	6
Marketing	4	16	Livestock	2	8
Medicinal plant	4	17	Local uses	2	8
Non-timber forest products	4	21	Medicinal	2	7
Trade	4	17	Medicinal uses	2	12
Agroforestry	3	13	Morocco	2	15
Biorefinery	3	11	Multiple purpose	2	12
Climate change	3	14	Myrrh	2	12
Commercialization	3	14	Natural resource use	2	13
Cultivation	3	15	Neglected and underutilized species	2	8
Essential oils	3	14	Non-timber forest product or Ntfp (2)	2	9
Food	3	19	On-farm conservation	2	9
Gum Arabic	3	19	Organic agriculture	2	13
Health	3	15	Public library	2	6

(continued)

Table 25.1 (continued)

Keyword	Occurrences	TLS	Keyword	Occurrences	TLS
Policy	3	19	Zipf's law	2	6
Rural women	3	17	Rural poverty	2	15
Traditional medicine	3	12	Slovenia	2	13
Indigenous knowledge	3	12	Sri Lanka	2	8
Agrobiodiversity	2	9	Subsistence	2	8
Antimalarial plants	2	3	Sustainable agriculture	2	11
Bangladesh	2	10	Sustainable management	2	10
Bibliometrics	2	6	Traditional	2	7
Bioprospecting	2	8	Traditional healers	2	8
Certification	2	10	Use value	2	10
Community-based conservation	2	9	Utilization	2	7
Contract farming	2	6	Vetiver	2	9
Cultural values	2	17	*Zanthoxylum armatum*	2	29
Income generation	2	9	Informant consensus factor	2	10

TLS total link strength

income, there would be multiple independent variables including market values, quantities, accessibility, or categories (rare, threatened, and endangered) of the plant species, not restricted to a single variable as "plant biodiversity."

25.2 Can all Plants Be Considered as Medicinal and Aromatic Plants, Which in Turn to Be Commercialized for Rural Development? What Kind of Socio-Economic Importance has Been Attributed to the Medicinal and Aromatic Plants from the Wild?

As stated above, approximately 20% of the total plant species have been evaluated for their therapeutic purposes, especially in developing countries, mostly in rural areas. For the commercialization processes, as alternative income, Reinten and Coetzee (2002) have well and clearly documented that plant species must have or provide opportunities regarding sustaining livelihoods, adding contributions for

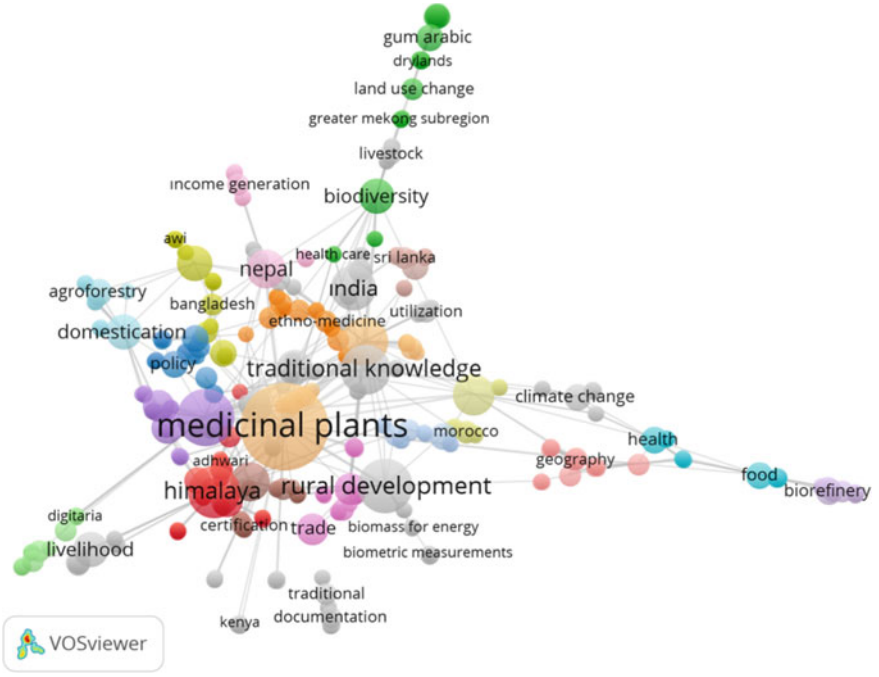


Fig. 25.3 Keywords of the rural development coupled with medicinal and aromatic plants of the present study

developing the rural development. Legislation and regulations regarding commercializing a species or possible introduction or opening to agriculture is strictly to be followed. It is worthy to highlight that practices during collection from wild should also be based on the legislation and regulations authorized by the official bodies for sustainable harvest. Hereby, the sustainable harvest or we can name "harvest without ending" of the plant species will be ensured (Moré et al. 2013).

With respect to the alterations in socio-economic status of locals in rural areas, medical relating herbal preparations, selection of the appropriate plant species compatible with ecological requirements of the region, cultivation of the right plant species, and gathering plants from the wild are key issues. Along with the compatible and acceptable regulations, better economy, augmented income, and raw material for manufacturing companies like improvements might provide additions or contributions to socio-economic structures of the rural people (Shankar and Rawat 2006).

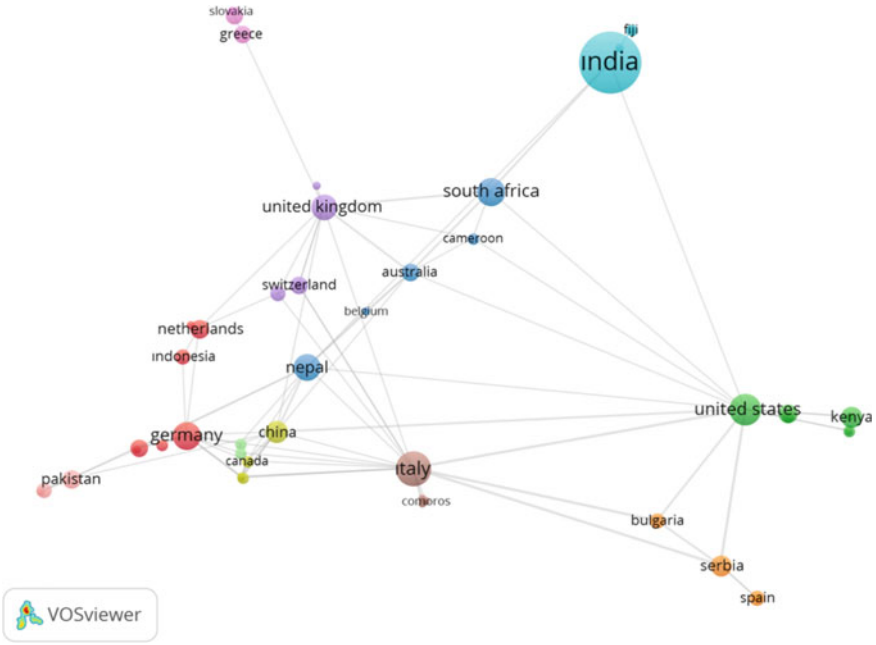


Fig. 25.4 Visualization of the countries with respect to the number documents disseminated

25.3 What has been Reported and What has Been Proposed for the Possible Rural Development: A General View of the Studies

Hereby with the section, the recommendations proposed by the authors were listed as follows:

1. Geographical information mediated multicriteria and multiobjective analyses might be applied for sustainable monitoring the plant species, such case in oregano harvest and regeneration (Irina et al. 2019).
2. The sine-quo-non roles of woman in transferring the traditional knowledge have been clearly noted. Along with the traditional knowledge of woman, product development and subsequently production process are the key for the progresses in rural development (Montanari and Bergh 2019).
3. As in all communities, sustainable harvest of the plants is of the core of the progress and conservation of the natural resources. Shah and Bhat (2019) observed that medicinal and plants should be considered regarding their statistics for ecological status of themselves, proposing categories for plants such as rare, endangered, and threatened species.

4. Shah et al. (2019) mention that a holistic approach should be addressed for complex issues regarding diversifying farm production along with the implementation of efficient marketing strategy in cooperation with local bodies, focusing the case of Tulsi as an alternative additional crop for possible additional income options.
5. Bakare et al. (2020) underline the significance of documentation, conservation, and awareness of plant-based natural products in treatment of animal diseases as a simple and cost-effective strategy, indicating that those mentioned strategies might matter in addressing the production problems in relevant sectors such as pharmaceutical, medicinal, cosmetics, culinary.
6. In the reports by Phondani et al. (2019), decision for the promising relevant species, standardization for the species seed production or propagation, introduction appropriate cost efficient but beneficial technologies and training programs for skill development of the rural have been listed for recommendations.
7. Kulak (2018) reviewed the relevant and plausible management strategies, viz. coherent strategies combined with appropriate infrastructure, governmental mediated managements, and encouragement of diversifying the agricultural practices. Prior to the decision of strategies, plant species compatible with the available ecological conditions and requirements should be introduced. After plant species and management strategies determination, for high yielding of the metabolites responsible or attributed roles in their uses by communities, new strategies, viz. harvesting time, post-harvest practices, and marketing should be addressed.

25.4 Sine-Quo-Non Roles of Woman with Respect to the Rural Development, as in all Society: Time for Preventing Development of "Bottleneck"

Empowering woman in society is a sine-quo-non requirement concerning with progress of a country. Highly effective words by Jawaharlal Nehru, the first Prime Minister of India, emphasize the considerable acts of woman for all nations, saying that "You can tell the condition of a nation by looking at the status of its women" (Sathiabama 2010). The Treaty of Rome (March 25, 1957) (Bock 2010) and The Beijing Declaration at the Fourth World Conference on Women (September 4–15, 1995) (Beijing Declaration 1995) pay equal for man and woman in society. With the directives and promotion of equally paid right after the Treaty of Rome, the position of the woman in Europe exhibited great improvements in the past decades but unfortunately great differences of gender is still available across many countries in different regions (Bock 2010). Unfortunately, more traditional and social dynamics in rural areas make woman more disadvantaged by restricting their personal and social development as well as being exposed to risk of poverty when get older or

heading single household with their children (Bock 2010). Even social and traditional barriers in front of woman, Quisumbing et al. (1996) defines woman as "the key to food security." Forbye, woman has been equipped with multiple functions, viz. assurance of household food security, income earners, and food access stabilizers in addition to the caretaker roles of households (Quisumbing et al. 1996; Sharaunga et al. 2016). In that context, as in all society, any effort without women with respect to attempts for rural development will surely be futile. That should be noted that women cannot be deemed as alternative in rural activities, on the contrary, women exhibit complementary roles. Ogunlela and Mukhtar (2009) revealed the striking roles of women in leading agricultural activities in Nigeria, making up 60–80% of the labor force even though women' contributions to rural development are seldom noticed.

25.5 Which Are Crucial Factors in Conversion of Raw Material into the Functional Material?

Conversion of raw material into functional material, viz. medicinal, aromatic, or cosmetic purposes is of the basic requirements of industrial bodies. The selection of the species might bring economic values for the rural people though true crop cultivation or gathering the true plant from wild. Herewith the issue, Lubbe and Verpoorte (2011) presented excellently with a list, proposing that following strategies to be addressed and then to be followed. Hereby, we can list the factors proposed by Lubbe and Verpoorte (2011) as identification what factors are crucial as major players in relevant industry, being compatible with regulations or meeting the desired requirements relating with raw materials, being awareness of forthcoming demands of industry, selection of the environmentally compatible crops coupled with market value, which might be potent in the competition of the market.

25.6 How to Decide the Right Plant Species, at Which Extent to Be Collected from Wild

The first and compulsory rule is the not to give damage to the wild conditions, specifically habitats of the living organisms. Strict rules have to be implemented for ensuring sustainable availability of the species by obeying the legislations and regulations of the authorities. Being sensitive and awareness of the endemic species, which is considered as wealth of nations and humanity, is of the first compulsory demands. For the selection of the right species, value-added crops but for those crucial factors in valuing a product have to be well-defined, must be put into use.

After ascertaining the right one, appropriate harvesting time for the sustaining next generations of the plants must be ensured. In that context, strict precautions such as preventing over-grazing or excessive collection of the plants must be taken. We should note that those given strategies can be achieved with the trained collectors, which make the training programs obligatory to be done by the official bodies (Moré et al. 2013).

25.7 Route from Beginning to the End: From Ethno-Botany Knowledge to the Industrial Uses

Indigenous knowledge, traditional knowledge, ethno-botany, and ethno-medicine are the key points of the route. In that context, a vast array of ethno-botanical studies is required for addressing the point regarding traditional knowledge to the modern industrial uses. Traditional knowledge is broadly portrayed as a cumulative theme of knowledge, experiences, practice, belief, and values handed down through generations with a history entity (Berkes 1999; Berkes et al. 2000; Ellis 2005). Of the multidisciplinary, ethno-botany is deemed as a science including botany, linguistics, and ethnography, specifically dealing with the traditional knowledge concerned with the plants and the wild environment (Szabó 1976; cited in Papp et al. 2014). Herewith the bibliometric analysis, it was revealed those different terms addressing the analogous targets, viz. traditional knowledge, traditional ecological knowledge, indigenous knowledge, local knowledge, quantitative ethno-botany have been reported in different researches. Specifically, ethno-pharmacology, ethno-biology, ethno-veterinary, folk medicine, and ethno-zoology were recorded (Table 25.2; Fig. 25.5). Pieroni and Quave (2014) underline that ethno-botany cannot just be considered as a recording list of plants with their uses, highlighting the comprehensive roles in how socio-ecological microsystems work. Experiences gained from the microsystem might lead to discovery of new drugs or drug development since the plants encompass large part of the systems, being of the significant components of the world cultural heritage (Sheng-Ji 2001).

Reappraising traditional knowledge and subsequently complementing with scientific knowledge can be relevant to innovation and rural change (Lado 2004), which in turn might contribute to rural development and additional livelihoods but with appropriate infrastructure, training, and related policies.

In addition to the manageable practices such as infrastructure, training programs, policy, policymakers, changing climatic, in an extent factor as a not controllable, might exhibit significant but adverse impacts on traditional crops of the relevant regions. Herewith, local people, we herein name as smallholder farmers, confront multiple stress factors that force them to adapt in response to changes, being more fragile and being affected disproportionately (Harvey et al. 2014). Nagayet (2005) has reported that smallholder farmers are estimated to be 400–500 million with an 85% of the world's farms.

Table 25.2 Extracted relevant keywords regarding ethno-botany and rural development (28 keywords out of 1935 keywords after setting a criterion of minimum occurrence to be 10)

Keyword	Occurrences	Total link strength
Ethno-botany	288	318
Medicinal plants	161	210
Traditional knowledge	114	143
Ethno-medicine	39	60
Traditional ecological knowledge	38	39
Traditional medicine	37	53
Indigenous knowledge	35	55
Conservation	34	51
Local knowledge	24	23
Biodiversity	23	30
Quantitative ethno-botany	22	20
Use value	21	28
Ethno-pharmacology	19	34
Ethiopia	15	26
Ethnobiology	15	18
Wild edible plants	13	16
Wild food plants	13	20
Benin	12	14
Fidelity level	12	26
Herbal medicine	12	9
Informant consensus factor	12	25
Folk medicine	11	18
Pakistan	11	15
Traditional healers	11	12
Bangladesh	10	17
Ethno-veterinary	10	15
Ethno-zoology	10	9
Livestock	10	12

25.8 Non-timber Forest Products: Not Limitation with Plant Species

Non-timber forest products (NTFPs) cover forest origin products except wood, viz. rattan, resins, essential oils, latex, nuts, species, fruits, seeds, leaves, game, fish, birds, eggs, and honey (Ros Tonen et al. 1995; Adam et al. 2013). Ingram et al. (2010) define NTFPs as products other than timber or products of biological origin, namely vegetables involving plants and fungi, and animal origin products such as meat insects

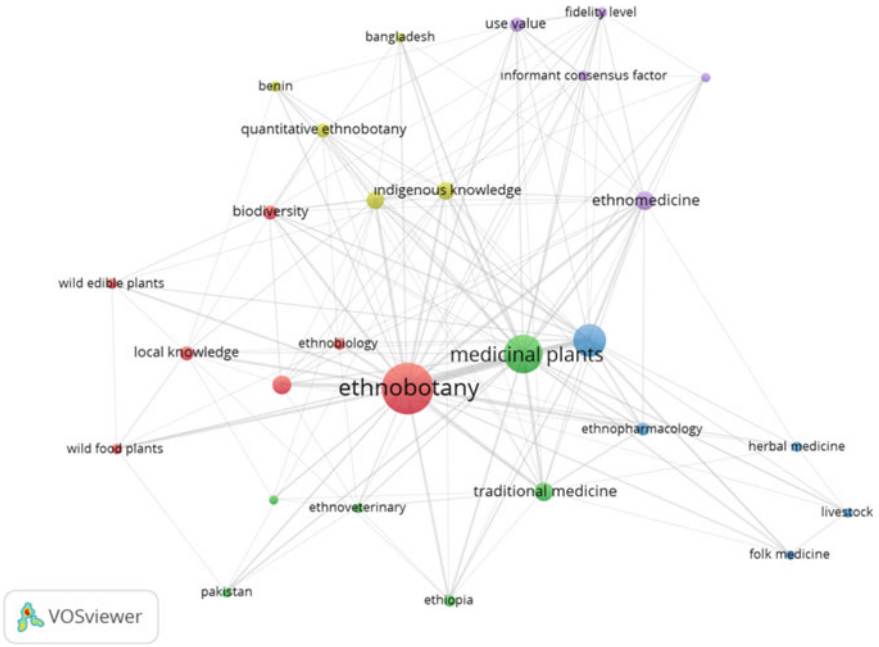


Fig. 25.5 Keywords of the ethno-botany with respect to the rural development

and forest fish (Table 25.3; Fig. 25.6). However, NTFPs have been neglected for long time and their uses as minor products have been considered as being primary interest of locals, which were deemed with lack of capital and technology (Homma 1992). For the last decades, increasing demands on attributes of NTFPs have been emerged in relation to the rural development and natural resources conservation (Arnold and Pérez 2001). In that context, the importance of NTFPs with respect to the rural livelihoods has been widely and globally acknowledged (Shackleton et al. 2019).

25.9 How Significant are Agricultural Policies to Promote the Rural Development?

Agricultural policies proposed by official authorities are/must be in charge of insurance of livelihoods of rural people in response to global shocks. Those policies should be justice—and integration of different needs—oriented for concept of sustainable development. Hereby, the responsibility of those policies and accordingly official covers scrutinized concepts, not including confronting ideas, nexus between science

Table 25.3 Extracted relevant keywords regarding NTFPs out of 4890 keywords

Keyword	Occ.	TLS	Keyword	Occ.	TLS
Non-timber forest products	573	1044	Diversity	8	13
Non-timber forest product	104	158	Environment	8	18
Conservation	85	216	Laos	8	26
Livelihoods	74	204	Natural regeneration	8	14
NTFP	74	148	Palms	8	23
Forest management	65	129	Participation	8	12
Biodiversity	61	158	Protected area	8	9
NTFPs	61	102	Remote sensing	8	12
Sustainability	59	123	Sustainable harvesting	8	14
Ethno-botany	48	120	Tanzania	8	14
Amazon	40	93	Invasive species	8	22
Medicinal plants	38	92	Biomass	7	10
Non-timber forest products (NTFPs)	37	50	Cost-benefit analysis	7	12
Agroforestry	34	67	Cultivation	7	18
Livelihood	32	71	Distribution	7	16
Ecosystem services	31	64	Economic analysis	7	12
Amazonia	30	67	Ethiopia	7	14
Cameroon	30	80	Forest degradation	7	16
Non-timber forest products	29	46	Forest income	7	12
Sustainable management	29	55	Fuel wood	7	14
India	27	75	Guyana	7	14
Sustainable development	25	54	GIS	7	14
Sustainable use	25	72	Household	7	11
Climate change	24	46	Household income	7	15
Domestication	23	50	Logging	7	15
Mexico	23	51	Pacific northwest	7	8
Tropical forest	23	63	Population dynamics	7	26
Extractivism	22	52	Restoration	7	16
Poverty	22	58	Sri Lanka	7	20
Rural livelihoods	22	51	Adansonia digitata	6	8
Demography	21	61	Benin	6	11
Forest policy	21	42	Brazil nuts	6	15
Nepal	21	47	Carbon sequestration	6	8
Forests	20	51	Charcoal	6	9
Policy	20	47	China	6	8

(continued)

Table 25.3 (continued)

Keyword	Occ.	TLS	Keyword	Occ.	TLS
Protected areas	20	52	Community	6	17
Traditional knowledge	20	40	Community forest	6	16
Deforestation	19	48	Community participation	6	16
Forest conservation	19	49	Community-based conservation	6	17
Harvesting	19	45	Development	6	16
Non-timber forest products (NTFP)	19	34	Ecotourism	6	11
Timber	19	63	Ecuador	6	15
Tropical forests	19	48	Extraction	6	19
Arecaceae	18	44	Firewood	6	16
Gender	18	54	Forest dependency	6	16
Management	18	42	Forest economics	6	14
Trade	18	52	Forest restoration	6	7
Community forestry	17	31	Gum Arabic	6	14
Non-timber forest product (NTFP)	16	32	Human ecology	6	18
Non-timber forest products	16	20	Hunting	6	13
Brazil	15	51	Joint forest management	6	12
Economic valuation	15	35	Land tenure	6	11
Forest	15	29	Land-use change	6	11
Land use	15	39	Lao Pdr	6	11
Markets	15	47	Local communities	6	11
Rattan	15	26	Market	6	9
Regeneration	15	33	Matrix population models	6	12
West Africa	15	35	Multiple use	6	16
Income	15	35	Non-timber forest product	6	8
Food security	14	37	Opportunity cost	6	9
Forest products	14	36	Palm	6	13
Forestry	14	25	Perceptions	6	8
Sustainable forest management	14	23	Plant management	6	13
Sustainable harvest	14	30	Plantations	6	11
Biodiversity conservation	13	24	Political ecology	6	9
Bolivia	13	47	Population structure	6	9
Governance	13	39	*Prunus africana*	6	16
Himalaya	13	30	Resilience	6	13

(continued)

Table 25.3 (continued)

Keyword	Occ.	TLS	Keyword	Occ.	TLS
Madagascar	13	26	Resource management	6	17
Marketing	13	29	Resource use	6	12
Matrix models	13	37	Rubber	6	26
Rural development	13	24	Secondary forest	6	7
Bertholletia excelsa	12	27	Silviculture	6	19
Brazil nut	12	34	Southeast Asia	6	11
Commercialization	12	29	Sustainable forestry	6	11
Non-timber products	12	16	Transaction costs	6	12
South Africa	12	37	Women	6	17
Indigenous knowledge	12	28	Indigenous communities	6	11
Commercialization	11	31	Indonesia	6	8
Forest certification	11	14	Agriculture	5	15
Ghana	11	19	Agroforestry tree products	5	11
Poverty alleviation	11	35	Antioxidant	5	3
Vulnerability	11	25	Aquilaria	5	9
Agarwood	10	15	Araceae	5	11
Cerrado	10	25	Bangladesh	5	7
Community forest management	10	15	Basketry	5	8
Ecology	10	19	Bhutan	5	8
Ethnomycology	10	19	Bromeliaceae	5	7
Forest resources	10	16	Certification	5	11
Genetic diversity	10	28	Chamaedorea	5	14
Local ecological knowledge	10	21	Community-based forest management	5	6
Natural resource management	10	23	Cites	5	15
Natural resources	10	22	*Dacryodes edulis*	5	9
Non-wood forest products	10	14	Defoliation	5	13
Peru	10	30	Economics	5	14
Redd+	10	26	Ecosystem management	5	7
Value chains	10	38	Ethnoecology	5	16
Indigenous people	10	28	Euterpe edulis	5	19
Africa	9	23	Extractive reserve	5	12
Bamboo	9	21	Fire	5	17
Brazilian Amazon	9	15	Foraging	5	9
Extractive reserves	9	24	Forest inventory	5	10
Forest governance	9	26	Forest use	5	13

(continued)

Table 25.3 (continued)

Keyword	Occ.	TLS	Keyword	Occ.	TLS
Forest structure	9	18	Fruit harvest	5	17
Handicrafts	9	20	Homegarden	5	10
Harvest	9	30	Household economy	5	10
Local knowledge	9	20	Kenya	5	14
Medicinal plant	9	18	Landscape management	5	10
Reforestation	9	20	Latin America	5	15
Smallholders	9	15	Leaf harvesting	5	16
Traditional ecological knowledge	9	15	Nature conservation	5	11
Value chain	9	10	*Phyllanthus emblica*	5	23
Western ghats	9	27	Rain forest	5	12
Wild edible plants	9	23	Rural households	5	13
Indigenous peoples	9	21	Safou	5	11
Institutions	9	23	Savanna	5	17
Anthropogenic disturbance	8	22	Savanna woodland	5	17
Atlantic forest	8	19	Selective logging	5	11
Central Africa	8	17	Socio-economics	5	10
Congo basin	8	15	Soliga	5	4
Disturbance	8	14	Species richness	5	8
Valuation	5	8	Succession	5	8
Vietnam	5	13	Tourism	5	18
Wood products	5	15	Tropical rain forest	5	15
Zimbabwe	5	24	Tropical rainforest	5	17

Occ. occurrences, *TLS* total link strength

and practical implementation, and neoliberal rationality. Finally, of the most important factors, local actors have responsibilities of dealing with the conflicting targets and interests, which are of the parts of rural development politics (Mölders 2014). In the case of Nigeria, Eze et al. (2010) clearly recommended and highlighted the importance of governmental agriculture financing policies in improving and enhancing the rural development, but noting that would come true as desired with strategically relevant investments in agriculture. With right but the policies to be followed, targets for augmented rural infrastructure, increased productivity, compatible competitiveness of the products, and also significant contribution to fight against corruption would be achieved.

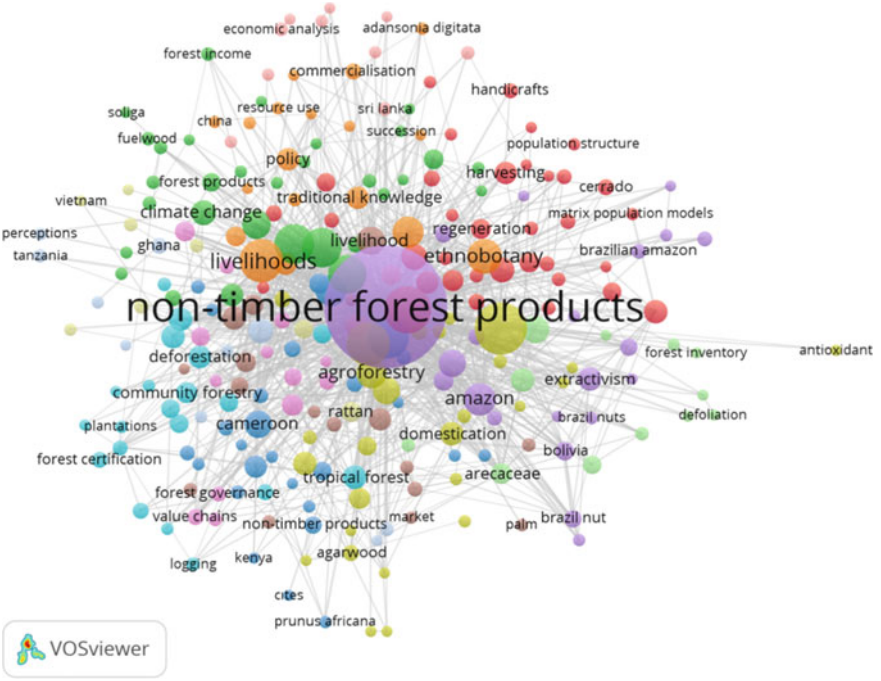


Fig. 25.6 Keywords of the NTFPs with respect to the rural livelihoods

25.10 Conclusion and the Way Forward

Along with the chapter, we can list the recommendations as conclusion and future perspective:

- Regional coherent strategies have to be implemented rather than national strategies.
- Micromanagement and compatible strategies for each plant species have to be implemented.
- In addition to normative strategies, training programs for the local actors are of the milestone for sustainable rural development
- Strong nexus between local and official bodies must be constituted. That nexus must be based on the transmission of scientific knowledge regarding extent, harvesting time and type, harvesting region of the plant species in order to save the right conditions for sustainability of the wild.
- With the use of geographical information systems, yield of plant species must be monitored for the sustainability of the harvests.
- A strict consensus involving competent experts in biology, geography, linguistics, and economists relating ethnography and ethno-botany must be provided for the neoliberal rationality.

- Finally, regulations regarding microfinance and women empowerment must be ensured, highlighting equality of the gender, pioneer role of woman and economic independency of the woman. In this context, imperative attempts are a must for preventing development of "bottleneck" in gender equality. In addition, new insight and ideas should be handled on the conflict between motherhood and work.

References

Adam YO, Pretzsch J, Pettenella D (2013) Contribution of non-timber forest products livelihood strategies to rural development in drylands of Sudan: potentials and failures. Agric Syst 117:90–97

Anriquez G, Stamoulis K (2007) Rural development and poverty reduction: is agriculture still the key? ESA Working, FAO, Rome, pp 07-02

Arnold JM, Pérez MR (2001) Can non-timber forest products match tropical forest conservation and development objectives? Ecol Econ 39(3):437–447

Bakare AG, Shah S, Bautista Jimenez V, Bhat JA, Dayal SR, Madzimure J (2020) Potential of ethno-veterinary medicine in animal health care practices in the South Pacific Island countries: a review. Trop Anim Health Prod 1–11

Berkes F (1999) Sacred ecology: traditional ecological knowledge and resource management. Taylor & Francis, Philadelphia and London

Berkes F, Colding J, Folke C (2000) Rediscovery of traditional ecological knowledge as adaptive management. Ecol Appl 10(5):1251–1262

Bock BB (2010) Personal and social development of women in rural areas of Europe. European Parliament. https://edepot.wur.nl/163660

Corlett RT (2016) Plant diversity in a changing world: status, trends, and conservation needs. Plant Diversity 38(1):10–16

Csáki C, Lerman Z (2001) The challenge of rural development in the EU accession countries: third world bank/FAO EU accession workshop, vol 504. World Bank Publications, Sofia, Bulgaria, pp 17–20

Çelik MA (2020) Kuraklık araştırmalarında yeni eğilimler, kullanılan teknikler ve kavramlar üzerine bibliyometrik ağ analizi. lnt J Geogr Geogr Edu 42:602–630

Declaration B (1995) Beijing declaration and platform for action fourth world conference on women. Paragraph 112

Donthu N, Kumar S, Pattnaik D (2020) Forty-five years of journal of business research: a bibliyometric analysis. J Bus Res 109:1–14

Ellis SC (2005) Meaningful consideration? A review of traditional knowledge in environmental decision making. Arctic 66–77

Eze CC, Lemchi JI, Ugochukwu AI, Eze VC, Awulonu CAO, Okon AX (2010) Agricultural financing policies and rural development in Nigeria (No. 352-2016-18042)

Guney İ, Öncü MA, Somuncu M (2020) Kafkas dağları için yeni araştırma eğilimleri: bibliyometrik bir analiz. Coğrafi Bilimler Dergisi 18(2):161–190

Harvey CA, Rakotobe ZL, Rao NS, Dave R, Razafimahatratra H, Rabarijohn RH, MacKinnon JL (2014) Extreme vulnerability of smallholder farmers to agricultural risks and climate change in Madagascar. Philos Trans R Soc B: Biol Sci 369(1639):20130089

Hendawy SF, Ezz El Din AA, Aziz EE, Omer EA (2010) Productivity and oilquality of *Thymus vulgaris* L under organic fertilization conditions. Ozean J Appl Sci 3:203–216

Homma AKO, (1992) The dynamics of extraction in Amazonia: a historical perspective. In: Nepstad DC, Schwartzman S (eds) Non-timber products from tropical forests: evaluation of a conservation and development strategy. Adv Econ Bot 9:23–32

Ingram V, Ndoye O, Iponga DM, Tieguhong JC, Nasi R (2010) In: de Wasseige C, de Marcken P, Bayol N, Hiol Hiol F, Mayaux P, Desclée B, Nasi R, Billand A, Defourny P, Eba'a R (eds) Non-timber forest products: contribution to national economy and strategies for sustainable management. The Forests of the Congo Basin: state of the forest

Irina LT, Javier BP, Teresa CBM, Eurídice LA, del Carmen CILM (2019) Integrating ecological and socioeconomic criteria in a GIS-based multicriteria-multiobjective analysis to develop sustainable harvesting strategies for Mexican oregano *Lippia graveolens* Kunth, a non-timber forest product. Land Use Policy 81:668–679

Kulak M (2018) Bibliometric analysis of studies in medicinal and aromatic plants for rural development. In: 17th international scientific conference engineering for rural development, Jelvaga

Kulak M, Ozkan A, Bindak R (2019) A bibliometric analysis of the essential oil-bearing plants exposed to the water stress: how long way we have come and how much further? Sci Hort 246:418–436

Lado C (2004) Sustainable environmental resource utilisation: a case study of farmers' ethnobotanical knowledge and rural change in Bungoma district, Kenya. Appl Geogr 24(4):281–302

Lou J, Tian SJ, Niu SM, Kang XQ, Lian HX, Zhang LX, Zhang JJ (2020) Coronavirus disease 2019: a bibliyometric analysis and review. Eur Rev Med Pharmacol Sci 24(6):3411–3421

Lubbe A, Verpoorte R (2011) Cultivation of medicinal and aromatic plants for specialty industrial materials. Ind Crops Prod 34(1):785–801

Luo J, Han H, Jia F, Don H (2020) Agricultural co-operatives in the western world: a bibliometric analysis. J Cleaner Prod 122945

Montanari B, Bergh SI (2019) Why women's traditional knowledge matters in the production processes of natural product development: the case of the green morocco plan. Women's Stud Int Forum 77:102275

Moré E, Melero R, Barata AM, Lopes V, Rocha V, Radusien J, Karpaviene B, Cetinkaya H, Sekeroglu N, Kulak M (2013) Relatório do projeto. Forest plants wild harvesting learning in Europe

Mölders T (2014). Multifunctional agricultural policies: pathways towards sustainable rural development. Int J Sociol Agric Food 21(1)

Nagayet O (2005) Small farms: current status and key trends. In: The future of small farms: proceedings of a research workshop. International Food Policy Research Institute, Washington DC, pp 355–367

Naguib NYM (2011) Organic vs chemical fertilization of medicinal plants: a concise review of researches. Adv Environ Biol 5:394–400

Ogunlela YI, Mukhtar AA (2009) Gender issues in agriculture and rural development in Nigeria: the role of women. Hum Soc Sci J 4(1):19–30

Papp N, Birkás Frendl K, Farkas Á, Czégényi D (2014) Hungarian ethnobotanical studies in Romania. Ethnobotany and biocultural diversities in the Balkans. Springer, New York, pp 29–44

Peng X, Dai J (2020) A bibliometric analysis of neutrosophic set: two decades review from 1998 to 2017. Artif Intell Rev 53(1):199–255

Pieroni A, Quave CL (2014) Ethnobotany in the Balkans: Quo vadis? Ethnobotany and biocultural diversities in the Balkans. Springer, New York, pp 1–9

Phondani PC, Bhatt ID, Maikhuri RK, Kothyari BP, Bhatt A, Purohit VK, Joshi P (2019) Exploring floriculture potential for sustainable livelihood of subsistence communities in Indian Himalaya. Nat Acad Sci Lett 42(1):39–44

Quisumbing AR, Brown LR, Feldstein HS, Haddad L, Peña C (1996) Women: the key to food security. Food Nutr Bull 17(1):1–2

Raparelli E, Lolletti D (2020) Research, innovation and development on *Corylus avellana* through the bibliyometric approach. Int J Fruit Sci 1–17

Reinten E, Coetzee JH (2002) Commercialization of South African indigenous crops: aspects of research and cultivation of products. Trends in new crops and new uses. ASHS Press, Alexandria VA, pp 76–80

Ros Tonen M, Dijkman W, van Bueren EL (1995) Commercial and sustainable extraction of non-timber forest products: towards a policy and management oriented research strategy. Tropenbos Foundation

Sathiabama K (2010) Rural women empowerment and entrepreneurship development, id: 2475

Shackleton CM, Mahonya S, Schreckenberg K (2019) Non-timber forest product use and market chains along a deforestation gradient in Southwest Malawi. Front Forest Glob Change 2:71

Shah GM, Ahmad F, Panwar S, Khadka MS, Ali A, Bisht S (2019) *Ocimum sanctum* an alternative additional livelihood option for the poor and smallholder farmers. Sustainability 11(1):227

Shah S, Bhat JA (2019) Ethnomedicinal knowledge of indigenous communities and pharmaceutical potential of rainforest ecosystems in Fiji Islands. J Integr Med 17(4):244–249

Shankar R, Rawat MS (2006) Medicinal plants activities for change in the socio-economic status in rural areas of North East India. Bull Arunachal Forest Res 22(1–2):58–63

Sharaunga S, Mudhara M, Bogale A (2016) Effects of 'women empowerment' on household food security in rural KwaZulu-Natal province. Dev Policy Rev 34(2):223–252

Sheng-Ji P (2001) Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. Pharm Biol 39(1):74–79

Szabó K, Zubay P, Németh Zámboriné É (2020) What shapes our knowledge of the relationship between water deficiency stress and plant volatiles? Acta Physiol Plant 42(8):1–11

Szabó TA (1976) A természettudományok és a néprajz határán (on the border of natural sciences and ethnography). Népism Dolg 36–40

Xie H, Zhang Y, Wu Z, Lv T (2020) A bibliometric analysis on land degradation: current status, development, and future directions. Land 9(1):28

Chapter 26
Significance of Medicinal Plants in Medzibodrozie Region, East-Southern Slovakia, for the Socio-Economic Stability of Rural Areas

Ivan Salamon, Maryna Kryvtsova, Michal Stricik, and Pavol Otepka

Abstract The main aim of the European Agricultural Model is to develop prosperity in the countryside. The model supports traditions, environment, and economic welfare. The agricultural business and management of land are focused on alternative crop production, enviro- and social-activities. This model is applied to Medzibodrozie region in East-southern Slovakia, which is created by the Tisa, the Latorica, and the Bodrog rivers (418 km^2 and 38,100 inhabitants). Several possibilities for expanding the agricultural production of the Medzibodrozie region are the cultivation of medicinal plants such as poppy, small fruits, energetic crops, and crops with tourist interest, e.g. the Tokay Wine Region. These agricultural activities have importance from several points of view: (1) rational (offering appropriate job opportunities for unemployed people), (2) production (better utilization of marginal land resources, e.g. salty soils, lower-quality soils in sub-mountainous or mountainous areas), (3) economic (from the viewpoint of market value the medicinal plants belong to the most effective crops), (4) environmental (application of bio-pesticides and mineral fertilizer based on natural zeolite), and (5) diversification (widens crop composition by adding the possibility of introducing new plant species with their own unique natural substance compositions).

Keywords Agricultural model · Biodiversity · Countryside · Medicinal herbs · Tourism

I. Salamon (✉)
Department of Ecology, Faculty of Humanities and Natural Sciences, University of Presov, 17th November St., 01, 081 16 Presov, Slovakia
e-mail: ivan.salamon@unipo.sk

M. Kryvtsova
Department of Genetics, Plant Physiology and Microbiology, Biological Faculty, Uzhhorod National University, 32, Voloshyn St., Uzhhorod 88000, Ukraine

M. Stricik
Department of Economy, Faculty of Business Economics in Kosice, University of Economics in Bratislava, 13, Tajovskeho St., 041 30 Kosice, Slovakia

P. Otepka
European Ecocycles Society, Matrai St. 36, Gyongyos 3200, Hungary

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_26

26.1 Introduction

The key objective of the European agricultural model is rural development—creating a coherent and long-lasting context that would guarantee the future of rural areas by promoting the creation and maintaining of jobs, with an emphasis on improving public welfare (Van Meijl et al. 2006).

At present, competitiveness in the primary sector depends on the economic directives that affect business and production decisions only partially. More and more important for the competitiveness of farms and regional systems are the environmental sustainability of production processes, area protection, food safety, and the primary sector's ability to provide environmental services (Otepka and Haban 2007).

European agricultural model supports

- strong commitment to traditions, where the objective of agriculture is not only to produce but also to protect the countryside and active rural communities and to create new jobs,
- strengthening the main rural sectors: agriculture and forestry,
- improving competitiveness in rural areas to provide job opportunities and well-being of people living in the countryside,
- preserving the environment, landscape, and rural heritage in Europe.

Changes in lifestyle increased social sensitivity to the environmental topics, and the quality of agricultural production are elements that offer new opportunities for business units and new tests on the road to the sustainable development of rural areas. The concept of multifunctionality of agriculture is becoming more important and is getting to the fore.

The European agricultural model is based exactly on multifunctional farms, which in addition to the main business activities based on the soil, and focuses on activities in the following fields (Šalamon 2000):

(a) alternative production (medicinal, aromatic and spice plants, herbalism, natural fabrics, and colours), tourist facilities and services-related (agritourism, food tasting, and catering), valorization of typical products, entertainment (hiking, horse riding, and other sports), hunting, cultural activities (local culture and habits demonstration, guided tours), local crafts, and environmental courses;

(b) environmental activities—an active role in actual territorial planning: the production of renewable energy and growing energetic crops, the maintenance and cleaning of sidewalks, trenches, streams, and small lakes, management of abandoned public forests, environmental activities focused on wild animals, preserving and protecting biodiversity, management, and maintenance of public parks and gardens;

(c) social activities (providing social services to citizens)—services at your fingertips: mixed goods shops, transport, post services, home care services, nursing services (day centres for the elders, kindergartens, and schools). These are activities that could be developed by multifunctional rural companies in smaller and larger villages.

26.2 Biodiversity Protection

Biodiversity is especially important for the balance of ecosystems. At the global level, it is threatened by industrial development, urban construction, deforestation, increasing agriculture specialization due to seed market orientation dominated by supranational groups as well as the monoculture trend (Plačková 2007).

Genetic heritage and biodiversity protection are some of the main goals of the development policy of the European Union, representing an excellent opportunity for agricultural companies. Biodiversity protection can be performed (Haban et al. 2007) in two ways:

- production interventions are based on the valorization of the typical and introduction of the cultivation and breeding of such crop and animal species that have been overlooked in recent years, and the introduction of plant production systems with low environmental impact.
- non-production interventions are based on the promotion of environmental improvement actions to improve the reproduction of plant and animal species (e.g. fences renovation and planting of trees, non-cultivated fields to be intended for the feeding of wild animals, etc.). All these activities rely on the EU rural development regulations, supported by subsidies.

26.3 The Area Among Rivers—Medzibodrozie Region

Islands mean an exciting feeling of something exotic, something safe where you can take shelter from the rest of the world. The Slovakia east-southern Island is a small one that is created by the Tisa, the Latorica, and the Bodrog rivers. This has a surface area of 418 km^2 (Fig. 26.1) and a population of 38,100 inhabitants (Midriak et al. 2008). It belongs to the regions with typical identity, nice picturesque landscape, and strong character. From the tourist point of view, the surrounding is not well known as yet.

However, this is the ideal place where visitors can get closer to nature and fulfil their expectations by wandering, riding a horse and a bike, bird-watching, rowing a boat, fishing, and hunting. On the other hand, visitors can make an unforgettable trip by visiting some romantic castles, mansions, churches, taste special dishes prepared from fresh fish and have a drink of regional wine in one of the typical wine cellars.

26.4 Growing of Medicinal, Aromatic, and Spice Plants

One of the possibilities of expanding agricultural production in the Medzibodrozie region is the development of large-scale cultivation of medicinal, aromatic, and spice plants. This special production follows worldwide efforts on improving population

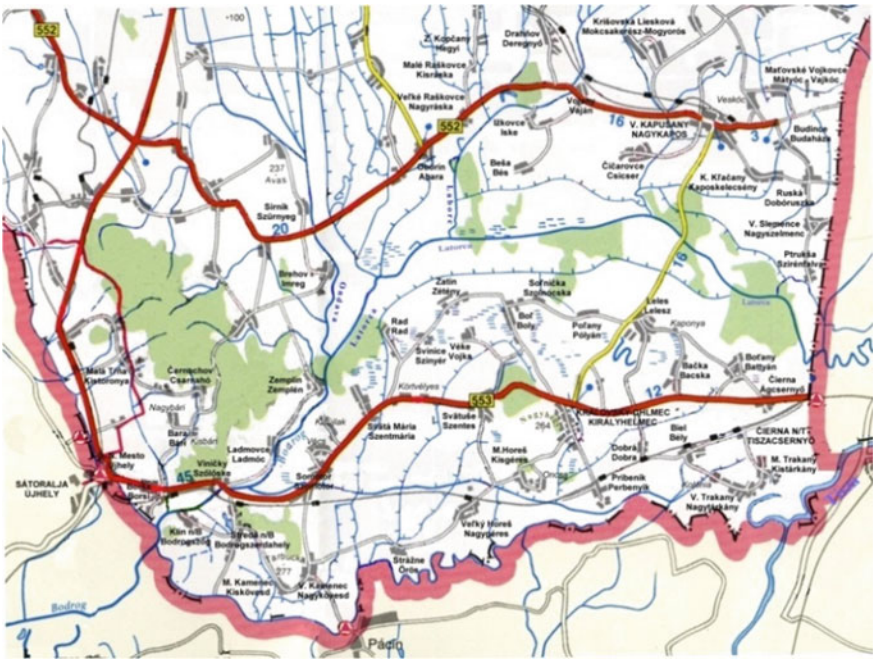


Fig. 26.1 Slovakia east-southern island

health, environmental quality, and biodiversity protection. This agricultural production falls within the sphere of specialized crop production, and it is the only way to ensure the necessary amount of high-quality material for processing and export (Šalamon and Gurková 2006).

Medicinal plant production has several meanings for overall crop production from the view of social aspects (Salamon 2001):

- Production (puts into use the reserves in the soil fund which is not suitable for usual plant production),
- Diversification (widens crop composition by adding the possibility of introducing new plant species with their unique cultivation processes),
- Economic (in terms of revenues, these plants are considered as some of the most profitable crops in agriculture),
- Social (increases employment and provides suitable and interesting job opportunities).

26.4.1 Large-Scale Cultivation of Special Crops

Nowadays, about 150 and 200 medicinal plants are used in official therapy and popular medicine, respectively. The medicinal, aromatic, and spicy plants are mainly

used in this country in phytotherapy, veterinary medicine, cosmetics, and food industry. These plants have additive, ecological, decorative, and sanitary-hygienic functions and positive influence on the water system, soil conservation, plant pasture for bees, phytoadditives, and natural substances (Salamon 2014a).

Large-scale cultivation of medicinal, aromatic, and spice plants can be realized by every agricultural subject on the practically whole area of Medzibrodrozie. The production basis within the principles of sustainable rural development is therefore very wide and has many possibilities to utilize the diversity of agricultural soils and its multifunctional value. This fact has to be always taken into account. On the other hand, herbs come from a wide range of species classified into various botanical families. For this reason why most of these plants are not demanding for particular soil-climatic conditions. The selection and subsequent cultivation of the species are practically unlimited for an agricultural company with an interest in large-scale production (Salamon 2014b).

Every plant species demands special agro-technical processes for large-scale cultivation. In practice, this means that it is necessary to obtain information about seeds germination biology, their subsequent sprouting, and growth, the ontogenetic phases of plant evolution, etc. From these facts, it can be deduced that the elaboration of a cultivation procedure based on the soil-climatic environment conditions is a process requiring a lot of research and experimental works (Salamon 2014b).

Cultivating medicinal, aromatic, and spice plants require an extensive amount of manual labour. Flower collectors and subsequent technology after the material is collected have been developed due to this reason. These machines and equipment have earned the attention deserved not only inland but also abroad.

The development of special crops is mostly limited by the shortage or unsuitable state of the capacities of drying plants in the agricultural subjects, interested in this activity. After minimizing tobacco cultivation areas, there are box dryers in many farms in the region of Medzibodrozie left unused. These could be used to dry these special crops.

An important perspective is mainly in the expansion of non-waste technologies, which means utilizing waste material after herb processing for animal feeding, production of organic fertilizers, and phyto-products used for crop protection. These are the global trends in the complex utilization of grown material, which should also be introduced in our country (Haban 1996).

On the other hand, selling the raw material on EU markets means a very interesting price. If a business partner is satisfied with the quality, then it is about tens of tons of deliveries. Direct profit is multiplied. In most cases, a long-lasting stable business relationship will also be created.

Large-scale cultivation of medicinal, aromatic, and spice plants in the area of Medzibodrozie began in the 1990s. Permanent cultivation areas of chamomile (*Matricaria chamomilla*), pot marigold (*Calendula officinalis*), Moorish mallow, yarrow (*Achillea collina*), red clover (*Trifolium pratense*) (cultivated for flowers), and later marjoram (*Origanum majorana*) (Haban et al. 2001) were annually on an area of 7.5 ha. In connection with the cultivation process and soil-climatic demands of various plant species, the suitability of this environment for production was

confirmed. Mechanization for harvesting and post-harvesting treatment—flowers harvester (type VZR-4), sorting device (type ST-1001), and tobacco dryer modified with pallets (type SIROKO)—was concentrated in the village "Streda nad Bodrogom" (Salamon 2007). Thanks to the use of high-quality races, a high content of therapeutically active ingredients crop was grown and exported abroad (e.g. Australia). The annual volume of funds earned from this special production was up to € 10,000.

26.4.2 Perspectives of New Cultivation Fields

Slovakia is one of the European countries in which particular attention has been devoted to the research of medicinal, aromatic, and spice plants in all its aspects, including the breeding and selection. Based on the study of pharmacodynamics properties of several medicinal crops, the chamomile variety "Lianka" and the peppermint variety "Kristinka" were bred at the University of Presov, Slovakia, between the years 2008–2013. Currently, both varieties have the certificates by the Community Plant Variety Office in Angers, France (No. 49433 and No. 46937). The chamomile variety is characterized by its high percentage of sequiterpenes (/-/-α-bisabolol [52–55%], chamazulene [18–19%], the low contents of /-/-α bisabololoxides A and B [<3%], and essential oil content is from 0.65 to 0.85%). The peppermint variety has very high content the menthol [70–75% of herbs and 80–85% of leaves] of essential oil [2.6%] into the dry raw material. There is a strong premise that the cultivation of these two varieties of medicinal plants throughout the European Union will be expanded in the coming years (Salamon et al. 2018).

Natural products, their derivates, and analogues represent over 50% of all drugs in clinical use, with higher plant-derived natural products providing approximately 25% of the total use (Fery 2002). The commercial value of drug products still derived directly from higher plants is considerable, and plants continue to be important sources of the new drugs and nutrition supplements. It is one way to start a profitable production of new/alternative crops.

New/alternative crops are those which are not normally produced in the Medzibodrozie areas. If the new crop is being transferred from somewhere nearby, at least something about its production techniques and markets may be known. Otherwise, the challenge of new crops is to discover what they produce and how the product can be sold (Salamon 1998).

New/alternative crops are a long term, high-risk adventure into the unknown with the possibility of better returns than current crops, eventually. Indulging in the new crop adventure can be like gambling. One should only gamble what one can afford to lose. As with gambling, the thrill of the high risks involved can be exhilarating. Though leuzea (*Rhaponticum carthamoides*/Willd/Iljin) and puncturevine (*Tribulus terrestris* L.) are introduced crops, these are commercially successful in this area (Salamon 2014a, b).

Both plants are a biogenic stimulant and adaptogenic containing phytohormones which affects the central nervous system. It produces increased physical and psychic

abilities, stimulates tired body muscles, improves the condition, and conciliates demonstration of nervousness, a disorder of insomnia, depression, and disgust. The medicinal attributes of Puncture Vine are due to the furostanol saponins, which have been shown to stimulate increases in natural testosterone levels. Concerning this fact, they are a stimulating effect on the immune, sexual and reproductive systems, with improved muscle building, their tonic action, vigour, vitality, stamina, and physical endurance (Salamon et al. 2006).

26.5 Poppy (*Papaver somniferum* L.) Production and its Heritage

Poppy (*P. somniferum* L.) is an oil plant that has been cultivated in the middle European countries for many years. The main purpose is a seed production used in the food industry, and the second purpose is poppy straw (including capsules), which is an important source for obtaining alkaloids (morphine) for medicinal products in the pharmaceutical industry.

26.5.1 Poppy Cultivation in Eastern Slovakia

Poppy as a traditional crop in the Medzibodrozie region and its production has a long-lasting history, especially to be cultivated in the private gardens. Farmers have preferred a combination of both methods of poppy production: seeds for food uses and dry capsules for the procession in pharmaceutical industry. Areas of poppy production in Slovakia were usually from 386 to 2714 hectares during the last 15 years. The yield of seed during the period was from 0.28 to 0.73 tons per hectare. Good agricultural practice and own registered Slovakian poppy varieties are a very suitable background for high yield potential of poppy seeds production (about 2 tons per hectare) (Fejer and Salamon 2014a).

The breeding and selection of poppy plants in Slovakia date back to 1948, and it was concentrated on the generation of non-specific varieties. The main aim was to find the generation of varieties with the high capacity to accumulate morphine in dry capsules together with high production of good quality blue-coloured seeds used mainly in food industry. Several varieties were registered as the result of long-time selection process with relatively high production potential of blue-coloured poppy seed. There have been already several different varieties of poppy cultivated and grown in Slovakia. Currently, six varieties of poppy with blue-coloured seed are officially registered in Slovakia: “Bergam”, “Gerlach”, Major”, “Malsar”, “Maraton”, and “Opal”. The only variety “Albin” has white-coloured seed. All of them are properly adapted to Central European agro-ecological conditions. The yield of poppy seed of the varieties has been varying from 0.28 to 0.73 t ha^{-1} in the past ten

years. However, the genetic yield potential of poppy seed within these varieties is up to 2.0 t ha^{-1} (Fejer and Salamon 2014b). All of these varieties are suitable primarily for food industry purposes, secondary for medicinal purposes.

Poppy capsules as a by-product or a secondary product of poppy seed production is also very important raw material to use in pharmaceutical industry. The straw yield of poppy within mentioned Slovakian varieties ranges from 300 to 500 kg ha^{-1}. Content of morphine in dry capsules varies between 0.3 and 0.6%. The straw of these poppy varieties is mostly used for morphine extraction; however, it does not meet the increasing demands of the pharmaceutical industry for the product. These varieties are also valued for their good tolerance to herbicides used in the cultivation period. Therefore, they are very suitable for large-scale production of poppy seeds.

Market of poppy straw material ranges between 55.3 and 1591.5 tons annually during the period of 1990–2015. However, real processing capacity of the pharmaceutical industry in Slovakia is much higher: about 4000 tons annually (Seneca Pharmaceuticals, Co. in Hlohovec, West Slovakia). The rest of raw material has to be imported, mainly from the Czech Republic. Actually cultivated varieties of poppy are able to accumulate from 0.4 to 0.6% of morphine. In reality, the marketed straw material contains only 0.3% of morphine in average. The influence of soil characteristics, the weather conditions of actual vegetative season, harvest and post-harvest technologies also affect seed and alkaloid yields.

26.5.2 Breeding and the Content of Poppy Morphine

With the increased poppy cultivation in 1948, breeding works had also begun. At this time, the breeding workstation in Sladkovicovo cooperated with the Slovak pharmaceutical company. Breeding was aimed to higher morphine content in dry capsules and their suitability for mechanized harvest and production of poppy seeds. The selection of new varieties was also realized in other localities of the country (the Eastern parts of Slovakia in Trebisov and Maly Saris).

The first Slovak poppy variety "Blankyt" was registered in 1967. It provided a high yield of blue-coloured seeds. Morphine content in dry capsules reached 0.40%. Since the registration of the first Slovak variety, eight more varieties of blue seeds were bred. They have a high yield potential of poppy seeds (2.0 t ha^{-1}) with the ability to accumulate 0.4–0.6% of morphine in dry capsules (Fejér 2007). The main advantage is their suitability for large-scale technology production. They are adapted to the agro-climatic conditions of Central Europe (Cihlář et al. 2008). Particularly appreciated is their high tolerance to used herbicides when compared with foreign, e.g. the Hungarian and Polish varieties. The variety "Albin" has white seeds and low morphine contents in capsules (around 0.3%). It is used for baking, and its taste reminds us walnuts.

The annual average of poppy straw that Seneca Pharmaceuticals, Co. in Hlohovec handles is 3840 tons (average of 1990–2015). The bulk of raw material was imported from the Czech Republic. Small growing areas in Slovakia are unable to cover their

needs. With 0.301% content of morphine, it is currently necessary to process 5500 tons of poppy raw materials to produce 18 tons of morphine. The actual interest in the varieties able to accumulate high levels of morphine (more than 1.0%) has increased. To obtain sufficient quantities of quality raw materials, several measures have been made. The company implements a variety from Hungary (1995–2002) containing 1.5% of morphine. In some cultivation areas, the Polish variety "Lazur" is grown. This accumulates about 1.0% of morphine. The possibilities of domestic breeding were also looked into. Selected and improved breeding materials can accumulate from 0.9 to 1.1% morphine. Poppy gene pool collection in the Gene Bank in Piestany provides genotypes with the ability to accumulate up to 2.0% or more of the alkaloid (Fejér, unpublished).

Opium poppy (*P. somniferum* L.) cultivation under Medzibodrozie conditions is not only a traditional plant but also a perspective crop in the structure of sowing for farmers. Concerning the existence of a Slovak pharmaceutical company that requires high-quality raw material, it is likely that the cultivation area will increase within the next five years, from 1500–2000 to 5000–8000 ha. A prerequisite for the expansion of poppy cultivation is a suitable variety for poppy straw production.

26.5.3 Using of Poppy Seeds by Households

Poppy seeds are used in many baked goods as a decorative garnish and as a paste of ground seeds. They are used in main course dishes and desserts. They are also the source of poppy seed oil. In Slovakia, seeds are used in various types of breads, cakes, and pastries which are often sprinkled on top with black and white poppy seeds (e.g. strudel, buns with poppy seeds and pastry, "opekance"—round yeast bread with poppy seeds, a traditional meal prepared for the Christmas Eve dinner, and various types of biscuits).

The average consumption of poppy seeds per head in Slovakia, the Czech Republic, and Austria is 300 g. In Germany and the former Soviet Union states, it is about 100 g poppy seeds (Vašák et al. 2010).

In connection with the consumption of poppy seeds, there is a study on possible harm due to the morphine content of the seed. The seeds themselves do not contain significant amounts of alkaloids. It may be contaminated with small particles of poppy straw and thus may show little amounts. Slovak varieties contain low to moderate content of morphine, so contamination is negligible, and consumption is not harmful to human health. Many of Slovak households have been growing poppy for their use in gardens. The law permits the cultivation in the area up to 100 m^2 without permission. After the harvest, the poppy capsules are emptied by Slovak housewives. Impurities are removed by washing with water, and seeds are dried (Fejer 2007).

26.6 Cultivation of Elderberry and Its Anthocyanins

Sambucus L. is a member of the Adoxaceae family. It consist of 5–30 different species of deciduous shrubs, small trees, and herbaceous perennial plants. European (black) elderberry (*Sambucus nigra* L.) has the highest economic importance, and selected natural populations are commonly used and distributed in Europe. This species naturally grows from the lowlands to the sub-mountain level throughout Slovakia. Plants grow in the forests landscape, in bushes, shambles, and near rivers. It is relatively resistant to poor soil, drought, heavy sunshine, and shade. Elderberry production increased from 18 to 77 ha localized in different parts of Slovakia, during 2005–2015. The most used cultivar: "Haschberg" is cultivated almost on 75 ha (e.g. 97%). Only 3% (about 2 ha) of the production area is used for cultivation of the other registered cultivars: "Bohatka", "Dana", "Sampo", and other minor cultivars (Fejer et al. 2015).

The most cultivated cultivar "Haschberg" has been selected in Austria, as a natural resource of anthocyanins. It is a shrub or small tree growing to up to 6–7 m. The trees start to flower in early June and produce a lot of pollen. Cultivar "Haschberg" is mostly windy pollinated or self-pollinated. The ripen fruit is bright, black-purple, round, sized from 4.0 to 5.5 mm in diameter. The inflorescence is pendulous, compact, and relatively dense. The fruits usually stay on the stem when ripen and finally take a dark burgundy colour. The pulp is juicy, dark red. Ripening of fruits usually starts in the second half of September and lasts quite a long time on the tree.

Anthocyanins (also anthocyans; from Greek: ἀνθός (anthos) = flower + κυανός (kyanos) = blue) are heteroglycosides composed of a sugar and an anthocyanidin. They are the most important components in elderberry fruit. The anthocyanins have significant antioxidant activity. The major five anthocyanidins in elderberry are cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5 diglucoside, cyanidin-3-sambubioside, cyanidin-3-glucoside, and cyanidin-3-rutinoside (Salamon et al. 2021). The flowers, but mostly the ripen fruits of elderberry, are widely used in food, cosmetic, and pharmaceutical industries as well as in the phytomedicine.

The amount of the accumulated anthocyanin as the fruit pigments highly depends on suitable environmental conditions, especially temperature, solar radiation, as well as cultivation and production management. Wu et al. (2004) found that total content of anthocyanins in elderberry fruit varied from 602 to 1265 mg 100 g^{-1}. The most cultivated cultivar: "Haschberg" accumulated about 737 mg of total anthocyanins per 100 g of fresh weight. They also found the presence of all five the most important anthocyanins: cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-sambubioside, cyanidin-3-glucoside, and cyanidin-3-rutinoside. Other minor anthocyanins were found only in trace quantities.

26.6.1 Lyophilization Technology for Anthocyanin Isolation

Distillation methods are often used (hydro-, steam- distillations, or water vapour) to extract natural compounds (secondary metabolites of plants, animals, and other organisms). They can extract volatile oils and solids to produce liquid and dry extracts. In both methods, among different types of solvents and various temperatures are used. This directly affects the stability and frequently the breakdown of some sensitive natural components. Based on this fact, lyophilization is suitable for isolating natural substances.

Lyophilization (freeze drying) is done using a simple principle of physics called sublimation. Sublimation is the transition of a substance from the solid to the vapour state, without first passing through an intermediate liquid phase. This technology is important in pharmaceutical, food, and cosmetic industries (Genovese 2015).

Because anthocyanins are unstable in different pH and degrade rapidly at higher temperature and lights, therefore the freeze-drying process was used for their stabilization. The optimal extraction method and the process of freeze drying were developed to obtain pure anthocyanins from elderberry fruits (*S. nigra* L.).

Before a successful lyophilization, it was necessary to dilute extracts with purified deionized water in quality that complies the European Pharmacopoeia (Aqua purificata PhEur). The ratio of 4.7:1 was optimal for an extract from the elderberry (Salamon et al. 2021).

A variety of lyophilization procedures were tested and optimized so that the resulting product is a good, dry powder. Finally, for lyophilization, a program for 36 h was used, where the temperature of freezing was lowered to −40 °C. The program was separated into eleven sections where the temperature and pressure changed. This program included four steps—loading, freezing, evacuating, and drying (Tomash et al. 2015).

The pharmaceutical company Medicproduct, Co. in Lipany (Slovakia) uses freeze drying to increase the shelf life of products, such as vaccines and other injectables. By removing the water from the material and sealing the material in a vial, the material can be easily stored, shipped, and later reconstituted to its original form for injection. Our new original research deals with optimize extraction and freeze-drying procedures to the natural components. The purpose is carrying out a dry, quality lyophilized product, which is then submitted to further analytical and biological testing.

26.6.2 Anthocyanin Lyophilizates Enhance Growth and Inhibit Apoptosis in Rat L6 Muscle Cells

Anthocyanins are water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH of the solution. These natural components occur in all tissues of some higher plants, including leaves, stems, roots, flowers, and fruits.

The characteristic dark purple-red colour of elderberry comes from anthocyanins, water-soluble polyphenolic belonging to the flavonoid family. In addition to this, medicinal plant species have been used in traditional medicine for centuries in North America, Europe, Asia, and North Africa. Elderberry has antioxidant activity, lowers cholesterol, improves vision, boosts the immune system, improves heart health, and has good activity in clinical trials for coughs, colds, flu, bacterial and viral infections, and tonsillitis (Fejer et al. 2015).

Anthocyanins are known for their antioxidant, anticancer, and anti-inflammatory activities and have been suggested to be beneficial in maintaining and building bone and muscle in clinical trials (Yarahmadi et al. 2014); however, their mechanism of action is not well understood.

It has been demonstrated that elderberry anthocyanin lyophilisate protects rat muscle cells (L6 cells) growth in vitro. The extract also prevented cell death and apoptosis. The extract also prevented cell death and apoptosis. Of the anthocyanins present in the extracts, cyanidin-3-glucoside was the most active and enhanced L6 growth by 200%. These data suggest that fruit extracts reduce muscle cell apoptosis and may be useful for development as a preventative treatment for sarcopenia (Wicks et al. 2018).

Sarcopenia is an age-related condition and causes loss in muscle mass and function in the elderly, causing serious morbidity and mortality. Old age and muscular dystrophy are associated with an increased rate of apoptosis in skeletal myocytes (Wicks et al. 2018).

In terms of a mechanism of action, gene expression analysis showed that BCE treatment of the myoblasts produced a dramatic increase BCL-2 mRNA expression >284 fold over controls, reduced Bax mRNA expression, and also increased expression of PPARγ mRNA. Both BCE and C3G altered the Bax/Bcl-2 ratio in favour of reduced apoptosis and increased SS-cell proliferation. Besides, BCE enhanced Sirtuin 1 mRNA twofold. SIRT1 is known as NAD-dependent deacetylase sirtuin-1, a protein encoded by the SIRT1 gene. Activation of SIRT1 has been shown to improve metabolism and induces protection against physiological and cognitive disturbances in old age (Mitchell et al. 2014). These data suggest that fruit extracts and anthocyanins reduce muscle cell apoptosis and may be useful for development as a preventative treatment for sarcopenia and for possible dietary treatment of Duchenne muscular dystrophy (DMD). In DMD, muscle apoptosis precedes necrosis, and markers of apoptosis such as a reduced Bcl-2 mRNA expression are seen as an indicator of muscle degeneration (Abdel-Salam et al. 2009).

Elderberry and their active components have been traditionally used for treating influenza and colds in western and eastern countries of Europe. The highly active flavonoids extracted from elderberry inhibit influenza A (virus H1N1 infection) comparable to the anti-influenza drug of Oseltamivir (the neuraminidase inhibitor). Flavonoid of elderberry extracts blocks viral entry by binding to H1N1 virions and inhibits several strains of the influenza virus in vitro. (Zakay-Rones et al. 1995).

Viral neuraminidase (NA) is found on influenza virus surfaces and enables viral release from host cells. The cyanidin-3-sambubioside extracted from elderberry fruits

is also a natural NA inhibitor (Swaminthan et al. 2013). The compound cyanidin-3sambubiocide, an anthocyanin flavonoid, displays potent NA inhibition. The extract displays inhibitory potential on the H5N1-type influenza A virus (avian influenza).

The elderberry natural components have the potential to be used as antiviral drugs, include novel virus SARS CoV-2 (against the pandemic COVID-19) because they block the function of viral neuraminidases of influenza virus, by preventing its reproduction by budding from the host cell.

26.7 Energy Production from Renewable Sources

Nature, and especially the Sun, is the richest source of energy. Increased attention is paid to renewable energy in the agrarian policy of developed countries. In line with the European trends, 12–15% of national energy consumption shall be covered by these sources, and the rural areas represent the ideal environment for their production (Petříková et al. 2006).

26.7.1 Biomass and Production of Energetic Crops

The term biomass includes various natural products as an outcome from the plant's photosynthesis process. Photosynthesis of green plants can capture 1–3% of the incident solar energy. When looking for new energy sources, the attention naturally focuses on biomass as a reliable and constantly renewing energy source. Biomass has the greatest potential for further development among all renewable forms of energy (Petříková et al. 2006). The main reason is that in many areas with a shortage of natural deposits of oil and coal it can replace these conventional fuels.

Main methods of production of plant biomass are as follows:

- cultivation of energetic crops for solid biomass production,
- growing of oilseeds for the industrial production of biofuels,
- using wood waste from wood processing factories and forests for energy production.

It is clear from the above-mentioned points that the crops in question are gradually transformed into cultivated crops important in connection with sustainable farming systems, the use of new resources, and the diversification of the rural economy in the Medzibodrožie region (Dobos and Novák 2008).

The production of energetic crops can also partially provide an aspect to the solution to the insistent socio-economic problems of the Gypsy population. Support for solutions and its results may soon reflect in the elaboration of the principles for rational settlement building for this ethnic group that will be without undesirable logging in protected areas.

For energetic purposes, we can use a large number of different plant species characterized by rapid growth and quality of produced biomass. Energetic crops can be divided into one-year plants, multiannual, and perennial non-woody energetic crops and trees (Porvaz 2005). These plants include oilseed rape, cereals, sunflower, corn, sugar beet, potatoes, cannabis, sorghum, common flax, poppy, and others. Multiannual and perennial energetic crops include Chinese silver grass, knotweed, phragmites, Jerusalem artichoke, sugar cane, burclover, reed canary grass, hollyhock, and others. For the establishment of fast-growing tree plantations, the best suitable plants are eucalypts, plane trees, aspen, locusts, willow, alder, and others, which are also suitable for the use at waterlogged areas that are very numerous in the Medzibodrozie area.

Energetic crop Chinese silver grass has been introduced in eastern Slovakia during the last few years. The yield potential of Chinese silver grass (*Miscanthus sinensis*) exceeds the potential of domestic species grown in our country, including fast-growing trees. The plant can be considered a significant source of raw construction materials, for industrial and energetic use. The study of the influence of factors of harvest, trimming, fertilization, and mechanical and morphological properties of plants has been examined by several authors (Porvaz 2005; Petříkova et al. 2006; Clifton-Brown and Lewandovski 2002; Kaack and Schwarz 2001). On well-stocked soil, the plant is well grown without fertilization in the first year of cultivation and does not go into further production. Harvested material is used as a mulch against damages caused by freezing. The production is collected only from the second year of cultivation. The harvest achieved in the cultivated fluvisol conditions is 30–34 t ha^{-1}.

26.8 Rural Tourism and Agritourism

Rural tourism represents a relatively new form of tourism in Slovakia. It means the realization of tourism in the countryside, excluding recreational activities that are connected to a rural environment and different from civilization's recreational activities (Otepka and Haban 2007). Its specificity consists of decentralization of accommodation facilities, which allows dispersal of tourists and thus to eliminate the negative impacts of a high concentration of people in tourist centres. It allows individual activities in the phase of offering the product and its implementation.

The concept of implementation of rural tourism activities means a return to nature in some way and return to the traditions and activities that directly reflect the ways of securing basic life needs (Dobos and Novak 2008). It is a special form of recreation in a rural environment, taking advantage of the variety of services that this environment provides (meadows, forests, ponds, rivers, local crafts, folklore, etc.). Those interested in rural recreation will be provided by services by making use of the free capacities of rural houses, business, accommodation, catering, sports and leisure facilities, and the countryside environment. Generally, it appropriately complements agricultural production, by providing agricultural products, recreational facilities of tenants, and free capacity of the facilities services, to holidaymakers. The quality of

rural tourism can be summarized in the following keywords: silence, greenery, the environment, relaxation, return to traditions, and contact with the local population, etc.

Agritourism is a business activity that is provided by the operator (multifunctional agricultural company, joint-stock Company, Ltd. Company, farmer, town, and region) to the tourists for relaxation in a rural environment. It includes specific activities of agribusinesses and farms according to local economic and natural conditions, focused on meeting the recreational needs of tourists.

It is a specific form of rural tourism which, in addition to the direct use of nature and the rural environment, is also characterized by a direct relationship to agricultural activities or to the objects with agricultural functions (Salamon 1998).

Regarding the type and the number of services provided, we can identify the following main types of agritourism with concretization of their use in the area of Medzibodrožie (Takač and Szilasi 2008):

- holidays at the farmer's yard (the use of mainly older yeoman houses)—the tenant, besides the accommodation, also provides other services to satisfy the customer's requirements (equine-assisted therapy, herbalism, aromatherapy, massages, etc.), to generate side income;
- gastronomy agritourism—in its foreground, there is a direct sale of regional specialties (mush "pencári", pancakes "lokše", goulash, red pepper stew, soup "halászlé" and "juchu", home butchers pork menu, wine tasting, etc.) to their guests, a regular circle of customers or restaurants with cymbal music and dancers of the Čardáš dance in pairs, queues, circles, or "solo-Hungarian" Čardáš dance, as a Verbunk dance;
- agritourism with the rental of holiday houses (farmer's, yeoman's or brick-built multipurpose houses with pillar facades) with a focus on fishing, hunting, mushroom picking, wine houses with vineyards, and wine cellars with or without provision of services;
- eco-agritourism, provided by eco-farms producing bio-products without the use of mineral fertilizers, pesticides, and heavy mechanization;
- ecotourism with an emphasis on nature wandering (walking, cycling, sailing, horseback riding, etc.) with an emphasis on observation and exploration, especially invaluable natural areas with extraordinary landscape scenery (a large number of protected areas of the Medzibodrozie), cultural (presentation of folklore traditions during holidays throughout the year: vineyard harvesting, harvest festival, construction of May trees with May festivals, local markets, etc.), and historical (the manor in Somotor, Streda nad Bodrogom, Leles, part of the Tokaj wine route, etc.).

The primary importance of agritourism for agricultural subjects is the acquired source of farms or enterprise income, which provides certainty for existence. It significantly finalizes the production of business entities by making it possible to monetize their products, accommodation capacities, even the technological process and the environment of the farm (farmyard), the fields, the meadows, and the landscape. It creates suitable conditions for the production of less fertile soils or even

endangered agricultural companies and farms. In the end, providing of agritourism services requires a certain degree of equipment of agricultural settlement and thus increases the overall standard of households in these often-remote areas, etc.

Agritourism has its importance also for the villages and towns (Otepka and Haban 2007). At most, it makes use of existing objects that ceased to serve their original purpose. It increases the level of equipment of the municipality (as a prerequisite for the development of rural tourism and agritourism) and creates additional sales of agricultural products as a stimulus for the expansion of production. It increases the income of the inhabitants of the village, the village itself, which can be reflected in its facilities. It creates the appropriate conditions for creating new job opportunities, revives, and maintains craftsmanship, folklore, and other traditions. It contributes to the utilization of the natural, cultural, and historical potential of the village and preserves its original landscape character.

26.8.1 Tokay Wine Region

The special climatic and environmental conditions of the Tokaj Region, also shaped by human activity exploiting these, gave way to a unique tradition of viticulture and wine-making (enology).

Tokaj wine region is a geographically closed area in the basin of the Bodrog river, north Slovakia Zemplín bordered to the south, and Hungary bordered by the confluence of the Tisza and Bodrog. Slovakia is delimited by law 907 ha. It is the smallest wine region in Slovakia (Pospišilová et al. 2005).

The volcanic slopes and wetlands create a special microclimate that favours the apparition of the "noble rot" (*Botrytis cinerea*). The landscape is characterized by a rich variety of building structures (terraces, supporting walls, dry-built stone fences, water cisterns, etc.). Thanks to the unique microclimate, cellar walls are colonized by a special cellar mould called *Gladosporium cellare*, which has a benign influence on the maturation of wines. Sessile oak growing in the higher ranges of nearby mountains provides an excellent raw material for wine barrels, which is a decisive factor for the maturing process, aroma, and colour of the wine.

The diverse socio-economic, cultural, ethnic, and religious background of the population of Tokay Region and last, but not least, the outstanding fame of Tokay wines have contributed to the establishment of the rich and varied cultural heritage of the region.

Typical names Tokay wines (Hronský 2016):

- Samorodné—if the wine is produced by ethanol fermentation from Tokay grape varieties, grown on special selected tassels if there are favourable conditions for mass creation of raisins are two types of autogenous wines: dry—grapes must have a sugar content of at least 21 °NM and made wine must have content natural sweetening and 10 g l^{-1}, and sweet—grapes must have a sugar content of at least

24 °NM and the wine produced shall have a natural content of residual sugar above 10 g l^{-1}.

- Selection—the wine to the ethanol fermentation after pouring the raisins harvested skilled special selected tassels must with a sugar content of at least 21 °NM. Depending on the quantity, added raisins are divided into 3–6 Tubes (Putnam—formerly wood, now and plastic container, which is used for carrying grapes, usually has a capacity of 20–60 l. The Tokaj region, this vessel with a capacity of 15 l is also used as a measure of the processing raisins).
- Mášláš—wine produced by ethanol fermentation of must or wine of the same vintage from qualified furlongs, poured on fermentation lees of wine Samorodné or Výber. It is marketed after two years of maturing, of which at least one year in a wooden cask.
- Forditáš—wine produced by ethanol fermentation of must or wine of the same vintage from qualified furlongs, poured on wine marc of raisins harvested for qualified hunts. It is marketed after two years of maturing, of which at least one year in a wooden cask.
- Selection Essence—wine obtained by ethanol fermentation of raisins qualified furlongs. At harvest, the grapes specially selected to be drenched immediately after the processing of the must from the defined vineyard, which contains at least 180 g l^{-1} of natural sugar and 45 g l^{-1} of sugar-free extract. It is marketed after three years of maturing, of which at least two years in cask.
- Essence—wine produced by slow fermentation samotok, which is obtained from separately collected raisins harvested for qualified hunts. The essence shall contain at least 450 g l^{-1} of natural sugar and 50 g l^{-1} of sugar-free extract. Wine has been marketed after three years of maturing, of which at least two years in cask.

26.9 Territory Care

The state of the Medzibodrozie region is getting worse. Intensified anthropological activities (production or civil), depopulation of rural areas, lack of protection, and climate changes in recent years due to polluting emissions are causing dramatic weather changes that often lead to natural catastrophes. The main cause is progressive soil erosion and inadequate control over surface waters (Terek and Maťaš 1983).

Territory care and risk prevention require detailed planning and broader interventions at the level of local government, management of multifunctional agricultural companies, and other business entities. Therefore, it is necessary to cooperate in the implementation of strengthening of slopes using engineering works to control erosion—cleaning of banks and river beds in the framework of flood prevention—optimization of surface water regime—alarm network and flood emergency system (Midriak et al. 2008).

26.10 Conclusions

In conclusion, it is important to emphasize that the new Common Agricultural Policy recognizes the essential role of agriculture in environmental protection and focuses its support in this sector towards the creation of an agricultural model based on environmental sustainability, food security, and territorial balance. Presented clauses point to the need for further development of the agro and food sector in this new trend, which already appears in the directives and strategies adopted by the European Union.

References

Abdel SE, Meguid IA, Korraa SS (2009) Markers of degeneration and regeneration in duchenne muscular dystrophy. Acta Myologica 28(3):94–100

Cihlář P, Vašák J, Pšenicka P, Mikšík V, Vlk R, Kose Z (2008) Intenzivní pěstování máku. In-Pestovanie maku. Zborník z odborného seminára, Piešťany, Februar 26:19–22

Clifton-Brown JC, Lewandovski I (2002) Screening *Miscanthus* genotypes in field trials to optimise yield and quality in Southern Germany. Europ J Agron 82(2):97–110

Dobos E, Novák P (2008) Súčasné využitie krajiny. In: Terek J, Dobos E (eds) Život medzi riekami Miskolc: Univerzitné vydavateľstvo, pp 181–187

Fejer J (2007) Nové trendy a perspektívy šľachtenia maku siateho (*Papaver somniferum* L.). In: Osivo a sadba (ed) VIII. odborný a vědecký seminář, sborník referátů. Praha, ČZU, pp 122–128

Fejer J, Salamon I (2014a) Poppy (*Papaver somniferum* L.) as a special crop in the Slovakian history and culture. Acta Horticult 1036:107–110

Fejer J, Salamon I (2014b) Agro-technology of the poppy: large-scale cultivation in Slovakia. Acta Horticult 1036:181–185

Fejer J, Salamon I, Grulova D, Michalek S, Zvalova M (2015) Elderberry (*Sambucus nigra* L.)—cultivation in Slovakia, identification and quantification of anthocyanins. Acta Horticult 1061:253–258

Fery FL (2002) New opportunities in Vigna. In: Janick J, Whipkey A (eds) Trends in new crops and new uses. ASHS Press VA, Alexandria, p 452

Genovese F, Altieri G, Admane N, Salamon I, Di Renzo GC (2015) Processing plants and technologies for a sustainable Mediterranean food chain. In: Vastola A (ed) The sustainability of agro-food and natural resource systems in the mediterranean basin. Springer Cham, p 397. https://doi.org/10.1007/978-3-319-16357-4_22

Habán M (1996) Pestovanie liečivých rastlín. Nitra: Agroservis, ÚVTIP Bratislava, p 135

Habán M, Bokoš J, Gurčík Ľ, Paška Ľ, Porhajaš V (2007) Manažér pestovania liečivých rastlín. Nitra: Vydavateľstvo Slovenskej poľno-hospodárskej university, p 100

Haban M, Černa K, Dančak I (2001) *Koreninové rastliny*. Nitra: Agroservis, p 145. ISBN 80-85330-95-4

Hronský V (2016) Slovak wine guide. The 1st Eds, Bratislava, Slovakia: slovart, p 256 ISBN: 9788055613406

Kaack K, Schwarz KU (2001) Morphological and mechanical properties of *Miscanthus* in region to harvesting, lodging, and growth conditions. Ind Crops Prod 112(2):145–154

Midriak R, Terek J, Šalamon I et al (2008) Potenciál krajiny a návrhy na využitie. In: Terek J, Dobos E (eds) *Život medzi riekami* Miskolc: Univerzitné vydavateľstvo, pp 187–207

Mitchell SJ, Montalvo AM, Mercken EM et al (2014) The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet. Cell Rep 6(5):836–843

Otepka P, Habán M (2007) Vidiecky turizmus a agroturizmus. Nitra: Ústav vedecko- technických informácií, p 120

Petřiková V, Sladký V, Strašil Z et al (2006) Energetické plodiny. Prof Press Praha, p 127
Plačková A (2007) Inváz ne rastliny ohrozujú ekosystémy na Slovensku. Ochrana Prírody Slovenska 2(4):10–12
Porvaz P (2005) Produkčné parametre ozdobnice čínskej (*Miscanthus sinensis*) v podmienkach VSN. Zborník vedeckých prác VÚRV—Ústav agroekológie v Michalovciach 21(1):81–85
Pospíšilová D, Sekera D, Ruman T (2005) *Ampelografia Slovenska.* ilustroval rastislav ŠIMORA. Modra: Výskumná a šľachtiteľská stanica vinárska a vinohradnícka p 368, ISBN 80-969350-9-7
Salamon I (1998) Plant genetic resources of selected medicinal plants in the East-Slovak lowland, their evaluation and use. Med Plant Rep 5(5):24–30
Salamon I (2001) Production of medicinal, aromatic and spicy plants in Slovak Republic and its perspective. Matieres Medicales XXI 1:5–15
Salamon I (2007) Large-scale production of Chamomile in Streda nad Bodrogom (Slovakia). Acta Hort 749:121–126
Salamon I (2014a) Aromatic and medicinal herbs of Slovakia. Med Plants Inter J Phytomed Relat Ind 6(3):155–160
Salamon I (2014b) Medicinal plants of high significance in Slovakia. Med Plants Inter J Phytomed Relat Ind 6(2):75–80
Salamon I, Fejer J (2014) Poppy cultivation and its legalization by the governmental law in Slovakia. Acta Hort 1036:119–121
Salamon I, Fejer J (2018) Certificate of the community plant variety office. No. 49433, Variety of German Chamomile (*Matricaria recutita* L.) "Lianka", In: Bulletin of CPVO, Angers, France, vol 4, p 76
Salamon I, Mariychuk R, Grulova D (2015) Optimal extraction of pure anthocyanins from fruits of *Sambucus nigra.* Acta Hort 1061:73–78
Salamon I, Grulova D, Hancianu M, Cioanca O (2015) Optimization of lyophilization technology for purification and stabilization of anthocyanins from elderberry fruits. Acta Hort 1061:245–252
Salamon I, Haban M, Otepka P, Habanova M (2018) Perspektives of small- and large-cultivation of medicinal, aromatic and spice plants in Slovakia. Med Plants Inter J Phytomed Relat Ind 9(4):261–267
Salamon I, Sezer ENS, Kryvtsova M, Labun P (2021) Antiproliferative and antimicrobial activity of anthocyanins from berry fruits after their isolation and freeze-frying. Appl Sci – MDPI 11(2096):1–15
Šalamon I (2000) Rozvojový program výroby a spracovania liečivých, aromatických a koreninových rastlín v Slovenskej republike. Michalovce: OVÚA, Grafex, 160 p
Šalamon I, Gurková A (2006) Možnosti rozvoja pestovania liečivých, aromatických a koreninových rastlín v agropodnikaní regiónu Východoslovenskej nížiny. Acta Facultatis Studiorum Humanitatis et Naturae Universitatis Presoviensis. Prírodné Vedy: Folia Geographica, XLV 10:506–511
Swaminathan K, Dyason JC, Maggioni A et al (2013) Binding of a natural anthocyanin inhibitor to influenza neuraminidase by mass spectrometry. Anal Bioanalyt Chem 405(20):6563–6572
Takač A, Szilasi I (2008) Architektúra a etnografia. In: Terek J, Dobos E, (eds) Život medzi riekami. Univerzitné vydavateľstvo, Miskolc, pp 117–131
Terek J, Maťaš E (1983) Structure of the landscape and hydrologic regime in the area "Medzibodrožie." Ekológia 2(6):407–413
Tomash J, Fejer J, Mariychuk R, Salamon I, Simko V (2015) Patent no. 288313 Methods of anthocyanine lyophilisation from small fruits of medicinal plants. Industrial property office of Slovak Republic, Banská Bystrica, Slovakia
Van Meijl H, Van Rheenen T, Tabeau A, Eickhout B (2006) The impact of different policy environments on agricultural land use in Europe. Agric Ecosys Environ 114(1):21–38
Vašák J et al (2010) Mák. Praha: Powerprint, 352 p

Wicks SM, Salamon I, Calderon AI, Carcache de Blanco E, Mahady GB (2018) Sarcopenia, diabetes and nutritional intervention. In: Bagchi D, Sreejayan N (eds) Nutritional and therapeutic intervention of diabetes and metabolic syndrome, 2nd edn. Elsevier Academic Press, London, San Diego, Cambridge, Oxford, pp 279–292

Wu X, Gu L, Prior R, McKay S (2004) Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. J Agric Food Chem 52:7846–7856

Yarahmadi M, Askari G, Kargarfard M et al (2014) The Effect of anthocyanin supplementation on body composition, exercise performance and muscle damage indices in athletes. Inter J Prevent Med 5(12):1594–1600

Zakay-Rones Z, Varsano N, Zlotnik M, Manor O, Regev L, Schlesinger M (1995) Inhibition of several strains of influenza virus in vitro and reduction of symptoms by an elderberry extract (*Sambucus nigra* L.) during an outbreak of influenza B in Panama. J Altern Compl Med 1(4):361–369

Chapter 27
Ecological Value, Cultivation and Utilization of Important Medicinal Plants (Sage, Oregano and Sideritis) in Greece

Alexandra D. Solomou, Kyriakos D. Giannoulis, Elpiniki Skoufogianni, Styliani Kakara, George Charvalas, and Antonios Kollimenakis

Abstract The Greek territory provides an ideal environment for growing plants and herbs, which are to be found in abundance, flourishing on mountain sides, where they grow naturally. Medicinal plants are considered as important factors in sustainable development, environmental protection, and public health. The aim of this chapter is to present comprehensive information about the diversity, ecological value, cultivation, and utilization of the economically important medicinal plants such as Salvia sp., Origanum sp., and Sideritis sp., in Greece, stressing their medical-environmental and economical values. The utilization of our natural wealth together with the conservation of biodiversity is issues of paramount importance. Valuable aides to these appear to be the medicinal plants which play a crucial role in both these sectors. Another important fact we should keep in mind is the potential use of medicinal plants and their unconventional applications in sustainable agriculture, enhanced by the selective and multiple biological properties of their essential oils. Numerous and valuable uses are more than possible as the diversity of medicinal plants presents new sustainable opportunities—financial and environmental—for agriculture, and this should be the motive to boost further studies concerning the cultivation of medicinal plants.

Keywords Sustainability flora · Agriculture · Uses · Biodiversity · Greece

27.1 Introduction

Greece as a "biodiversity hotspot" is known for its high plant species diversity, 5885 species and 2000 subspecies (native and naturalized), representing 6760 taxa, belonging to 1087 genera and 184 families (Dimopoulos et al. 2013, 2016). Compared

A. D. Solomou (✉) · S. Kakara · A. Kollimenakis
Hellenic Agricultural Organization "DEMETER",
Institute of Mediterranean and Forest Ecosystems, Athens, Greece

K. D. Giannoulis · E. Skoufogianni · G. Charvalas
Laboratory of Agronomy and Applied Crop Physiology, Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Volos, Greece

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4_27

to other areas of the globe, the position of Greece is highly rated, as it provides a variety of habitats, a rich geological history, and ideal climatic conditions. As the world is rapidly developing and demographic changes are escalating, new challenges appear having to do with the use of available natural resources. In areas having favorable climatic conditions, the use of natural resources is a priority (Bogers et al. 2006), and Greece is one such place where medicinal and aromatic plants (MAPs) are an important natural resource (Lange 2001; Mateescu et al. 2014).

The Greek National agro-food sector is still developing and MAPs are expected to greatly contribute to the Greek rural economy. In 2013, MAPs covered only 0.04% of the country's cultivated land (Ministry of Rural Development pers. comm.) Fortunately, nowadays the demand for MAPs is on the rise. The Common Agricultural Policy (CAP) has played a crucial role in crop selection as developments in CAP (2007–2013) augmented the culture and production of MAPs in Greece. One such important development was the "release" policy (decoupling) of subsidies from production and another was their conversion to area subsidies based on historical production (RE HERB 2013).

Nowadays, MAPs are considered as important factors in sustainable development, environmental protection, and public health. Since antiquity their beneficial role has been recognized and traditional medicine has been based on their pharmaceutical properties. Even beekeepers utilize these plants in honey, pollen, and bee-glue production during spring. Used fresh, frozen or dry, also transfused into oils and essences, these plants nowadays concern a large part of food, cosmetic, and pharmaceutical industries (Miguel et al. 2004; Bogers et al. 2006).

Hence, the value of MAPs in curing or in maintaining health has been known and recognized throughout time but this is not their only property. Based on their use, we can divide them into groups:

a. Raw material group—for essential oil extraction
b. Culinary spices group (non-leafy parts)—used for flavoring/seasoning
c. Culinary herb group (leafy or soft flowering parts)—used for flavoring/seasoning
d. Medicinal group—many synthesized medicines are patterned after plant extracts which provide the basis of modern drugs.
e. Miscellaneous—MAPs as ingredients of cosmetics, dyes, disinfectants, insect repellents, etc.

It is noteworthy that the cultivation of MAPs poses a serious means of income, especially for small-scale farmers, who strengthen their livelihood by this trade. In this way, they can choose to enhance their farming activities with more successful results in the foreseeable future, ostracizing poverty. Greece is an ideal place for MAPs' cultivation because of its rich flora such as *Melissa sp., Rosmarinus sp., Origanum sp., Ocimun sp., Chamomilla sp., Salvia sp., Mentha sp., Sideritis sp., Lavandula sp., Thymus sp., Satureja sp.* (Solomou et al. 2016, 2019). In Greece, the most important cultivations for their medicinal and economic properties are those of *Salvia sp., Origanum sp.,* and *Sideritis sp.*

The aim of this chapter is to present comprehensive information about the ecological value, diversity, cultivation, and utilization of the economically important medicinal plants *Salvia sp., Origanum sp.,* and *Sideritis sp.,* in Greece, stressing their medical-environmental and economical values.

27.2 Methodology

A literary research was conducted, using scientific databases such as Scopus, Web of Science, and Google Scholar. Wanting to identify, appraise, select, and synthesize all high quality research evidence, an effort was made in order to review and consolidate the existing research on the ecological value, diversity, cultivation and usage of *Salvia sp., Origanum sp.,* and *Sideritis sp.* in Greece. Toward this aims, a systematic methodology was applied focused on the following phases:

a. The creation of keywords.
b. The systematic search (Harrison et al. 2014).

27.3 Types and Plant Morphology

27.3.1 Sage

Sage (Fig. 27.1) is a perennial subshrub native to the Mediterranean region but now found in most places throughout the world. The genus Salvia is classified in angiosperms, dikotylon plants belonging to the family of Lamiacae. Salvia as a

Fig. 27.1 Salvia plants

genus includes nearly 900 plant species (Walker et al. 2004). In Greece, there are 23 native species of the genus Salvia L. of which three are endemic (Karousou et al. 1998). From 23 species, three of them (*Salvia officinalis*, *S. pomifera,* and *S. sclarea*) are used as spices or as ingredients of remedies and their range has to do with the climatic conditions of each area (Skoufogianni et al. 2017).

The morphological description and distribution of the most important species of Sage in Greece are presented in Table 27.1.

Table 27.1 Morphological description and plant distribution of *Salvia* species

Species	Morphological description	Distribution
Salvia officinalis L.	It is a small aromatic evergreen shrub. Its stem is square, multibranched, fluffy, 30–50 cm high, with bulbar or lanceolate leaves, serrated, fluffy, and green. It grows both in warm and cold regions (islands, mainland Greece) and n calcareous, medium fertility, and arid fields	Ionian Islands, North Central Greece, North Pindos, North-East Greece, South Pindos
Salvia pomifera L.	It is a shrub, 0.5–1 m high. There are many glandular stems on the top. Leaves are petiolate, oval or oblong oval, a little serrated. Its widely known as bitter sage (Cretan sage) which grows in South Greece at an altitude of 0–500 m, in W. Crete up to 1200 m, in stony hillsides (semi-shrub plant), 1 m high, with smooth round galls on stems, which are eaten by the Arabs and said to quench thirst. In old times, villagers collected these galls at May Day and they boiled them in sugar	East Aegean islands, East Central Greece, Kiklades, Kriti and Karpathos, Peloponnisos, Sterea Ellas, West Aegean islands
Salvia sclarea L.	It is commonly known as erythranthis sklarea, gorgogiannis, etc. It is located in stony places in Thrace, Macedonia, Epirus, Thessaly, Peloponnesus and Ionian islands. It is also native to France in limestone hills, in Provence and in the seaside Alps. When it is cultivated, it can reach a height of 1 to 1.5 m	East Aegean islands, Ionian Islands, North Central Greece, North Pindos, North-East Greece, Peloponnisos, South Pindos, Sterea Ellas, West Aegean islands

Fig. 27.2 Oregano plants

27.3.2 Oregano

Oregano (Fig. 27.2) is a perennial flowering plant belonging to the Lamiaceae family (Makri 2002). This genus includes more than 70 species, 49 taxa (species and subspecies), and it is commonly found in all Mediterranean countries and also in the temperate zones of Asia and America (Kintzios 2002). One species (*Origanum onites)* and two subspecies (*O. vulgare subsp. hirtum and O. vulgare subsp. viridulum)* are found in Greece and their range has to do with the climatic conditions of each area.

The morphological description and distribution of the most important species of Oregano in Greece are presented in Table 27.2.

27.3.3 Sideritis

Sideritis (Fig. 27.3) is a genus of flowering plants belonging to the Lamiaceae family. The genus *Sideritis* comprises about 150 species distributed in the Mediterranean region (González-Burgos et al. 2011). In Greece, 11 species (*Sideritis clandestine, Sideritis curvidens, Sideritis euboea, Sideritis lanata, Sideritis montana, Sideritis perfoliata, Sideritis purpurea, Sideritis raeseri, Sideritis scardica, Sideritis sipyleam* and *Sideritis syriaca*) and one subspecies (*Sideritis perfoliata* subsp. athoa) are found in Greece and their range has to do with the climatic conditions of each area.

The morphological description and distribution of the most important species of *Sideritis* in Greece are presented in Table 27.3.

Table 27.2 Morphological description and plant distribution of Origanum species

Species and subspecies	Morphological description	Distribution
Origanum vulgare subsp. hirtum (Link) Ietsw	Its height usually ranges from 30–80 cm. Its shoots are upright, square. The leaves are relatively short, ranging from 1.5–2.5 cm and their color is gray-green. The flowers are arranged in a complex white inflorescence. The seeds are small in size, and in particular less than 1 mm	East Aegean islands, East Central Greece, Ionian Islands, Kiklades, Kriti and Karpathos, North Aegean islands, North Central Greece, North Pindos, North-East Greece, Peloponnisos, South Pindos, Sterea Ellas, West Aegean islands
Origanum vulgare subsp. viridulum (Martrin-Donos) Nyman	Perennial herbaceous plant with woody shoots at the base that reaches 40 cm in height. The stem is erect, hairy, square in cross section. The leaves green oval and the flowers are white. The inflorescence is formed at the top of the shoots. It blooms from July to September	East Central Greece, North Aegean islands, North Central Greece, North Pindos North-East Greece, Peloponnisos
Origanum onitesL.	It is a perennial aromatic shrub, which can reach a height of up to 50 cm. The shoots are upright. The large leaves range from 10–20 × 5–12 mm and are two per vertebra, ellipsoid to broadly ovate. Stands out from the white inflorescence. The seeds are small in size, and specifically less than 1 mm. They are smooth, elliptical in shape, and brown in color	East Aegean islands, Ionian Islands, Kiklades, Kriti and Karpathos, North Aegean islands, Peloponnisos, Sterea Ellas, West Aegean islands

27.4 Crop Ecology and Cultivation of Medicinal Plants

27.4.1 Sage

27.4.1.1 Crop Ecology

There are three sage species, members of the genus Salvia that are mainly found in Greece: *S. fruticosa S. promifera* and *S. officinalis. S. officinalis* grows only in the northwest part of the mainland at altitudes between 600 and 950 m. The mounts Voras, Pinovo, Vermion, Vourinos, Smolikas, Mitsikeli, and Timfi are the main zones where *S. officinalis* is part of the mixed deciduous one. It is located mainly in stony soils at an altitude of 1200 m, especially on Crete island and also it can withstand both high and low temperatures. The Ionian and Aegean islands are the natural places

Fig. 27.3 *Sideritis* plant

where *S. fruticosa* coexists with macchies and phrygana. It grows at altitudes lower than 1000 m, rarely up to 1350 m. In the area of Southern Peloponnese and on several Aegean islands, it co-occurs with *S. pomifera* which grows at altitudes lower than 1350 m. It forms extended populations in the macchies and phrygana as well as in openings of pine forests (Karousou et al. 2000). The ideal soil for growing sage is soil that should drain well, and the ideal pH should range between 6 and 6.5 (Katsiotis and Hatzopoulou 2015). The salvia can be propagated either by cutting and micropropagation or by seed. Most of the time, the seed is not used due to the low germination.

27.4.1.2 Cultivation

Sage is a perennial woody shrub with elongated leaves, a woody square shoot, and vertebrate flowers cultivated in various countries (Govahi et al. 2015; Laborda et al. 2013). Sage is compatible with organic cultivation practices. Sage inoculation with mycorrhiza affects its dry biomass positively (Geneva et al. 2010). The most important species is *Salvia officinalis* (sage), a semi-shrub perennial species, whose height varies from 30–80 cm. Its flowers are large 2–3 cm, violet in sparse vertebrae per 3–6 flowers that form a simple apex. Flowering begins in April and ends in the first ten days of May.

Sage grows in both cold and warm areas and at an altitude of 0–1500 m. Furthermore, sage is very resistant to cold up to −25 °C.

Sage can grow in various soil types, but prefers medium-textured, well-drained calcareous with a pH 6.2–6.4. Very light sandy soils are not suitable because apart from the fact that the growth of the plants is delayed, in case of rain the fine grains

Table 27.3 Morphological description and plant distribution of *Sideritis* species

Species	Morphological description	Distribution
Sideritis clandestina (Bory and Chaub.) Hayek (Taygetos's tea)	Perennial herbaceous plant, up to 40 cm high. Its stem is simple or branched, and the leaves are fuzzy, with a shadowy hue, oblong—speared, intact or saw—shaped, the lower leaves with petiole and the upper epiphytic or with petiole. The calyx is bell-shaped, covered by dense bristles	Peloponnisos
Sideritis perfoliata subsp. *athoa* (Papan and Kokkini) Baden (Athos's tea)	Native to mount Athos and Samothraki. It is a perennial herbaceous plant, up to 40 cm high. The stem is upright, straight or branched and woody at its base. The leaves are lanceolate and flowers are yellow in color. Also, the calyx is bell shaped	North Aegean islands and North-East Greece
Sideritis curvidens Stapf	Annual herbaceous plant, up to 5–15 cm high. A white thin fuzz covers all the parts of the plant. With one or more straight green stems. The flowers are white and rarely purple	East Aegean islands—present, East Central Greece, Ionian Islands, Kiklades, Kriti and Karpathos, North Aegean islands, North Central Greece, North-East Greece,Peloponniso, Sterea Ellas, West Aegean islands
Sideritis euboea Heldr (Euboa's tea)	Perennial herbaceous plant, up to 30–50 cm, with a dense and white fuzz all over the whole parts of the plant. Its stem is strong, simple or sometimes branched. The leaves are elongated and the flowers are yellow. The calyx is tubular which ending in teeth and has fluff	West Aegean islands
Sideritis lanata L.	Annual herbaceous plant, with white thin fuzz all over the parts of the plant. The flowers are white with black spots	East Aegean islands, Kiklades, North Aegean islands, North-East Greece, Peloponnisos
Sideritis montana L.	Annual or biannual herbaceous plant, up to 35 cm. The stem is upright straight and woody in the base. The leaves are simple and the flowers are yellow	East Aegean islands, East Central Greece, Ionian Islands, North Aegean islands, Peloponnisos, South Pindos, Sterea Ellas, West Aegean islands

(continued)

Table 27.3 (continued)

Species	Morphological description	Distribution
Sideritis perfoliata L.	It is a perennial herbaceous plant, up to 30–50 cm high. The stem is green, straight or branched and woody at its base. The leaves are lanceolate and flowers are yellow	North Aegean islands, North-East Greece, South Pindos, North Pindos
Sideritis purpurea Benth	Perennial herbaceous plant up to 30 cm high, with lanceolate leaves. The flowers are yellow, white or purple	East Central Greece, Ionian Islands, North Central Greece, North-East Greece, Peloponnisos, South Pindos, Sterea Ellas, West Aegean islands
Sideritis raeseri Boiss and Heldr (Parnassos's tea)	Perennial herbaceous plant, up to 40 cm high, with a thin stem and lanceolate leaves and yellow colored flowers. The calyx ends in teeth	North Central Greece, Peloponnisos, Sterea Ellas and South Pindos
Sideritis scardica Griseb. (Olympus's tea)	Perennial herbaceous plant, with a simple or branched stem The leaves are lanceolate, intact or slightly saw—sshaped with white fuzz. The flowers are yellow colored and the calyx is probably bell-shaped and covered with dense bristles	North Central Greece, North Aegean islands and East Central Greece
Sideritis sipylea Boiss.	Native to mounts of Samos, Chios, Lesvos and Ikaria Perennial herbaceous plant. A dense white fuzz covers all the parts of the plant. The flowers are yellow with some brown spots	East Aegean islands
Sideritis syriaca L. (Malotira)	Perennial herbaceous plant, up to 50 cm. Its stem is usually simple, strong, upright, covered with dense white fuzz. The leaves are oblong—lanceolate, and the flowers are yellow. The calyx is tubular and ends in teeth	Kriti and Karpathos

of sand adhere to the lower leaves of the plants, where they remain for a long time, thus degrading the quality of the product. Also, unsuitable are heavy and cohesive soils that retain a lot of moisture.

Sage has low input requirements (nitrogen, phosphorus, potassium, and water). In irrigated crops, it suffers from the weeds.

Fig. 27.4 Sage cultivation

Sage can be propagated by seed in an outdoor seedbed. The amount needed to sow 1 m^2 is 8–10 gr, and each gram contains about 150 seeds. The seed must be 1–3 years old, as to be able to germinate. The best time to make seedlings is the beginning of August as long as it is watered frequently and protected from excessive temperature. Finally, sowing can be done directly in the field in rows, by hand or with machines, using 3–5 kg ha^{-1}. It can also be propagated by implants (Fig. 27.4), which are parts of annual shoots usually 10–12 cm long, planted to take root in a mixture of soil or manure and sand (1:1). The planting distances are 5 × 10 cm.

The best time to take place the planting in the field is autumn (October–November), but it can also be done in spring (February–March). Planting distances between the lines are 1 m and on the lines 0.6–0.7 m.

The recommended annual fertilization is 70–80 kg N ha^{-1}, 80–100 kg K $ha^{-}{}_{1,}$ and 80–100 kg P ha^{-1} in case of conventional cultivation, or manure or similar approved preparations in organic cultivation (Maloupa et al. 2013). Irrigation is required only at the time of planting and then the crop is grown under dry conditions (Maloupa et al. 2013).

In a previous study, we had seen the positive effect of the use of *mycorrhiza* on *Salvia* biomass. The plant–fungi symbiosis explores the soil volume beyond the depletion zone which results in increased yield (Ye et al. 2017). The extrametrical hyphae produced by the fungi acts as root extensions contributing to the enhancement of the root system for efficient absorption of nutrients and water as well, in larger area (Bagyaraj and Reddy 2000). These results indicate a biological way which could help in the reduction of the used agrochemicals and could lead to a sustainable cultivation of medicinal plants, even in abandoned soils of low fertility.

Basic cultivation care is the control of weeds, which is done through carvings, weeding, and the use of herbicides. Soil cover using fabric ground cover constitutes a method of weed control where plants can have a protected grow. On the other hand, the use of plastic films is not recommended as it favors the development of high values of humidity and temperature favoring the development of diseases to the root system.

When the soil-climatic conditions are suitable and the right treatments are made, the lifespan of sage is 13–15 years. The right time for the harvest is when the plant is at the start of the flowering period where the concentration of essential oils is maximized; Marquard and Kroth 2001). Harvest starts in April in the case of lowlands while in the mountainous areas in June and July. During the establishment year, only one harvest takes place, while in the second year and after, there are 2–3 harvests. Harvesting is done manually, and it is recommended to be done in the afternoon.

Drying can be done in the shade in sheds but the most efficient drying is achieved in hot air dryers, where the plant material is constantly in contact with dry air, while the moist air is pushed with ventilators in the opposite direction.

During the establishment year, the yield in dry leaves is about 1000 kg ha^{-1}, while from the second year onward it amounts to about 4000 kg ha^{-1}, which is harvested under the two-crop harvests regime. The essential oil yield, after the second year, can reach up to 75 kg ha^{-1}.

27.4.2 Oregano

27.4.2.1 Crop Ecology

Oregano as a crop is in high demand both as a conventional and as an organic crop (Asdal et al. 2006). The quality and quantity of oregano play an important role regarding its commercial value. A very important factor in order to have a proper oregano cultivation is that the genetic resources of oregano come from natural habitats so that it is fully adapted to the soil and climatic conditions of the area. The evaluation of local oregano varieties for the selection of the appropriate plant propagating material should be done with criteria such as resistance to low temperatures, resistance to disease, and biotic stress (Sivicka et al. 2015). Oregano is a plant with a wide range of temperature resistance but the ideal temperatures range between 18 and 22. It prefers sunny places in order to increase the content of essential oils. When it comes to soil, oregano prefers well-drained soils. It is a drought-tolerant plant without any nutrient demand but reacts very well to the addition of potassium nitrogen and phosphorus. Finally, its flowering begins in June and the seed production takes place in August (Droushiotis and Della 2002; Asdal et al. 2006).

27.4.2.2 Cultivation

Oregano can be propagated by seed, by cuttings as well as by implants from the mother plantation (Fig. 27.5). When propagated natively, the seed, after being mixed with sand because it is too small, is sown in a greenhouse to produce seedlings at the end of July. Kuris et al. (1980) reported that oregano propagation by seed may result in delayed annual production. The implants are planted in the field in spring or autumn, depending on the area. Kuris et al. (1980) reported that *Lamiaceae* implants make a root system relatively easily, which is characteristic of this family. Even from the second year after crop establishment, oregano can be propagated by implants, which are planted in the field, such as bed seedlings. It is usually resistant to fungal and insect infestations. During the winter, the aboveground part of the plant dries and sprouts in the spring. It is better to be established in autumn or in early spring. Planting takes place in a row distance of 50−80 cm and plant distance of 30−40 cm (3.5–4.5 plants m^{-2}). Before planting, a deep plowing should take place as to reduce weed population.

In case of fertilization, most of the farmers in Greece are using 50–65 kg of nitrogen and 60–80 kg of phosphorus per hectare, during the plantation and every autumn throughout the year and 100–150 kg N ha^{-1} each May (Omer 1999; Ozgoven et al. 2006). In a recent study that it was conducted in Greece (Giannoulis et al. 2020), it is reported that nitrogen fertilization (using urea 40–0–0) of 300 kg per hectare in one dose around the middle of May increased the harvested yield and also the produced essential oil.

Although oregano is grown in dry conditions, in cases where there is water, it is good to have 1–2 irrigations in May and in early June, because lack of water as well

Fig. 27.5 Oregano cultivation

as lack of nitrogen could cause an increase in abscisic acid in the leaves which results in the closure of the stomata and a decrease in perspiration (Davis 1994).

It is necessary to control weeds either by mechanical means or by the use of herbicides. However, great care is required because there are no approved herbicides for the cultivation of oregano and in addition many of them cause toxicity to young plants (Karamanos 1992).

The harvest takes place during the flowering season, which varies in altitude and climate. Harvesting can be done either manually or mechanically. The drying which ensures a better product is in the shade. This is followed by rubbing and sieving to remove foreign matter, which degrades its quality.

Dry yield gets the maximum values at the third growing year due to its perennial nature. This yield ranges between 1.5 t ha^{-1} and 4 t ha^{-1} (Sotiropoulou and Karamanos 2010). In the case of the essential oil, it has been reported that the age effect of crop plays an important positive role (Kokkini et al. 1994), with values obtained in the third growing period approaching those expected from mature Greek oregano plants (on the average between 2 and 7.5%). The average values of oil concentration recorded for oregano varied within the range of 1.1–8.2% (v/w) (Baser et al. 1994; Kokkini et al. 1994; Karamanos and Sotiropoulou 2013).

27.4.3 Sideritis

27.4.3.1 Crop Ecology

Sideritis sp. is a very hardy plant that blooms from July to August. Populations of Sideritis sp. thrive in mountainous areas of high altitudes, over 1000 m, usually on sunny slopes with high inclination (Solomou et al. 2019). It is drought-resistant, has low nutrient requirements, and the ideal soil pH is between 6.9 and 8. It can be found at high altitudes on rocky soils preferably slightly alkaline (Koutsos 2006). The range of low temperatures that tea can withstand is between −5 and −10 (Katsiotis and Chatzopoulou 2015).

27.4.3.2 Cultivation

Sideritis sp. grows in a variety of soils with a pH ranging from 6.0 to 8.0. It usually prefers rocky and calcareous soils but can also grow in stony soils (Fig. 27.6). Also, the high temperature at daytime and the low temperature at night at such habitats seem to have a positive effect (Koutsos 2006). Other species grow in soils filled with limestone gravel and in stony areas, either alone or in plant communities.

As a cultivated plant, it could be characterized as hardy in climatic conditions because it withstands at very low temperatures and it has not special requirements in soil conditions, since it thrives best in moderately mountainous limestone soils. The plants thrive at altitudes ranging from 500 to 2000 m above sea level. The location

Fig. 27.6 *Sideritis* cultivation

and conditions of the areas that can be used for cultivation should be similar to those of their natural habitat in order to keep the product quality. In such conditions, the cultivated plants, which are already stressed because they are genetically adapted to a different environment, are less vulnerable to pests and diseases, and require less cultivation practices. Thereby, organic farming becomes more visible and economical (Katsiotis and Chatzopoulou 2015).

Sideritis sp. is characterized as a dry crop, and this property is given by its dense and deep root system. However, if there is the possibility to get water, it takes advantage of it. Nevertheless, special care must be taken so that it does not stand around the area of the root system, because fungal diseases will develop and they will destroy the crop. The water requirements depend on the climatic conditions, the mechanical composition of the soil, the physical properties of the soil, the height of the groundwater level, the cultivation technique (plant density, nitrogen fertilization, etc.). Water requirements are determined empirically by the yield of each area from the adequacy of irrigation water, evaporation, and soil moisture.

Fields of high sun exposure and relatively high soil slope, which do not retain moisture and no plant rot is observed in spring are ideal for the cultivation of *Sideritis*. In higher altitude fields, the plants enter at flowering stage later, which helps the drying procedure due to the better prevailed temperatures of April–May. Furthermore, in fields of medium altitude (low parts of the valleys) rot is usually observed in spring, while in lowland fields except the appearance of rot there are conditions that are more ideal for the appearance of insects in larger populations, while the crop has a much shorter productive life. Therefore, the typical climate of the mountain fields where the plants grow naturally is the one to which *Sideritis* is adapted.

Its propagation can be done either inherently by seed, by cuttings. However, vegetative propagation is recommended, although siderite seeds germinate easily, as

seed propagation creates great inhomogeneity of plant material in growth, flowering season and quantity of flowering stems. When cuttings are used, planting is done manually only. Nevertheless, planting can be done with tobacco or tomato transplanting machines when seedlings are used and large non-sloping fields are to be planted (Mylonas 2017).

Sideritis is a perennial crop, which lasts from 5–12 years in the same field. For this reason, before planting, the field must necessarily be properly prepared. During the summer period, before planting, a deep plowing must take place and just before planting, depending on the nature of the soil, milling, or a light plowing and mulching, to destroy the weeds, to cover the fertilizer, and to facilitate planting (Goliaris et al. 1999). When it is grown in mountainous, sloping, stony soils, no special soil handling is required, but it is hand-cultivated as far as possible.

Two seasons are considered suitable for planting, in autumn (October–November) and in the end of winter or beginning of spring (February–March). It is stated that planting can be done with distances of 0.7–1 m between the rows and 0.5–0.6 m on the rows (Maloupa et al. 2013). If the planting take palace in spring and the soil is dry, it is suggested that irrigation of the new plants be carried once or twice, in order to minimize the losses. The technique of covering soil is suggested only during the first and second year of crop establishment, due to the fast grow of the plants, which will cover the surface of the field in the coming years and therefore the herbaceous plants will not be able to find space or light to grow (Koutsos 2006).

There are no experimental data on fertilization requirements. However, unpublished experimental data carried out in Greece, shown nice results by the addition of 30–40 kg N ha^{-1}, and 40–50 kg P ha^{-1} applied in late autumn. When the plantation is lively, fertilization should be avoided as to reduce the large amount of nitrogen in the soil, which results in large plant growth and reduced quality of the product.

Sideritis is a crop, which utilizes water in small doses, but should not remain in the roots of the plant, as it is sensitive to rot.

The best harvest season is the stage of full blooming, when the flowering stems begin to be wooded, because it seems that the leaf's content in essential oil, and thus in aroma is the largest (Koutsos 2006). Depending on the conditions of each year, harvesting usually takes place in June—August. At harvest, the entire inflorescence is cut and underneath a part of the stem, about 10–15 cm long (Mylonas 2017).

Sideritis can be cultivated in the same field for 5–8 years from which both second and third year are the most productive, while from the fourth year and onward the production begins to decrease. In a full year of production, yields in dry product reach 1000–1500 kg dry weight per hectare (Dordas 2012), while the content of essential oil is very low and ranges between 0.05 and 1%.

Drying in the sheds is done either by spreading or in small bundles hanging upside down in a shady, cool place. Drying takes 5–8 days without the use of technical means. If the drying does not take place in shade or when the type of metal of the shed is not the appropriate, then the plants become discolored and their quality is degraded (Gabrieli and Kokkalou 1990).

27.5 Utilization of Medicinal Plants

27.5.1 Sage

Sage is one of the most gifted and rich in properties herb in Greece and used since very ancient times. The ancient Greek considered it a sacred plant and had dedicated it to the God Zeus. The active substances are essential oil (contains 1.8 cineole, β-myrcene, α- and β-pinene, α- and β-thujone, camphor), tannins, bitter agents, terpenoids, flavonoids, and phenolic acids (http 1).

The uses of this medicinal plant concern the treatment of various ailments. It is used as an infusion or concoction for gargles, in order to sooth a sore throat, laryngitis, bronchitis, etc. Its herbal tea is a good digestive remedy (especially helpful when fatty meats were consumed), and also a tonic. Another medicinal remedy concerns irregular menstruation as its estrogenic activity also reduces hot flashes and sweating. It has been known to reduce blood sugar, so its use is beneficial in cases of diabetes and also useful in cases of muscular spasms (http 2).

Also, sage (mainly essential oil) improves memory and information processing. According to research published in the Journal Pharmacological Biochemical Behavior, people with Alzheimer's disease should have sage in their diet (http 2).

Another property is the allelopathic effect of the plant. This is the ability of the plant to release chemical compounds in the environment, which affect in a positive or negative way the developments of other plants or microorganisms (Cheng and Cheng 2015).

27.5.2 Oregano

Oregano is an aromatic herb primarily known for its use as a condiment (mixture of dry leaves and inflorescences) and lately it is "re-found" as a potent medicinal plant. Oregano was used in herbal medicine even by the ancient Greeks. Hippocrates, the ancient Greek physician and "father of medicine," used it for its valuable medicinal properties to treat many various medical problems.

The essential oil of Oregano presents a high content in carvacrol, thymiol, menthol, lemon, pinene, camphene, caryophyllin, rosmarinic acid, and triterpenes. The plant also contains phenolic acids, tannins, resins, flavonoids, sterols, vitamins A, B, and C, fatty acids, proteins, and trace elements (calcium, phosphorus, iron, magnesium, sodium, potassium, zinc) (Baydar et al. 2004; Bozin et al. 2006). All these components are responsible for the plant's antioxidant properties in food (e.g., lard-sunflower oil) and its benefits concerning the fight against antibiotic resistant pathogenic bacteria, viruses, fungi, parasites, insects—both in vitro and in human food (meat and poultry products, eggs, milk) as well as animal food and wastes (Chami et al. 2005; Tajkarimi et al. 2010).

Oregano covers a wide range of medicinal properties as it is known to fight microbial and fungal toxin production. There is a whole list concerning its further properties: anti-inflammatory, analgesic, antiarthritic, antiallergic, anticarcinogenic (Conforti et al. 2008), antidiabetic, cardioprotective, gastroprotective, hepatoprotective, and neuroprotective (properties of carvacol). Other aspects worth considering are those having to do with its metabolic, synergistic, and mechanistic values (Bozin et al. 2006; Friedman 2014).

The therapeutic value of Oregano has been known since antiquity, firstly used in the Chinese medicine and later on in ancient Egypt (together with another similar herb, marjoram) in the embalming process of the mummies. Ancient Greeks also used Oregano herbal tea for colics and externally for skin conditions/irritations.

The essential oil of Oregano acts as a barrier against the growth of Aspergillus niger and A.flaus at 400 mg/ml, while *A. ochraceus* is inhibited at 600 mg/ml. The germination of fungal spores is obstructed by 600 mg/ml of oregano oil and (apart from *A. ochraceus*) with 700 mg/ml of thyme oil (Paster et al. 1990).

Under aerobic conditions, 250 mg/ml of Oregano ess.oil inhibited—up to an extent—the growth of *Staphylococcus aureus* and *Salmonalla typhimurium*, being also very effective against *Campylohacter jejun* and *Clostridium sporogenes*.

To continue with the very interesting and useful virtues of *Oregano vulgare* ssp. hirtum, in dilution 1/4000 or even 1/50000 there was a considerable decrease in bacterial growth rates. The same essential oil exhibits high levels of cytotoxicity against four permanent animal cell lines, including two derived from cancerous human growths (Paster et al 1990; Sivropoulou et al. 1996).

Carvacrol, a member of phenols, is known as a potent inhibitor of bacterial growth, causes a total obstruction in the development of fungus colonies: *Penicillium digitatum, Fusarium solani* var. coeruleum and *Botrytis cynerea* (Chami et al. 2005). Furthermore, the oil of *Origanum vulgare* presents a stronger antibacterial action against Gram-negative bacteria (*Esherichia coli, Salmonella typhimurium, Proteus, Yershia enterocolitica, Serratia marcenscens, Pseudomonas flourescens,* and *Pseudomonas putida*), as well as Gram- positive bacteria (*Micrococcus sp., Sarcina flana, Staphylococcus aureus, Bacillus lecheniormis, Bacillus thuringiensis,* and *Listera innocua*), compared to the essential oils of similar plants while their biosynthetic precursors c-terpinene and p-cymene were inactive (Sivropoulou et al. 1996; Marino et al. 2001).

The essential oils of Origanum vulgare are known a strong antibacterial action toward *Stenotrophomonas maltophilia* (Sarac et al. 2009). Moreover, the rich quantity of carvacrol in Oregano vulgare shows the same strong hostile activity against the bacteria *E.coli, S.aureus, Bacillus megaterium,* and *Salmonella badar* (Remmal et al. 1993).

This knowledge and the findings of these studies encourage further research on the effectiveness of oregano's essential oil against other nondiscriminatory pathogens, and those causing malignant diseases (Manohar et al. 2001).

27.5.3 Sideritis

Mountain tea, or Sideritis, has been known since the time of Dioscorides and Hippocrates. Mountain tea (Sideritis sp.) is a very popular herb in all Mediterranean countries, especially in the winter months. Its nutritional value is remarkable and along with the unique aftertaste, it also contributes to the hydration of the body. Sideritis contains terpenoids (iridoids and caurans), phenolic derivatives (flavonoids, phenolic acids, phenylethanoic glycosides), fatty acids (Paliogianni 2007), and mineral content (Romanucci et al. 2017).

Species of the genus Sideritis (which in Greek means "iron-made") has been used since antiquity for medicinal purposes due to its antimicrobial, anti-inflammatory and antirheumatic properties (Vasilopoulou et al. 2013).

The mountain tea—as it is commonly known in Greece is a very well-known herb of the Greek nature having many beneficial properties. By boiling the stems, leaves and/ or flowers in a pot of water we have a beverage suitable against common cold and inflammation of the upper respiratory system. Another recent breakthrough is the fact that recent surveys show its contribution to the fight against Alzheimer's disease (Hofrichter et al. 2016). Also, it is noteworthy that Sideritis is administered to prevent anemia and osteoporosis, while studies are being done on its possible inhibitory effect on the growth of cancer cells. In folk medicine, it is used as a sedative, hypnotic, warming for colds, but also against indigestion (http 3).

27.5.4 Medicinal Plants for Helminth Plant Parasitic Nematode Control

Plant parasitic nematodes are among the most damaging groups of plant parasites in numerous crops throughout the world. Members of the phylum Nematoda are thought to be one of the most diverse types of organisms on earth (Wang 1999), comprising over 26,650 described species (Hugot 2001), while there are approximately 4100 known species of plant parasitic nematodes (Decraemer and Hunt 2006).

PPN parasite a wide range of crops and their control can be extremely challenging (Bird et al. 2009). In the past, control of PPN in European countries was mainly limited to chemical soil fumigation (methyl bromide), use of nematicides, and unselective pesticides. Although highly efficient in controlling PPN (Pinkerton 1986), these methods pose a major environmental threat due to the high volatility of chemicals and fumigants (Yates et al. 2003). Therefore, there is a great need to develop less toxic alternative techniques (Chaudhary 2013). In the search for alternatives to chemical control of PPN, the potential nematicidal activity of phytochemicals is being studied (Mohammad Akhtar 1994). These compounds can have a great potential for nematode control, either as nematicides themselves or as model compounds for the development of chemically synthesized derivatives (Chitwood 2002).

In recent years, the nematicidal properties of several medicinal plants have been tested successfully (Pandey and Karla 2010) (Chaudhary 2013) (Nasiou and Giannakou 2018) and it has been revealed that essential oils extracted from plants produce secondary compounds that provide protection against nematodes and pests in general (Isman 2000). Essential oils consist of organic volatile compounds found in a variety of plants and they are mixtures (of mainly terpenoids) that may contain over 300 different compounds (Sell 2006). These terpenoids on essential oils provide an important defense strategy against nematodes (Abdel-Rahman et al. 2013).

In particular, it has been demonstrated that essential oils extracted from Oregano (*Origanum vulgare*) had significant activity to reduce the populations of *Bursaphelenchus xylophilus*, the Pine wood nematode that infects pine trees and causes the pine wilt disease (Kong et al. 2006). Moreover, after the increasing dispersion of B. xylophilus in Portugal in 1999, studies on the nematicidal activity of *Origanum vulgare* showed strong lethal effects to pinewood nematode (Bird et al. 2009; Barbosa et al. 2010). Essential oils derived from *O. vulgare* showed strong nematicidal activity on *Meloidogyne incognita* (Ntalli et al. 2010), one of the most economically important plant parasitic nematode species with a wide host range. Finally, tests on the nematicidal activity of the essential oils of Greek *Sideritis cladestina* showed that even though it contains a high number of constituents, it showed poor activity, suggesting (Ntalli et al. 2010).

27.6 Socioeconomic Benefits of Medicinal Plants

The cultivation of medicinal plants presents a special category of with multiple benefits for health, ecosystems, and the society. Medicinal plants contribute both to the improved conservation status of local species and neighboring ecosystems and to socioeconomic benefits through their significant importance both for local, regional, and national economies (Marshall et al. 2006). The multiple benefits of these cultivations can be divided into two greater categories relevant to the effects for farmers and local and national economy as well as their conservation and biodiversity benefits relevant to specific ecosystem services (Cruz et al. 2011). Due to the wide geographical diversity in the dispersion of medicinal flora, there is not a single methodological approach for estimating their associated benefits.

Considering the parallel conservation benefits arising from the medicinal plant cultivations, two categories of socioeconomic benefits can be further divided into (a) their *direct-use values* and (b) their *indirect non-use values.* Firstly, the direct-use value of the field cultivations products is principally assessed on available market prices based on the provision of updated market prices of relevant products. On the other hand, the indirect non-use values are examined mainly in relation to their conservation potential. The positive external non-market benefit of the proposed cultivations in Mediterranean ecosystems is assessed through the use of various valuation methodologies (Brehm et al. 2010; Akanni et al. 2019). As a result, the net

added value of the proposed cultivations can be extracted taking into account their overall sustainability potential.

The function of those ecosystem services can be enhanced by re-assuring the sustainable adjustment of the medicinal plant cultivations and the preservation of specific indicators related to parameters of all four categories of ecosystem services (provisioning, regulating, supporting, cultural). In addition, the "environmental benefits" highlighted through the evaluation of ecosystem services, determine to a high extent the welfare level achieved through the conservation and boost of those cultivation as well as their socioeconomic well-being on specific Mediterranean regions. The quantification and evaluation of those benefits are derived both through the use and application of various specialized methodologies as well as through secondary available sources and market prices (Brehm et al. 2010).

Recent studies (Grigoriadou et al. 2020) have contributed to the development of guidelines for the sustainable conservation of medicinal plant cultivations contributing to the cost-effective preservation of the specific cultivations. Similar initiatives can lead to the development of National Strategic Plans for incorporating investment potentials under the National Strategic Reference Framework and potential CAP subsidies and other funding sources.

27.7 Economic Values of Medicinal Plants

According to FAO (2011), approximately 400.000 tonnes of MAPs are traded annually with a total value of 1.3 billion US$, while in Europe the total economic value reaches approximately 0.325 billion US$. More than 70% of the plant species used in herbal medicines, cosmetics, and other plant-based products are harvested from the wild, and the demand for them is globally increasing (Leaman 2006). In Europe, at least 2.000 MAP species are traded commercially and as many as 1300 species are considered native to Europe (FAO 2011). Approximately 65.000 ha of MAPs are cultivated in the EU while according to available data the average amount of annual MAPs cultivated in Greece reaches approximately 2.300 hectares with a net production of 3000 tonnes (Papapanagiotou et al. 2001).

The annual cost of an average conventional cultivation of MAPs in Greece ranges from approximately 31 €/hectare (*Salvia officinalis*) to 33 €/hectare (*Origanum vulgare*) and incorporates main categories such as plantation and machinery costs, multiplication material, land and labor cost (Maloupa et al. 2013). According to latest market prices, the average commercial price of MAPs ranges from 7.5 €/kg (*Salvia officinalis) to* 8 €/kg (*Origanum vulgare* and *Sideritis* sp.). In regard to their gross generated revenue prices range from 96 €/hectare (*Origanum vulgare* and *Sideritis* sp.) to 300 €/hectare for *Salvia officinalis* (Super Green Label Foods 2019).

27.8 Risks of Medicinal Plants

27.8.1 Habitat Destruction

The creation of new drugs is largely based on medicinal plants (Nalawade et al. 2003; Hamilton 2004). It is estimated that there are 50,000 species of herbs used worldwide for medicinal purposes (Bentley 2010). In America most prescription drugs come from medical plants while in the European Union over 1300 drugs are based on medicinal plants that most often come from wild resources (Balunas and Kinghorn 2005). Thus, the intense demand for medicinal plants has often led to excessive harvesting and destruction of natural habitats. It has been estimated that due to these practices over 20% of medicinal plants are almost extinct in nature while over 15,000 species of medicinal plants are in immediate danger (Bentley 2010) The countries with the highest rate of plant species loss are China, Uganda, Nepal, and Kenya (Heywood and Iriondo 2003; Hamilton 2008; Zerabruk and Yirga 2012). This constant extinction of medical plants due to habitat destruction has deprived humanity of many medicines because habitats are being destroyed more quickly than scientists can investigate them, so it has been calculated that we lose an important medicine every 2 years.

27.8.2 Biopiracy

Many medicinal properties of plants have been known for centuries through the traditions of common plants. Many times this biological knowledge is exploited with great profit. Biopiracy is defined as the practice by private companies to patent traditional herbal remedies and exploit them at a high profit without returning anything to their local communities of origin (DeGeer 2002; Mgbeoji 2006). Biopiracy is more common with scientists from more affluent countries conducting research in poorer countries, most of which are former colonies of old empires. Biopiracy has been widely criticized by human rights activists, academics, and governments for violating scientific ethics and universal justice (Mushita and Thompson 2007). A well-known example of global piracy is the attempted patent by the American company Ricetec of the Indian basmati rice, which after protests by the Indian government was modified (Isaac and Kerr 2004).

27.8.3 Overharvesting

The over-collection of wild medicinal plants has been demanding for years a great deal for the survival of the species. The growing demand for pharmaceutical plants is putting a lot of pressure on some selected high value populations. Several of the

popular medicinal plant species have slow growth rates, in limited geographical areas (Nautiyal et al. 2002). Therefore, they are in greater danger. The weakening of laws governing the use of natural resources is one of the causes of threat to medicines (Kala et al. 2006). Studies have shown that almost 15,000 species of medicinal plants are immediately endangered by overharvesting (IUCN 2007).

27.8.4 Loss of Knowledge

Local knowledge about the use of medicinal plants in the area is slowly declining and, in some areas, has been lost. This loss is typical in areas close to urban centers. Without knowledge of the usefulness of medicinal plants, communities have little reason to preserve and protect species.

27.9 Prospects and Challenges for the Sustainability of Medicinal Plants

Considering the multiple socioeconomic benefits of medicinal plants, their sustainable cultivation and exploitation could contribute to several Sustainable Development Goals (SDGs) such as health and well-being, conservation of biodiversity and reduction of poverty. Specifically, medicinal plants can contribute to farmers' welfare through income generation and increased demand for further processing by associated markets such as cosmetics and food industry. It should be noted that due to the specific nature and wide geographical and taxa dispersion of medicinal plants, it is often hard to define their precise total economic value viewed from a holistic and ecosystemic point of view. However, there are also several challenges associated with their sustainable use and cultivation. Extensive medicinal plant cultivation is coupled with land conversion and habitat degradation and the increase in demand for medicinal plants is putting pressure on natural resources, posing around a quarter of those species are under threat (FAO 2011). Therefore, it would be essential for future studies to define those crucial levels at which the overall generated welfare levels are balanced by those conservation challenges.

It is noteworthy to be referred that medicinal plants in the coming decades will be very important in the field of medical system for the management of human diseases. About 100 million people in the European Union use traditional, complementary or herbal medicines. It is estimated that 80% of the world's population relies on traditional medicine for primary health care. Over the last decade, there has been one great interest in research on medicinal plants is re-emerging as a source of potential herbal medicines. Over the last decade, there is a great demand for research concerning medicinal plants whose medicinal value is re-assets show it is very important that advanced research should be done for the development of new herbal medicines and better cultivation methods (Shakya 2016; Jamshidi et al. 2017).

References

Abdel-Rahman FH, Alaniz NM, Saleh MA (2013) Nematicidal activity of terpenoids. J Environ Sci Health 48:16–22

Akanni JK, Oduola LI, Ayanronke AM, Ajibola OR (2019) Review on the Socioeconomic Value of Medicinal Plants. Int J Public Health 4:76

Asdal A, Galambosi B, Bjorn G, Olsson K, Pihlik U, Radušiene J, Porvaldsdottir E, Wedelsback Bladh K, Zukauska I (2006) Spice- and medicinal plants in the Nordic and Baltic countries. Conservation of genetic resources. Report from a project group at the Nordic Gene Bank. NGB. Alnarp Norway, p 157

Bagyaraj DJ, Reddy BJD (2000) Arbuscular mycorrhizas in sustainable agriculture. In: Rajak RC (ed) Microbial biotechnology for sustainable development and productivity. Scientific Pub, Jodhpur India, pp 43–53

Balunas MJ, Kinghorn AD (2005) Drug discovery from medicinal plants. Life Sci 78:431–441

Barbosa P, Lima AS, Vieira P, Dias LS, Tinoco MT, Barroso JG, Pedro LG, Figueiredo AC, Mota M (2010) Nematicidal activity of EOs and volatiles derived from portuguese aromatic flora against the pinewood nematode. J Nematol 2:8–16

Baser K, Özek T, Kürkçüoglu M, Tümen G (1994) The essential oil of *Origanum vulgare* subsp. hirtum of Turkish origin. J Essent Oil Res 6:31–36

Baydar H, Sagdic O, Ozkan G, Karadogan T (2004) Antibacterial activity and composition of essential oils from *Origanum thymbra* and Satureja species with commercial importance in Turkey. Food Control 15:169–172

Bentley R (2010) Medicinal plants. Domville-Fife Press, London, pp 23–46

Bird DM, Williamson VW, Abad P, McCarter J, Danchin EGJM, Castagnone-Sereno P, Opperman CH (2009) The genome of root-knot nematodes. Annu Rev Phytopathol 47:333–351

Bogers RJ, Craker LE, Lange D (2006) Medicinal and aromatic plants: Agricultural, commercial, ecological, legal, pharmacological and social aspects (Wageningen UR Frontis Series). Springer, Netherlands

Bozin B, Dukic NM, Simin N, Anackov G (2006) Characterization of the volatile composition of essential oils of some Lamiaceae spices and the antimicrobial and antioxidant activities of the entire oils. J Agric Food Chem 54:1822–1828

Brehm JM, Maxted N, Martins-Loução MA, Ford-Lloyd BV (2010) New approaches for establishing conservation priorities for socio-economically important plant species. Biodivers Conserv 19:2715–2740

Chami N, Bennis S, Chami F, Aboussekhra A, Remmal A (2005) Study of anticandidal activity of carvacrol and eugenol in vitro and in vivo. Oral Microbiol Immunol 20:106–111

Chaudhary KK (2013) Nematicidal activity of Eritrean weed plants against the root-knot nematode Meloidogyne incognita. Nematropica 43:207–215

Cheng F, Cheng Z (2015) Research Progress and the use of plant allelopathy in agriculture and the physiological and ecological mechanisms of allelopaphy. Front Plant Sci 6:1–16

Chitwood DJ (2002) Phytochemical-based strategies for nematode control. Annu Rev Phytopathol 40: 221−249

Conforti F, Ioele G, Statti GA, Marrelli M, Ragno G, Menichini F (2008) Antiproliferative activity against human tumor cell lines and toxicity test on Mediterranean dietary plants. Food Chem Toxicol 46:3325–3332

Cruz A, Benedicto J, Gil A (2011) Socio-economic benefits of Natura 2000 in Azores Islands–a case study approach on ecosystem services provided by a special protected area. J Coast Res 64:1955–1959

Davis GJ (1994) Managing plant nutrients for optimum water efficiency and water conservation. Adv Agron 53:85–120

Decraemer W, Hunt DJ (2006) Structure and classification. Plant Nematol pp 3–32

DeGeer ME (2002) Biopiracy: the appropriation of indigenous peoples' cultural knowledge. New Eng J Int Comp L 9:180–208

Dimopoulos P, Raus T, Bergmeier E, Constantinidis T, Iatrou G, Kokkini S, Strid A, Tzanoudakis D (2013) Vascular plants of greece: an annotated checklist. Berlin, Botanic Garden and Botanical Museum Berlin-Dahlem, Athens, Hellenic Botanical Society, p 372

Dimopoulos P, Raus T, Bergmeier E, Constantinidis T, Iatrou G, Kokkini S, Strid A, Tzanoudakis D (2016) Vascular plants of Greece: an annotated checklist. Suppl Willdenowia 46:301–347

Dordas C (2012) Medicinal and aromatic plants. In: Modern Education (ed), Thessaloniki, ISBN 978-960-357-107-0

Droushiotis D, Della A (2002) Genetic resources of medicinal and aromatic plants in Cyprus with emphasis on the selection, evaluation and management of Origanum dubium. Report of a Working Group on Medicinal and Aromatic Plants. pp 39–41

FAO (2011) Health and wealth of medicinal and aromatic plants. Available online at http://www.fao.org/3/a-i2473e.pdf

Friedman M (2014) Chemistry and multibeneficial bioactivities of carvacrol (4-isopropyl-2-methylphenol), a component of essential oils produced by aromatic plants and spices. J Agric Food Chem 62:7652–7670

Paster N, Juven BJ, Shaaya E, Menasherov M, Nitzan R, Weisslowicz H, Ravi U (1990) Inhibitory effect of oregano and thyme essential oils on moulds and food borne bacteria. Lett Appl Microbiol 11:33–37

Gabrieli C, Kokkalou E (1990) A glucosylated acyflavone from *Sideritis raeseri*. Phytochem 29:681–683

Geneva MP, Stancheva IV, Boychinova MM, Mincheva NH, Yonova PP (2010) Effects of foliar fertilization and arbuscular mycorrhizal colonization on *Salvia officinalis* L. growth, antioxidant capacity, and essential oil composition. J Sci Food Agric 90:696–702

Giannoulis KD, Kamvoukou CA, Gougoulias N, Wogiatzi E (2020) Irrigation and nitrogen application affect Greek oregano (*Origanum vulgare* ssp. hirtum) dry biomass, essential oil yield and composition. Ind Crops Prod 150:112–392

Goliaris A, Koutsos T, Chatzopoulou P (1999) Production and comparative study of phenotypes of Greek mountain tea (Sideritis sp. L.) hybrids and their parents. Medicinal Plant Report 6:1–7

González-Burgos E, Carretero ME, Gómez-Serranillos MP (2011) Sideritis spp.:Uses, chemical composition and pharmacological activities—a review. J Ethnopharmacol 135:209–225

Govahi M, Ghalavand A, Nadjafi F, Sorooshzadeh A (2015) Comparing different soil fertility systems in sage (*Salvia officinalis*) under water deficiency. Ind Crops Prod 74:20–27

Grigoriadou K, Krigas N, Lazari D, Maloupa E (2020) Sustainable use of mediterranean medicinal-aromatic plants. In- Feed Additives. Academic Press London, UK, pp 57–74

Hamilton AC (2004) Medicinal plants, conservation and livelihoods. Biodivers Conserv 13:1477–1517

Hamilton AC (2008) Medicinal plants in conservation and development: case studies and lessons learned. In: Kala CP (ed) Medicinal plants in conservation and development. Plantlife International Publisher Salisbury UK, pp 1–43

Harrison PA, Berry PM, Simpson G, Haslett JR, Blicharska M, Bucur M, Turkelboom F (2014) Linkages between biodiversity attributes and ecosystem services: a systematic review. Ecosyst Serv 9:191–203

Heywood VH, Iriondo JM (2003) Plant conservation: old problems, new perspectives. Biol Conserv 113:321–335

Hofrichter J, Krohn M, Schumacher T, Lange C, Feistel B, Walbroel B, Pahnke J (2016) Sideritis spp. extracts enhance memory and learning in Alzheimer's β-amyloidosis mouse models and aged C57Bl/6 mice. J Alzheim Dis 53:967–980. http://mediplantepirus.med.uoi.gr/pharmacology/plant_details.php?id=172, http://enallaktikidrasi.com/2016/04/faskomilo-idiotites-xrisi/, http://mediplantepirus.med.uoi.gr/pharmacology/plant_details.php?id=151

Hugot JP (2001) Biodiversity in helminths and nematodes as a field of study: an overview. Nematology 3:199–208

Isaac GE, Kerr WA (2004) Bioprospecting or biopiracy? J World Intellect Prop 7:35–52

Isman (2000) Plant essential oils for pest and disease management. Crop Prot 19:603–608

IUCN Species Survival Commission Medicinal Plant Specialist Group (2007) Why Conserve and manage medicinal plants? Available online at www.iucn.org/themes/ssc/sgs/mpsg/main/Why.html

Jamshidi F, Lorigooini Z, Amini-Khoe H (2017) Medicinal plants: past history and future perspective. J Herb Med Pharmacol 7:1–7

Kala CP, Dhyani PP, Sajwan BS (2006) Developing the medicinal plants sector in Northern India: challenges and opportunities. J Ethnobiol Ethnomed 2:1–15

Karamanos AJ (1992) Cultivation of selected aromatic and medicinal plants. In: Identification, preservation, adaptation and cultivation of selected aromatic and medicinal plants suitable for marginal lands of the mediterranean region (Progress Report of the EEC CAMAR-Programme No 8001-CT91–0104, August 1991-January 1992)

Karamanos AJ, Sotiropoulou DEK (2013) Field studies of nitrogen application on Greek oregano (Origanum vulgare ssp. hirtum (Link) Ietswaart) essential oil during two cultivation seasons. Ind Crop Prod 46:246–252

Karousou R, Hanlidou E, Kokkini S (2000) The sage plants of Greece: distribution and infraspecific variation. The Genus Salvia Harwood Academic Publishers, The Netherlands, pp 27–46

Karousou R, Vokou D, Kokkini S (1998) Distribution and essential oils of *Salvia pomifera* subsp. pomifera (Labiatae) on the island of Crete (S Greece). Biochem Syst Ecol 26:889–897

Katsiotis S, Chatzopoulou P (2015) Aromatic medicinal plants and essential oils, thessaloniki. Kyriakidi. Greece, Publisher, p 656 (in Greek)

Kintzios SE (2002) Oregano—The genera Origanum and Lippia. Agricultural University of Athens, Athens, Greece

Kokkini S, Karousou R, Vokou D (1994) Pattern of geographic variation of Origanum vulgare trichomes and essential oil content in Greece. Biochem Syst Ecol 22:517–528

Kong JO, Lee S-M, Moon Y-S, Lee S-G, Ahn Y-J (2006) Nematicidal activity of plant essential oils against Bursaphelenchus xylophilus (Nematoda: Aphelenchoididae). J Asia Pac Entomol 9:173–178

Koutsos T (2006) Aromatic and Medicinal plants. Edt Ziti p 349

Kuris A, Altman A, Putievsky E (1980) Rooting and initial establishment of stem cuttings of oregano, peppermint and balm. Hort Sci 13:53–59

Laborda R, Manzano I, Gamón M, Gavidia I, Pérez-Bermúdez P, Boluda R (2013) Effects of *Rosmarinus officinalis* and *Salvia officinalis* essential oils on *Tetranychus urticae* Koch (Acari: *Tetranychidae*). Ind Crops Prod 48:106–110

Lange D (2001) Trade in medicinal and aromatic plants: a financial instrument for nature conservation in Eastern and Southeast Europe? In: Heinze B, Bäurle G, Stolpe G (eds) Financial instruments for nature conservation in Central and Eastern Europe (BfN-Skripten, No. 50) German Federal Agency for Nature Conservation Bonn Germany, pp 157–171

Leaman DJ (2006) Sustainable wild collection of medicinal and aromatic plants: development of an international standard. In: Bogers RJ, Craker LE, Lange D (eds) Medicinal and aromatic plants: Agricultural, commercial, ecological, legal, pharmacological and social aspects. Wageningen UR Frontis Series 17, Springer, The Netherlands, pp 97–107

Makri O (2002) Cultivation of Oregano. In: Kintzios SE (ed) Oregano, the genera Origanum and Lippia. Taylor and Francis, London and New York pp 153-162

Maloupa E, Grigoriadou K, Lazari D, Krigas N (2013) Cultivation, processing and quality assurance of Greek aromatic and medicinal plants. Greece. Available online at http://www.moa.gov.cy/moa/ari/ari.nsf/all/C810E0B75DCCE0F0C2257B6D003A569D/$file/open_access_gewee_kall_fitwn.pdf?openelement

Manohar V, Ingram C, Gray J, Talpur NA, Echard BW, Bagchi D, Preuss HG (2001) Antifungal activities of origanum oil against *Candida albicans*. Mol Cell Biochem 228:111–117

Marino M, Bersani C, Comi G (2001) Independence to study the antimicrobial activity of essential oils from Lamiaceae and Compositae. Int J Food Microbiol 67:187–195

Marquard R, Kroth E (2001) Anbau und Qualitätsanforderungen Ausgewählter Arzneipflanzen, (Cultivation and Quality Requirements of Selected Medicinal Plants) Agrimedia, Bergen Dumme

Marshall E, Schreckenberg K, Newton A (2006) Commercialization of non-timber forest products in Mexico and Bolivia: factors influencing success. Research conclusions and policy recommendations for decisionmakers, UNEP-WCMC, Cambridge, United Kingdom

Mateescu I, Paun L, Popescu S, Roata G, Sidoroff M (2014) Medicinal and aromatic plants—A statistical study on the role of Phytotherapy in human health. Bull UASVM Animal Sci Biotech 71:14–19

Mgbeoji I (2006) Global biopiracy: patents, plants and indigenous knowledge. UBC Press, Vancouver

Miguel G, Simões M, Figueiredo AC, Barroso JG, Pedro LG, Carvalho L (2004) Composition and antioxidant activities of the essential oils of Thymus caespititius, *Thymus camphoratus* and *Thymus mastichina.* Food Chem 86:183–188

Mohammad Akhtar IM (1994) Potentiality of phytochemicals in nematode control: a review. Bioresour Technol 48:189–201

Mushita A, Thompson CB (2007) Biopiracy of biodiversity: global exchange as enclosure. Africa World Press, Trenton

Mylonas C (2017) The influence of environmental factors on the production of tea (*Sideritis raiseri*) in the area of Vrynaina. (Graduate thesis), University of Thessaly, Volos

Nalawade SM, Sagare AP, Lee CY, Kao CL, Tsay HS (2003) Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. Bot Bull Acad Sin 44:79–98

Nasiou E, Giannakou IO (2018) Effect of geraniol, a plant-based alcohol monoterpene oil, against Meloidogyne javanica. Eur J Plant Pathol 152:701–710

Nautiyal S, Rao KS, Maikhuri RK, Negi KS, Kala CP (2002) Status of medicinal plants on way to VashukiTal in Mandakini Valley, Garhwal, Uttaranchal. J Non-Timb for Prod 9:124–131

Ntalli N, Ferrari F, Giannakou I, Menkissoglu-Spiroudi U (2010) Phytochemistry and nematicidal activity of the essential oils from 8 greek lamiaceae aromatic plants and 13 terpene components. J Agric Food Chem 58:7856–7863

Omer EA (1999) Response of wild Egyptian oregano to nitrogen fertilization in a sandy soil. J Plant Nutr 22:103–114

Ozgóven M, Ayanoglu F, Ozel A (2006) Effects of nitrogen rates and cutting times on the essential oil yield and components of *Origanum syriacum* L. var. bevanii. J Agron 5:101–105

Paliogianni A (2007) Study of volatile ingredients of Greek wines and spirit.-Production of biofunctional wines based on a plant of the genus *Sideritis*. Agricultural University of Athens

Pandey R, Karla A (2010) Inhibitory effects of vermicompost produced from agro-waste of medicinal and aromatic plants on egg hatching in *Meloidogyne incognita* (Kofoid and White) Chitwood. Curr Sci 98:833–835

Papanagiotou E, Papanikolaou K, Zamanidis S (2001) The cultivation of aromatic and medicinal plants in Greece: 1 The economic dimension. Agriculture 1:36–42

Pinkerton JN (1986) Control of Meloidogyne chitwoodi in commercially grown Russet Burbank potatoes. Plant Dis 70:860–863

RE.HERB (2013) Recording, documentation, promotion and distribution of the aromatic and therapeutic herbs, plants and plant products, 10/10/2013. Available online at http://reherb.eu/sites/default/files/Market%20Research%20Re%20Herbs%20EN

Remmal A, Bouchikhi T, Rhayour K, Ettayeby M, Tantaoui-Elaraki A (1993) Improved method for the determination of antimicrobial activity of essential oils in agar medium. J Essent Oil Res 5:179–184

Romanucci V, Di Fabio G, D'Alonzo D, Guaragna A, Scapagnini G, Zarrelli A (2017) Traditional uses, chemical composition and biological activities of *Sideritis raeseri* Boiss. & Heldr. J Sci Food Agric 97:373–383

Sarac N, Ugur A, Duru ME, Varol O (2009) Antimicrobial activity, antioxidant activity and chemical composition of *Origanum onites* L. and *Origanum vulgare* L. ssp. hirtum from Mugla (Turkey). Acta Hortic 826:397–404

Sell CS (2006) The chemistry of fragrances: from perfumer to consumer. Royal Society of Chemistry, Cambridge UK

Shakya AK (2016) Medicinal plants: future source of new drugs. Int J Herb Med 4:59–64
Sivicka I, Zukauska I, Balode A, Adamovics A (2015) Fresh and air-dry biomass of oregano (*Origanum vulgare* L.) accessions. In: Nordic view to sustainable rural development: proceedings of the 25th NJF Congress. Nordic Association of Agricultural Scientists. pp 46–51
Sivropoulou A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T, Arsenakis M (1996) Antimicrobial and cytotoxic activities of origanum essential oils. J Agric Food Chem 44:1202–1205
Skoufogianni E, Solomou AD, Kamperllari F, Danalatos NG (2017) Ecology, cultivation, composition and utilization of *Salvia officinalis* L. In greece: a review. Global Adv Res J Agric Sci 6:449–455
Solomou A, Martinos K, Skoufogianni E, Danalatos N (2016) Medicinal and aromatic plants diversity in greece and their future prospects: a review. Geography 4:9–20
Solomou A, Skoufogianni E, Mylonas C, Germani R, Danalatos N (2019) Cultivation and utilization of "Greek mountain tea" (Sideritis spp.): current knowledge and future challenges. Asian J Agric Biol 7:289–299
Sotiropoulou DE, Karamanos A (2010) Field studies of nitrogen application on growth and yield of Greek oregano (*Origanum vulgare* ssp. hirtum (Link) Ietswaart). Ind Crop Prod 32:450–457
Super Green Label Foods project (2019) Available online at https://supergreenlabelfoods.eu/media/SGLF_guidebook_EL.pdf
Tajkarimi MM, Ibrahim SA, Cliver DO (2010) Antimicrobial herb and spice compounds in food. J Food Control 21:1199–1218
Vasilopoulou CG, Kontogianni VG, Linardaki ZI, Iatrou G, Lamari FN, Nerantzaki AA, Gerothanassis IP, Tzakos AG, Margarity M (2013) Phytochemical composition of '"mountain tea"' from Sideritis clandestina subsp. clandestina and evaluation of its behavioral and oxidant/antioxidant effects on adult mice. Eur J Nutr 52:107–116
Walker JB, Sytsma KJ, Treutlein J, Wink M (2004) Salvia (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of Salvia and tribe Mentheae" . Am J Bot 91:1115–1125
Wang DY (1999) Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. Proc Royal Soc b: Bio Sci 266:163–171
Yates SR, Gan J, Papiernik SK (2003) Environmental fate of methyl bromide as a soil fumigant. Rev Environ Contam Toxicol 177:45–122
Ye Y, Xiaohong O, Guang Y, Yunsheng X, Meilan C, Lanping G, Dahui L (2017) Arbuscular mycorrhizal fungi regulate the growth and phyto-active compound of *Salvia miltiorrhiza* Seedlings. Appl Sci 7:68
Zerabruk S, Yirga G (2012) Traditional knowledge of medicinal plants in Gindeberet district, Western Ethiopia. S Afr J Bot 78:165–169

Index

H. M. Ekiert et al. (eds.), *Medicinal Plants*, Sustainable Development and Biodiversity 28, https://doi.org/10.1007/978-3-030-74779-4

D

Q

R