Tariq Aftab
Khalid Rehman Hakeem *Editors*

Medicinal and Aromatic Plants

Healthcare and Industrial Applications

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(1908–1999)

A great philanthropist, thinker, visionary, and founder-chancellor of ***Jamia Hamdard****, New Delhi, India.*

Preface

In prehistoric times, humans undoubtedly acquired nature's benefits by discovering medicinal and aromatic plants (MAPs) that were food and medicine. Today, a variety of available herbs and spices are used and enjoyed throughout the world and continue to promote good health. As the benefits from medicinal and aromatic plants are recognized, these plants will have a special role for humans in the future. The enormous demand in botanicals results in a huge trade from local to international level. Until the last century, production of botanicals relied to a large degree on wild collection. However, utilization and commerce of wild plant resources are not detrimental in themselves, For example, the increasing commercial collection, largely unmonitored trade, and habitat loss lead to an incomparably growing pressure on plant populations in the wild. Throughout time, medical care has continually progressed, moving from illnesses to vaccinations and new medicines along with improved healthcare facilities that can more accurately diagnose and treat health problems. Advancements in modern medicine and medical care have enabled people to live longer and healthier lives. New medicines from plant materials and antibiotics from microflora have defeated most diseases. Medicinal and aromatic plants are of high priority for conservation action as wildcrafting will certainly continue to play a significant role in their future trade: the sustainable commercial use of their biological resources may provide a financial instrument for nature conservation. The international market is also quite welcoming for MAPs and essential oils. The increasing environment- and nature-conscious buyers encourage producers to produce high-quality essential oils. There has been growing preference for organic and herbal-based products in the world market. Similarly, the world is shifting from crude export to processing of herbal products and essential oils.

The present book covers a wide range of topics, discussing the role of MAPs in healthcare and their industrial uses. Moreover, this will be a unique reference book on the topic highlighting various healthcare, industrial, and pharmaceutical applications that are being currently used on immensely important MAPs and its future prospects. In this volume, we highlighted the working solutions as well as open problems and future challenges in MAPs research. We believe that this book will initiate and introduce readers to state-of-the-art developments and trends in this field of study.

The book comprises 27 chapters, most of them being review articles written by experts, highlighting wide range of topics, discussing the role of MAPs in health-care, industry, and their pharmaceutical applications. We are hopeful this volume would furnish the need of all researchers who are working or have interest in this particular field. Undoubtedly, this book will be helpful for general use of research students, teachers, and those who have interest in MAPs.

We are highly grateful to all our contributors for accepting our invitation and for not only sharing their knowledge and research but for venerably integrating their expertise in dispersed information from diverse fields in composing the chapters and enduring editorial suggestions to finally produce this venture. We also thank the Springer Nature team for their generous cooperation at every stage of the book's production.

Lastly, thanks are also due to well-wishers, research students, and authors' family members for their moral support, blessings, and inspiration in the compilation of this book.

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About the Editors

Tariq Aftab received his Ph.D. from the Department of Botany at Aligarh Muslim University, India, and is currently an Assistant Professor there. He is recipient of the prestigious Leibniz-DAAD Fellowship from Germany, Raman Fellowship from the Government of India, and Young Scientist Awards from the State Government of Uttar Pradesh (India) and Government of India. After completing his doctorate, he worked as Research Fellow at the National Bureau of Plant Genetic Resources, New Delhi, and as Postdoctoral Fellow at Jamia Hamdard, New Delhi, India. Dr. Aftab also worked as Visiting Scientist at Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, and in the Department of Plant Biology, Michigan State University, USA. He is a member of various scientific associations from India and abroad.

He has edited 7 books with international publishers, including Elsevier Inc., Springer Nature, and CRC Press (Taylor & Francis Group), co-authored several book chapters, and published over 50 research papers in peer-reviewed international journals. His research interests include physiological, proteomic, and molecular studies on medicinal and aromatic plants.

Khalid Rehman Hakeem, PhD is Professor at King Abdulaziz University, Jeddah, Saudi Arabia. After completing his doctorate (botany; specialization in plant eco-physiology and molecular biology) from Jamia Hamdard, New Delhi, India, in 2011, he worked as a Lecturer at the University of Kashmir, Srinagar, for a short period. Later, he joined Universiti Putra Malaysia, Selangor, Malaysia, and worked there as Postdoctoral Fellow in 2012 and Fellow Researcher (Associate Professor) from 2013 to 2016. Dr. Hakeem has more than 10 years of teaching and research experience in plant eco-physiology, biotechnology and molecular biology, medicinal plant research, plant-microbe-soil interactions, as well as in environmental studies. He is the recipient of several fellowships at both national and international levels; he has also served as a Visiting Scientist at Jinan University, Guangzhou, China. Currently, he is involved with a number of international research projects with different government organizations.

So far, Dr. Hakeem has authored and edited more than 50 books with international publishers, including Springer Nature, Academic Press (Elsevier), and CRC Press. He also has to his credit more than 110 research publications in peer-reviewed international journals and 60 book chapters in edited volumes with international publishers.

At present, Dr. Hakeem serves as an editorial board member and reviewer of several high-impact international scientific journals from Elsevier, Springer Nature, Taylor and Francis, Cambridge, and John Wiley Publishers. He is included in the advisory board of Cambridge Scholars Publishing, UK. He is also a fellow of Plantae group of the American Society of Plant Biologists; member of the World Academy of Sciences; member of the International Society for Development and Sustainability, Japan; and member of Asian Federation of Biotechnology, Korea. Dr. Hakeem has been listed in Marquis Who's Who in the World between 2014 and 2019. Currently, Dr. Hakeem is engaged in studying the plant processes at eco-physiological as well as molecular levels.

Chapter 1
Medicinal Plants and Herbal Drugs: An Overview

Burhan Ahad, Waseem Shahri, Humeera Rasool, Z. A. Reshi, Sumaiyah Rasool, and Tufail Hussain

1.1 Introduction

Plants apart from being a source of food are also sources of non-food industrial products. Such crops are typically cultivated on a large scale for the production of fine chemicals or specialty products. Medicinal plants are one such category of plants being an incredible treasure of chemical compounds which find diverse applications in human healthcare. A medicinal plant according to the World Health Organization is any plant which, as whole or in one or more of its organs, either contains such substances that can be used for synthesis of useful drugs or contains such substances with medicinal properties that can be used directly for therapeutic purposes or can affect human health (Farnsworth and Soejarto 1997). Such plants as whole or their parts such as roots, stem, leaves, stem barks, fruits or seed can be applied in control or treatment of a disease. These medically active non-nutrient substances or chemical components present in these plants are often referred to as phytochemicals or bioactive chemicals or active principles (Liu 2004; Doughari et al. 2009).

Partly based on their use in traditional medicine, an impressive number of modern drugs have also been isolated from these natural plant species. Various phytochemicals serve as imperative drugs, which are currently used across the globe to cure a variety of perilous diseases.

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Due to diversity of phytochemicals present in each plant, single plant may be applied in treatment of various disease conditions. Traditionally used medicinal plants and their components have been found useful in treatment of several ailments including asthma, fever, constipation as well as infections of urinary tract, gastrointestinal and biliary systems and skin (Saganuwan 2010). The plants are applied in different forms such as poultices, concoctions of different plant mixtures, infusions as teas or tinctures or as component mixtures in porridges and soups administered in different ways including oral and nasal (Adekunle and Adekunle 2009).

The history of medicinal plant utilization for therapeutic purposes dates back to antiquity. Medicinal plants form the mainstay of herbal drugs. These plants have been an integral part of the ancient traditional medicine systems, e.g. Chinese, Egyptian and Ayurvedic (Sarker and Nahar 2007). Medicinal plant products have over the years assumed a very central stage in healthcare system and as an alternative source of health beneficial drugs.

The rise in acceptability of medicinal plants is primarily due to the increasing inefficacy and side effects of many modern synthetic drugs such as increase in bacterial resistance (Smolinski et al. 2003). According to the World Health Organization (WHO), about two-thirds of world population in developing countries rely on plant-based traditional medicines and herbal drugs for primary healthcare requirements.

Medicinal plants contain a curative capacity used for the treatment of major and minor human disease (Oksman-Caldentey 2007) and serve as raw material for synthesis of diverse products ranging from traditional to modern medicines. Regular scientific research have highlighted the importance and contribution of many plant families, i.e. Asteraceae, Apocynaceae, Liliaceae, Rutaceae, Caesalpiniaceae, Solanaceae, Piperaceae, Ranunculaceae, Apiaceae, Sapotaceae, etc., and their bioactive compounds in therapeutic arena thus constituting very important part of natural wealth (Table 1.1). Therapeutic activities of medicinally important bioactive components obtained from these plants range from successful preventions and treatment of diseases, improvement in illnesses symptoms, as well as beneficial regulation of the physical and mental status of the body.

However, the production of plants as raw material for fine chemicals needs to take into consideration certain aspects such as to know how trade in these raw materials and ingredients is organized and regulated nationally and internationally, identifying the current trends in the sector and what future demands might exist, determination of desired crops based on market demand, employability of the cultivation requirements and investigating the potential need for the production of the final product. Production of natural as well as recombinant bioactive products of commercial importance has attracted substantial attention over the past few decades (Canter et al. 2005).

Traditional use of medicinal plants for herbal drugs is well established and widely acknowledged as safe and effective. To promote the proper use of traditional medicine and medicinal plant products, medicine strategy that focuses on policy, safety/quality/efficacy, access and rational use of traditional medicine should be in place (WHO 2013). Unfortunately, rapid explosion in human population put more demands on the use of natural herbal health remedies.

Table 1.1 Some medicinal plants, their parts used and therapeutic use

Plant name	Family	Part used	Therapeutic use
Acacia tenuiflora	Fabaceae	Bark	Wound healing, cough, common cold
Acanthospermum hispidum	Asteraceae	Leaves	Cardiovascular disease, cancer, inflammation, allergies
Achyrocline satureioides	Asteraceae	Inflorescence, seeds	Antispasmodic, digestive and gastrointestinal disorders
Aconitum chasmanthum	Ranunculaceae	Rhizome	Rheumatism, hypertension
Acorus calamus	Araceae	Rhizome Leaves	Epilepsy, chronic dysentery, intermittent fevers and tumours
Allium cepa	Liliaceae	Bulbs	Antimicrobial, antioxidant, anti-inflammatory, antidiabetic, anti-hypertensive
Allium sativum	Liliaceae	Bulbs	Antiarthritic, expectorant, febrifuge, stimulant
Artemisia absinthium	Asteraceae	Leaves	Fever, depression, memory loss, liver diseases, intestinal spasms
Artemisia annua	Asteraceae	Leaves	Antimalarial, anti-inflammatory, anticancer
Aloe vera	Asphodelaceae	Leaf cathartic	Wound healing, anti-inflammatory
Althaea officinalis	Malvaceae	Root	Coughs, skin irritation, gastritis ulcers
Atropa belladonna	Solanaceae	Root, leaves, flower, seeds	Hepatic disorders, peptic ulcer, motion sickness
Baccharis trinervis	Asteraceae	Stem, leaf	Hepatic diseases
Bacopa monnieri	Scrophulariaceae	Stem	Antioxidant, anti-inflammatory, antiarthritis, anti-stress and antiulcergenic
Bauhinia candicans	Fabaceae	Leaf	Hypoglycaemic, analgesic, anti-inflammatory, nephroprotective
Brassica nigra	Solanaceae	Seed	Stimulant, diuretic and purgative
Calendula officinalis	Asteraceae	Leaf flower	Rheumatic pains, antiseptic
Cannabis sativa	Cannabaceae	Leaf	Antidiarrhetic, sedative, anticancer
Carthamus tinctorius	Asteraceae	Flower, seed	Heart disease, stroke, diabetes, scarring
Capsicum frutescens	Solanaceae	Fruit	Analgesic, vasodilator
Chenopodium ambrosioides	Amaranthaceae	Whole plant	Liver disorders, contusions, lung infections, analgesic, antifungal
Cinchona officinalis	Rubiaceae	Bark	Haemorrhoids, varicose veins and leg cramps
Cinnamomum zeylanicum	Lauraceae	Bark	Diabetes, flu, intestinal disorders
Cinchona pubescens	Rubiaceae	Bark	Antiparasitic, antipyretic

(continued)

Table 1.1 (continued)

Plant name	Family	Part used	Therapeutic use
Cleome rutidosperma	Cleomaceae	Leaves	Analgesic and anti-inflammatory
Commiphora mukul	Burseraceae	Stem	Astringent, anthelmintic, anti-inflammatory, diuretic, depurative, anodyne, thermogenic, antiseptic, nervine tonic, aphrodisiac, stimulant and diaphoretic
Crocus sativus	Iridaceae	Stigma	Asthma, cough, whooping cough (pertussis) and to loosen phlegm (as an expectorant)
Curcuma longa L.	Zingiberaceae	Rhizome	Anti-inflammatory, antioxidant
Cymbopogon flexuosus	Poaceae	Leaves	Anti-inflammatory, analgesic, anti-fungal
Dioscorea deltoidea	Dioscoreaceae	Rhizome	Rheumatoid arthritis, menstrual cramps, stomach cramps
Digitalis purpurea		Leaves	Cardiovascular, cytotoxic, antidiabetic, antioxidant, insecticidal, immunological, hepato-, neuro- and cardioprotective effects
Embelia ribes Burm.	Myrsinaceae	Fruit	Tapeworm infestation
Eupatorium odoratum	Asteraceae	Root	Bleeding disorders, appendicitis
Evodia lepta	Rutaceae	Root	Asthma
Ferula asafoetida regel.	Apiaceae	Root gum	Cough, jaundice, antispasmodic, gastritis, laxative and rheumatism
Foeniculum vulgare	Apiaceae	Seed	Diaphoretic, diuretic, carminative, expectorant, febrifuge, stomachic, stimulant, appetizer, cardiac stimulant
Geranium sessiliflorum	Geraniaceae	Whole plant	Hypoglycaemic
Ginkgo biloba	Ginkgoaceae	Leaf	Cerebral vasodilator
Gnaphalium indicum	Asteraceae	Flower	Cough, bronchitis
Heliotropium indicum	Boraginaceae	Root	Back pain
Hibiscus sabdariffa	Malvaceae	Flower	Diuretic, hypertension, liver diseases and fevers
Hyoscyamus albus	Solanaceae	Leaves, seeds	Sedative, analgesic (small dose), hallucinogenic (large dose
Inula racemosa	Asteraceae	Root and rhizome	Anti-inflammatory, carminative, diuretic, antiseptic, chronic bronchitis, rheumatism, stomach troubles
Kaempferia galanga	Zingiberaceae	Rhizome	Gastric ulcer, chest pain

(continued)

Table 1.1 (continued)

Plant name	Family	Part used	Therapeutic use
Malva sylvestris	Malvaceae	Leaves, flower	Expectorant, digestive, emollient, respiratory disorders
Mentha piperita	Lamiaceae	Leaves	Carminative, spasmolytic, antitumour, antidiabetes, anti-nociceptive
Nasturtium officinale	Brassicaceae	Whole plant	Antiscorbutic, diuretic, depurative, purgative, hypoglycaemic, expectorant, stimulant, bronchitis
Ocimum sanctum	Lamiaceae	Leaves, stem, seeds, flower, root	Anticancer, antidiabetic, antifungal, hepatoprotective, cardioprotective, analgesic, antispasmodic
Papaver somniferum	Papaveraceae	Flower, seeds	Analgesic, sedative
Picrorhiza kurroa	Plantaginaceae	Root	Dyspepsia, anti-allergic, anti-asthmatic, hepatoprotective, wound healing, vitiligo
Piper nigrum	Piperaceae	Dried fruits	Anti-inflammatory, antihypertensive, anti-thyroids, hepatoprotective, anticonvulsant, appetizer, antihistaminic, counterirritant, antiflatulent
Podophyllum hexandrum	Berberidaceae	Root stocks	Jaundice, typhoid fever, dysentery, chronic hepatitis, rheumatism, ulcers, gonorrhoea
Rauvolfia serpentina	Apocynaceae	Root, bark	Hypertension, insomnia, constipation, fever
Rheum emodi	Polygonaceae	Root	Purgative, astringent, laxative, liver stimulant diuretic
Rosa laevigata	Rosaceae	Roots, leaves, fruits	Astringent, anticancer, antibacterial, carminative, stomachic
Sapindus mukorossi	Sapindaceae	Fruit	Cold, epilepsy, constipation, nausea
Saussurea costus	Asteraceae	Root	Asthma, cough, dysentery, eczema, cholera
Syzygium aromaticum	Myrtaceae	Floral bud	Nausea, liver, bowel and stomach disorders
Taraxacum officinale	Asteraceae	Leaf, flower, root	Depurative, hepatoprotective, diuretic
Taxus wallichiana	Taxaceae	Root, stem, leaves	Common cold, pain, fever, cough, antispasmodic, diaphoretic, narcotic and purgative, asthma, bronchitis, rheumatism
Valeriana jatamansi Jones	Valerianaceae	Root, rhizome	Epilepsy, hypochondriasis, hysteria, nervous unrest and skin diseases
Withania somnifera	Solanaceae	Root, flower	Hypertension, asthma, diabetes, arthritis and uterine sedative
Zingiber officinale	Zingiberaceae	Rhizome	Influenza, cholic haemorrhagia

1.2 Taxonomical Aspects and Botanical Characteristics of Medicinal Plants

The knowledge of medicinal plants begins with the classification (systematics/taxonomy) and description (anatomy/morphology) of these plants, especially in view of their healing or simply other useful properties.

1.2.1 Plant Systematics and Taxonomy

Medicinal plants are highly diverse with respect to their botanical characteristics, belonging to various families of plant kingdom, which, often, comprise characteristic similar active principle components (owing to biosynthetic pathway similarities). For instance, Solanaceae comprises of several alkaloid-containing species, while as species of Labiateae are characterized by occurrence of large number of essential oil ingredients (tobacco, belladonna, etc.).

Like all plants, the species remains the basic taxonomic unit of medicinal plants with genus comprising of related species. For differentiating dissimilar populations among wild-growing species, the categories *subspecies* (subsp.), *variety* (var.) and *form* (f.) are used. In some variants of medicinal plants of same species, different secondary metabolite profile may arise due to mutation. In some instances, genetic variations affect species' chemical constituents. These are termed *chemical races* or *chemodemes*. Chemical races have been detected in numerous species, and variant chemical substances include, for instance, cyanogenetic glycosides in *Prunus communis*, alkaloids in *Duboisia* species and cardiac glycosides in *Digitalis purpurea*. In an eco-botanical sense, both natural and cultivated species are divided into well-distinguished *infraspecific varieties* (Terpo 1992). Cultivars are differentiated usually according to their features valued by human societies.

Correct identification forms the first and foremost component in study and utilization of medicinal plants, and as such there should be greater emphasis on the correct reproducible experiments (Bennett and Balick 2008) required for identification of plants and that correctly identified vouchered plant should be deposited in recognized herbaria.

Botanical sciences like plant systematics, plant morphology, physiology, molecular traits and off late chemo-differentiation based on plant metabole information as well as ecological characters have been assisting in multiple ways in the correct identification and description of medicinal plants. High-refined morphological systems contemplate more on plant morphological traits and assess plants as per hereditary and evolutionary principles. In addition to these, recent trends in underlying biological processes, the study of the gene structure, function and the mechanism by which genes are replicated and transcribed to control the metabolism in botanical classification (in Molecular Systematics), have established themselves as efficient tools to supplement correct identification of medicinal plants (Minelli 1993) and seem to have somewhat eased role of secondary metabolites in plant systematics

(Singh 2004). This multidisciplinary collaborative approach not only helps in correct identification but also opened promising perspectives for the breeding of highly powerful chemo-cultivars of medicinal taxa.

1.2.2 Plant Morphology

Plant morphology involves external appearance, i.e. external form, arrangement and relationships with anatomy, i.e. the internal structure.

The implications of morphological structures in medicinal plants have, however, remained intact because synthesis and accumulation of active chemical constituents are cells, tissues and organs specific, e.g. in *Datura* and *Hyoscyamus*, tropane alkaloids are synthesized in root system and then transported to aerial shoot system of plant. Similarly in Lamiaceae family members, glandular hairs are sites of accumulation of essential oils (Mothes et al. 1985). There is great reliance of pharmacognosy, on the morpho-anatomic characteristics, i.e. on presence or absence of a trait, so as to determine crude drug identity and eliminate adulterations. Histological structures such as starch grains, polygonal crystalloids and secretory structures such as glandular hairs, lactiferous vessels, schizolysigenous cavities, etc. also facilitate drug identification even in the dried crude drugs. The morphological features of plants (e.g. density of glandular cells) may also vary according to growth and development stage as well as under the influence of ecological factors.

1.2.3 Plant Physiology

Plant physiology is an experimental science which observes effect of environment as well as variations in hereditary on the plant life-processes and uses this information to explain plant behaviour. The production and variation of active principles in medicinal plants is mainly linked to plant physiology (Robbins et al. 1957). The metabolic processes change dynamically from juvenile to adult stage which in turn influences synthesis and accumulation of biologically active chemical constituents.

1.2.3.1 Primary and Secondary Metabolism

The sum of multitude of chemical reactions and processes occurring in every plant cell at any single moment is called metabolism. While primary metabolism results in formation of carbohydrates, proteins, lipids and nucleic acids as primary compounds, secondary metabolites are produced mainly during different stress conditions, resulting in a wide range of compounds that are not found in all species. They are used for special purposes, e.g. defence, or to cope with a specific environmental need (Graham et al. 2006). These secondary metabolites produced in medicinal plants are put to utilization by humans for various health benefits.

In contrast to universally occurring substances, secondary metabolites are referred frequently as special principles because of their distinct role in the plant metabolism (Vagujfalvi 1992). These compounds are synthesized frequently during altered natural conditions by special tissues and organs to serve a specific function; hence, within plant, their occurrence is not ubiquitous. The capability to synthesize multitude of bioactive principles with beneficial properties for humans forms the very base for their utilization as herbal drugs, where the humans adapt the very same compounds which the plants use for themselves (Daniel 2006). For instance, for protection from harms in humans caused by oxidative or free radical, the antioxidants synthesized in plants which confer protection against similar attacks in them can be used by man.

The differences which make medicinal plants distinguishable from others are mainly those of the physiological activity. The physiological basis of synthesis or accumulation of bioactive ingredients characterizes speciality of a taxon. Since same chemical constituents can be produced in different ways, their synthetic pathways are characteristic of any plant. For example different plant families can synthesize structurally similar naphthoquinones, e.g. synthesis of lawsone in Balsaminaceae and Lythraceae and plumbagin in Droseraceae, Ebenaceae and Apocynaceae (Hegenauer 1986). The qualitative changes that occur in medicinal plants under environmental pressure particularly the ability to change their synthesis (chemical components) is remarkable feature of their physiology and such changes are observed mainly in metabolism of terpenoids containing plant species.

1.3 Bioactive Chemical Substances

Medicinal plants display wide-range pharmacological activities such as anti-inflammatory, antibacterial and antifungal properties (Okwu and Ekeke 2003) and hence have marked their importance in human health. The medicinal plants owe this value to presence of certain chemicals which invokes a certain physiological response in human body. These phytochemicals are widely distributed in plant kingdom and perform many physiological and ecological functions. The chief role of these bioactive secondary products in plants is to help them in combating various types of abiotic and biotic stresses such as chemical defence against pathogens, predators, pathogens and allopathic agents (Naikoo et al. 2019). The medicinally important active ingredients of the medicinal plants are secondary metabolites. They are relatively small chemical compounds, with ubiquitous distribution in plant kingdom, though their role in the life of plants in the most cases is not known.

The main chemical reactions involved in synthesis of these metabolites in medicinal plants are oxidation, reductions, substitutions and condensation reactions. Medicinal plants synthesize and accumulate a great variety of these phytochemicals such as phenolic compounds, flavonoids, alkaloids, tannins and terpenes (Edeoga et al. 2005) and find their use for therapeutic purposes or as precursors for synthesis of useful medicines (Sofowara 1993). A large diversity of these biologically active chemical constituents exists and produces a definite physiological action in the human body (Edeoga et al. 2006).

In most medicinal plants, stems and leaves are found to be rich in most and varied secondary metabolites with marked physiological activity (Sofowara 1993).

The secondary metabolites in medicinal plants are mainly divided in to three sets, namely, phenolic compounds, terpenoids/terpenes and alkaloids.

1.3.1 Phenolic Compounds

Phenolics, phenols or polyphenolics (or polyphenol extracts) are chemical components that occur ubiquitously in plant kingdom as natural colour pigments responsible for the colour of fruits of plants. They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators and thus are applied in the control of human pathogenic infections (Puupponen-Pimia et al. 2008). They are low molecular weight, ranging from simple single aromatic-ringed compounds to complex and large polyphenols. Plant phenolics range from simple one-ringed phenols to lignin, a huge polymeric structure composed of phenylpropanoid units cross-linked to each other through heterogeneous chemical bonds. The biologically active chemical constituents found in plants belonging to phenolic group vary according to their functional group, which may be hydroxyl, aldehydes or carboxylic group. Most phenolic secondary metabolites are aromatic compounds with C6-C1 carbon skeleton, having a carbonyl group attached (Dewick 2002). They can be synthesized in plants principally by shikimic acid pathway via which pool of aromatic amino acids are synthesized, which in turn are converted into diverse phenolic compounds while some other phenolic compounds are synthesized via polyketide pathway such as quinones and orcinols (Fig. 1.1).

Phenolic compounds synthesized from both these pathways are rather common, e.g. flavonoids, pyrones and xanthones (Bruneton 1999). The shikimate pathway, which starts with erythrose-4-phosphate and phosphoenolpyruvate, is a major biosynthetic route for secondary metabolism. Chorismate which is formed at the end of this pathway acts as substrate for all succeeding products thus forming an important branching site in metabolism (Mustafa and Verpoorte 2005). These intermediate products form the basis for synthesis of great variety of phenolic compounds, e.g. phenylalanine is a common precursor for tannins and lignans (Wink 2000) (Fig. 1.2). Phenolic compounds in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase.

The phenolic group includes metabolites derived from the condensation of acetate units (e.g. terpenoids), those produced by the modification of aromatic amino acids (e.g. phenylpropanoids, cinnamic acids, lignin precursors, hydroxybenzoic acids), coumarins, flavonoids and tannins.

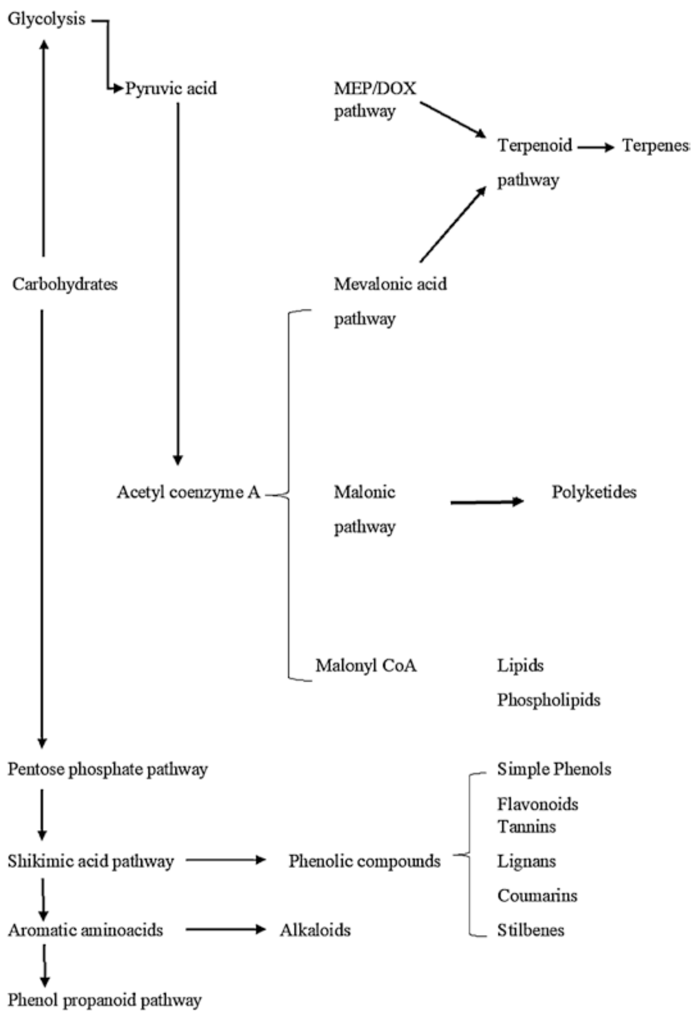

Fig. 1.1 Principal biosynthetic pathways of secondary metabolites

The main groups of phenolics found in the medicinal plants are simple phenolics (phenols, phloroglucinols, etc.), coumarins, flavonoids, tannins, lignans, phenylpropanoids, etc. and the subgroups of these larger groups. The presence of phenols is considered as pathogen growth inhibitor. Protonated phenol acts as antioxidant, anticlotting, anti-inflammatory and immune enhancer agents. Phenols block specific inflammation causing enzymes and also are known to protect platelets from clumping. Polyphenols are considered to prevent malignant tumour development and progression (Okwu 2004). Likewise plant flavonoids are potential cancer chemoprotective agents (Okwu and Okwu 2004) and are also known to reduce the risk of heart diseases (Cook and Samman 1996). In addition flavonoids are known as potential agents for protection against free radicals, allergies, inflammation, platelet

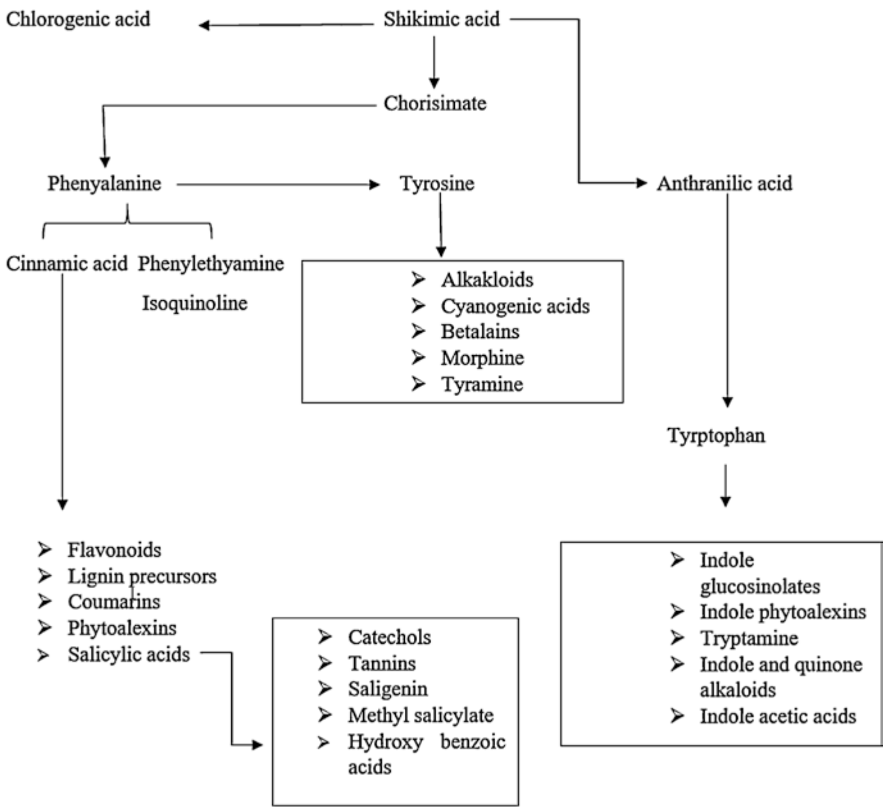

Fig. 1.2 Biosynthesis of diverse phenolic compounds from amino acids via shikimic acid pathway

aggregation, ulcers, hepatotoxins and tumours (Uruquiaga and Leighton 2000; Okwu and Omodamiro 2005).

1.3.1.1 Simple Phenolics

Phenolic groups are ubiquitous among plants, although free phenols are rare. Simple phenolics are compounds having at least one hydroxyl group attached to an aromatic ring, e.g. catechol. Some representatives of simple phenolics are phenylpropanoids (anethole, estragole, myristicin, asarone, etc.) which are defined as secondary metabolites derived from phenylalanine, having a C6C3 carbon skeleton and most of them are phenolic acids (cinnamic acid, *o*-coumaric acid, *p*-coumaric acid, caffeic acid and ferulic acids) which are often the ingredients alone or mainly together with terpenoids of essential oils (Fig. 1.3). Some of these compounds are toxic like asarone and myricetin. Some simple phenolics and their sources in medicinal plants are phloroglucinol derivatives such as hyperforin used as antidepressant found in

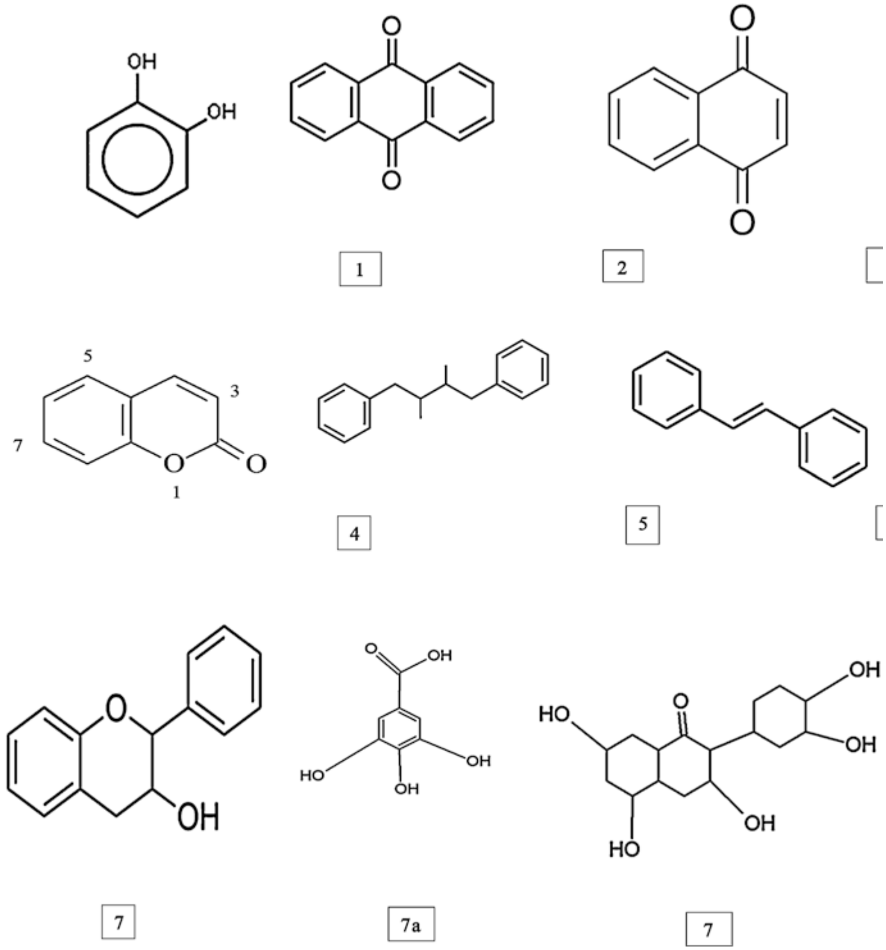

Fig. 1.3 Phenolic compounds occurring in medicinally important plants. 1. Simple phenol 2. Anthraquinones 3. Naphthoquinones 4. Coumarin 5. Lignans 6. Stilbenes 7. Tannins 7a. Hydrolysable tannins 7b. Condensed tannins. Phenolic compounds occurring in medicinally important plants

Hypericum perforatum and albaspidin and filicic acid in *Dryopteris filix-mas*, lupulone and humulone (sedatives) in *Humulus lupulus*, diuretic antiseptic arbutin in *Arctostaphylos uva-ursi* and salicin, an anti-inflammatory, in *Salix fragilis*.

1.3.1.2 Anthraquinones

Anthraquinones are derivatives of phenolic and glycosidic compounds derived from anthracene ring with keto group at position nine and ten, and different functional groups such as hydroxyl, aldehyde, methoxy, carboxylic acid group and so on may substitute at various positions giving variable oxidized derivatives such as anthrones and anthranols (Maurya et al. 2008; Firn 2010) (Fig. 1.3). They are found in families Fabaceae, Polygonaceae, Rubiaceae and Xanthorrhoeaceae and exhibit diuretic, laxative, estrogenic and immunomodulatory activities. Some of the common anthraquinones are aloin A in *Aloe vera*, damnacanthal and rubiadinin in *Cassia sp.* and emodin in *Rheum rhaponticum.*

1.3.1.3 Naphthoquinones

They are also phenolic secondary metabolites compounds found in plant kingdom. Chemically naphthoquinone is an organic compound from naphthalene with one aromatic ring fused to quinone subunit derived from naphthalene (Fig. 1.3). The common and wide spread naphthoquinones are lawsone, juglone and plumbagin. In plants they occur in glycosidic and reduced forms. Naphthoquinones display very significant pharmacological properties such as antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory and antipyretic. Naphthoquinones are found in members of Avicenniaceae, Droseraceae, Ebenaceae, Juglandaceae, Nepenthaceae and Plumbaginaceae families (Ramawat et al. 2004). *Lawsonia inermis* has naphthoquinones with hepatoprotective activity. *Juglans regia* and *Lithospermum erythrorhizon* have naphthoquinone derivatives attributed with antibacterial, fungicidal, cytostatic and anticarcinogenic effects.

1.3.1.4 Tannins

Tannins (or *tannoids*) are a class of polyphenolic biomolecules that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids. In this process, tannin molecules cross-link the protein and make it more resistant to bacterial and fungal attack. Typically, tannin molecules require at least 12 hydroxyl groups and at least five phenyl groups to function as protein binders. Tannins are classified often into two large groups: the hydrolysable and non-hydrolysable or condensed tannins. Hydrolysable tannins have central carbohydrate molecule such as glucose or polyhydric alcohol esterified partially or completely with phenolic acids such as gallic acid and hexahydroxydiphenic, forming gallotannins and ellagitannins. Condensed tannins, also known as proanthocyanidins, are polymers of the flavanol units bound by carbon-carbon bonds (Fig. 1.3).

Some of the common sources of tannins are *Quercus* sp., *Crataegus monogyna*, *Acacia catechu*, *Hamamelis virginiana*, *Rosa gallica*, *Krameria triandra*, *Vitis vinifera* and *Rhus hirta*. They have antioxidant, astringent and styptic properties owing to which they are used to treat pharyngitis, tonsillitis, skin eruptions and haemorrhoids.

1.3.1.5 Coumarins

Distributed throughout the plant kingdom, they form large class of secondary metabolic compounds. Simple coumarins are biogenetically derived from shikimic acid, via cinnamic acid. They occur at high concentrations in some essential oils, such as *Cassia* oil (87,300 ppm), *Cinnamon* bark oil (7000 ppm) and lavender oil. Structurally, coumarins have a benzene ring joined to a pyrone ring, hence classified as members of the benzopyrone family. More than 1400 type of coumarins distributed across more than 40 different families of angiosperms have been isolated (Fig. 1.3).

Different types of coumarins found in medicinal plants are simple coumarins, biscoumarins, furocoumarins, dihydrofurocoumarin and phenylcoumarins (Venugopala et al. 2013). Their bacteriostatic and antitumour properties have put them in category of novel therapeutic agents. Coumarin and its derivatives have shown promise as potential inhibitors of cellular proliferation in various carcinoma cell lines. They also have antioxidant, antibacterial, antiviral, antifungal, anticoagulant, anti-inflammatory, anti-hyperglycaemic and anti-adipogenic properties. The families with high incidence of coumarins are Asteraceae, Apiaceae, Fabaceae, Rutaceae, Oleaceae and Thymelaeaceae, respectively (Venugopala et al. 2013).

1.3.1.6 Lignans

They are dimeric compounds of two molecules of phenyl propane derivatives (Fig. 1.3). Some of the known lignans are podophyllotoxin in *Podophyllum peltatum* having potent anticancer effect. Similarly flavanolignans found in *Silybum marianum* have hepatoprotective effect. Lignans are present in many plants like in *Vixcum album* and *Eleutherococcus senticosus* having adaptogenic effect.

1.3.1.7 Stilbenes

Stilbenes are polyphenolic compounds like flavonoids having C6-C2-C6 structure and are found in *Pinus*, *Madura* and *Eucalyptus* (Fig. 1.3). Other major sources are grape, soya and peanut. These are produced in plants in response to bacterial, fungal or viral attack. They have known cardioprotective properties and can also inhibit offsetting of atherosclerosis. The most common stilbene is resveratrol, having cancer antiprogression properties.

1.3.1.8 Flavonoids

Flavonoids are brightly coloured compounds generally present in plants as their glycosides. “Flavonoid” is a larger group which refers to various subgroups. They represent the largest polyphenol group with ubiquitous distribution in plant kingdom and are synthesized predominantly via phenylpropanoid pathway. They play important role in plant colour, light stress and pathogens and are known potential health-promoting compounds responsible for variety of pharmacological activities.

Flavonoids are synthesized in plants in response to microbial infection, and activity of these hydroxylated phenolic substances is structure dependent. The structural class, degree of hydroxylation, conjugations, substitutions and degree of polymerization determine the chemical nature of flavonoids (Ramawat 2007). They can be divided into a variety of classes such as flavonols (e.g. quercetin, kaempferol, myricetin and fisetin), flavones (e.g., flavone, apigenin and luteolin), flavanones (e.g. hesperetin and naringenin) and others.

More than 8000 naturally occurring flavonoids have been characterized from various plants. Chemically, flavonoids have the general structure of a 15-carbon skeleton consisting of two aromatic rings (A and B) which are joined together by a three-carbon unit that may or may not form a third ring (C). Therefore, their structure is also referred to as C6-C3-C6 (Fig. 1.4).

In most cases, B ring is mostly attached ring to C ring at position 2, but it can bind also at position 3 or 4. The patterns of this B-C rings attachment, together with the structural features of the ring B and the patterns of hydroxylation and glycosylation of the three rings, make the flavonoids one of the most diversified groups of the secondary metabolites occurring in the nature. Flavonoids are further subdivided into following subgroups.

Flavones

Flavones in C ring have ketone group at position 4 and double bond between positions 2 and 3. Most flavones have hydroxyl group in position 5 of the A ring, while the hydroxylation at position 7 of ring A or 3 and 4 of B ring varies according to the taxonomic classification of the particular plant (Fig. 1.5).

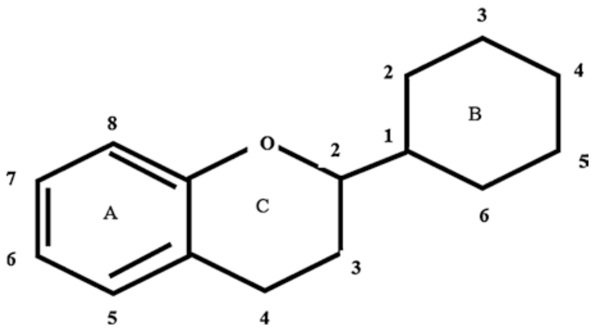

Fig. 1.4 Basic skeleton of flavonoid

Flavanols

They are similar to flavones but lack keto group in C ring and have hydroxy group attached at position 3 in the C ring (Fig. 1.5). Epicatechins and catechin, the two isomers, are widely occurring flavanols present in edible plants and are found in high levels at green tea, cocoa powder and red wine. They have antioxidant properties with beneficial effects on immunity and cardiac health.

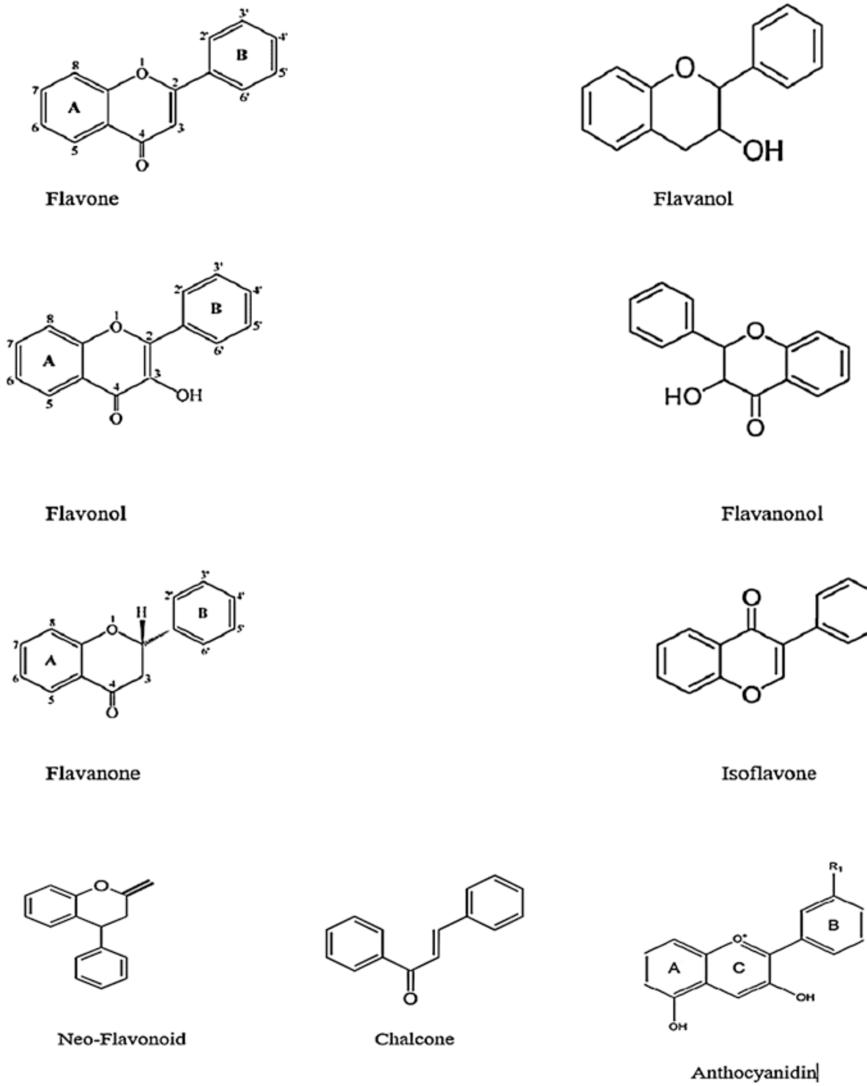

Fig. 1.5 Structure of different types of flavonoids

Flavonols

The flavonol is similar to flavanol but has a double-bonded oxygen atom attached to position 4 in C ring and also has one double bond between position 2 and 3 in C ring. Flavonols are found mostly as *O*-glycosides-aglycons (Fig. 1.5). For example, quercetin is the most abundant flavonol present in many plant foods with proven antioxidant effects.

Flavanones

They are also known as dihydroflavones and are close structurally to flavones but have saturated C ring., i.e. they don't have double bond between positions 2 and 3 in C ring (Fig. 1.5). An important flavanone is naringenin found in citrus species. Naringenin is known for anticancer and liver protective effects.

Flavanonols

Flavanonols are hydroxyl derivatives of flavanones and are also called dihydroflavonols; they are a highly diversified and multi-substituted subgroup (Fig. 1.5).

Isoflavones

The isoflavonoids are rearranged flavonoids in which the B ring is attached to position 3 of the C ring (Fig. 1.5). The transformation of flavanones such as naringenin or liquiritigenin to isoflavones (daidzein or genistein) occurs normally, which is duly mediated by cytochrome P-450-dependent enzyme, respectively. Simple isoflavones such as daidzein and coumestans affect reproduction of grazing animals. Owing to their high estrogenic activity, they are also known as phytoestrogens. Isoflavones also have anticancer, antiangiogenic and antioxidant properties (Dixon and Ferreira 2002; Dixon and Sumner 2003). Isoflavone consumption reduces risks of breast and prostate cancers and additionally also helps in limiting cardiovascular diseases, postmenopausal disorders, osteoporosis and prevention of postmenopausal disorders (Lamartinier 2000; Uesugi et al. 2001). Genistein and daidzein – phytoestrogens – are some common isoflavones and can effect reproduction of grazing animals.

Neoflavonoids

They have the B ring attached to position 4 of the C ring (Fig. 1.5). Some of the known neoflavonoids are calophyllolide from *Calophyllum inophyllum* and dalbergichromene extracted from *Dalbergia sissoo*. They are used as precursors of antidiabetic and anticancer drugs.

Anthocyanidins

Anthocyanidins are common plant pigments, the sugar-free counterparts of anthocyanin. They have two double bonds in positive C ring (Fig. 1.5). They are based on the flavylium cation, an oxonium ion, with various groups substituted for its hydrogen atoms.

Chalcones

Chalcones are open C-ringed flavonoids; they are classified as flavonoids because they have similar synthetic pathways (Fig. 1.5).

1.3.2 Terpenes

The functional diversity of chemicals in medicinal plants is best demonstrated by terpenes. In plants more than 30,000 terpenes have been identified. All terpenes have basic five-carbon isoprene repeating unit giving rise to structures that may be divided into isopentane units (Fig. 1.6) by which they are defined and by which they may be easily classified. There are two biosynthetic routes that produce main terpene building block, i.e. isopentenyl diphosphate (IPP): mevalonic acid pathway (MVA) and methylerythriol-4-phosphate (MEP) or 1-deoxy-D-xylulose (DOX) pathway. The first biosynthetic pathway, i.e. MVA, occurs in the cytosol, producing sesquiterpenes (Janses and de Groot 2004; Oudin et al. 2007). The key intermediate, six-carbon compound, mevalonic acid (MVA) formed in the process by enzyme mediated reduction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) is converted to IPP with the loss of carbon dioxide, and subsequently IPP and its interconvertible isomeric form dimethylallyl pyrophosphate (DMAPP) are incorporated directly into cholesterol. These two isomers are condensed together by enzyme prenyltransferases in a sequential manner producing diversity of products such as squalene, geranyl, farnesyl and phytoene, all being direct precursors of the major terpene families.

In MEP, IPP is derived from 1-deoxyxylulose 5-phosphate (1-DXP), formed from the glycolytic intermediates pyruvate and glyceraldehyde 3-phosphate. The key step in the biosynthesis is the DXP skeletal rearrangement and reduction using NADPH to form 2C-methylerythritol 4-phosphate (MEP) which then by sequential removal of three molecules of water is converted to IPP. Here, IPP is formed in the chloroplast, mainly for the synthesis of more volatile mono- and diterpenes. Various classes of terpenes classified by the number of five-carbon units are monoterpenes, sesquiterpenes, diterpenes, triterpenes and saponins (Fig. 1.6).

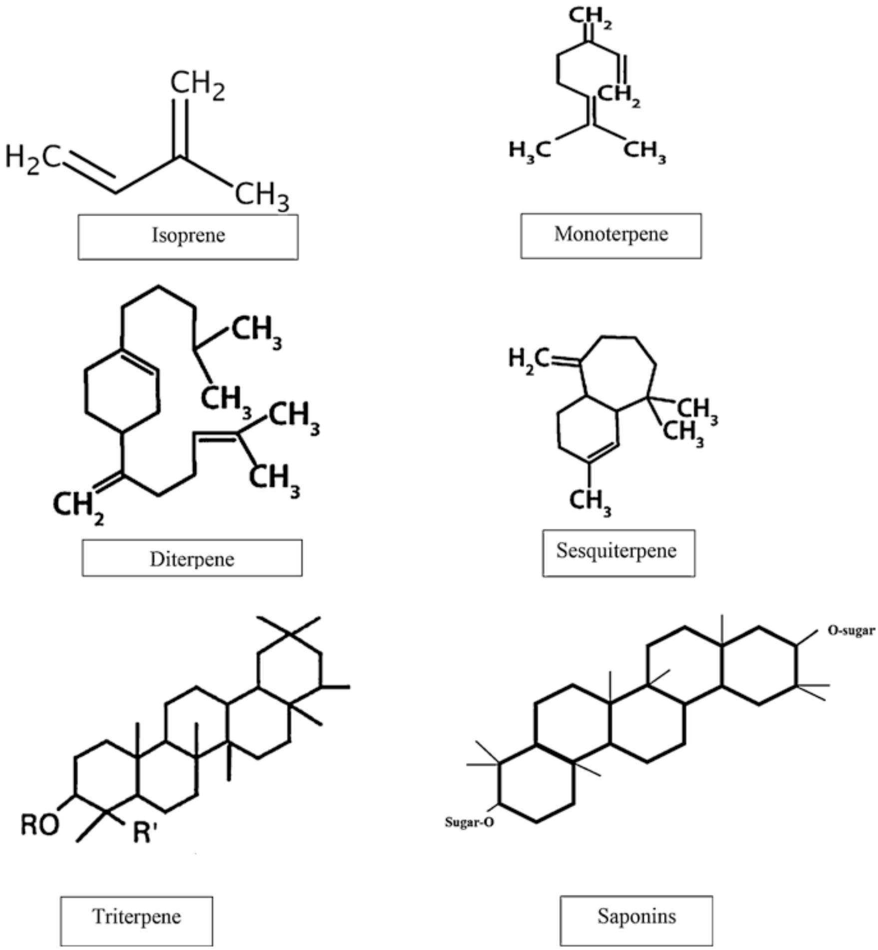

Fig. 1.6 Structure of basic isoprene and different types of terpenes

1.3.2.1 Monoterpenes

Monoterpenes can be divided into two groups: volatile monoterpenes and nonvolatile (iridoids) monoterpenes. Volatile monoterpenes are components of many essential oils and are found in many families such as Apiaceae, Lamiaceae, Asteraceae, etc. Monoterpenes may be linear type noncyclic, cyclic and bicyclic compounds. They may have double bonds and acetoxy carbonyl, hydroxyl and other types of substituents. They mainly occur in angiosperm families and some gymnosperms. Some predominantly occurring monoterpenes are linalyl acetate, geraniol and linalool in *Lavandula angustifolia*; α-, β-thujone in *Salvia officinalis*; menthol and menthone in *Mentha piperita*; linalool in *Ocimum basilicum*; thymol and carvacrol in *Thymus vulgaris*; oregano in *Origanum vulgare*; geraniol in *Cymbopogon citratus* and α-, β-pinene in *Pinus*.

Nonvolatile monoterpenes are monoterpene glycosides with D-glucose predominantly being the sugar part. The main groups are the lactons having a cyclopentan-c-pyran-ringed, normal iridoids (iridane skeleton) and seco-iridoids – a six-membered lacton ring. In a special case only ester-bound iridane skeleton are found. They are the ingredients of valepotriates of *Valeriana* species. Hapagoside in *Harpagophytum procumbens* has choleretic, anti-inflammatory, appetite-stimulating and analgesic effects. Oleuropein and oleacin in *Olea europaea* are high-content seco-iridoids having hypotensive effect and are traditionally used to enhance the renal and digestive elimination. Sweroside and amarogentin, found in *Centaurium erythraea*, *Gentiana lutea* and *Menyanthes trifoliata*, are class of bitter seco-iridoids used to enhance the appetite. *Lamium album* and *Galium verum* also contain significant amount of iridoids such as aucubin and asperuloside.

1.3.2.2 Sesquiterpenes

Sesquiterpenes having molecular formula C15H24 comprise of three isoprene units, and their biosynthesis occurs via farnesyl pyrophosphate (FPP). Sesquiterpenes are colourless lipophilic compounds and may be acyclic or contain rings, including many unique combinations with most functional forms being cyclic. The 15 number long carbon backbone chain of farnesyl pyrophosphate gives rise to several ring systems which together with the different substituents such as ketones and lactones provide a large group of natural products. The most typical nonvolatile sesquiterpene lactons are germacranolide, eudesmanolide, guaianolide and elemanolide found in *Inula helenium*. Artemisinin found in *Artemisia annua* is used in making an important antimalarial drug. *Matricaria* contains sesquiterpene lactons.

1.3.2.3 Diterpenes

Diterpenes have chemical formula $C_{20}H_{32}$ and comprise of four isoprene units. In plants they are produced by activity of HMG–COA reductase with geranyl pyrophosphate being a primary intermediate. They form the basis for some important biological compounds such as phytol, retinal and retinol. They are known to be antimicrobial and anti-inflammatory. Tanshinones I and II found in *Salvia miltiorrhiza* are used against angina pectoris. Marrubiin found in *Marrubium vulgare* is an expectorant. Diterpenes found in *Taxus brevifolia* contain the anticarcinogenic taxol in the bark.

1.3.2.4 Triterpenes

Triterpenes are **c**omposed of three terpene units, biosynthetically derived from squalene, a 30 carbon acyclic hydrocarbon having molecular formula $C_{30}H_{48}$. Triterpenes exist in great variety and have reasonably complex structure.

Around 200 skeletons broadly divided on basis number of rings have been identified, with pentacyclic structures generally dominating ones. Some important pentacyclic triterpenes found in plants are oleanane, ursane and lupane, all of which are promising anticancer agents.

1.3.2.5 Saponins

They are C27 steroids composed of glycone or sugar part and aglycone or geenin (triterpene) part and are found in families like Amaryllidaceae, Liliaceae, Dioscoreaceae, Solanaceae and Scrophulariaceae. The triterpene steroid aglycone part is linked to sugar or glycone oligosaccharide chains of different size and complexity. They are produced as part of self-defence by plants.

Azadirachtin in *Azadirachta indica*, licorice in *Glycyrrhiza glabra*, limonins and the cucurbitacins are some known saponin triterpenes.

Steroids are also modified triterpenes and have profound importance as hormones (androgens such as testosterone and estrogens such as progesterone), coenzymes and provitamins in animals. All plant steroids hydroxylated at C3 are sterols.

1.3.3 Alkaloids

Alkaloids occur predominantly though not exclusively in the dicotyledonous species. Alkaloids generally occur as organic acid salts in plants, with few occurring as sugar and glycosides, e.g. solanine in *Solanum*; some as amides, e.g. piperine; and few esters of organic acids, e.g. cocaine. A few alkaloids mainly weak basic in nature occur freely in nature such as nicotine. More than 12,000 types of alkaloids are known to occur in about 150 plant families, the important being are Ranunculaceae, Solanaceae, Apocynaceae, Papaveraceae, Fabaceae and Rutaceae (Wink 2000; Ramawat 2007). Alkaloids are present in specific plant parts such as root (*Atropa belladonna*), bark of stem (*Cinchona*), etc. or can occur in whole plant. Chemically alkaloids are nitrogen-containing low molecular weight cyclic organic compounds; the N originates from one of the essential amino acids (Fig. 1.7). However alkaloids like the other secondary metabolites cannot be simply classified.

They are roughly distinguished into three groups: real, proto- and pseudo-alkaloids (Hegnauer 1963). The alkaloids having N atom in a heterocyclic ring or

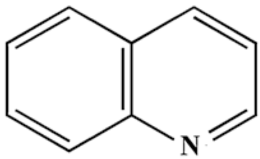

Fig. 1.7 Basic structure of alkaloid

ring system with N originating from one of the essential amino acids are true alkaloids, e.g. nicotine, atropine, etc., while as in proto-alkaloids N atom, which also arise from amino acids, is not in a ring system, e.g. ephedrine and mescaline. The precursors of these types of alkaloids are l-tyrosine and l-tryptophan. Pseudo-alkaloids are alkaloid-like substances whose carbon skeleton does not arise directly from amino acids. These include steroid- and terpene-like alkaloids such as caffeine and theophylline.

Alkaloids have found their use as stimulants, pharmaceuticals, narcotics and even poisons because of their physiological activities. Alkaloids are used as basic medicinal agents for their analgesic, bactericidal, antispasmodic, stimulating and at the same for sedative effects (Stary 1998). Some of the alkaloids used clinically include codeine and morphine as analgesics, colchicine as gout suppressants, vinblastine as anticancer agent, sanguinarine as antibiotic and scopolamine as sedative. The caffeine, which is present in coffee and tea, and nicotine, which is present in all tobacco preparations, are widely consumed alkaloids (Ramawat 2007). Some of the alkaloid-containing plant families are the following.

Apocynaceae (Dogbane family): This family contains tryptophan-derived alkaloids having strong anticancer properties (Aniszewski 2007). Some of the typical alkaloids belonging to species of this family are quinine, reserpine, ibogaine, rescinnamine, deserpidine, tabersonine, ervatine, menilamine and coronaridine (Srivastava et al. 2001; Macabeo et al. 2005).

Asteraceae: With more than 2000 species belonging to this family, its members contain many different tryptophan- and ornithine-derived alkaloids. The most common alkaloids are apigenin, intergerrimine, retrorsine, senecionine and usaramine (Pelser et al. 2005; Aniszewski 2007).

Loganiaceae: This family has more than 100 species containing mainly tyrosine-derived medicinally important alkaloids such as brucine, isosungucine, strychnine, sungucine, curare and isostrychnopentamine (Lansiaux et al. 2002; Frederich et al. 2004).

Papaveraceae: Poppy family has about 250 plant species producing many tyrosine-derived alkaloids such as berberine, codeine, morphine, thebanine, narcotine, narceine, papaverine, sanguinarine, cholidonine and chelerythine (Aniszewski 2007).

Rutaceae This family contains more than 900 species, containing both histidine- and anthranilic acid-derived alkaloids, e.g. pilosine, pilocarpine, fagaronine, isodecarpine, rutaecarpine, cuspareine, galipinine, dihydroevocarpine and evodiamine are l-histidine-derived alkaloids, while acronycine, acutine, dictamine, rutacridone, melicopicine, bucharidine, perfamine, helietidine and kokusaginine are anthranilic acid-derived alkaloids. (Nazrullaev et al. 2001; Chen et al. 2005).

Solanaceae This family has more than 2000 species abundant in ornithine-derived alkaloids, e.g. cuscohygrine, hyoscine and hyoscyamine. Some other alkaloids such as capsaicin have phenylalanine as precursor, while anabasine and nicotine both have nicotinic acid as precursor (Schwarz et al. 2005).

Boraginaceae This family with about 2000 species contains ornithine-derived alkaloids such as europine, indicine-N-oxide and ilamine and several pyrrolizidine-derived alkaloids used as sedatives, analgesic as well as sudorific remedies and for skin disease treatment (Haberer et al. 2002; Siciliano et al. 2005).

Fabaceae The species of this family contain mostly lysine-derived alkaloids such as angustifoline, castanospermine, epi-lupanine, swainsonine, etc. (Przybylak et al. 2005) as well as some tryptophan-derived alkaloids, namely, serine and eseramine.

Amaryllidaceae This family contains plant species with tyrosine-derived alkaloids such as lycorine, galanthine, haemanthamine, ycorenine, maritidine, oxomaritidine and vittatine (Herrera et al. 2001; Forgo and Hohmann 2005).

Berberidaceae The plant species of this family produce tyrosine-derived alkaloids such as glaucine, berberine, hydroxycanthin and berbamine widely used against asthma, whooping cough, uterine bleeding, pharynx tumours and diabetes (Khamidov et al. 2003; Aniszewski 2007).

Ranunculaceae The plant species of this family biosynthesize tyrosine-derived alkaloids such as hydrastine, berberine and fangchinoline (Erdemgil et al. 2000) and terpenoid-derived alkaloids such as acorone, aconitine, cammaconine, coryphidine, nepelline, songorine, secokaraconitine, methyllycaconitine and delcorinine (Salimov 2001; Sultankhodzaev et al. 2002).

Liliaceae The plant species of this family produce tyrosine-derived alkaloids, for instance, autumnaline, kreysigin and floramultine (Aniszewski 2007) and steroidal alkaloids such as cyclopamine, jervine, protoveratrine A and O-acetyljervine used as expectorant and antitussive (Jiang et al. 2005).

1.4 Cultivation and Good Agricultural Practices for Medicinal Plants

The wild-harvested resources of medicinal plants are widely considered more efficacious than those that are cultivated. However increased cultivation eases harvest volume pressure on wild medicinal plants and contributes towards recovery and restoration of these wild resources. Moreover, the scope for improvement of medicinal plant products as quality drugs primarily stems from their good cultivation, where factors like good site selection, good seed material, nutrient input, harvest and post-harvest techniques and management form the merits for their commercialization as well as provide opportunities to solve the problems encountered in medicinal plant production such as low bioactive ingredient content, pesticide and toxic contamination and misidentification of biological origin. The efficiency of cultivation of these species is fundamentally dependent on the productivity of the plant biomass within which the active principles are synthesized and frequently accumulated. The quantity and composition of bioactive chemical components are important preconditions for their utilization and therefore also intensively investigated botanical domains.

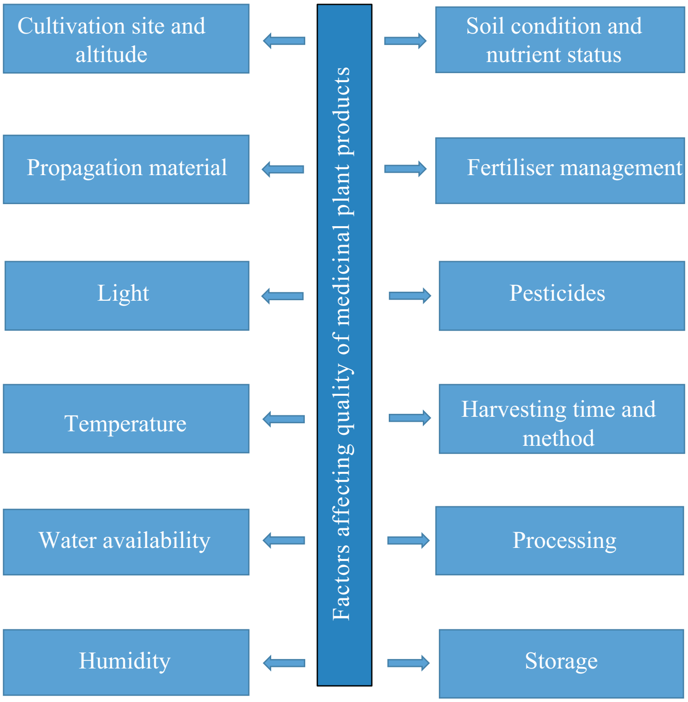

Fig. 1.8 Factors affecting growth of medicinal plants and quality of bioactive chemical constituents

Controlled cultivation practices are designed to improve yield of secondary metabolites and ensure stability in their production by making available better and optimal environmental factors such as light, temperature, humidity, water, soil and other additives such as nutrients, fertilizers and pesticides (Fig. 1.8).

There is need for intensive care and management in cultivation of medicinal plants depending upon the quality of plant material, the duration and conditions of cultivation required. Traditional methods of cultivation should be preferred in case of absence of any scientific published or documented cultivation data.

Good agricultural practices, including apt rotation of selected medicinal plants should be followed according to environmental suitability and other growth requirements to ensure quality production and facilitate the standardization of herbal drugs. Conservation agriculture techniques which aim for efficient use of natural resources should be followed through integrated management of available water, soil and biological resources in combination with external inputs which contribute to sustained agricultural production and also environmental conservation. The biosynthesis of active principles and their concentration in medicinal plants is also correlated with certain other physiological and environmental factors. Some of the most important factors influencing growth of medicinal plants as well as quality and content of their active chemical constituents and hence medicinal plant products are the following.

1.4.1 Seeds and Other Propagation Materials

Seeds and any other propagating material should be of good quality having available record performance and breeding history. This material should be free from diseases and contamination as much as possible so as to promote healthy growth and development of plant and their chemical products. The quality of propagation material should conform to the regional regulations and be well documented. Substandard, counterfeit and adulterated propagation materials must be avoided.

1.4.2 Site Selection

Site selection is the most critical factor in the cultivation of medicinal plants so as to meet requirements of ecological homogenous groups. Cultivation site influences quality of medicinal plant products mainly related to the active constituents owing to difference in soil, climate, eco-geographical and other factors. The impact of past land uses on the cultivation site, including the planting of previous crops and any applications of plant protection products, should be evaluated. Among other factors while considering site selection, altitude and slope has an important bearing impact on cultivation of medicinal plants. Altitude/elevation of selected site/region has a profound influence on successful medicinal cultivation. With increase in the altitude, there is decrease in atmospheric pressure and temperature, while an increase in light intensity, relative humidity and wind velocity occurs.

Thus, change in pattern of vegetation occurs with change in height due to variation in the climatic conditions, e.g. *Atropa belladonna*, *Datura innoxia* and *Catharanthus roseus* show high active principle content at high altitudes. Alkaloid content in *Aconitum lobelia* and pyrethrum in *Pyrethrum* increases with increase in altitude, while in peppermint, decrease in alkaloid content occurs.

Slope face also determines diurnal light intensity and temperature both having pronounced effect on growth and development of medicinal. Latitude is another factor effecting biomass composition, e.g. *Atropa belladonna*.

1.4.3 Light

Light being the main external energy source for continuity of plant life affects photosynthesis, vegetative growth, stomata opening and closure, seed germination and flowering in medicinal plants. Day light length has profound bearing on growth and development of medicinal plants. Light intensity also plays an important regulatory role in performance improvement in a number of medicinally plants. Dry sunny weather coupled with more than 14 hours of day light conditions increase the proportion of glycosides in *Digitalis* and those of alkaloids in *Atropa*, *Mentha*, *Coptis*, *Glycyrrhiza* and *Humulus*. Some other plants such as *Pelargonium, Citronella* and *Pogostemon* shows nearly 25% increase in essential oil production when grown under less than 12 h of daylight conditions. In crops like *Solanum, Digitalis, Rauvolfia* and *Dioscorea*, effects of photoperiodic cycles (long days) have been clearly established (Chatterjee 1996).

Higher content of menthol in *Mentha* is obtained when plants are subjected to ample sunshine; in contrast to this, low light intensity promotes higher active chemical constituent production in *Cephaelis ipecacuanha*, thereby necessitating need for artificial shading. Similarly in *Coptis japonica*, berberine content increases by 15% when 75 of the incident light is screened. Similarly, 40% reduction in light intensity results in increase of alkaloid and quinine by 30% in *Cinchona*.

1.4.4 Temperature

The role of atmospheric temperature on growth and development of medicinal plants is well established. Temperature like in other plants is the major influencing factor in medicinal plant cultivation. The rate of photosynthesis and respiration are both affected by temperature, with both increasing with increase in temperature up to a certain degrees. Likewise the sudden temperature decrease results in ice crystals formation in plant intercellular spaces leading to cell death and plant death due to desiccation. In many terpene- and alkaloid-producing medicinal plants, increasing temperature up to a maximum (prior to physiological damage) favours increase in production of these secondary metabolites. Temperature also affects the secondary metabolite composition of medicinal plants. For instance, morphine content in *Papaver somniferum* increases while as total principle content decreases at low temperature. Low temperature also favours increase in yield and total pyrethrin contents in *Pyrethrum*.

1.4.5 Atmospheric Humidity

Atmospheric humidity affects various aspect of growth and development in medicinal plants by affecting photosynthesis and under abnormal conditions leads to production of more secondary metabolite content. Water evaporation and condensation along with precipitation depend upon relative humidity which thereby affects form, structure and transpiration in these plants.

1.4.6 Water Supply

Water supply is the most vital factor influencing medicinal plant cultivation. Plant morphology and physiology are highly influenced by water availability. Water supply should be in accordance to needs of individual medicinal plant. There should be controlled irrigation and drainage so as to ensure that plants are neither under-watered nor over-watered.

A very complex relationship exists between availability of water and optimum medicinal plant production though differences in adaptation of these plant species to water stress exist. Consideration of per-plant response vis-a-vis a whole field response is very critical. Water stress though in general might not be beneficial, but correctly timed stress of appropriate intensity can be productive with regard to secondary metabolite synthesis (Simon 1999). Water stress stimulates more secondary metabolite accumulation in *Catharanthus* and *Rauvolfia.* An increase in alkaloid content is observed in *Atropa* and *Datura* under moisture stress conditions. In contrast, increased water availability supresses alkaloid accumulation in *Cinchona* sometimes even leading to zero alkaloid content.

1.4.7 Soil

Soil being one of the most important natural resources supporting plant growth provides anchorage, nutrients and mechanical support to plants. The optimal growth of medicinal plants is ensured in soil containing appropriate amounts of inorganic and organic nutrients. Other conditions such as soil type, moisture retention, drainage, pH and fertility will depend on target medicinal plant. Adequate soil moisture and moderate nutrient status generally meet the requirements for growing most medicinal plants. However some medicinal plants such as *Withania* and *Cymbopogon* can also thrive well on less fertile soils. Medicinal plants are adapted generally to a wide range of soil pH and texture, e.g. increase in yield is observed in *Cymbopogon* and *Cassia*, when transformed from light soils to loam and clay loam. *Vetiveria* is unique for its tolerance to soil alkalinity and periodic flooding and water logging of field conditions which have no adverse effect on total oil yield and its composition. *Cinchona* and *Coptis* have preference for acidic soils (5–6 pH); whereas species like *Aloe, Vetiveria* and *Commiphora* are grown in soils with high pH.

1.4.8 Fertilizers

The key factor in successful cultivation of medicinal plants is fertilizer management. Even though each plant has its own specific requirement of nutrients, the use of correct type of fertilizers, chemical or organic, in correct quantities is often necessary to obtain good yield. The application of NPK has resulted in higher biomass as well as active principle content production in a variety of medicinal plants. For instance, application of nitrogen fertilizers results in an increase in content of diosgenin in *Dioscorea*, alkaloid in *Atropa* and essential oils in *Cymbopogon* grasses, *Vetiveria* and *Mentha*. Increase in secondary metabolite production is obtained in medium levels of N, P and K.

1.4.9 Maintenance and Protection

The field management practices should be guided by growth and development characteristics of a particular medicinal plant or its specific part destined for medical use. The timely application of measures such as topping, bud nipping, pruning and shading may be used to control the growth and development of the plant, thereby improving the quality and quantity of the medicinal plant material being produced. There should be in place integrated pest management with little to no use of herbicides and pesticides. Different types of pest control methods such as hand picking to remove insects, pruning, burning and trapping of pests need to be employed. Chemical methods of pests control involve use of chemical pesticides such as insecticide (DDT, gammaxene, parathion, malathion), fungicides (Bordeaux mixture, chlorophenols), herbicides (2, 4-dichlorophenoxy acetic acid, sulphuric acid) and rodenticides (warfarin, red squill). However advanced plant breeding techniques involving genetic manipulations result in production of hybrid varieties production, which are resistant to fungal and bacterial attack, can prove more useful. In addition to these factors, hybridization both natural as well as human engineered and greenhouse effect influence growth, development, production, quality, content and quantity of medicinal plant and their constituent bioactive chemicals.

1.4.10 Harvesting

The mass production of quality crude drugs with desirable active principle contents (i.e. composition and high amounts) has scientific basis similar to other commercial plants and involves sustainable harvesting such as expert collection practices, time of harvesting, material to be harvested, harvesting techniques, harvesting equipment, proper processing and drying, packaging and correct storage.

Medicinal plants should be harvested during the optimal season to ensure the ample production of quality medicinal plant materials. The harvest time varies with plant type as well as plant part to be used. Harvesting should be done under best possible conditions, avoiding rain, dew and high humidity, so as to prevent any possible deleterious effects due to increased moisture levels. The biologically active ingredient concentration varies with plant growth and developmental stage, thus harvesting time should be determined by peak quality and quantity of active chemical constituents rather than vegetative yield.

There should be well-documented, easily available information regarding appropriate harvest timing of a particular medicinal plant. Utmost care should be taken during harvest, so as to avoid mixing of weeds, foreign matter or toxic plants with the harvested medicinal plant materials. Harvesting tools and other machines should be kept clean and so to reduce chances of contamination from soil and other materials. Contact of harvested material with soil should be avoided to the possible extent so as to reduce the microbial load.

The harvested raw medicinal plant materials should be transported promptly in clean, dry conditions. They may be placed in well-aerated containers and carried to a central point for transport to the processing facility.

Any mechanical damage or compacting of the raw medicinal plant materials, as a consequence, for example, of overfilling or stacking of sacks or bags that may result in composting or otherwise diminish quality should be avoided. Decomposed medicinal plant materials should be identified and discarded during harvest, post-harvest inspections and processing, in order to avoid microbial contamination and loss of product quality.

1.4.11 Post-Harvest Processing

Prior to primary processing, there should be inspection of raw medicinal plant material which may include visual inspection for cross-contamination by untargeted medicinal plants or plant parts, foreign matters as well as organoleptic evaluation, such as size, colour, taste, odour and damage. Appropriate measures of primary processing are dependent on the individual materials. These processes should be carried out in conformity with national and/or regional quality standards, regulations and norms. As far as possible, standard operating procedures should be followed. If modifications are made, they should be justified by adequate test data demonstrating that the quality of the medicinal plant material is not diminished. Harvested or collected raw medicinal plant materials should be promptly unloaded and unpacked upon arrival at the processing facility. Medicinal plant materials that are to be used in the fresh state should be harvested/collected and delivered as quickly as possible to the processing facility in order to prevent microbial fermentation and thermal degradation. The materials may be stored under refrigeration, in jars and in sandboxes, or using enzymatic and other appropriate conservation measures immediately following harvest/collection and during transit to the end-user must be followed. The use of preservatives should be avoided. If used, they should conform to national and/or regional regulations for growers/collectors and end-users.

Wherever the medicinal plant product is to be used in dry form, its moisture level needs to be kept as low as possible so as to minimize damage from microbial infestation. Information regarding the appropriate moisture content for particular medicinal plant materials should be made available from pharmacopoeias or other authoritative monographs. There are many ways by which medicinal plants are dried: by direct sunlight, in ovens, by indirect fire, in the open air in drying frames, baking, lyophilization or infrared devices. To avoid damage to biologically active constituents, the humidity and temperature should be controlled. There is considerable effect of method and temperature used for drying on the quality of the resulting medicinal plant product. For instance, in case of plant material containing volatile oil, low temperature for drying is preferred, and shade drying is employed to minimize flower and leaf colour loss. There should be avoidance of drying medicinal plants on bare ground and that it should also be ensured that drying is uniform to reduce chances of mould formation.

For indoor drying, drying temperature and its duration, humidity and other conditions should be determined by the part of plant used as well as its natural constituents.

Specific processing is required by certain medicinal plant materials so as to improve purity, prevent damage from infestation and enhance efficacy. The most common specific processing practices include pre-selection, root and rhizome skin peeling, steaming, pickling, natural fermentation, distillation, roasting, treatment with lime and fumigation. Processing procedures involving building of specific shape formation also impacts quality of medicinal plant material and subsequent products. Antimicrobial treatments of raw and processed medicinal plant materials by various methods must be declared, and the materials must be labelled as required and should be conducted in accordance with standard operating procedures and national and/or regional regulations.

1.5 Medicinal Plant Products (Herbal Drugs) and Safety and Quality Concerns

Plants being rich sources of phytochemicals are generally free from side effects, and because of better human body compatibility, herbal medicines are widely acknowledged as alternative to synthetic drugs (Fennel et al. 2004; Yadav and Dixit 2008). Herbal drugs or plant drugs are medicines that contain a chemical compound or more usually mixtures of chemical compounds derived from plants that act individually or in combination on the human body to prevent diseases and to maintain or restore health. These plant products are used in raw form, or chemical compounds are isolated from these natural products in the laboratory, and sometimes new drugs are synthesized by combining different isolated compounds before being put to use. Isolation of vast number of herbal drugs isolated from natural sources is done by traditional methods in non-standardized manner (Rizvi et al. 2009). This coupled with varied locations of plant growth, problems of different vernacular names and absence of standard procedure for cultivation, harvesting and post-harvesting often leading to addition of impurities thereby deteriorating quality and effectiveness of final medicinal products, where the use in some cases can also result in adverse drug reaction. In recent times emphasis is being laid on the adherence of certain standards for herbal drug safety to minimize adulteration and maintain quality of these herbal products (WHO 1999).

The integration of medicinal plant products/herbal medicine into modern medical practices needs to take into account the interrelated issues of quality, safety and efficacy. Quality is the utmost issue as it affects safety and efficacy of medicinal pant products. Product quality is influenced by extrinsic, intrinsic and regulatory factors. Species differences, organ specificity, diurnal and seasonal variations can affect the qualitative and quantitative production and accumulation of active chemical constituents in the source medicinal plants. Extrinsic factors which contribute to the quality of herbal products include environmental factors, method employed in collection and harvesting, post-harvest stages, manufacturing practices, unintended contamination and intentional adulteration. Among intrinsic factors, difference in species and their specific ecological requirements and biochemical process operative in them affect the quality of medicinal products. Quality of herbal medicine can also be attributed to regulatory practices, with unregulated practices often leading to decline in quality of products.

1.5.1 Adulteration of Herbal Drugs

Drug adulteration, intentional or direct, usually includes those practices where herbal drug is either completely or partially substituted with other inferior products, which may or may not have any therapeutic value. Adulteration is generally of two types, deliberate and undeliberate. Deliberate or direct adulteration is wherein, with genuine crude material, intentional substitution by different materials is carried out. This practice is encouraged mainly by traders who are unwilling to pay premium prices for superior quality herbs and are more inclined to procure cheaper products, thereby encouraging producers and traders to sell inferior quality herbs. In addition to this, another factor which promotes adulteration of herbal drugs is their rarity. Undeliberate or unintentional adulteration is due to confusion of common vernacular name of herbal drugs. Also due to absence of proper evaluation means, an authentic drug partially or fully devoid of the active ingredients may enter the market. This occurs mostly in case of volatile oils. Such adulteration and substitution lead to poor quality and batch-to-batch inconsistency.

In deliberate adulteration, drugs are generally adulterated or substituted with substandard, inferior or artificial drugs. Substitution with substandard commercial varieties involves use of adulterants which are cheaper cost-wise and are substandard but resemble morphologically and chemically with the original crude drug used. Another type of deliberate adulteration is substitution with superficially similar inferior drugs which involves use of inferior quality drugs which have little or no chemical or therapeutic value and are used owing to their morphological resemblance with actual products. Substitution with artificially manufactured substance is another form of direct adulteration where adulterant resembling original drug is manufactured artificially and used for the costlier drugs. Many a times there is substitution by "exhausted" drugs which involves adulteration of the herbal plant material with the same plant material but devoid of the active constituents. This is mainly done in volatile oil containing drugs like coriander clove, fennel and caraway. In addition to these types, many times synthetic chemicals are used as substitutes to augment natural character of the already exhausted drug.

For instance, citral is added to citrus oils like lemon and orange oils. In some cases, some vegetative parts of miniature plants growing along with medicinal plants are added. The drugs which are in the form of powders are frequently adulterated. For example, dextrin is added in *Ipecacuanha*, exhausted ginger in ginger, red sanders wood in capsicum powder and powdered bark adulterated with brick powder.

Some other factors also affect quality of drugs, e.g. growing conditions, geographical sources, processing, storage, etc. Drug quality is also influenced by deterioration to which crude drugs are often prone to, especially during post-processing stage such as storage resulting in a loss of active principles and production of non-active and sometimes even toxic metabolites. Physical factors such as temperature, humidity, oxygen level and light alone or in combination are also responsible for bringing about deterioration. Elevated temperatures and humidity by accelerating enzymatic activities lead to physical appearance changes and decomposition of the herbs. High oxygen levels cause oxidation of the active ingredients in drugs often resulting in resinification or rancidification of essential oils.

Dried herbs are prone to contamination with bacterial and fungal spores which is accompanied usually by the growth of moulds, evident by changes in appearance of the plant material, breakdown in some cases and even production of smell.

1.5.2 Quality Control of Medicinal Plant Products/Drugs

The process involved in maintaining quality as well as validity of manufactured entity is referred to as quality control. Quality control of drugs whether herbal or synthetic is of paramount importance for its safety and efficacy (Balandrin et al. 1993; Chan 2002). Herbal drug quality can be defined as the drug status which is determined by identity, purity, content and other physico-chemical or biological properties and by the manufacturing and storage processes. For the traditional medicines, quality control involves the study of traditional information and method of identification and quality assessment of a drug and its interpretation in terms of modern assessments. Herbal drugs denote plant parts or crude products derived from plants which are converted by simple means of processing such as harvesting, drying and storage into pharmaceuticals. Herbal drug quality is influenced by various factors such as plant material being naturally and chemically variable, existence of plant chemo cultivars, their inherent complexity as well as the limitations of simple analytical techniques for identification and characterization of bioactive constituents coupled with the fact that herbal drugs are mixture of many constituents whose chemical nature is still unknown. The preparations of crude products is also influenced directly by the primary processing and production of the medicinal plant product or herbal drug and prerequisites for adequate quality assurance system during all stages of herbal drug manufacturing right from cultivation, harvesting, primary processing, handling, storage, packaging to distribution. As these processes are susceptible to variation caused by differences in geographical location, differences in growth, duration of growing season and time of harvesting, so is variation expected in quality of these drugs. Since contamination and deterioration can occur at any one of these stages, therefore to maintain the quality and potency of an individual herbal product, it is extremely important to establish good agricultural, harvesting and manufacturing practices to ensure quality and stability of these drugs (Shimmer et al. 1998; Jacobson et al. 2001; Kasper 2001).

All medicines, however, whether they are of synthetic or of plant origin, should fulfil the basic requirements of being efficacious and safe, and this can be achieved by suitable clinical trials.

The quality control in herbal drugs generally is based on three important pharmacopoeia parameters: the correct identification of the plants, whether the product is in pure form and whether the content active constituents are within the defined limits.

The correct identity of the plant material or crude material is pivotal in establishing quality control of herbal drugs. Both macro- and microscopic examinations are employed to ascertain the correct identity and also can voucher specimens be used as reference sources.

Sometimes markers can be used which are, by definition, chemically defined constituents that are of interest for control purposes, independent of whether they have any therapeutic activity or not (Rates 2001).

Purity is of prime importance as being linked closely to safe usage of herbal drugs and deals with factors such as evaluation of contaminants, ash values, heavy metals, aflatoxins and pesticides, etc. To prove purity, criteria such as type of preparation, sensory properties, physical constants, adulteration, contaminants, moisture, ash content and solvent residues have to be checked. Several improved analytical methods such as photometric analysis, high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and gas chromatography (GC) are employed to establish the constant composition of herbal preparations. Depending upon whether the active principles of the preparation are known or unknown, different concepts such as "normalization versus standardization" have to be applied in order to establish relevant criteria for uniformity.

Content or assay is the most difficult assessment of quality control as active constituents in most herbal drugs are not known. A vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy (UV/VIS), TLC, HPLC, GC, mass spectrometry (MS) or a combination of GC and MS (GC/MS) are employed for ascertaining this parameter (Goldman 2001).

Another important parameter in quality control is standardization. Standardization involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations. There is ample variation in terms of chemical composition, quality and therapeutic effect in the crude extracts obtained from plants. Standardized extracts are high-quality extracts containing the consistent levels of specified compounds, and these high-quality extracts are subjected to thorough quality controls during all phases of the growing, harvesting and manufacturing processes. Since in absence of any regulatory definition of standardization of dietary supplements, the word “standardized” on a supplement label does not necessarily indicate product quality. In case of the unknown active principles, marker substance(s), which in herbal drugs are chemically defined constituents important in determining quality of finished product, should be established for analytical purposes of standardization. Ideally, the chemical markers chosen would also be the compounds that are responsible for the botanical’s effects in the body.

There are two types of standardization: “true” standardization, where a certain known phytochemical constituent is known to have activity. For instance, the highly concentrated flavones and terpenes in *Ginkgo* which are now considered as phytopharmaceuticals, which no longer represent whole herb, are more effective in most cases than plant as whole. The other type of standardization is where in the presence of certain percentage of marker compounds is guaranteed by the manufacturers; these are not indicators of herb quality or its therapeutic activity.

The other important parameter in quality control of medicinal plant products or drugs is validation. Both in developed and underdeveloped countries, the major public health concern vis-a-vis the use of herbal products is their validation so as to avoid or reduce the selling of adulterated herbal medicines. Thus for any herbal products having potential to cure and reduce the disease severity, it is essential to certify scientific validation and periodic monitoring of its quality and efficacy in order to control or limit introduction of its adulterated and impure forms.

Validation is the process of proving that an analytical method is acceptable for its intended purpose for pharmaceutical methods, and depending upon whether analytic method is quantitative or qualitative, it in both cases includes studies on specificity, precision, accuracy, precision, range, detection and quantitative limits.

1.6 Conservation and Sustainable Use of Medicinal Plants: Threats, Issues and Strategies

The use of plant-based products or herbal medicines in healthcare along with new nutritional trends and evolving markets led to increase in demand of these raw materials. Harvesting of wild medicinal plants on small scale occurring mainly for local use and on large scale for supplying to the global markets on commercial basis (Zlobin 2012) has put these species under tremendous pressure even threatening their existence. There is growing concern in conservationist vis-a-vis ever-increasing extinction threats to medicinal plants world over. Both natural and anthropogenic activities are reasons for causing a plant species to become endangered. Since natural ecosystem plays fundamental role in successful existence conservation of species, thus among all the factors, habitat destruction is the most serious cause of species extinction or slipping into endangered categories (McKenzie et al. 2009). The other factors which threaten existence of species include introduction of invasive alien species, over-exploitation, climate change, outbreak of diseases and increase in pollution level and fire outbreaks (Miththapala 2006; Zlobin 2012). Genetic changes occurring naturally (mutation, speciation and evolution) as well as human-induced genetic modifications (intended to increase concentrations of active medicinal compounds) are also having profound impacts on species existence (WWF and IUCN 1997). These threats to species existence are further exacerbated by destructive harvesting techniques, grazing, tilling and swamp drainage. Furthermore uncontrolled trade in the form of the export of vast majority of medicinal plants to industrialized countries, due to local restrictions in use of native species for medicinal purposes, is pushing many rich medicinal plant regions to the brink of ecological and economic catastrophe (Lange 1998). Weak or non-existent regulation coupled with lack of transparency in law enforcement is a major contributor to this crisis (Mulliken 2000). There has been a huge transformation from collection of the medicinal plant for satisfying local needs to trade because of the increased global demand for herbal drugs due to realization of shortcoming, no holistic nature and side effects of holistic drugs. This combination of enticement of short-term profits and lax regulation which led to the over-exploitation of wild medicinal plant stocks, without any consideration to sustainability (Hachfeld and Schippmann 2000), has put medicinal plant diversity under tremendous existence pressure.

These factors had brought issues of conservation of medicinal plants to the forefront of public discourse (Switzer et al. 2003). The conservation strategies thus thrive to ensure continuance of these natural plant races by identifying and evaluating employment of modern scientific methods (Johns and Eyzaguirre 2000; Baricevic et al. 2012).

A multitude of strategies are being employed for conservation of medicinal plants. These include ex situ conservation, in situ conservation, improved and enhanced management of wild demes, improved cultivation, public awareness programmes, fair and transparent trade monitoring and national and international legislation and regulation. The general medicinal plants conservation strategy from system's view point comprises of in situ and ex situ conservation. In practice, all other mentioned subsystems can be closely connected.

The in situ conservation strategy ensures that the plants continue to grow and evolve in their natural habitats. These measures can be applied in various types of species recovery and restoration programmes. Since most medicinal plants are endemic species, the secondary metabolites which attribute them medicinal values are produced in them in response to those natural endemic environmental conditions involving intricate relationship network with other existing species in the community, and as such these metabolites under culture conditions may not be expressed in same content, quality and quantity. In situ conservation thus aims to conserve not only indigenous plants but whole communities (Long et al. 2003; Gepts 2006), thereby strengthening the link between conservation of resources and their sustainable use by increasing the diversity amount that can be conserved. In situ conservation thus rather being species oriented is ecosystem oriented and focuses on establishing protected areas.

Ex situ conservation is an effective complement to in situ conservation. Ex situ conservation aims to cultivate and naturalize threatened, low-abundance, slow-growing, over-exploited and disease-susceptible species to ensure their continued survival and increase their density. Ex situ approaches involve propagation, breeding and conservation of medicinal plants in controlled and managed ecosystems.

Here, vegetative propagules and seeds of rare and threatened medicinal plants are collected from the wild ecosystem and bred in a controlled environment in order to ensure adequate stocks. This approach relies on basic understanding of ecological requirements of medicinal plants for their preservation and maintenance often involving their cultivation in botanical gardens, herbal gardens, seed banks and gene banks and other agrosystems (Shengji 2001) and at the same time resulting in promotional and dissemination of ethnopharmacological knowledge as well as sustainable management for conservation (Innerhofer and Bernhardt 2011; Salako et al. 2014). This is important for sustenance of medicinal plant resources used for producing large quantities of human beneficial drugs, and it is often an immediate action taken to sustain medicinal plant resources (Pulliam 2003; Swarts and Dixon 2009). Wild-growing medicinal plant species may not retain high potency when grown away from their natural habitats in managed gardens but can have their reproductive materials selected and stored for future replanting.

1.7 Conclusions

Medicinal plants despite being used since prehistoric times still have incalculable unknown and unexplored potential for human healthcare. There has been dynamic and continuous expansion in the role of medicinal plants and their products with changing times. This has led to the need for expanded research in medicinal plants to get insights into promising leads from these natural products in efforts to produce new beneficial drug entities. Close collaboration between various scientific and clinical domains needs to be established with a common endeavour – production of quality, safe and efficacious products. However, issues like conservation of both ethno-botanical data and biodiversity must be addressed so as to minimize loss of genetic resources and also not to upset coexisting balance between plants and humans which other can result in myriad ecological problems. The accumulated knowledge regarding cultivation and therapeutic use of medicinal plants needs to be disseminated across the world and down to generations as well as making it a worthwhile contribution to human healthcare.

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Chapter 2
Secondary Metabolites in Medicinal and Aromatic Plants (MAPs): Potent Molecules in Nature's Arsenal to Fight Human Diseases

Deepu Pandita and Anu Pandita

2.1 Introduction

Nature has created perfectly complex bioactive chemical compounds that no synthetic chemist could ever imagine (Brower 2008). Plants act as "biosynthetic laboratory" for innumerable active molecules that use physiological effects and provide plants their medicinal assets. Medicinal plants are sources of medicines, and different traditional systems of medicine are available from time immemorial of human civilization (Teiten et al. 2013; Verpoorte et al. 2006). According to the World Health Organization (WHO), beyond 80% of global population in developing countries relies mostly on plant-based traditional drugs and medicines for primary healthcare necessities (Vines 2004). The World Health Organization (WHO 1993) accepted significance of traditional medicine-based novel drug discovery in the contemporary medicines. In the West, 25% of pharmaceutical molecules have plant origin (Payne et al. 1991). Till 1991, only 5000 species out of 250,000–300,000 were investigated for medicinal properties (Payne et al. 1991). The broad investigation on the medicinal plants from 500,000 existing diversities of the plant kingdom will lead to sequestration of the novel bioactive phytocompounds for the forthcoming drug molecule repository findings in the modern medicine system (Fowsiya and Madhumitha 2017; Ngo et al. 2013). The oldest record of plant based medicines was 5000–3000 BC in Alps from Ötzi by Iceman (Inoue and Craker 2014). Some 40,000 plant species find ethnomedicinal use (Trease and Evans 2002). Out of 20,000 Indian medicinal plant species, 800 were phytochemically used for treatment of diseases (Kamboj 2000). Modern synthetic medicines and antibiotics have

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become available only during the last 150 years (Bellamy and Pfister 1992; Hänsel et al. 2007; Mutschler et al. 2008; Schmeller and Wink 2008). Before that humans mostly relied on plant-based natural drugs. The goldmine of well-recorded medicinal and aromatic plants is available for traditional Chinese, Indian (Ayurvedic, Siddha, and Unani), African, Kampo, American, European, and Australian herbal medicine systems (Bruneton 2002; Chevallier 2001; Duke 2002; Saller et al. 1995; Tyler 1993, 1994; Van Wyk and Wink 2004, 2015; Van Wyk et al. 2015; Wagner et al. 2007; Weiß and Fintelmann 2002; Wichtl and Bisset 2000; Wiesenauer 2012). In industrialized countries complementary and alternative medicine (CAM) systems were adapted by 65% of the population (Baser 2005). For promotion of the appropriate utilization of the traditional medicinal/CAM systems, the World Health Organization has expanded Traditional Medicine Strategy focusing on policy, safety/quality/efficacy, access, and rational use of traditional medicinal/CAM systems (WHO 1998, 2002, 2013). The cure of infections and syndromes in humans with herbal medicines involves low molecular weight active natural plant secondary metabolites (Bruneton 2002; Chevallier 2001; Duke 2002; Saller et al. 1995; Tyler 1993, 1994; Van Wyk and Wink 2004, 2015; Van Wyk et al. 2015; Wagner et al. 2007; Weiß and Fintelmann 2002; Wichtl and Bisset 2000; Wiesenauer 2012; Bown 1995; Bejeuhr 1994, 1998).

Secondary metabolites of plants are chemical compounds produced from pathways of the primary metabolism. Secondary metabolites are "bioactive compounds eliciting pharmacological or toxicological effects in man and animals." Albrecht Kossel proposed the concept of secondary metabolites for the first time and received Nobel Prize for Physiology or Medicine in 1910 for this pioneering research (Jones and Kossel 1953). Later on secondary metabolites were termed end products of nitrogen metabolism by Czapek, produced due to secondary modifications, for instance, deamination (Bourgaud et al. 2001). The secondary metabolite-rich medicinal plants with wide array of biological and pharmacological properties act as an alternate route for the drug discovery. Plant metabolome contains approximately 4000 compounds, and there are over 2,000,000/2,140,000 known plant secondary metabolites in plant kingdom with different functions (Bino et al. 2004; McMurry 2015; Sepulveda et al. 2003). Morphine was isolated from opium poppy plant in 1804 by Friedrich Serturner which led to the concept that plant bioactive compounds have therapeutic properties (Busse 2006; Jonsson et al. 1988; Kinghorn 2001; Samuelsson 2004).

Medicinal and aromatic plants (MAPs) include variety of species which produce numerous natural plant-based compounds essential for mankind. A lot of plants are bestowed with the traits of both the aromatic and medicinal plants, but all plant kingdom members do not own this twofold quality. Essential oils and aromatic chemicals may possess antibacterial, anti-inflammatory, expectorant, and sedative properties (Krings and Berger 1998; Rowe 2004). There are various instances of non-aromatic but medicinal plants which produce active metabolites with pharmacological properties, for instance, *Digitalis* spp. Some other plants, for instance, *Jasminum* spp., are principally known as aromatic plants and used because of their valuable aroma (Barata et al. 2011). Medicinal plants, aromatic plants, and medicinal and aromatic plants (MAPs) are three different groups of plants with prominent differences as given in Table 2.1.

Table 2.1 Medicinal plants, aromatic plants, and medicinal and aromatic plants (MAPs)

Medicinal plants	Aromatic plants	Medicinal and aromatic plants (MAPs)
Medicinal plants constitute an independent unit in plant systems of economic botany (Simpson and Conner-Ogorzaly 1986), e.g., *Digitalis* spp.	Aromatic plants have pleasant taste and odor and include spices, condiments, and flavoring plants, e.g., *Jasminum* spp.	MAPs are bestowed with the properties of both the medicinal plants as well as the aromatic plants
Biologically active substances or drug constituents with medicinal value by Western standards can be isolated from the medicinal plants (Farnsworth and Solejarto 1991)	Essential oils, flavors, and fragrances can be extracted from aromatic plants at industrial level	MAPs include products ranging from traditional and modern medicines, healthcare products, and food industry to cosmetics (Hornok 1992)

2.2 Primary Metabolites Versus Secondary Metabolites

On the basis of the biological prerequisites in plant kingdom, naturally occurring bioactive compounds may be generally categorized into the primary metabolites and secondary metabolites (SMs). Primary metabolic pathways, for instance, Calvin cycle, glycolysis, Krebs cycle, and photosynthesis, in plants synthesize primary metabolites mainly carbohydrates, lipids, amino acids, proteins, and nucleic acids with the help of chlorophylls (Weinberg 1971). The primary metabolites are ubiquitous in plant kingdom which play vital biological functions like reproduction, growth, and development required for basic metabolism and plant survival (Fraenkel 1959). With secondary metabolism, the medicinal plants harvest a wide range of special, small, active principles of Secondary metabolites which do not make molecular skeleton of plant but play special roles in plant metabolism including defensive and self-defense functions in plants by interacting with ecosystems, specialized structures, and in reproduction (Graham et al. 2006; Stamp 2003; Vágujfalvi 1992). Secondary metabolites show low abundance, specificity, and non-ubiquitous nature. Some secondary metabolites known as accessory health factors are exploited for promoting public health in addition to basic nutrition (Combs et al. 1996; Diplock 1999; Krzyzanowska et al. 2009; Wahle et al. 2009). The variance between primary and secondary metabolites is indistinct, as metabolites like amino acid and sterol intermediates are overlapping with mosaic nature suggesting biosynthesis through common biochemical pathways. Primary metabolite amino acids act as a secondary metabolite as well. The secondary metabolite sterols are part of primary structural framework of cells (Verpoorte et al. 2000; Yeoman and Yeoman 1996).

2.3 Classification of Secondary Metabolites

Above 200,000 known plant-derived secondary metabolites are subdivided into nitrogen and nonnitrogen compounds on the basis of diversity in chemical structure, composition, function, and biosynthesis (McMurry 2015; Sepulveda et al. 2003).

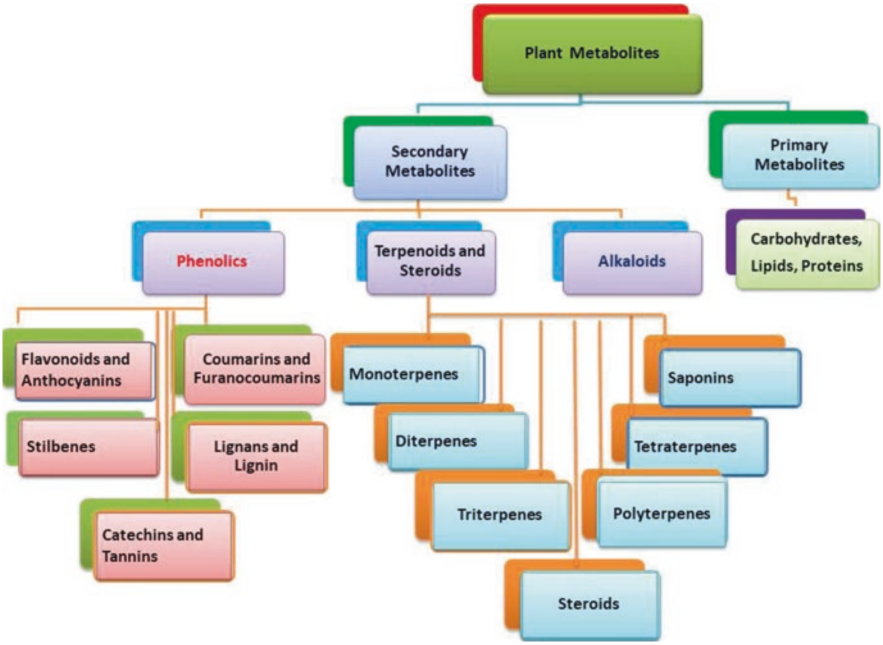

Fig. 2.1 General classification of secondary metabolites

The nitrogen secondary metabolites consist of alkaloids, whereas nonnitrogen secondary metabolites comprise terpenoids, steroids, and phenolics produced by biosynthetic pathways of the malonic, shikimic, and mevalonic acid (Poiroux-Gonord et al. 2010; Wink 2015). The alkaloids (12,000) have one or more nitrogen atoms synthesized from the amino acids. The phenolic compounds (8000) are biosynthesized by pathway of the shikimic acid or malonate/acetate (Rodney et al. 2000). The general classification of secondary metabolites is given in Fig. 2.1.

A: Nitrogen-Free Secondary Metabolites

2.3.1 Terpenoids and Steroids

At present, over 35,000 terpenoid and steroid compounds are characterized (Thirumurugan et al. 2018). Terpenoids have diverse range of distinct structures, whereas steroids (C27) share tetracyclic carbon skeleton and are modified terpenoids derived from the triterpene lanosterol (Teuscher and Lindequist 2010; Thirumurugan et al. 2018).

2.3.1.1 Terpenoids/Terpenes

Terpenoids are the most essential, common worldwide, and major group of secondary metabolites among the main plant species. The name terpenoid has originated from turpentine acquired from pine resins (Breitmaier 2006). Around 25,000 terpenoids are derived biosynthetically from five-carbon precursor isopentenyl diphosphate (IPP) by mevalonic acid biosynthetic pathway (Rodney et al. 2000; Thirumurugan et al. 2018). The chemical nature of terpenoids is unsaturated hydrocarbon backbone in liquid form typically accessible in plant resins and essential oils (Firn 2010). The basic building block of terpenoid structure includes branched five-carbon (C5) unit known as the isoprenoid biosynthesized from acetyl-CoA or 3-phosphoglycerate. Repetitive coupling of the isoprene C5 unit gives rise to diverse categories of terpenoid molecules, for instance, hemiterpene (C5), monoterpene (C10) (geraniol), sesquiterpene (C15) (farnesol), diterpene (C20) (geranylgeraniol with having two geraniols), triterpene (C30) (squalene having two sesquiterpenes), teraterpene (C40) (carotenoids with two diterpene units), and polyterpenes (rubber with many isopentenyl pyrophosphates). Terpenes show cytotoxic activities against bacteria, fungi, insects and vertebrates, and infections (Teuscher et al. 2012). In plants terpenes play different roles in plant defense growth and development (Ashour et al. 2010), stem elongation, seed germination, flowering, seed dormancy, senescence, photo-oxidation (Sharoni et al. 2004), and other functions (Aerts and Mordue 1997; Davies et al. 2007; Thomas et al. 2005).

Monoterpenes

Monoterpenes with an aromatic fragrance are broadly found in various gymnospermic and angiospermic families such as Asteraceae, Burseraceae, Apiaceae, Lamiaceae, Dipterocarpaceae, Myricaceae, Poaceae, Myristicaceae, Verbenaceae, and Rutaceae and resin of conifers. In angiosperms, monoterpenes attract pollinators. Monoterpenes can be isolated as essential oils from plants which find use in aroma therapy and phytomedicines for treatment of rheumatism, infections, cold, flatulence, and spasms, as stomachic, and for improvement of taste (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015). Monoterpenes are volatile and non-volatile (iridoids). Volatile monoterpenes are part of essential oils which are mixtures of monoterpenes, sesquiterpenes, and phenylpropanoids. Monoterpenes with double bonds can be cyclic, non-cyclic, and bicyclic linear type with substituents of hydroxyl, carbonyl, and acetoxy. Thujone monoterpenes (*Tanacetum vulgare*, *Artemisia absinthium*, *Thuja spec.*) alkylate proteins of neuronal signal transduction and cause neuronal disorders (Teuscher and Lindequist 2010; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015). Sabinene and sabinol (*Juniperus sabina*) bind with protein SH groups and change their conformation. Dialdehydes polygodial and warburganal (*Polygonum hydropiper*, *Drimys aromatica*, *Warburgia salutaris*) and cynaropicrin, helenalin, lactupicrin, and parthenolide sesquiterpene

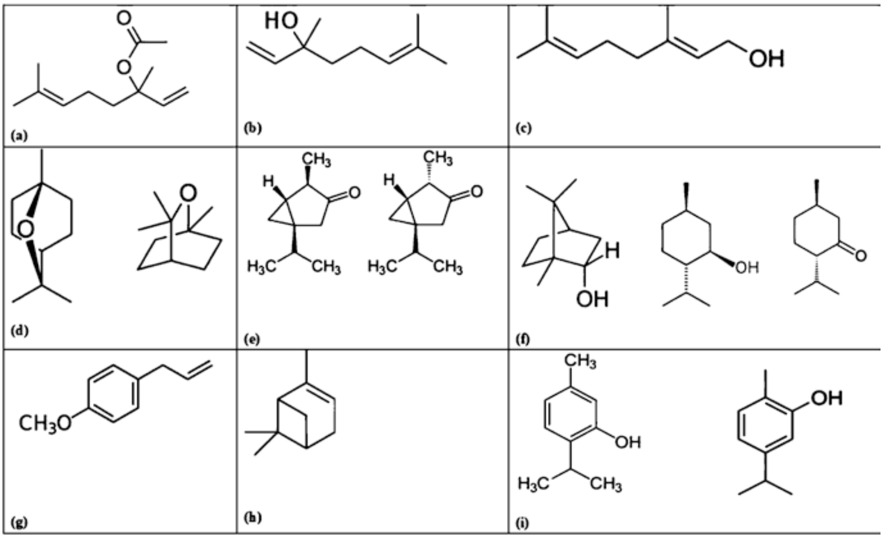

Fig. 2.2 Chemical structures of some monoterpenes: (**a**) linalyl acetate (*Lavandula angustifolia*; (**b**) linalool (*Ocimum basilicum*, *Lavandula angustifolia*); (**c**) geraniol (*Lavandula angustifolia*, *Cymbopogon citratus*); (**d**) eucalyptol/1,8-cineole (*Rosmarinus officinalis*); (**e**) (−)-α-thujone, (+)-β-thujone (*Salvia officinalis*); (**f**) borneol, menthol, menthone (*Mentha spicata*, *M. piperita*); (**g**) estragole (methyl chavicol) (*Ocimum basilicum*); (**h**) pinene (*Pinus* spp.); (**i**) thymol, carvacrol (*Thymus* spp., *Monarda punctata*, *Origanum vulgare*, *Satureja Montana*)

lactones (Asteraceae, Apiaceae, Menispermaceae, Magnoliaceae, Lauraceae and fern pteridophytes) (Teuscher et al. 2012; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015) have anti-inflammatory properties. Sesquiterpene lactones (*Achillea*, *Matricaria*, *Arnica*, *Parthenium*) behave as anti-inflammatory, antibacterial, expectorant, antifungal, and antiparasitic (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015). Monoterpenes of *Mentha* sp. can be used as relaxants against spasms and cramps (Teuscher et al. 2012; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015). The chemical structures of some of the important monoterpenes are drawn in Fig. 2.2.

Diterpenes

Diterpenes have a lot of forms. Cyclic compounds are produced from geranylgeranyl pyrophosphate. Several central diterpene skeletons include abietane, kaurane, clerodane, cembrane, taxane, and tiglane. The species of genera like *Croton*, *Daphne*, *Euphorbia*, and *Thymelaea* contain highly toxic diterpenes. Tanshinones I and II (*Salvia miltiorrhiza*) aid in urea excretion and angina pectoris, marrubiin (*Marrubium vulgare*) is expectorant and choleretic; forskolin (*Coleus forskohlii*), grindelic acid (*Grindelia squarrosa*, *G. robusta*, *G. humilis*),

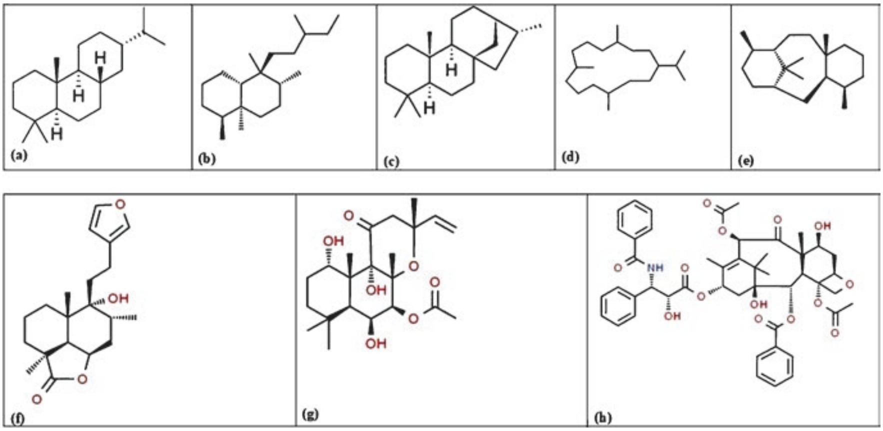

Fig. 2.3 Basic structure skeletons of diterpenes: (**a**) abietane, (**b**) clerodane, (**c**) kaurane, (**d**) cembrane, (**e**) taxane. Chemical structures of some diterpenes: (**f**) marrubiin (*Marrubium vulgare*), (**g**) forskolin (*Coleus forskohlii*), (**h**) taxol (*Taxus brevifolia*)

and taxol (*Taxus brevifolia*) possess anticarcinogenic potential. Phorbol esters (Euphorbiaceae and Thymelaeaceae) have toxic, purgative, and tumor-promoting effects (Teuscher and Lindequist 2010; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015), whereas phorbol esters of *Daphne mezereum* produce laxatives and blister-forming drugs (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010); romedotoxin and atractyloside (Ericaceae, *Pieris*, *Gaultheria*, *Ledum*, *Kalmia*, *Rhododendron*) are neurotoxic and cause bradycardia, hypotension, or death (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010); and atractyloside (*Atractylis gummifera*) blocks energy supply of cells and organisms (Wink and van Wyk 2010). The chemical structures of some diterpenes are drawn in Fig. 2.3.

Triterpenes

Triterpenes which are mainly the glycoside and ester members are produced from 30C atoms comprising squalene present in certain fish oils and some plants. The important triterpene skeletons of medicinal plants are ursane, lupine, and oleanane pentacyclic triterpenes and tetracyclic dammarane which may contain double bonds, carboxylic groups and hydroxyl groups (ursolic acid, oleanolic acid), or oxo groups. Saponins occur as triterpenes and steroids in various species. Hederacoside C (*Hedera helix*) causes skin disorders, whereas asiatic acid (*Centella asiatica*) causes venous insufficiency and skin disorders. Protoprimulagenin A and echinocystic acid (*Primula veris*) treat cough and skin disorders. Presenegenin glycosides (*Polygala senega)* have antitussive effects against cough. Bioactive steroids phytoecdysones (*Achyranthes*, *Ajuga*, *Podocarpus*, *Rhaponticum*, *Silene*, *Vitex*) show

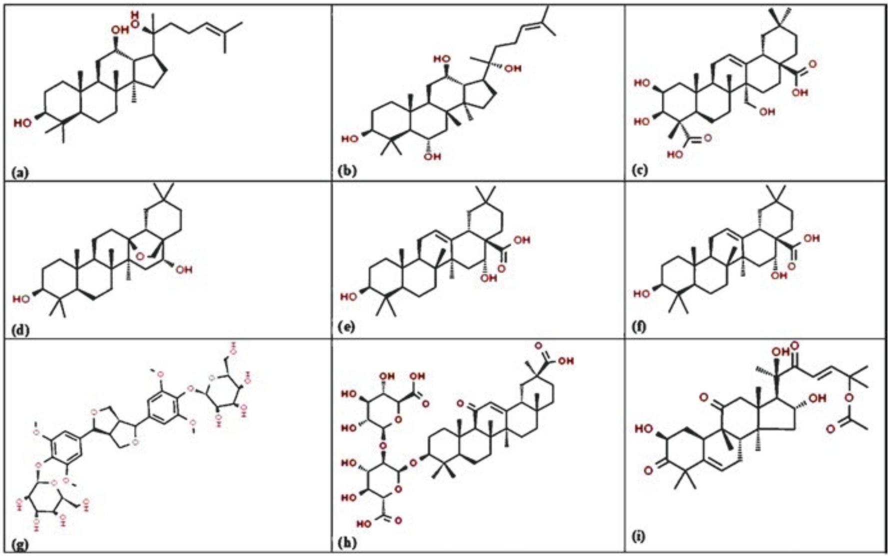

Fig. 2.4 Chemical structures of some triterpenes: (**a**) protopanaxadiol (*Panax ginseng*), (**b**) protopanaxatriol (*Panax ginseng*), (**c**) presenegenin glycoside (*Polygala senega*), (**d**) protoprimulagenin A (*Primula veris*), (**e**) echinocystic acid (*Primula veris*), (**f**) faradiol (*Calendula officinalis*), (**g**) eleutheroside I-M (*Eleutherococcus senticosus*), (**h**) glycyrrhizin (*Glycyrrhiza glabra*), (**i**) cucurbitacins (Cucurbitaceae)

antiherbivore defense (Wink and van Wyk 2010). Steroidal glycosides, cucurbitacins (Cucurbitaceae), show cytotoxic, diuretic, abortifacient, and anti-rheumatism effect and inhibit tumor growth and nasopharyngeal carcinoma and muscle pain (Teuscher and Lindequist 2010; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). The chemical structures of some triterpenes are drawn in Fig. 2.4.

Tetraterpenes

Carotenoids (600 naturally occurring) which denote the most significant tetraterpenes with documented health benefits are extremely lipophilic compounds associated with biomembranes. Carotenoids can be acyclic (such as lycopene) or have one or two cyclic rings (such as β-carotene and provitamin A). Tetraterpenes in chloroplasts function as accessory pigments which play a central role in photosynthesis and give plants protection from UV radiation. Carotenoids in nutrients and drugs are potent antioxidants (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Carotenoids are vitamin A precursors to produce retinal and retinoic acid. Some carotenoids are ABC transporter inhibitors which when applied together with cytotoxic drug lead to reversal of drug resistance (Eid et al. 2012). The chemical structures of some tetraterpenes are drawn in Fig. 2.5.

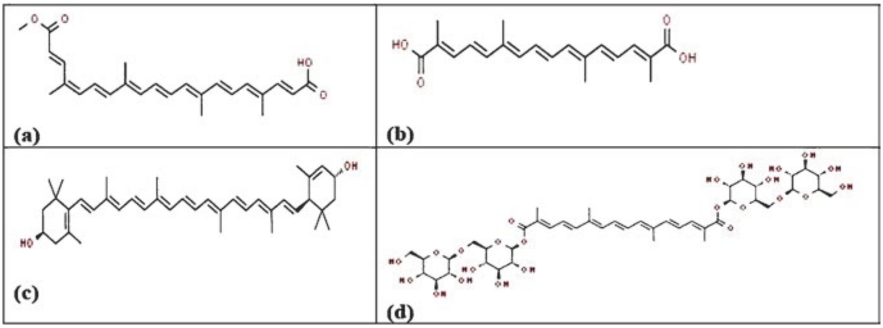

Fig. 2.5 Chemical structures of some tetraterpenes: (**a**) bixin (*Bixa orellana*), (**b**) crocetin, (**c**) crocin (*Crocus sativus*), (**d**) lutein (*Tagetes erecta*)

Polyterpenes

These consist of isoprene units which are 100–10,000 in number. Polyterpenes are noticeable in latex of families of Apocynaceae, Euphorbiaceae, Moraceae, Asteraceae, and Sapotaceae. Rubber (*Hevea brasiliensis*) or gutta-percha of family Euphorbiaceae is commercially used polyterpene (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010).

Saponins

Saponins are bioactive components which produce foam over the surface on shaking with water due to drop in surface tension caused by grouping of the lipophilic sugar moiety (Guclu-Ustundag and Mazza 2007). The saponins occur as steroid glycosides or polycyclic triterpenes (Matsuura 2001) in plants, for instance, *Gynostemma* or *Bupleurum*, *Panax*, *Salvia*, *Astragalus*, Boussigaultia, and litchi, and include cardiac glycosides with additional five- or six-membered cardenolide or bufadienolide ring functional groups and steroidal alkaloids. Steroidal saponins from garlic and allied species exhibit antifungal and antitumor activity and blood coagulation effects (Purmova and Opletal 1995). Steroid saponins are typical for a number of monocot families and Araliaceae, Plantaginaceae, Fabaceae, Solanaceae, and Scrophulariaceae dicot members. Triterpene saponins are copious in a number of dicot families, for instance, Amaranthaceae, Phytolaccaceae, Caryophyllaceae, Poaceae, Ranunculaceae, Primulaceae, and Sapotaceae and lacking in gymnosperms (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Cardenolides occur in Apocynaceae (*Apocynum*, *Periploca*, *Nerium*, *Strophanthus*, *Xysmalobium*, *Thevetia*), Celastraceae (*Euonymus*), Brassicaceae (*Erysimum*, *Cheiranthus*), Plantaginaceae (*Digitalis*), Convallariaceae (*Convallaria*), and Ranunculaceae (*Adonis*). Bufadienolides are found in Asparagaceae (*Urginea*), Crassulaceae (*Kalanchoe*), and Ranunculaceae (*Helleborus*) (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015). Cardiac glycosides are neurotoxic. Isolated cardiac

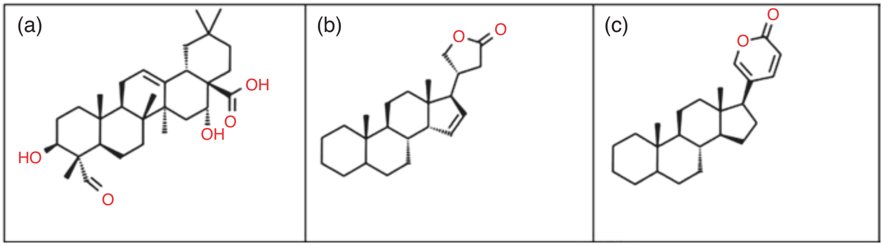

Fig. 2.6 Chemical structures of some saponins: (**a**) quillaja saponins (*Quillaja saponaria*), (**b**) cardenolide (*Apocynum*, *Nerium*, *Periploca*, *Strophanthus*, *Thevetia*, *Xysmalobium*), (**c**) bufadienolide (*Urginea*, *Kalanchoe*, *Helleborus*)

glycoside phytomedicine can be used for treatment of patients with cardiac insufficiency (Mutschler et al. 2008; Teuscher et al. 2012; Teuscher and Lindequist 2010; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Diosgenin, cincholic acid, gypsogenin, acetic acid, oleanolic acid, and hecogenin saponins show hypolipidemia and anticancer properties (Guclu-Ustundag and Mazza 2007). The saponins find use in traditional medicine as anti-infection (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015). Saponins aggravate Nervus vagus in the stomach inducing secretion of water in bronchia. Saponin phytomedicines (*Hedera helix*, *Primula veris*) act as secretolytic agents (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Saponins inhibit lipid peroxide formation in cardiac muscles; change function of liver enzymes; reduce coagulation of blood, cholesterol levels, and blood sugar content; stimulate immune system (Xu et al. 1996); and show chemotherapeutic value (Hecht 2004). Mussaendoside O saponins isolated from *M. pubescens* possess anti-toxicity, hemolytic, and immunopromotive activities (Thakur et al. 2011). The chemical structures of some of the central saponins are drawn in Fig. 2.6.

2.3.1.2 Steroids

Plant steroids include several subgroups such as cardenolides and bufadienolides cardiac glycosides, ecdysterols, sterols, pregnane derivatives, steroid saponins, and steroid alkaloids. Pregnane derivatives occur in *Asclepias* spp. and *Vitex agnus-castus*, and cardiac glycosides occur in several plants (such as *Digitalis*). The β-sitosterol and sigmasterols are ubiquitous in plants in free and ester or glycoside forms. *Urtica dioica* has huge number of sitosterols, and *Cucurbita pepo* contains seven sterols which affect benign prostate hypertrophy. *Dioscorea floribunda*, *Dioscorea composita*, *Dioscorea deltoidea*, and *Dioscorea mexicana* have diosgenin and tigogenin, *Agave sisalana* has hecogenin, and *Trigonella foenum-graecum* has diosgenin glycosides. Diosgenin and hecogenin form initial materials in hormone-steroid industries. The length and composition of cardiac glycoside sugar moiety and substituents on steroid skeleton impacts cardiac activity of glycosides. Purpurea glycosides A–E and lanatosides A–E are glycosides of digitoxigenin, digoxigenin, and gitoxigenin in *Digitalis purpurea* and *Digitalis lanata*. The various species of *Strophanthus* such as *S. kombe*, *S. gratus*, *S. hispidus*, and *S. sarmentosus*, *Convallaria majalis*, and *Adonis vernalis* have strophantidin glycosides. *Drimia maritima* mainly possess bufadienolides like proscillaridin, and *Helleborus odorus* has hellebrigenin as the chief element of aglycones of the cardiac glycosides.

2.3.2 Phenolics

Phenolic compounds contain a low molecular weight single aromatic ring attached by at least one hydroxyl functional group besides, very large, complex and multi-ringed species and produced by shikimate or malonate pathway (Goldberg 2003; Taiz and Zeiger 2002). Polyphenols with multi-phenolic rings and phenolic OH groups occur in most drugs with antioxidant, sedating, anti-inflammatory, antimicrobial, antiviral, and wound-healing properties (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Above 8000 polyphenolic compounds are reported and are further subclassified into flavonoids and non-flavonoids (Crozier et al. 2006; Somasegaran and Hoben 1994). Condensed tannins follow either pathway, while ellagitannins are produced by only shikimate pathway (Salminen and Karonen 2011). Moieties phenylpropanoids and polyketides on condensation generate flavonoids, chalcones, stilbenes, catechins, and anthocyanin compounds having two aromatic rings with phenolic hydroxyl or methoxyl groups (Dewick 2001; Harborne and Baxter 1993). A number of simple phenolics and their plant sources are listed in Table 2.2.

2.3.2.1 Flavonoids and Anthocyanins

Flavonoids, generic name, are the major group of natural polyphenol secondary metabolites with over 7000 flavonoid chemical structures (2000 known) and 500 occurring in free state abundantly present in wide range of plants

Table 2.2 Sources of some simple phenolics

Plant sources	Simple phenolics
Dryopteris filix-mas and other *Dryopteris* species (Polypodiaceae)	Filicic acid, aspidinol, albaspidin which are the taenicide phloroglucinol derivatives
Mallotus philippinensis (Euphorbiaceae)	Antidepressant phloroglucinol derivatives
Hypericum perforatum (Hypericaceae)	Hyperforin
Humulus lupulus	Humulone and lupulone
Cannabis sativa (Cannabaceae)	Cannabinoids with hallucinogenic compound THC (Δ9-tetrahydrocannabinol)
Arctostaphylos uva-ursi (Ericaceae)	Arbutin (D-glucoside of methyl hydroquinone)
Salix purpurea, *S. fragilis*, *S. alba*, *Filipendula ulmaria*	Anti-inflammatory salicin

(Andersen and Markham 2006; Evans 2009; Harborne and Herbert 1999; Yonekura-Sakakibara et al. 2008). Flavonoids, secondary metabolites, have low molecular weight aromatic structural skeleton with chroman ring having an aromatic ring at 2, 3, and 4 positions (Robards and Antolovich 1997). Flavonoids are hydrophilic compounds and complement the hydrophobic carotenoids (Rice-Evans et al. 1997). Flavonoids are divided on the basis of degree of unsaturation and oxidation level of central 3C bridge in flavone skeleton between phenyl groups of flavonoids into various subgroups, for instance, flavanols (epicatechin, catechin, proanthocyanidins), flavanones (naringenin, hesperetin, eriodictyol), flavonols (quercetin, myricetin, kaempferol, isorhamnetin), flavanonols (dihydroquercetin, dihydromyricetin), flavones (apigenin, luteolin), isoflavones (daidzein, genistein), anthocyanidins (delphinidin, cyanidin, malvidin, pelargonidin, petunidin), flavan-3-ols, and proanthocyanidins (Le Marchand 2002; Rhodes 1996). Flavonoids give color to flower petals which attracts the insect pollinators and act as chemical messengers in plants for regulation of physiological function of plant cell. Quercetin, kaempferol, and quercetin flavonols are broadly dispersed over 70% of plant species (Karr 2007). Flavan-3-ols are most complex group in structure and include monomeric catechin, epicatechin, and oligomeric and polymeric proanthocyanidins (Crozier et al. 2006). Flavones and allies are yellow in color and widely distributed in nature along with Polygonaceae, Leguminosae, Rutaceae, Umbelliferae, and Asteraceae. The action of *Chamaemelum nobile*, *Glycyrrhiza glabra*, and *Ginkgo biloba* flavonoids-based drugs is known. *Betula pendula*, *Sambucus nigra*, *Calendula officinalis*, *Tilia cordata*, *Equisetum ramosissimum*, *Leonurus cardiaca*, and *Passiflora edulis* contain flavonoids and have been included in British Pharmacopoeia (Montanher et al. 2007; Serafini et al. 2010). Naringin, neoeriocitrin, and neohesperidin are bitter in taste, while dihydroflavonol taxifolin 3-O-acetate, 6-methoxytaxifolin, and 6-methoxyaromadendrin 3-O-acetate are sweet (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Flavonoids give pigmentation to leaves and fruits, attract and repel insects, and protect plants from herbivores (Iwashina 2003; Koes et al. 2005). Anthocyanins-based blue or red dye is influenced by glycosylation degree, H ion concentration, and existence of some metals in cell vacuole (Dewick 2001). Anthocyanins possess antioxidant value and find use in phytomedicine or nutraceuticals to avoid ROS-linked health syndromes (Teuscher and Lindequist 2010).The chemical structures of some of the important flavonoids are drawn in Fig. 2.7.

2.3.2.2 Stilbenes

Stilbenes have basic skeleton of C6-C2-C6 and are small, heartwood constituents widely distributed in *Pinus*, *Eucalyptus*, and *Madura* (Seigler 1998). Phytoalexins are produced under pathogen attack (Crozier 2008). Para-hydroxylated resveratrol is the most commonly occurring stilbene in nature such as in *Picea*, *Pinus*, Fabaceae, Myrtaceae, and Vitaceae. Resveratrol possesses antioxidant, antibacterial,

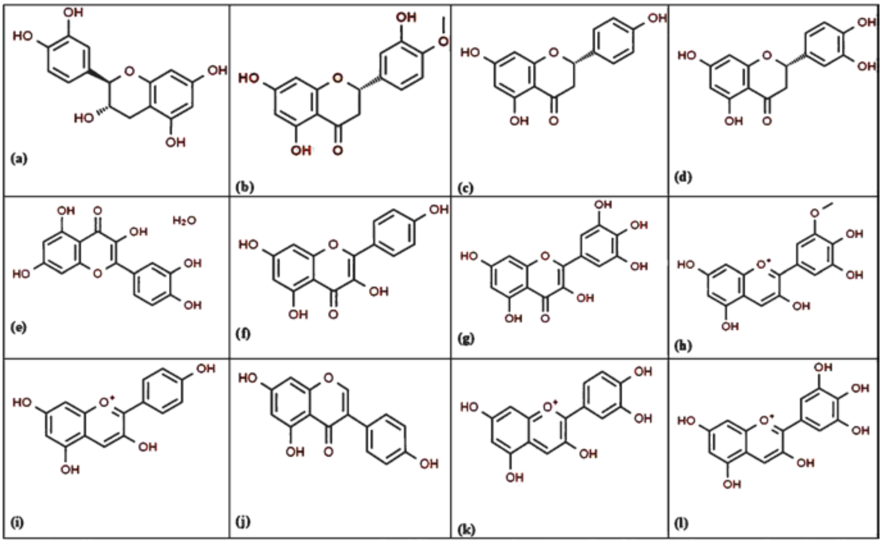

Fig. 2.7 Chemical structures of some flavonoids: (**a**) catechin, (**b**) hesperetin, (**c**) naringenin, (**d**) eriodictyol, (**e**) quercetin, (**f**) kaempferol, (**g**) myricetin, (**h**) petunidin, (**i**) pelargonidin, (**j**) genistein, (**k**) cyanidin, (**l**) delphinidin

antifungal, and estrogen-like potential, improves lipid profile and glucose levels in high-fat diets, and makes several drugs and nutraceuticals (Gehm et al. 1997; Rocha et al. 2009; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Isoliquiritigenin and glyceollin II inhibit mitochondrial monoamine oxidase and uncouple mitochondrial oxidative phosphorylation. Chalcone O-glycoside phloridzin of *Pieris* and *Rhododendron* inhibits the transport of glucose at biomembranes (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010).

2.3.2.3 Catechins and Tannins

Catechins make a distinct flavonoids class which frequently undergo dimerization to procyanidins or polymerization to oligomeric procyanidins. Tannins which precipitate proteins have two main types: hydrolyzable tannins and non-hydrolyzable or condensed tannins. The conjugate or condensed tannins or proanthocyanidins have structures according to oligomeric flavonoid precursors with more than 10 OH groups. The tannin-protein interactions allow application of plants with catechins in phytotherapy. *Crataegus monogyna* can be used in heart complications (Teuscher et al. 2012; Teuscher and Lindequist 2010; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Hydrolyzable tannins are formed from gallic and hexahydroxydiphenic acids and have two types, gallotannins (made of gallic acid) and ellagitannins (made of ellagic acid). Geraniin ellagitannins were isolated from *Geranium robertianum* and *G. maculatum*, and tellimagrandins 1 and 2 were isolated from *Punica granatum*, *Quercus alba*, and *Filipendula ulmaria* (Catarino et al. 2017; Evans 2009; Yi et al. 2004). Gallotannins have esters between gallic acid and sugars along with numerous gallic acid moieties linked by ester bonds. Gallotannins find wide distribution in bark, leaves, and fruits of plants. Gallotannins can be condensed with catechins having numerous phenolic hydroxyl groups to form stable protein-tannin complexes and show interaction with various protein targets in microbes and animals (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). *Quercus infectoria* and other *Quercus* spp., *Rosa gallica*, *Alchemilla glabra*, *Hamamelis virginiana*, *Krameria triandra*, *Potentilla erecta*, *Crataegus monogyna*, *C. laevigata*, *Polygonum bistorta*, *Rubus* spp., *Geum urbanum*, *Fragaria vesca*, *Uncaria tomentosa*, *Vitis vinifera*, *Acacia catechu*, *Corylus avellana*, and *Rhus hirta* are source of tannins. *Camellia sinensis* and *Hamamelis virginiana* contain both hydrolyzable and non-hydrolyzable tannins (Puneet et al. 2013). Tannins are used both in traditional and modern phytotherapy and have antioxidant, anti-inflammatory, anti-diarrheal, antiparasitic, antibacterial, antifungal, cytotoxic, and antiviral potential. *Agrimonia*, *Krameria*, *Alchemilla*, *Potentilla*, *Fragaria*, *Ribes*, *Quercus*, and *Sanguisorba* have anti-inflammatory and anti-infection properties (Teuscher et al. 2012; Teuscher and Lindequist 2010; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010).

2.3.2.4 Coumarins and Furanocoumarins

Coumarins and furanocoumarins (FC) are derived from building blocks of phenylpropanoids (benzo-α-pyrone, lactone of O-hydroxycinnamic acid). Over 700/1000 aromatic structures of coumarins have been determined (Dewick 2001). Coumarins occur in 150 species of 30 families. Richest sources of coumarin are *Dipteryx odorata*, *Melilotus* spp. and *Galium odoratum* (Hoffmann 2003), *Atropa belladonna*, *Daphne mezereum*, *Datura stramonium*, *Ruta graveolens* and *Aesculus hippocastanum*, and *Anthoxanthum odoratum* (Poaceae) and Rosaceae, Apiaceae, Asteraceae, Rutaceae, and Fabaceae families (Evans 2009). Aesculetin in *Aesculus hippocastanum*, melilotsid in *Melilotus officinalis*, khellin (furanochromone) and visnadin in *Ammi visnaga*, *A. majus*, *Hieracium pilosella*, *Angelica archangelica*, *Galium odoratum*, *Dipteryx odorata*, and umbelliferone, scopoletin, umbelliferone, and herniarin coumarins are common in plant kingdom. Furanocoumarins have a 3rd furan ring and linear (psoralen) and angular (angelicin) types. These are abundantly found in Apiaceae (up to 4%), Rutaceae, and *Psoralea bituminosa* (Fabaceae) (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Coumarins are anti-inflammatory, antimicrobial, anticoagulant, anti-edemic, anticancer, and anti-Alzheimer's (Teuscher et al. 2012; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010; Xu et al. 2015). Furanocoumarins are mutagenic and carcinogenic. 8-MOP is used for treatment of psoriasis and vitiligo (Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010).

2.3.2.5 Lignans and Lignin

Lignans are complex dimeric structures of phenylpropanoids with four subtypes (dibenzylbutane derivatives, dibenzyl butyrolactones, monoepoxy lignans, and bisepoxylignans) and occur mainly in *Achillea lingulata* (Asteraceae) (Saeidnia et al. 2011), *Cedrus deodara* (Pinaceae) (Sharma et al. 2008), and *Fagara heitzii* (Rutaceae) (Mbaze et al. 2009). Lignans are antimicrobial and antifungal (Seigler 1998). *Cedrus deodara* containing wikstromal, dibenzyl butyrolactol, and matairesinol are cytotoxic (Sharma et al. 2008).

Vixcum album and *Eleutherococcus senticosus* lignans have adaptogenic potential. Podophyllotoxin which is an inhibitor of microtubule formation with potent anticancer effect occurs in *Anthriscus*, *Linum*, and *Podophyllum peltatum*. Pinoresinol and allied compounds are cAMP phosphodiesterase inhibitors, insecticidal, cytotoxic, and immune modulating (Teuscher and Lindequist 2010; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Silybin, silandrin, and silychristin flavanolignans from *Silybum marianum* (Asteraceae) are hepatoprotective and useful in poisoning of *Amanita* and damages due to liver cirrhosis. *Schisandra chinensis* (Schisandraceae) is effective against hepatitis (Teuscher et al. 2012; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010).

B. Nitrogen-Containing Secondary Metabolites

2.3.3 Alkaloids

Alkaloids represent the most active and are composed of organic compounds (protein molecules) carbon, oxygen, and hydrogen with one or several nitrogen atoms in a heterocyclic ring structure (true alkaloids) or in a side chain (pseudoalkaloids). The true alkaloids have N-atom (originated from essential amino acids) in a heterocyclic ring. Sometimes phosphorus, chlorine, sulfur, and bromine also exist (Hegnauer 1963; Nicolaou et al. 2011). There are over 12,000 recognized natural alkaloids present in 20% of plant species, and their basic structure contains basic amine group (Thirumurugan et al. 2018). The nitrogen of the nitrogen-based alkaloids protect plant cells from bacteria, viruses, or microorganisms, herbivore attacks, ecological turbulences, and climatic changes. On the basis of alkaloid ring structures, subgroups such as aromatics, acridones, bisindoles, carbolines, ergots, ephedras, imidazoles, indolizidines, indoles, manzamines, oxindoles, phenylethylamines, phenylisoquinolines, purines, pyrrolidines, piperidines, pyrrolizidines, pyridines, pyrroloindoles, quinozolines, quinolines, and tetrahydroisoquinolines are available (Dewick 2001; Tadeusz 2015). Alkaloids are predominant in roots and seeds of families such as Chenopodiaceae, Boraginaceae, Berberidaceae, Lauraceae, Menispermaceae, Magnoliaceae, Convolvulaceae, Ranunculaceae, Fumariaceae, Papaveraceae, Rutaceae, Apocynaceae, Rubiaceae, Loganiaceae, and Solanaceae and subfamilies such as Leguminosae, Lobelioideae, and Senecioneae

(Evans 2009). The alkaloids have pharmacological properties of central nervous system (CNS) stimulation, anesthesia effects (Madziga et al. 2010), anticancer and chemotherapeutic potential of the mutagenic paclitaxel or camptothecin and vinca alkaloids, and have antibacterial, antifungal, and antiviral nature (Teuscher and Lindequist 2010; Wink and van Wyk 2010). Different alkaloids, codeine and sanguinarine (Papaveraceae) and pyrrolizidine (Asteraceae), show various biological effects (Crozier 2008; Roberts et al. 2010). Berberine (Berberidaceae genera such as *Argemone mexicana*, *Phellodendron amurense*, *Xanthorhiza simplicissima*) has anti-inflammatory, antiviral, antibacterial, and anticancer effects (Agyapong et al. 2013; Kim et al. 2010; Zha et al. 2010; Zhang et al. 2010). Cocaine (Erythroxylaceae genera such as *Conium maculatum*, *Sarracenia flava*) are neurotoxins (Hajek et al. 2013; Panter et al. 2013), and vinblastine, vinorelbine, vincristine, and vindesine (Apocynaceae genera such as *Catharanthus roseus* G.) have antitumor and antidiabetic effects and lower high blood pressure (Jordan and Leslie 2004; Moudi et al. 2013). The reserpine lowers high blood pressure (Reyburn et al. 2009); tomatine (*Solanum lycopersicum*) has anticancer, antifungal, and immune effects (Gao and Hu 2010; Morrow et al. 2004; Tomsik et al. 2013); and strychnine (*Strychnos nux-vomica*) has anticonvulsant and pesticide effects (Dopham et al. 2014; Jensen et al. 2006; Umukoro et al. 2013). The cytisine (*Cytisus laburnum*) acts as acetylcholine agonist (Porreca et al. 1983; West et al. 2011). Quinines (*Cinchona succirubra*, *Cinchona calisya*, *Cinchona ledgeriana*) are antimalarial, antipyretic, and anti-inflammatory (Adnyana 2013; El-Tawil et al. 2010; Fewell and Roddick 1993; Mwita et al. 2012; Reyburn et al. 2009), whereas atropine (*Mandragora officinarum*) has muscarinic antagonist, anti-myopia, and anti-cholinergic value (Gu et al. 2011; McBrien et al. 2013).

2.3.3.1 Ornithine-Derived Alkaloids

Tropane alkaloids (TAs) are predominant in families of Solanaceae and Erythroxylaceae. The main TA representatives are L-hyoscyamine (its raceme mixture is atropine) and hyoscine (L-scopolamine). Hyoscyamine is CNS stimulant, whereas hyoscine has sedative effect and occurs in *Atropa belladonna*, *Datura stramonium*, *D. sanguinea*, *D. innoxia*, *D. metel*, *Duboisia myoporoides*, *D. leichhardtii*, *Hyoscyamus niger*, *H. muticus*, *Mandragora officinalis*, *Physalis*, *Physochlaina*, *Salpichroa*, *Scopolia carniolica*, *S. japonica*, and *Schizanthus* (Solanaceae). Cocaine, cinnamylcocaine, and α-truxilline are analgesic, and CNS stimulant TAs are present in *Erythroxylum coca* and *E. novogranatense* (Erytroxylaceae). Pyrrolizidine alkaloids have toxic nature, cause hepatic tumors, and occur in *Doronicum*, *Eupatorium*, *Petasites*, *Senecio*, *Tussilago*, *Alkanna*, *Echium*, *Lithospermum*, and *Symphytum*. TA shows parasympatholytic properties, spasmolysis, and loss of motion in the gastrointestinal tract, bladder, and bronchia and inhibits glandular secretions, tachycardia, and accommodation disturbance. TA has a long history of generating hallucinations and intoxication.

Hyoscyamine and scopolamine produce hallucinations and central paralysis and find use as narcosis premedication (Van Wyk and Wink 2004, Wink and van Wyk 2010; Teuscher and Lindequist 2010; 2015; Van Wyk et al. 2015; Teuscher et al. 2012; Wink 2000). Scopolamine cures travel sickness. *Datura* herbal cigarettes were used to cure asthma and respiratory disorders. Atropine cures smooth muscle spasms in GI and urinary tract, gall ducts, and bronchia, bradycardia arrhythmia, and hyperhidrosis and is used as antidote in parasympathomimetic poisoning (Mutschler et al. 2008; Van Wyk and Wink 2004, 2015; Van Wyk et al. 2015; Wink and van Wyk 2010; Teuscher and Lindequist 2010; 2015; Teuscher et al. 2012; Wink 2000).

2.3.3.2 Lysine-Derived Alkaloids

Lysine-derived alkaloids occur as quinolizidine alkaloids (QA) (sparteine, cytosine, lupanine, and anagyrine) in *Cytisus scoparius*. QA are neurotoxins. Examples of few lysine-derived alkaloids are lobeline, lobelanine, lobelanidine (*Lobelia inflata*), piperine (*Piper nigrum*), nicotine (*Nicotiana tabacum*, *N. glauca*, *N. rustica*), and pelletierine (*Punica granatum*). Sparteine treats heart arrhythmia and induces uterus contraction during delivery in humans. The crooked calf disease occurs in female pregnant animals on eating anagyrine-rich herbs (Teuscher et al. 2012; Teuscher and Lindequist 2010; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink 2000; Wink and van Wyk 2010).

2.3.3.3 Phenylalanine and Tyrosine-Derived Alkaloids

Alkaloids of this subgroup arise from phenylalanine and tyrosine. The phenethylamine proto-alkaloids such as mescaline in *Lophophora williamsii*, cathinone (central nervous system stimulant) in *Catha edulis*, and ephedrine in *Ephedra distachya*, *E. sinica*, and *E. gerardina* cure asthma and hay fever. *Colchicum autumnale* has highly toxic colchicine proto-alkaloid which finds use against gout and chromosome multiplication. Hydrastine and berberine isoquinoline alkaloids in *Hydrastis canadensis* cure uterine hemorrhage. *Fumaria officinalis* is choleretic. Phenantridine, protopine, and other alkaloids occur in *Chelidonium majus*, *Escholtzia californica*, *Sanguinaria canadensis*, and *Papaver rhoeas*. Morphine, codeine, thebaine, and papaverine occur in *Papaver somniferum*. Morphine and derivative and opium are traded as illegal drugs.

Various phenylalanine-derived alkaloids of *Galanthus*, *Leucojum*, and *Narcissus* find use in Alzheimer's disease. *Cephaelis ipecacuanha* and *C. acuminate* have emetine, psychotrine, and cephaline. Tubocurarine used as a muscular relaxant is present in *Chondrodendron tomentosa* and *C. candicans*. *Peumus boldus* has antihepatoxic and anti-inflammatory boldine.

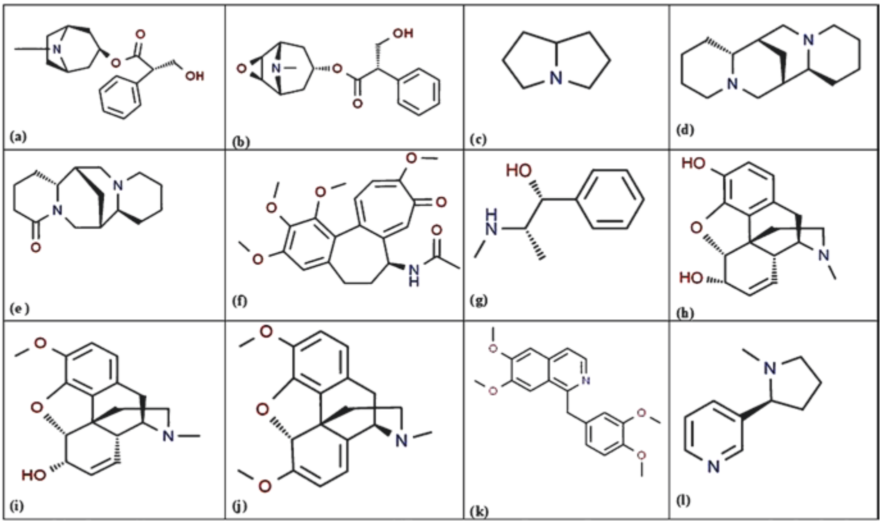

Fig. 2.8 Chemical structures of some alkaloids: (**a**) hyoscyamine, (**b**) hyoscine, (**c**) pyrrolizidine, (**d**) sparteine, (**e**) lupanine, (**f**) colchicine, (**g**) ephedrine, (**h**) morphine, (**i**) codeine, (**j**) thebaine, (**k**) papaverine, (**l**) nicotine

2.3.3.4 Indole Alkaloids

Indole alkaloids derived from tryptophane or tryptamine amino acids are classified into smaller and larger groups. Smaller groups are simple indole alkaloids with substituents on indole ring, e.g., psilocybin in *Psilocybe aztecorum* and toxic cholinesterase inhibitor physostigmine in *Physostigma venenosum*. The indole alkaloids' larger groups can be combined with monoterpene building units. Ergonovine, ergotamine, ergometrine, ergocristine, ergocryptine, and ergocornine of *Claviceps purpurea* are biosynthesized from dimethylallyl pyrophosphate. Monoterpene indole alkaloids are largest alkaloid group. Strictosidine which serves as an intermediate compound of over 2000 indole alkaloids is produced from tryptamine and secologanin (Bruneton 1999).

The chemical structures of some of the important alkaloids are drawn in Fig. 2.8.

2.4 Functions of Plant Secondary Metabolites

Plants exist on Earth from beyond 400 million years and survived and evolved through challenges of the drastic conditions of external abiotic and biotic stresses from herbivores, microbial pathogens, and internal agents. The terminology "secondary" suggests that molecules are less essential for plants, but as a matter of fact, these metabolites play vital functions in plants' adaptation and protection from various biotic or abiotic stresses (Fraire-Velázquez and Balderas-Hernández 2015; Harborne 1999; Pavarini et al. 2012). As a defense mechanism against herbivores and bacteria, fungi, and viruses, thousands of secondary metabolites with different

Table 2.3 Functions secondary metabolites perform in plants

Secondary metabolites	References
Chemical defense mechanism and adaptation of plants to their environments, help plants recover from injury	Harborne (1993), Stamp (2003), Seigler (1998), Crozier et al. (2006), Gershenzon and Mary (1983), Singh (2004), Herms and Mattson (1992) and Taiz and Zeiger (2002)
Protect plants from microbial infections and other pathogen attacks by their repellant or toxic nature to defend against predators, herbivores, pathogens, diseases, and allelopathic agents	Harborne (1993), Seigler (1998), Stamp (2003), Singh (2004), Crozier et al. (2006), Bennett and Wallsgrove (1994), Gershenzon and Mary (1983), Herms and Mattson (1992) and Taiz and Zeiger (2002)
Contributes to specific odors (smell, color, taste) in plants, attracting pollinators in pollination and seed-dispersing animals	Harborne (1993), Stamp (2003); Crozier et al. (2006), Seigler (1998) and Bennett and Wallsgrove (1994)
Function as signaling molecules in plant-plant competition and plant-microbe symbioses or ecological interactions, metal transport, competition	Bennett and Wallsgrove (1994) and Demain and Fang (2000)
Protect plants from ultraviolet radiation	Herms and Mattson (1992) and Taiz and Zeiger (2002)
Tannins contribute to drought tolerance by increasing elastic resilience in plant cell walls	Herms and Mattson (1992) and Taiz and Zeiger (2002)

structures have evolved during plant development (Schmeller et al. 1997; Seigler 1998; Teuscher et al. 2012; Teuscher and Lindequist 2010; Wink 1988, 1993, 2003, 2008, 2010; Wink et al. 1998). Alkaloids have defense toxic chemicals against herbivorous predators and target neuroreceptors at molecular levels or modulate neuronal signal transduction (Teuscher and Lindequist 2010; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink 2000; Wink and van Wyk 2010). Plant secondary metabolites symbolize stimulating library of bioactive molecules sieved by natural selection (Wink 2010). SMs function as antioxidants, antibiotic, antifungal, antiviral, UV protectants of DNA and photosynthetic apparatus, anti-germination phytotoxics, and signal compounds to fascinate pollinators and seed-dispersal agents (Dewick 2001; Hartmann 2007; Mattoo et al. 1999; Seigler 1998; Teuscher et al. 2012; Teuscher and Lindequist 2010; Wallace 2007; Wink 1988; Wink 2003). SMs perform significant ecological roles in plants (Bennett and Wallsgrove 1994), some of which are enlisted in Table 2.3.

2.5 Biological Potential and Health Benefits of Secondary Metabolites of MAPs

Ever since the primeval period, man has used plant life as medicine for curing diseases. The traditional medicine systems are most realistic, effective, and naturally available primary sources of treatment for human disease and ailments (Hosseinzadeh et al. 2015; Madhumitha et al. 2016; Manivel et al. 2009; Roopan and Khan 2010). Nature has a huge repository of valuable drug sources and continues to deliver lead compounds (Grabley and Thiericke 1999; Tulp and Bohlin 2004). Higher plant species (50,000–70,000) find place in traditional and modern medicinal systems throughout the world (Leaman 2008). Pandita et al. (2013a, 2013b) documented 86 medicinal herbs and 105 medicinal tracheophytes from Jammu province (India) with ethnobotanical utilization against a range of health disorders and reviewed the pharmacological and phytochemical properties of monocotyledonous and dicotyledonous antidiabetic medicinal plants of Jammu and Kashmir UT (India) (Pandita et al. 2015a, 2015b. Before the recognition of efficacy of pharmacological active compounds of medicinal plants, the 'Doctrine of Signatures' stating that plant or plant parts resembling body parts can be used to treat disorders of those body parts, used to be practised (Pandita et al. 2016). Secondary metabolites among the natural products are of medicinal value (Dobson 2004). Humans use SMs as medicines, spices, perfumes, arrow poisons, and toxins (Wink 2010). Plant fruits and vegetables contain biologically active phytonutrients used as pharmaceuticals or nutraceuticals and derivatives of secondary metabolism (Bartosz 2003; Bourgaud et al. 2001). Saponins lower cholesterol levels (Aazami et al. 2013), and phenolics show antioxidant and anticarcinogenic effect (Waghorn 2008; Waghorn et al. 1994). Taxol is isolated from *Taxus brevifolia* bark. Taxus species' leaves contain 10-deacetylbaccatin III which acts as substitute of anticancer taxol compounds. Hundred compounds with taxan-skeletons are reported in *Taxus* spp. (Baloglu and Kingston 1999; Oberlies and Kroll 2004). Carotenoids and tocopherols protect against cardiovascular and eye diseases, and ascorbic acid, phenolics, and polyphenols boost immune system and lower cancer risk (Cho et al. 2007). Phenolic compounds are present in all types of fruits, vegetables, tea leaves, and green plants and possess antioxidant, antimicrobial, anticancer, antiseptic, anti-inflammatory (Pengelly 2004; Rauha et al. 2000; Santos et al. 2000), antiviral (Perez et al. 2003), vasodilatory (Padilla et al. 2007), neuroprotective (Nichenametla et al. 2006), fungicidal (Prats et al. 2007), bactericidal (Okunade et al. 1997), anti-atherosclerosis (Tsuda et al. 1997), and cardioprotective effect and improve metabolic disorders (Kishimoto et al. 2013). Epigallocatechin-3-gallate (EGCG) polyphenol in green tea has protective role at cellular levels due to antioxidant potential, and high green tea consumers show reduced risk of Parkinson's disease (Checkoway et al. 2002; Hu et al. 2007) and cognitive impairment (Kuriyama et al. 2006; Suh et al. 2009). Flavonoids are active constituents of numerous herbal medicines (Teuscher et al. 2012; Van Wyk et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). Flavonoids are anti-inflammatory, antiallergic, antithrombotic and vasoprotective, antitumor, anticancer, antiviral, and antioxidant, protect the gastric mucosa, and inhibit low-density lipoprotein oxidation which in turn prevents cardiovascular disorders (Guardia et al. 2001; Hertog et al. 1995; Hooper et al. 2008; Karr 2007; Montanher et al. 2007; Scalbert et al. 2005; Serafini et al. 2010). Isoflavones resemble female sex hormone estradiol hence known as phytoestrogens. They show estrogenic and antioxidant potential and impede tyrosine kinases, thus preventing cancers and menopause in females or osteoporosis-related complications. *Glycine max* and *Trifolium pratense* isoflavones are sold by way of nutraceuticals (Van Wyk

Table 2.4 MAPs and their secondary metabolites and pharmacological properties

Botanical name	Family	Secondary metabolites	Pharmacological effects	References
Acanthospermum hispidum	Asteraceae	Acanthospermol galactoside, flavones, caffeic acid, cis-germacranolides, melampolides, β-caryophyllene	Effective against cardiovascular diseases, allergies, and microbes, anticancer, anti-inflammation	Edewor and Olajire (2011)
Achillea millefolium Linn.	Asteraceae	Sesquiterpenes, flavonoids, cynaroside I, and cosmosiin II	Anti-inflammatory, antiulcer, anticancer, antispasmodic	Ali et al. (2017) and Falk et al. (1975)
Aesculus hippocastanum L.	Hippocastanaceae	*α*- and *β*-escin, protoaescigenin, vanillic acid, prosapogenin, gallic acid, barringtogenol C, hyperoside, protocatechuic acid, chlorogenic acid, hesperidin, quercetin, kaempferol, procyanidin A2	Anti-inflammatory, anti-edemic, antitumor, antiviral, antioxidative, cytotoxic, antidiabetic, gastroprotective, antisecretory, neuroprotective, anticancer, antibacterial, antiobesity, anti-peripheral vascular disorders, anti-SARS-CoV	Bombardelli et al. (1996), Sarikurkcu et al. (2020), Yang et al. (1999), Wilkinson and Brown (1999), Idris et al. (2020) and Prasad et al. (2020)
Aframomum melegueta roscoe K. Schum	Zingiberaceae	Hydroxyphenyl alkaloids	Anti-inflammatory, antimicrobial, antidiabetic, antiobesity, anti-arthritic, antihypertensive and analgesic, antinociceptive, anticancer, anti-heart disease	Amadi et al. (2016)
Ageratum conyzoides Linn	Asteraceae	β-Farnesene, precocene I, β-caryophyllene, eupalestin, kaempferol, sinensetin, quercetin, β-sitosterol, stigmasterol, echinaline, brassicasterol, linalool, lycopsamine, limonene, eugenol	Anti-asthma, anti-inflammatory, antitumor, antispasmodic, antioxidant, hemostatic, analgesic, anti-diarrheal, wound healing, antimalarial, anti-hyperglycemic, antiulcer, antimicrobial	Shailajan et al. (2013) and Okunade (2002)
Agropyron repens (L.) P. Beauv.	Poaceae	Flavonoids, saponins, monoterpenes (carvacrol, carvon, trans-anethole, thymol, menthol), sesquiterpenes	Hypoglycemic, diuretic, hypolipidemic, anti-inflammatory, antimicrobial	Al-Snafi (2015)

(continued)

Table 2.4 (continued)

Botanical name	Family	Secondary metabolites	Pharmacological effects	References
Allium cepa Linn.	Liliaceae	S-trans-prop-1-enyl cysteine sulfoxide (PECSO)	Antimicrobial, analgesic, antioxidant, anti-inflammatory, antihypertensive, antidiabetic, hypolipidemic, immunoprotective	Jones et al. (2004) and Teshika et al. (2018)
Allium sativum Linn.	Liliaceae	S-Allyl cysteine sulfoxide (ACSO), alliin (cysteine sulfoxide), allicin (cysteine thiosulfinate)	Anti-arthritic, digestive, expectorant, febrifuge, antithrombotic, antitumor, hypoglycemic, stimulant, antihypertensive, anti-atherosclerosis, antidiabetic, antimutagenic	Manoharachary and Nagaraju (2016), Thomson and Ali (2003), Sato and Miyata (2000), Jakubowski (2003) and Corzo-Martínez et al. (2007)
Amomum cardamomum L./*Elettaria cardamomum* (L.) Maton	Zingiberaceae	α-Pinene, β-pinene, sabinene, myrcene, α-phellandrene, linalool, limonene, 1,8-cineole, terpinolene, terpinene, cymene	Antioxidant, anticancer, anti-inflammatory, antibacterial, antifungal, antiproliferative, antidiabetic, antiviral	Abu-Taweel (2018)
Ancistrocladus korupensis	Ancistrocladaceae	Naphthylisoquinoline alkaloids korundamine A, getrimine B, korupensamine A–E, michellamine A–E, youndamines A and B	Anti-AIDS, antimalarial, fungicidal, larvicidal	Hallock et al. (1998)
Andrographis paniculata (Burm. F.) Nees	Acanthaceae	Andrographolide, neoandrographolide, 14-deoxyandrographolide	Anticancer, anti-diarrheal, anti-arthritic, anti-HIV, anti-rheumatoid, antipyretic, anti-hepatitis, anti-inflammatory, cytotoxic, antimalarial, antioxidant, hepatoprotective	Tajidin et al. (2019)

Botanical name	Family	Secondary metabolites	Pharmacological effects	References
Anethum graveolens L. (dill)	Apiaceae	Monoterpenes (carvone and limonene), anethole, p-anisaldehyde, α-phellandrene, dill ether, myristicin, coumarins, flavonoids (quercetin, isoharmentin), phenolic acids, and steroids	Carminative, galactogogue, stimulant, stomachic, antisecretory, antiulcer, antioxidant, antibacterial, antihyperlipidemic, diuretic, mucosal protective, cures urinary complaints, piles, and mental disorders	Jana and Shekhawat (2010)
Angelica archangelica Linn.	Apiaceae	β-Phellandrene, furocoumarins (angelicin, bergapten), xanthotoxin, umbelliprenin, R-pinen, β-phellandren, imperatorin, osthole, umbelliferone, δ-3-caren, limonene, R-phellandren	Stimulant, expectorant, diaphoretic, antiviral, anti-inflammatory, anti-dementia, antianxiety activity, antitumor, antifungal, anti-epileptic, hepatoprotective	Kaur et al. (2020)
Artemisia annua Linn.	Asteraceae	Artemisinin, arteanuin, artemether, arteether, artemetin, casticin, chrysoplenetin, cirsilineol	Antimalarial, anticancer, anti-inflammatory, antitumor	Weathers and Towler (2012), Mahomoodally and Fakim (2013), Ferreira et al. (2010), Efferth (2015), Wang et al. (2019) and Woldemichael and Wink (2001)
Atropa belladonna Linn.	Solanaceae	Atropine, hyoscyamine, scopolamine	Antispasmodic, muscarinic, mydriatic, anti-cholinergic, anti-myopia, anti-inflammatory, anti-peptic ulcer, anti-Parkinson's, anti-bronchial spasms	Okigbo et al. (2008), McBrien et al. (2013), Gu et al. (2011) and Singh and Sharma (2020)
Bacopa monnieri	Scrophulariaceae	Bacoside, bacoside A3, bacopaside II, bacopaside I, bacopaside X, bacopasaponin C, bacopaside N2, luteolin, cucurbitacins, apigenin	Antioxidant, sedative, anti-inflammatory, antipyretic, analgesic, anti-arthritic, anti-depression, antiulcer, anti-epilepsy, antitumor, anti-leprosy, anti-hyperglycemic, memory enhancing, anti-epileptic, anticancer, anti-Parkinsonian, anti-Alzheimer's	Sharma et al. (2015), Pareek and Kumar (2014), Kishore and Singh (2005) and Russo and Borrelli (2005)

(continued)

Table 2.4 (continued)

Botanical name	Family	Secondary metabolites	Pharmacological effects	References
Boswellia sp.	Burseraceae	Frankincense di- and triterpenoids	Antineoplastic, inflammatory, anti-Alzheimer's, antidiabetic	Moussaieff and Mechoulam (2009), Brendler et al. (2018) and Byler and Setzer (2018)
Calendula officinalis	Asteraceae	Faradiol-3-O-palmitate, faradiol-3-O-myristate, faradiol-3-O-laurate, arnidiol-3-O-palmitate, arnidiol-3-O-myristate, arnidiol-3-O-laurate, calenduladiol-3-O-palmitate, calenduladiol-3-O-myristate	Anti-inflammatory, immunostimulant, antibacterial, antiviral, antiprotozoal, and antineoplastic, antispasmodic and anti-spasmogenic, antiproliferative	Qiu et al. (2001) and Neukirch et al. (2004)
Cinnamomum osmophloeum	Lauraceae	Lipopolysaccharides, cinnamaldehyde, phenolic acids	Antimicrobial, antidiabetic, hypolipidemic, anti-inflammatory	Jakhetia et al. (2010) and James (2012)
Cleome rutidosperma	Cleomaceae	Terpenes, alkaloids, flavonoids	Analgesic and anti-inflammatory	Abdullah et al. (2016) and Edeoga et al. (2005)
Coffea arabica Linn.	Rubiaceae	Caffeine, xanthines Trigonelline, chlorogenic acids, rutin, quercetin, and kaempferol glycosides	Psychostimulant, central nervous system (CNS) and peripheral nervous system (PNS) and metabolic stimulant, antioxidant	Monteiro et al. (2020)
Crocus sativus L.	Iridaceae	Crocin, picrocrocin, crocetin, carotenes, safranal, mangicrocin, xanthone, anthocyanin, zigzantin, lycopene, quercetin, kaempferol, zeaxanthin, alkaloids, saponins	Anti-asthma, antitumor, anticancer, memory enhancer, antioxidant, antiatherosclerotic, anti-inflammatory, antidepressant, hepatoprotective, immunomodulatory, antidiabetic, antihyperlipidemic, cardiovascular improver	Pandita (2020)
Curcuma longa	Zingiberaceae	Curcumin	Anti-Alzheimer's, choleretic, anti-arthritic, anti-inflammatory, antioxidant	Goutam (2011) and Ng et al. (2006)

Botanical name	Family	Secondary metabolites	Pharmacological effects	References
Cymbopogon flexuosus	Poaceae	Citral, geranium, geranyl acetate, neral, β-myrcene	Anti-inflammatory, antimicrobial, analgesic, antifungal, cytotoxic, anti-rheumatic, antitussive	Boukhatem et al. (2014), Han and Parker (2017) and Adukwu et al. (2016)
Datura stramonium	Solanaceae	Atropine, tropane alkaloids, hyoscamine, scopolamine, tigloidin, aposcopolamine, apoatropine, hyoscyamine N-oxide	Muscarinic, anti-cholinergic, anti-myopia, anti-skin infections, antispasmodics, antifungal, anesthetics, bronchodilators, anti-cholinergic, anti-asthma, anticancer, antimicrobial, anti-inflammatory, relieves ear pain	McBrien et al. (2013), Gu et al. (2011), Soni et al. (2012) and Singh and Sharma (2020)
Digitalis purpurea	Plantaginaceae	Cardenolides (digoxin and digitoxin)	Cardiotonic, anticancer, treats congestive heart failure	Verma et al. (2018)
Dioscorea species	Dioscoreaceae	Diosgenin, protodioscin, methyl protodioscin, parrisaponin, dioscin, progenin III (prosapogenin A of dioscin), progenin II	Antimicrobial, antifungal, antimutagenic, hypoglycemic, and immunomodulatory, anticancer, antidiabetic	Padhan and Panda (2020)
Foeniculum vulgare Mill.	Apiaceae	Furocoumarins imperatorin, psoralen, bergapten, xanthotoxin, isopimpinellin, quercetin, isorhamnetin 3-O-α-rhamnoside, kaempferol	Antimicrobial, antioxidant, analgesic, anti-inflammatory antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycemic, and estrogenic	Ruberto et al. (2000), Eun and Jae (2004) and Singh and Sharma (2020)
Ferula asafoetida Regel.	Apiaceae	Coumarin derivatives ferulic acid esters, ferulic acid	Anthelminthic, antispasmodic, antimicrobial, antiseptic, laxative, diuretic. Used in cough, jaundice, gastritis, and rheumatism	Moghaddam and Farhadi (2015)
Foeniculum vulgare Mill.	Apiaceae	Furocoumarins psoralen, imperatorin, bergapten, xanthotoxin, quercetin, isopimpinellin, kaempferol	Diaphoretic, diuretic, carminative, expectorant, febrifuge, stomachic, stimulant, appetizer, cardiac stimulant, vermifuge	Nassar et al. (2010)

(continued)

Table 2.4 (continued)

Botanical name	Family	Secondary metabolites	Pharmacological effects	References
Galanthus nivalis	Amaryllidaceae	Galantamine, agglutinin	Anti-Alzheimer's, against memory impairment, anti-feline coronavirus	Bores et al. (1996), Howes and Houghton (2009), Hsieh et al. (2010) and Prasad et al. (2020)
Glycyrrhiza glabra L.	Fabaceae	Triterpene, saponin, glycyrrhizic acid, glycyrrhizin, (iso)liquiritin apioside isomers	Antioxidant, antimicrobial, anti-inflammatory, cytotoxic, demulcent, expectorant, flavoring agent for drugs, anti-SARS-CoV	Zhou et al. (2019), Van Wyk and Wink (2004, 2015), Prasad et al. (2020), and Van Wyk et al. (2015)
Huperzia serrata	Lycopodiaceae	Huperzine A	Anti-Alzheimer's disease	Skolnick (1997) and Houghton et al. (2006)
Matricaria chamomilla	Asteraceae	Herniarin, umbelliferone	Spasmolytic, anti-inflammatory, antibiotic	Eliasova et al. (2004)
Mentha arvensis Linn.	Lamiaceae	Monoterpenoids, sesquiterpenoids, menthol, sitosterol, sucroside, menthofurolactone, glycosides, menthone	Useful in fever, cold, cough, indigestion, influenza, asthma, antitumor, antidiabetic, antinociceptive, anti-inflammatory, antifungal, antibacterial, antifertility	Mkaddem et al. (2009), Qian and Wang (2010), Lim et al. (2012), Sharma et al. (2017), Zhao et al. (2018) and Kalemba and Synowiec (2019)
Mentha piperita Linn.	Lamiaceae	Hesperidin, rosmarinic acid, buddleoside, didymin, diosmin, menthol, and menthone	Carminative, spasmolytic, antidiabetic, antitumor, antinociceptive, antibacterial, and antifungal	Zhao et al. (2018) and Kalemba and Synowiec (2019)
Ocimum sanctum L. *Ocimum tenuiflorum* L.	Lamiaceae	Eugenol, rosmarinic acid, carvacrol, oleanolic acid	Anticancer, antidiabetic, analgesic, antifungal, hepatoprotective, anti-arthritic, cardioprotective, chemopreventive, antispasmodic	Prakash and Gupta (2005), and Singh and Chaudhuri (2018) and Flegkas et al. (2019)

Botanical name	Family	Secondary metabolites	Pharmacological effects	References
Panax (ginseng) *species*	Araliaceae	Ginsenosides (panaxoside)/triterpenoids with dammarane skeleton like protopanaxadiol and protopanaxatriol or ginseng saponins	Against cardiac ailments, dyspepsia; aphrodisiac; boosts immune system; antimicrobial, antifungal, anticancerous, antiallergic, anti-Alzheimer's, anti-amnesia, anti-Parkinson, anti-dementia, antidiabetic, antiobesity	Yue et al. (2006), Radad et al. (2004), Park et al. (2003), Leung and Wong (2010), Rudakewich et al. (2001), Joo et al. (2005) and Kaneko and Nakanishi (2004)
Papaver somniferum L.	Papaveraceae	Morphine, thebaine, papaverine, narceine, codeine	Analgesic, narcotic, antitussive, antidepressant; morphine – acts on myenteric plexus, reduces shortness of breath	Heal and Taylor Robinson (2010), Lee et al. (2011), Okigbo et al. (2008), Rozov-Ung et al. (2014) and Simera et al. (2010)
Physostigma venenosum	Fabaceae	Physostigmine	Anti-Alzheimer's	Julian and Pikl (1935), McCaleb (1990) and Sitaram et al. (1978)
Piper nigrum Linn.	Piperaceae	Piperine	Anti-inflammatory, antihypertensive, appetizer, anticonvulsant, antihistaminic, anti-thyroids, hepatoprotective, counterirritant, antiflatulent	Damanhouri and Ahmad (2014)
Rosa laevigata	Rosaceae	Daucosterol, euscaphic acid, betulinic acid, rosamutin, tormentic acid, rubuside	Astringent, anticancer, antibacterial, carminative, stomachic	Mehboob et al. (2017)

(continued)

Table 2.4 (continued)

Botanical name	Family	Secondary metabolites	Pharmacological effects	References
Rosmarinus officinalis	Lamiaceae	Rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid, diosmin, carnosol, epirosmanol, apigenin, diosmin, rosmanol, eriocitrin, luteolin, 3'-O-β-D-glucuronide, hesperidin, isoscutellarein 7-O-glucoside	Antibacterial, antiviral, antioxidant, and anti-inflammatory, analgesic, expectorant, diuretic, antispasmodic, anti-rheumatism, antidepressant, antidiabetic, antitumor, neuroprotective, cholinergic, anti-dyslipidemia, anti-cerebral ischemia, anti-hepato-nephrotoxicity	Andrade et al. (2018) and de Macedo et al. (2020)
Scrophularia ningpoensis	Scrophulariaceae	Aucubin, catalpol, harpagide, harpagoside, cinnamic acid	Antifungal, anti-arthritic, anti-rheumatic, diuretic, antipyretic, febrifuge, antihypertensive, antimalarial	Wang et al. (2010), Tasdemir et al. (2008), Jeong et al. (2008) and Li et al. (2000)
Solanum nigrum Linn.	Solanaceae	(+) Syringaresinol (II), solasodine, (+)-medioresinol (III), scopoletin (IV), nicotine, tetracosanoic acid (V), solanine, beta-sitosterol (IV), steroidal glycosides, β-solamarine, solamargine and solasonine, solanidine	Anti-rheumatism antifungal, sedative, anti-inflammatory, anticonvulsant, stimulant, anticancer, antioxidant, hepatoprotective, antiulcer, antihyperlipidemic, antidiabetic, antibacterial, antitumor, teratogenic	Kenny et al. (2013), Mohsenikia et al. (2013), Jabamalairaj et al. (2019) and Morillo et al. (2020)
Stevia rebaudiana	Asteraceae	Stevioside	Antimicrobial, antiviral, antifungal, antihypertensive, anti-hyperglycemic, antitumor, anti-inflammatory, anti-diarrheal, diuretic, anti-human rotavirus, anti-HIV, hepatoprotective, and immunomodulatory	Singh and Sharma (2020)
Tagetes minuta L.	Asteraceae	Quercetin-3-methyl ether, quercetin-3,6-dimethyl, quercetin, axillarin-7-O-β-Dglucopyranoside	Anti-leishmanial, antimalarial, antispasmodic, antimicrobial, antiseptic, antioxidant, cytotoxic, stomachic, diaphoretic, sedative, antihelminthic	Musayeib et al. (2014) and Gakuubi et al. (2016)

Botanical name	Family	Secondary metabolites	Pharmacological effects	References
Theobroma cacao	Malvaceae	Theobromine, epicatechins, tocopherols, tocotrienols	Diuretic, myocardial stimulant, vasodilator, antibacterial, antiviral, anti-inflammatory, antioxidant, antiallergic, antithrombotic, vasodilatory	Cerri et al. (2019)
Thymus vulgaris L.	Lamiaceae	Thymol, carvacrol linalool, geraniol, α-terpineol, rosmarinic, trans-thujan-4-ol/terpinen-4-ol, caffeic acids	Antiseptic, spasmolytic, antioxidant, antitussive, antimicrobial	Kosakowska et al. (2020)
Trachyspermum ammi	Apiaceae	Thymol, para-cymene, α- and β-pinene, and γ-terpinene	Antispasmodic, diuretic, antioxidant, antinociceptive, antihypertensive, antilithiasis, antitussive, nematicidal	Bairwa et al. (2012)
Trigonella foenum-graecum Linn.	Fabaceae	Trigonelline, saponins, diosgenin	Aphrodisiac, carminative, astringent, demulcent, suppurative, aperients, diuretic, emollient, anti-inflammatory	Mehrafarin et al. (2010)
Vitex negundo Linn.	Verbenaceae	Viridiflorol, β-caryophyllene, sabinene, 4-terpineol, globulol, protocatechuic acid, oleanolic acid, flavonoids	Anti-arthritic, anodyne, appetizer, cephalic, cardiac, astringent, emmenagogue, demulcent, febrifuge, expectorant	Vishwanathan and Basavaraju (2010), Singh et al. (1999) and Surveswaran et al. (2007)
Vitis vinifera		Resveratrol	Anti-Alzheimer's	Marambaud et al. (2005)
Withania somnifera	Solanaceae	Withanine, withananine, somniferine, somnine, withanolides, withaferin A	Antioxidant, antineoplastic, antifibrotic, anti-inflammatory, antiproliferative. Useful in cardiovascular, amnesia, neurodegenerative disorders	Brant (2016), Bharti et al. (2016) and Chauhan and Mehla (2015)
Zingiber officinale Rosc.	Zingiberaceae	Gingerols, zingiberene, geranial, geranyl acetate	Anti-rheumatoid arthritis and osteoarthritis, anti-dyspepsia, anti-anorexia, antispasmodic, anti-diarrheal	Sasidharan et al. (2012) and Badreldin et al. (2008)

et al. 2015; Van Wyk and Wink 2004, 2015; Wink and van Wyk 2010). A summary of various MAPs and their secondary metabolites and pharmacological effects is presented in Table 2.4.

2.6 Conclusion

This chapter discusses the secondary metabolites, biological activities, and medicinal properties of herbal medicines of innumerable MAPs. There has been tremendous in last decade. Secondary metabolites are antibiotic, antifungal, and antiviral and defend plants from various kinds of pathogens. These possess UV-absorbing potential and thus prevent leaf damage from harsh light, and forage grasses (alfalfa) express estrogenic compounds and affect animal fertility (Bennets et al. 1946). Scientific research on MAPs will boost their consumption and demand and is opening new horizons in the potential of herbal-based medicines and other natural products. Secondary metabolites with notable abovementioned positive pharmacological and biological activities find important place in the healthcare system and act as naturally derived molecule substitutes to most of the synthetic medicines and can be efficiently utilized and exploited in the current medicinal research for the drug discovery against dreaded diseases including cancer, coronaviruses like SARS-CoV and FCoV, AIDS, and Parkinson's and Alzheimer's disease.

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Chapter 3
Biodiversity, Management and Sustainable Use of Medicinal and Aromatic Plant Resources

Amrina Shafi, Farhana Hassan, Insha Zahoor, Umer Majeed, and Firdous A. Khanday

3.1 Introduction

Medicinal and aromatic plants (MAPs) are gaining popularity globally as a source of raw material for pharmaceuticals and traditional healthcare system (Azaizeh et al. 2003; Kandari et al. 2012). More than 85% of herbal medicines used in traditional healthcare systems are derived from medicinal plants (Prasad and Bhattacharya 2003; Phondani et al. 2014) and ensure the livelihoods of millions of people (Phondani et al. 2011). Medicinal plants are globally valuable sources of new drugs (Chen et al. 2010; Chacko et al. 2010; Hamilton 2004). There are over 1300 medicinal plants used in Europe, of which 90% are harvested from wild resources; in the United States, about 118 of the top 150 prescription drugs are based on natural sources (Balunas and Kinghorn 2005). Furthermore, up to 80% of people in developing countries are dependent on herbal drugs for their primary healthcare, and over 25% of prescribed medicines in developed countries are derived from wild plant species (Hamilton 2004). With the increasing demand for herbal drugs, natural health products and secondary metabolites of medicinal plants, the use of MAPs is growing rapidly throughout the world (Chen et al. 2010).

Medicinal plants offer alternative remedies with tremendous opportunities to generate income, employment and foreign exchange for developing countries (Rawat and Uniyal 2004). Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent, alleviate or cure several human diseases (Dhar et al. 1999). Consumption of herbal medicines is widespread and increasing in recent years, and approximately 80% of the people in developing

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countries depend on traditional medicines for primary healthcare needs (Phondani et al. 2014). The global market for the medicinal plants and herbal medicine is estimated to be worth US$800 billion a year.

MAP species is increasing with the expanding growth of the human population (Maikhuri et al. 2003). As a result, indiscriminate collection of the MAPs from the wild is occurring, which has threatened the status of several high-value MAP species. This outcome has been widely recognized, and threatened wild species need to be brought under cultivation (Schippmann et al. 2002). As such, production through cultivation can reduce the pressure on wild medicinal plant populations and maintain uniformity in production. Also, the present approach will prevent environmental degradation and loss of genetic diversity in the wild (Dhar et al. 2000; Kala 2005). However, indiscriminate collection and overharvesting of MAP species from their natural habitats have adversely affected their availability. In a wider context, there is a growing demand for plant-based medicines, health products, pharmaceuticals, food supplements, cosmetics, etc. in the national and international markets. Conservation and sustainable use of medicinal plants are issued on which immediate focus is required in the context of conserving biodiversity and promoting and maintaining the health of local communities, besides generating productive (Shinwari and Qaiser 2011).

Therefore, approaches for cultivation, sustainable harvesting and protection against existing threats should be developed for the conservation of MAPs and livelihood enhancement of local communities in that region. This has become a priority agenda as part of meeting the international obligations under the biodiversity convention (Rao et al. 2003). As such, MAP cultivation is emerging as a sector of self-employment (Maikhuri et al. 2001) and an option for livelihood enhancement versus the cultivation of traditional food crops (Nautiyal and Nautiyal 2004; Phondani et al. 2011). Therefore, promoting the cultivation of medicinal and aromatic plants in the farmer's field was attempted (1) to evaluate the yield and cost-benefit analysis of cultivated MAP species and (2) to provide the technical skill to farmers.

The increasing demands for herbal medicines by consumers in both developing and developed countries have renewed interest in the multinational pharmaceutical industry in bio-prospecting. But the lack of national legislation or effective international agreements on conservation and sustainable use of biodiversity has resulted in 'slaughter harvesting' of medicinal plants and massive depletion of biodiversity (Phondani et al. 2011). Medicinal plants are globally valuable sources of herbal products, and they are disappearing at a high speed. This chapter reviews global trends, developments and prospects for the strategies and methodologies concerning the conservation and sustainable use of MAPs resources to provide a reliable reference for the conservation and sustainable use of medicinal plants. We emphasized that both conservation strategies (e.g. in situ and ex situ conservation and cultivation practices) and resource management (e.g. good agricultural practices and sustainable use solutions) should be adequately taken into account for the sustainable use of medicinal plant resources. We have further recommended that biotechnical approaches (e.g. tissue culture, micropropagation, synthetic seed technology and molecular marker-based approaches) should be applied to improve yield and modify the potency of medicinal plants. The present chapter provides a practical example of economic potential and research on MAPs, which may help policy planners at the national and international levels in linking livelihood and socio-economic development with natural resource conservation.

3.2 Medicinal and Aromatic Plants Diversity

Medicinal plants are grouped for any commercial purposes in the broader category of 'medicinal and aromatic plants' (MAPs), covering not only plants used medicinally but also for neighbouring and overlapping purposes, for instance, as foods, condiments and cosmetics (Schippmann et al. 2002). The term 'botanicals' is becoming commonly used for a wide range of plant-based products. In terms of the number of species individually targeted, the use of plants as medicines represents by far the biggest human use of the natural world. Plants provide the predominant ingredients of medicines in most medical traditions. There is no reliable figure for the total number of medicinal plants on Earth, and numbers and percentages for countries and regions vary greatly (Schippmann et al. 2002). More than a tenth of the plant species (over 50,000 species) are used in pharmaceutical and cosmetic products. However, the distribution of medicinal plants across the world is not uniform, and medicinal herbs are mainly collected from the wildlife population. Indeed, the demand for wildlife sources has increased by 8–15% per year in Europe, North America and Asia in recent decades (Verma and Singh 2008). The toxicity and adverse effects of conventional and allopathic medicines have also been important factors in the sudden increase in population demand an increase in the number of herbal drugs manufactures as well as a reduction in the use of chemical drugs (Rasool Hassan 2012). Among these uses, medicinal plants play a central role, not only as traditional medicines used in many cultures but also as trade commodities that meet the demand of often distant markets. Nowadays the centuries-old tradition of medicinal plants' application has turned into a highly profitable business on the world market. Numerous herbal products have been released like patented medical goods, food additives, herbal teas, extracts, essential oils, etc. (Lange 2002, 2004). Traditional ethnomedicinal knowledge is a particular and valuable form of indigenous knowledge; it is a cultural asset that can be used for the recognition and preservation of valuable species as well as the habitats in which they occur (Jules et al. 2008; Gaikwad et al. 2011).

The World Health Organization (WHO) defines traditional MAPs as natural plant materials which are used at least or in the absence industrial processing for the treatment of diseases at a local or regional scale (WHO 1999, 2007a, b, 2009, 2010, 2013; Tilburt and Kaptchuk 2008). Traditional herbal medicine has been used in developing and developed countries for thousands of years because it is natural and causes comparatively fewer complications (Wichtl 2004). Today, according to the WHO, more than 80% of the world's population rely more often on traditional drugs, mainly plants, serving as the main source of health care (Ganesan 2008).

Currently, it is estimated that over 50% of the available drugs are somehow derived from medicinal plants (Yarnell and Abascal 2002; Harvey 2008). Phytotherapy is widely being used across the world on a constantly growing basis. Therefore, the global trend of synthetic compounds has turned to herbal drugs, which we can refer to it as a return to nature to prevent diseases and pains. Nature has been served as the source of medicinal herbs (Fabricant and Farnsworth 2001).

MAPs are important for the livelihoods of poor communities all over the world. Even today, hundreds of millions of people, mostly in developing countries, derive a significant part of their subsistence needs and income from gathered plant and animal products (Firenzuoli and Gori 2007; Singh 2002). The great majority of species of medicinal plants are used only in Folk Medicine. Traditional Scholarly Medical Systems employs relatively few: 500–600 commonly in Traditional Chinese Medicine (Pei 2001); 1430 in Mongolian Medicine (Pei 2002b); 1106–3600 in Tibetan Medicine (Pei 2001, 2002a, b); 1250–1400 in Ayurveda (Singh 2002); 342 in Unani; and 328 in Siddha (Verma and Singh 2008). The number of plant species that provide ingredients for drugs used in Western Medicine is even fewer. Despite the small number of source species, drugs derived from plants are of immense importance in terms of numbers of patients treated. The value of medicinal plants to human livelihoods is essentially infinite. They make fundamental contributions to human health. Medicinal properties in plants are mainly due to the presence of secondary metabolites which the plants need in their natural environments under particular conditions of stress and competition and which perhaps would not be expressed under monoculture conditions. Active-ingredient levels can be much lower in fast-growing cultivated stocks, whereas wild populations can be older due to slow growth rates and can have higher levels of active ingredients. While it can be presumed that cultivated plants are likely to be somewhat different in their properties from those gathered from their natural habitats, it is also clear that certain values in plants can be deliberately enhanced under controlled conditions of cultivation (Uniyal et al. 2000).

More than one-tenth of plant species are used in drugs and health products, with more than 50,000 species being used. However, the distribution of medicinal plants is not uniform across the world (Huang 2011; Rafieian-Kopaei 2013). For example, China and India have the highest numbers of medicinal plants used, with 11,146 and 7500 species, respectively, followed by Colombia, South Africa, the United States, and another 16 countries with percentages of medicinal plants ranging from 7% in Malaysia to 44% in India versus their total numbers of plant species (Hamilton 2003; Marcy et al. 2005; Srujana et al. 2012).

3.3 Various Factors Responsible for MAPs Exploitation

Demand for a wide variety of wild species of MAPs is increasing with the growth in human needs, numbers and commercial trade. With the increased realization that some wild species are being overexploited, several agencies are recommending that wild species be brought into cultivation systems (BAH 2004). There is no reliable estimate for the number of medicinal plants that are globally threatened, variously calculated as 4160 or 10,000 (Schippmann et al. 2002). A highly conservative estimate states that the current loss of plant species is between 100 and 1000 times higher than the expected natural extinction rate and that the Earth is losing at least one potential major drug every 2 years (Pimm et al. 1995). According to the International Union for Conservation of Nature and the World Wildlife Fund, there are between 50,000 and 80,000 flowering plant species used for medicinal purposes worldwide. Among these, about 15,000 species are threatened with extinction from overharvesting and habitat destruction (Bentley 2010), and 20% of their wild resources have already been nearly exhausted with the increasing human population and plant consumption (Ross 2005). Hence, there is a necessity to strike a balance between conservation and utilization of these medicinal plants (Rajasekharan and Ganeshan 2002). The Global Checklist of Medicinal Plants (GCLMP; U. Schippmann, 2014, personal communication) recorded 21,524 taxa globally in 2010 (Biodiversity Indicators Partnership 2010), a number that constantly increases as further research records novel uses and additional species. The current reliance of commercial drugs on plant sources is more, since they exclude semi-synthetic and synthetic medicines based on naturally occurring compounds and estimates the future potential for 540 to 23,490 new drugs discovered from the world's flora, based on the current rate of drug discovery and development from plants and given the range of estimates of global plant species diversity (Bentley 2010). The threats are degradation of habitat due to expanding human activity, forest decline, destructive collection of plant species, invasion of exotic species that compete with native species, increased spread of diseases, industrialization, overexploitation, human socioeconomic change and upheaval, changes in agricultural practices, excessive use of agrochemicals, natural and manmade calamities, genetic erosion, etc. (Fig. 3.1). Different primary and secondary factors pose a threat to many MAPs and their wild populations (Fig. 3.1) are:

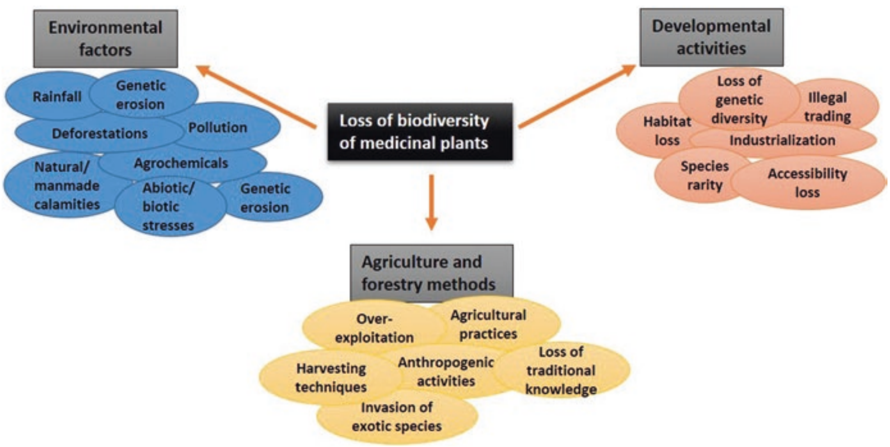

Fig. 3.1 Various factors responsible for loss of medicinal plant biodiversity

- *Intensive and increasing commercial collection:* MAPs can have other uses than as sources of medicines, and the threats from overharvesting may be due, or partly due, to the collection for purposes other than medicinal. One reason why MAPs have become increasingly threatened has been the weakening of customary laws that traditionally have regulated the use of natural resources. Such laws have often proved to be easily undermined by modern socioeconomic forces (Pant 2002). To satisfy the regional and international markets, the plant sources for expanding local, regional and international markets are harvested in increasing volumes and largely from wild populations (Lange 2008).
- *Destructive harvesting techniques:* MAPs collectors are untrained, and almost half of the material collected by untrained manpower is wasted. Therefore, there is a need to find ways to harvest medicinal plants sustainably from the wild. Major reasons for the loss of biodiversity are that most people live below the poverty line and harvest natural resources mindlessly to subsidize their meagre incomes (Tali et al. 2019; Lee et al. 2018). A further threat may be destructive harvesting techniques. To harvest the bark by felling the whole tree or to collect the fruits by cutting whole twigs may damage the plant significantly or even destroy it. Harvesting the roots or rhizomes of a plant is destructive per se.
- *Genetic erosion:* Overexploitation of medicinal plant resources may result in a decline in genetic diversity. In areas with high diversity in MAPs, this may lead to problems of genetic erosion. The loss of genetic diversity may cause enormous problems in the context of cultivation aspects (Tali et al. 2019). This erosion among wild plants is very poorly documented, and many species of medicinal plants known to have become globally extinct are very few, and conservationists are advised to avoid exaggerated claims in this respect. Many of the threats to medicinal plant species are similar to those causing endangerment to plant diversity generally.
- *Issues of accessibility:* Poorer members of local communities can face additional problems of loss of access to medicinal plants due to the privatization or nationalization of land (Ekor 2014). There is a major trend today in many developing countries towards stricter individual ownership of land and plant resources, replacing older forms of tenure and resource rights in which poorer people could be less excluded. Loss of access through nationalization can occur with the creation of more strongly protected types of the conservation area.
- *Anthropogenic activities:* The use of plants for medicinal uses, grazing and fodder now imposes a high pressure on the plant biodiversity, with implications for longer-term sustainability; with some species under such continuous pressure, they are likely to become extinct soon. Anthropogenic activities and biodiversity conflict with each other (Arunachalam et al. 2004). People choose species only because of their own needs and hence put pressure on rare species (Ahmad et al. 2002). The major social problems responsible for the enormous anthropogenic pressures on the vegetation in the region are the increased population, prevailing poverty, lack of awareness and poor education, which combine to increase the competition for and overexploitation of the natural plant resources. It can also be attributed to the high market value of medicinal species (Khan et al. 2013). Given the tremendously

growing world population, increasing anthropogenic activities, rapidly eroding natural ecosystem, etc., the natural habitat for a great number of herbs and trees are dwindling and of per capita consumption has resulted in unsustainable exploitation of Earth's biological diversity, exacerbated by climate change, ocean acidification and other anthropogenic environmental impacts (Rands et al. 2010).

- *Dwindling sources of traditional knowledge:* Knowledge of MAPs, as once embedded in tens of thousands of indigenous cultures, is rapidly disappearing. Every year, the total of human knowledge about the types, distribution, ecology, methods of management and methods of extracting the useful properties of medicinal plants is declining rapidly – a continuation of a process of loss of local cultural diversity that has been underway for hundreds of years (Joshi and Joshi 2000). There has, of course, been great growth in scientific information about MAPs in recent decades, but in many ways, this has proved poor compensation, because such information is often inaccessible at the field level or is irrelevant to the problems faced by land managers or collectors or growers of medicinal plants. Knowledge of the natural world is typically a very important part of the knowledge-worlds of rural people following more traditional ways of life. It is therefore not surprising that the revitalisation of traditional systems of medicine can be high on the agendas of those promoting local and indigenous cultures; a trend was seen in many parts of the world.
- *Global habitat loss and alteration:* An essential factor that poses a significant threat to wild populations and species is the continuing global destruction of habitats. The major direct threats to medicinal plants across the Himalaya are generally agreed to be habitat loss (including deforestation), habitat fragmentation, overgrazing by domestic stock, burning and unsustainable harvesting. More local direct threats include pressures from tourism, mining and construction. Climate change is a threat to many medicinal plants in the Himalaya. The Intergovernmental Panel on Climate Change has indicated that global warming will be particularly pronounced at high altitudes, especially at lower latitudes (as the Himalaya). However, habitat loss and alteration also affects the populations of medicinal plants in Europe or North America. Also, extensive destruction of the plant-rich habitat as a result of forest degradation, agricultural encroachment, urbanization, etc. is another factor, thus challenging their existence (Tali et al. 2019).
- *Largely unmonitored trade:* The use of botanical raw material is in many cases much cheaper than to use chemical alternative substances. As a consequence, there is an enormous demand in botanicals resulting in large quantities of MAPs traded into urban centres from rural areas in developing countries, and also regionally and internationally. According to the data of the Food and Agricultural Organization (FAO) at the United Nations annually, the flora bares irretrievable losses which destroy the natural resources and the ecological equilibrium (FAO 2010). Four thousand to 10,000 medicinal species were endangered of disappearing at the beginning of this century (Edwards 2004). Lange (2006) observed that the majority of internationally traded MAPs are raw or semi-processed and of wild origin. Just 3% of the world's well-documented medicinal flora has been evaluated for global conservation status by 2010, and the proportion of medicinal

plants flora considered to be threatened appeared to have remained relatively stable. The trade-in botanicals are largely unmonitored. In general, MAPs are collected in few countries (a very cheap source for botanicals) rather within their whole area of distribution.

- *Genetic diversity:* Another aspect to consider is the genetic diversity of the species that are in demand. The plants used in the phytopharmaceutical preparations are obtained mainly from the naturally growing areas. The genetic diversity of medicinal plants in the world is getting endangered at an alarming rate because of ruinous harvesting practices and overharvesting for production of medicines, with little or no regard to the future (Lee et al. 2018). Long before non-sustainable harvest practices lead to the extermination of a whole species, selection of favoured growth forms and concentration on certain harvesting areas which may hold certain ecotypes will lead to a degradation of genetic diversity of the wild populations.
- *Species rarity:* It is used to assess the extinction risk of medicinal plants and to identify those species most at risk of extinction, before the commencement of conservation efforts (Figueiredo and Grelle 2009). It is necessary to determine how rare each species is and in which ways rare species differ from one another. Not all medicinal plants are affected in the same way by harvesting pressures (Wagh and Jain 2013; Andel and Havinga 2008). Overexploitation, indiscriminate collection, uncontrolled deforestation and habitat destruction all affect species rarity but are insufficient to explain individual species susceptibility or resilience to harvest pressure. Multiple biological characters correlate with extinction risks, such as habitat specificity, distribution range, population size, species diversity, growth rate and reproductive system.

Species threat status of MAPs The Red List status of taxa is one of the most widely used indicators for assessing the condition of ecosystems and their biodiversity (Artsdatabanken 2010). It also provides an important tool in establishing priorities for species conservation. At the global scale, the primary source of information on the conservation status of plants and animals is The IUCN Red List of Threatened Species TM (the IUCN Red List), while numerous national Red List initiatives within the European region include many more plant species and often contain a wealth of additional information (IUCN 2011a, b). The IUCN Red List Categories and Criteria are designed to determine a taxon's relative risk of extinction, with the main purpose of cataloguing and highlighting those taxa that are facing a higher risk of extinction. The IUCN Red List provides taxonomic, distribution, ecological, threat and conservation status information on taxa that have been evaluated using the *IUCN Red List Categories and Criteria* (IUCN 2012a). The IUCN Red List Categories are based on a set of quantitative criteria linked to population trends, population size and structure and geographic range. There has been very limited and fragmented published work on only a few IUCN Red List plant species (Ali 2008; Alam and Ali 2009; Ali and Qaiser 2011), so one can find very few comparators to evaluate endangered and critical plant species at a national level (Ali 2008). There are nine categories, ranging from Not Evaluated (NE), where a species has not been evaluated against the Red List Criteria; Least Concern (LC), for species that are not threatened; and to Extinct (EX), for species that have disappeared from the planet1. The extinction risk of a taxon may be assessed at any scale from global to regional, national or even sub-national level. A taxon can have a different category in the global IUCN Red List than in a regional Red List. To avoid an over- or underestimation of the regional extinction risk of a taxon, the Guidelines for Application of IUCN Red List Criteria at Regional and National Levels (IUCN 2012b, 2014) should be applied.

3.4 Utilisation and Conservation Strategies of MAPs

The conservation and sustainable use of MAPs have been studied extensively (Chen et al. 2016; Schippmann et al. 2006; Schippmann 2013). Various sets of recommendations have been compiled regarding their conservation, including the establishment of systems for species inventorying and status monitoring and the need for coordinated conservation practices (Schippmann et al. 2006). Conservation of biological diversity involves protecting, restoring and enhancing the variety of life in an area so that the abundance and distribution of species and communities contribute to sustainable development (Hamilton 2004). The ultimate goal of conservation biology is to maintain the evolutionary potential of species by maintaining natural levels of diversity which is essential for species and populations to respond to long- and short-term environmental changes. MAPs are of high priority for conservation action, as their wild-crafting will certainly continue to play a significant role in future trade. Accordingly, it is necessary to ensure the sustainability of their wild-collection through developing and implementing adequate general and species-specific management programmes which should cover trade monitoring and guidelines for sustainable collection and may be supported by an adequate certification (Walter 2002). This requires coordinated conservation work at regional, national or even global level involving conservationists, scientists, governmental authorities, producers, traders and the processing industry. Medicinal plants contribute to health, income, agroforestry system, cultural identity and livelihood security (Khan et al. 2013). Hence there is a need for conservation, cultivation, maintenance and assessment of germplasm for future use (Fig. 3.2). Some of the steps used for conservation and sustainable use of MAPs (Fig. 3.2) are discussed as follows:

- *Small-scale cultivation:* It requires low economic inputs can be a response to declining local stocks, generating income and supplying regional markets. This can be a more secure income than from wild-harvest, which is notoriously inconsistent. For farmers that integrate MAP into agroforestry or small-scale farming systems, these species can provide a diversified and additional source of income to the family. Home gardens are increasingly a focus of medicinal plant propagation and introduction programmes intended to encourage the use of traditional remedies for common ailments by making the plant sources more accessible (Agelet et al. 2000).

Fig. 3.2 Strategies invilved in conservation and proper utilisation of medicinal and aromatic plants

- *Large-scale cultivation:* Commercialization is both necessary and potentially harmful to farmers. It is necessary for that without it the market for products is small and the opportunity does not exist for rural people to generate income. A degree of product domestication is therefore desirable. On the other hand, commercialization is potentially harmful to rural people if it expands to the point that outsiders with capital to invest come in and develop large-scale monocultural plantations for export markets. Rural people may benefit from plantations as a result of available employment and hence off-farm income (Laird and Pierce 2002). However, plantations may also distort market forces to their advantage, for example, by imposing low wages which will restrict the social and economic development of local people. The major beneficiaries of large-scale exports will probably be the country's elite and, perhaps, the national economy. To meet the requirements of expanding regional and international markets healthcare products and needs of growing populations, large quantities of medicinal plants are harvested from forests (Laird and Pierce 2002).
- *In situ or on-site conservation:* It involves maintaining genetic resources in their natural habitats, i.e., within the ecosystem to which it is adapted, whether as wild or crop cultivar in farmer's field as components of the traditional agricultural systems (Figueiredo and Grelle 2009). The key operational steps for establishing

in situ gene banks for the conservation of prioritized medicinal plants include threat assessment, the establishment of a network of medicinal plant forest reserves, involving local stakeholders, botanical, ecological, trade and ethnomedicinal surveys, assessing intraspecific variability of prioritized species, designing species recovery programmes, the establishment of a medicinal plant seed centre, etc. Conclusively, no in situ conservation project can succeed without the complete cooperation and involvement of local people (Forest et al. 2007). Most MAPs are endemic species, and their medicinal properties are mainly because of the presence of secondary metabolites that respond to stimuli in natural environments and that may not be expressed under culture conditions (Coley et al. 2003). In situ conservation of whole communities allows us to protect indigenous plants and maintain natural communities, along with their intricate network of relationships (Gepts 2006). Additionally, in situ conservation increases the amount of diversity that can be conserved (Forest et al. 2007) and strengthens the link between resource conservation and sustainable use (Long et al. 2003). In situ conservation efforts worldwide have focused on establishing protected areas and taking an approach that is ecosystem-oriented, rather than species-oriented (Ma et al. 2012). Successful in situ conservation depends on rules, regulations and potential compliance of medicinal plants within growth habitats (Volis and Blecher 2010).

- *Ex situ conservation:* It involves conservation of biodiversity outside the native or natural habitat where the genetic variation is maintained away from its original location. The ex situ genetic conservation fulfils the requirement of present or future economic, social and environmental needs. Conservation also includes propagation and assessment of molecular diversity (Haven et al. 2006). This includes a combination of methods, depending on factors such as geographic sites, biological characteristics of plants, available infrastructure and network having access to different geographical areas, human resources and number of accessions in a given collection (Rajasekharan and Ganeshan 2002). It is done especially for those overexploited and endangered medicinal plants with slow growth, low abundance and high susceptibility to replanting diseases (Yu et al. 2010). Ex situ conservation aims to cultivate and naturalize threatened species to ensure their continued survival and sometimes to produce large quantities of planting material used in the creation of drugs, and it is often an immediate action taken to sustain medicinal plant resources (Pulliam 2000; Swarts and Dixon 2009). Many species of previously wild medicinal plants can not only retain high potency when grown in gardens far away from the habitats where they naturally occur but can have their reproductive materials selected and stored in seed banks for future replanting (Hamilton 2004).
- *In vitro regeneration* include plant/explant growth, maintenance under disease-free condition, retention of regenerative potential, genetic stability and ensuring that there is no damage to the live material. In vitro multiplication protocols for fast propagation of several red-listed MAPs and recalcitrant taxa that are difficult to propagate through conventional means would be very useful. It offers several advantages over the in vivo method: (a) great savings in storage space

and time, (b) possibility of maintaining species for which seed preservation is impossible or unsuitable, and (c) disease-free transport and exchange of germplasm since cultures are maintained under phytosanitary conditions (Natesh 2000). Even more important is the reintroduction of in vitro raised material into their natural habitat and monitoring its performance over several years, to ensure fidelity concerning active compounds or the marker chemical, vis a vis the parents (Natesh 2000).

- *Botanic gardens:* It plays an important role in ex situ conservation (Havens et al. 2006), and they can maintain the ecosystems to enhance the survival of rare and endangered plant species. Although living collections generally consist of only a few individuals of each species and so are of limited use in terms of genetic conservation (Yuan et al. 2010), botanic gardens have multiple unique features. They involve a wide variety of plant species grown together under common conditions and often contain taxonomically and ecologically diverse flora (Primack and Miller-Rushing 2009). Botanic gardens can play a further role in medicinal plant conservation through the development of propagation and cultivation protocols, as well as undertaking programmes of domestication and variety breeding (Sharrock and Jones 2009; Sharrock et al. 2014). The Botanical Garden Conservation International (BGCI) 2000 database indicates that there are about 1846 botanic gardens.
- *Cultivation practice:* Although wild-harvested resources of medicinal plants are widely considered more efficacious than those that are cultivated, domestic cultivation is a widely used and generally accepted practice (Gepts 2006; Joshi and Joshi 2014; Leung and Wong 2010). Cultivation provides the opportunity to use new techniques to solve problems encountered in the production of medicinal plants, such as toxic components, pesticide contamination, low contents of active ingredients and the misidentification of botanical origin (Raina et al. 2011). Cultivation under controlled growth conditions can improve the yields of active compounds, which are almost invariably secondary metabolites, and ensures production stability. Cultivation practices are designed to provide optimal levels of water, nutrients, optional additives and environmental factors including temperature, light and humidity to obtain improved yields of target products (Wong et al. 2014).
- *Good agricultural practices (GAP):* GAP for medicinal plants have been formulated to regulate the production, ensure quality and facilitate the standardization of herbal drugs (Chan et al. 2012). A GAP approach ensures high quality, safe and pollution-free herbal drugs (or crude drugs) by applying available knowledge to address various problems (Muchugi et al. 2008). GAP include comprehensive items, such as the ecological environment of production sites, germplasm, cultivation, collection and quality aspects of pesticide detection, macroscopic or microscopic authentication, chemical identification of bioactive compounds and inspection of metal elements (Makunga et al. 2008). Many countries actively promote the implementation of the GAP. For example, Chinese authorities have promoted GAP for the growth of commonly used herbal drugs in regions where those medicinal plants are traditionally cultivated (Ma et al. 2012).

- *Organic farming:* It has received increasing attention for its ability to create integrated, humane and environmentally and economically sustainable production systems for medicinal plants (Rigby and Cáceres 2001; Macilwain 2004). The aims of organic farming of medicinal plants include producing material with better quality and high productivity and ensuring the conservation and sustainable utilization of those plants. The defining characteristic of organic farming is the non-use of synthetic fertilizers, pesticides and herbicides, which are not allowed according to many current organic certification standards in Europe and North America (Macilwain 2004). Organic farming is benign to the environment and relies upon farm-derived renewable resources to maintain biological processes of medicinal plants and the ecological balance of habitats (Macilwain 2004). Organic farming of medicinal plants is becoming increasingly important in the long-term development and sustainability of medicinal plants (Suresh 2010).
- *Good harvesting practices:* The wild collection is sustainable as long as the amount of medicinal plant material of a given species collected each year in a certain region does not exceed the annual, natural increase of the species in the same location. If collection exceeds the natural increase over several consecutive growing seasons, the species may become regionally threatened (Panday and Savita 2017). For medicinal plants with limited abundance and slow growth, destructive harvesting generally results in resource exhaustion and even species extinction (Panday and Savita 2017). Therefore, the sustainable use of medicinal plants should be considered, and good harvesting practices must be formulated. Root and whole-plant harvesting are more destructive to medicinal plants (e.g. herbs, shrubs and trees) than collecting their leaves and flowers or buds. For herbal drugs made of whole plants or roots, using their leaves as a remedy can be a benign alternative. In the case of MAPs, conservation concepts and management measures have to meet both (1) future supply and (2) the provisions of species conservation.
- *Natural reserves:* The degradation and destruction of habitats is a major cause of the loss of medicinal plant resources (Camm et al. 2002). Natural reserves are protected areas of important wild resources created to preserve and restore biodiversity (Rodriguez et al. 2007). Around the world, more than 12,700 protected areas have been established, accounting for 13.2 million km^2, or 8.81% of the Earth's land surface (Huang et al. 2002). Conserving medicinal plants by protecting key natural habitats requires assessing the contributions and ecosystem functions of individual habitats (Liu et al. 2001). It is impossible to designate every natural wild plant habitat as a protected area, owing to cost considerations and competing for land uses (Kramer and Havens 2009). A wild nursery is established for species-oriented cultivating and domesticating of endangered medicinal plants in a protected area, natural habitat or a place that is only a short distance from where the plants naturally grow (Strandby and Olsen 2008). Natural reserves and wild nurseries are typical examples to retain the medical efficacy of plants in their natural habitats (Strandby and Olsen 2008).
- *Cryobanks for conservation*: Cryopreservation of plant cells and meristems is an important tool for long-term storage of germplasm or experimental material

without genetic alteration using minimum space and maintenance. The development of methods to store apical meristems in liquid nitrogen successfully is needed to aid in the conservation of genetic resources. Cryobanks are meant for storage of germplasm. For long-term preservation, cryogenic storage at ultralow temperatures under liquid nitrogen (− 150 to −196 °C) is the method of choice. Several types of in vitro raised materials such as meristems/shoot tips, cell suspensions, protoplasts, somatic embryos and pollen embryos of MAPs species have been studied from the cryopreservation perspective (Natesh 2000).

- *Low-temperature germplasm storage:* Preservation by under-cooling has recently been applied to plant tissue cultures. The objective of this approach is to maintain tissues at low temperatures (−10 to −20 °C) but in the absence of ice crystallization. The plant tissues are immersed in immiscible oil, and the emulsion thus formed can be undercooled to relatively low temperatures thereby circumventing ice formation, one of the most injurious consequences of low-temperature storage (Chauhan et al. 2019). Recently, vitrification, simplified freezing and encapsulation-dehydration methods have been used for the storage of valuable germplasm. These new procedures may replace freeze-induced cell dehydration by removal of all or a major part of freezable water from cells at room temperature or 0 °C.
- *Seed storage modules:* Usually seeds, being natural perennating structures of plants, represent a condition of suspended animation of embryos and are best suited for storage (Schoen and Brown 2001). By suitably altering their moisture content (5–8%), they can be maintained for relatively long periods at low temperatures (−18 °C or lower). It is now possible to store materials other than seed, such as pollen or clones obtained from elite genotypes/cell lines with special attributes, in vitro raised tissues/organs or genetically transformed material (Schoen and Brown 2001). Seed banks offer a better way of storing the genetic diversity of many medicinal plants ex situ than through botanic gardens and are recommended to help preserve the biological and genetic diversity of wild plant species (Li and Pritchard 2009). The most noteworthy seed bank is the Millennium Seed Bank Project at the Royal Botanic Gardens in Britain (Schoen and Brown 2001). Seed banks allow relatively rapid access to plant samples for the evaluation of their properties, providing helpful information for conserving the remaining natural populations (Li and Pritchard 2009).
- *Geographical prioritization:* Prioritization of places for the conservation of Himalayan medicinal plants is essential. Achievement of conservation and sustainable use for Himalayan medicinal plants requires geographical prioritization, to focus conservation attention on key sites (Tali et al. 2019). Priority sites will vary according to the particular interests, remits and capabilities of concerned individuals or groups.
- *Preserve indigenous knowledge*: In 2004 the United Nations Conference on Trade and Development (UNCTAD) warned that a biodiversity crisis is being accompanied by a cultural diversity crisis and the weakening of the customary laws that traditionally regulated the use of natural resources. The growing awareness of the loss of indigenous knowledge and the implications of this was

reflected in questionnaire responses. Some conservationists interested in MAPs should become engaged with the commercial sector, both because it is the pressures of trade that are responsible for so much MAP endangerment and also, more positively, because of the opportunities which engagement with industry and consumers presents. Conservationists can become engaged in various ways, including helping to formulate and promote appropriate standards, supplying relevant information to the parties involved, and also putting parties in touch with each other (Msuya and Kideghesho 2009).

- *Community-based conservation:* The community level is the critical level for the conservation of MAPs. Recognition of important areas for plant conservation (including sustainable use) at the community level should be based on community values and knowledge. Participatory research and planning between communities and scientists can be useful for identifying priority sites for improving the management of medicinal plants and for working out how this might be achieved (Berkes 2003). Different community perspectives (local healthcare, income, culture; men, women, etc.) should be considered in making decisions on the use and management of medicinal plants. Traditional doctors associations, religious leaders and indigenous groups can provide encouragement and guidance to communities seeking to conserve their medicinal plants. The herbal industry can assist communities to achieve conservation of medicinal plants through making agreements that specify fair and assured prices for medicinal materials from sustainable sources (Infield 2001). Natural resource managers can assist communities to conserve medicinal plants through making agreements that provide benefits in the buffer zones of protected areas (e.g. assistance with the cultivation of medicinal plants) or rights of sustainable use within forest reserves. Governments can assist communities to conserve medicinal plants by providing appropriate policy frameworks (natural resource management, traditional medicine, community and cultural development, standards in the herbal industry). They also have responsibilities concerning education, training, advisory services and central scientific services (e.g. herbaria, etc.). Non-governmental organizations (NGOs) can play catalytic roles through developing innovative conservation case studies, especially at the critical community level, and contributing analyses to identify best practice. At a local level, botanic gardens can provide communities with valuable horticultural expertise and market information. The transfer of cultivation methodologies to farmers and other stakeholders via training initiatives should be encouraged, as should the development of harvester organizations and best practice horticultural knowledge shared between these. Additionally, investments in technology transfer, research, training and capacity building can make the private sector voluntarily respond to environmental management (Tali et al. 2019).
- *Traditional management practices:* They have an immense contribution to the conservation of medicinal plants and other resources. These traditional practices, which are mostly based on cultural norms and religious beliefs, are the basis for sustainable use and conservation of biodiversity. Although they have long been neglected by official conservation policies, they have proved effective, as

acknowledged in literature (Kajembe et al. 2003; Colding and Folke 2001; Saj et al. 2006). The reality that the traditional management practices exist and enhance conservation implies that the *protectionist model*, which prohibits access to resources by local communities in protected areas, is sometimes uncalled for. There is no point of prohibiting the use of natural resources if that use is harmless to a resource.

3.5 Role of Biotechnology in the Protection of Endangered MAPs

Biotechnological methods seem appropriate ones with their potential for multiplication, selection and protection of medicinal plants. The development of genetic engineering has led to the feasibility of large-scale biosynthesis of natural products, and advancements in tissue culture and fermentation of medicinal plants have opened new avenues for the large-scale and highly efficient production of desirable bioactive compounds. Biotechnological approaches are convenient for use of cells, tissue, organs or entire organism which grow and develop in vitro controlled conditions and can be subjected to in vitro and genetic manipulations (Khan et al. 2009) to obtain desired substances (Rao and Ravishankar 2002). These methods are especially appropriate and reasonable to apply when the targeted species have high economical or trade value, or when plant resources are limited concerning the availability of wild area or good healthy plants, or when the plants are difficult to grow (Verpoorte et al. 2002).

- Tissue culture (including plant cell and transgenic hairy root culture) is a promising alternative for the production of rare and high-value secondary metabolites of medical importance (Rao and Ravishankar 2002). Micropropagation via tissue encapsulation of propagules can not only facilitate storage and transportation but also promotes higher regeneration rates (Baker et al. 2007). When the amounts of normal seeds are insufficient for propagation, synthetic seed technology, defined as artificially encapsulated somatic embryos (or other tissues) that could be used for cultivating in vitro or ex vitro, is a feasible alternative (Lata et al. 2008; Zych et al. 2005). The micropropagation is considered to have the greatest commercial and economical importance for the rapid propagation and ex situ conservation of rare, endemic, and endangered medicinal plants (Khan et al. 2009; Tasheva and Kosturkova 2012). Micropropagation, cell and callus cultures, metabolic engineering and genetic manipulations are especially appropriate for species which are difficult to propagate in vivo (Verpoorte 1999).
- In vitro technologies offer some or most of the following advantages: easier extractions and purification of valuable substances from temporary sources; new products which may not be found in nature; absence of various environmental and seasonal effects, automation, better control of the biosynthetic pathways and

flexibility in obtaining desired product; shorter production cycles; and cheaper less costly products. Here should also be mentioned the potential of the sophisticated techniques of genetic engineering, which might be applied respecting the rules of contained use (Khan et al. 2009; Verpoorte et al. 2002). At present, the methods of plant cell and tissue cultures have found many proper sites for application in the medicinal plant's utilization. The achieved results and the confidence for further success drive the efforts for wider application of plant biotechnologies in more spheres concerning medicinal plants (Tripathi and Tripathi 2003). At present, there is a long list of research groups worldwide investigating hundreds of medicinal species. Various success procedures and recipes for many of these species have been developed. Furthermore, breeding improvements can be carried out using molecular marker-based approaches applied at the genetic level, and the time required for breeding may be significantly shortened (Baker et al. 2007; Rao and Ravishankar 2002; Lata et al. 2008).

- Biotransformation and its ability to release products into the cells or out of them provide an alternative method of supplying valuable natural products that occur in nature at low levels. Generally, the plant products of commercial interest are secondary metabolites, which in turn belong to three main categories: essential oils, glycosides and alkaloids (Sajc et al. 2000). Plant cell cultures as biotransformation systems have been highlighted for production of pharmaceuticals, but other uses have also been suggested as a new route for synthesis, for products from plants difficult to grow, or in short supply, as a source of novel chemicals. It is expected that the use, production of market price and structure would bring some of the other compounds to a commercial scale more rapidly, and in vitro culture products may see further commercialization (Mulabagal and Tsay 2004). The application of molecular biology techniques to produce transgenic cells and to affect the expression and regulation of biosynthetic pathways is also a significant step towards making in vitro cultures more generally applicable to the commercial production of secondary metabolites (Mulabagal and Tsay 2004).
- Gene transfer may be direct when isolated desired DNA fragments are inserted into the cell most often by electrical field or adhesion. This method is less used in medicinal plants. Indirect genetic transformation of plants uses DNA vectors naturally presenting in plant pathogens to transfer the isolated genes of interest and to trigger special metabolic pathways (Giri and Narasu 2000). *Agrobacterium rhizogenes* induces the formation of 'hairs' at the roots of dicotyledonous plants. Genetically modified 'hairy' roots produce new substances, which very often are in low content. Hairy roots are characterized by genetic stability and are the potential highly productive source for valuable secondary metabolites necessary for the pharmaceutical industry (Sevon and Oksman-Caldentey 2002). Manipulations and optimization of the productivity of the transformed hairy roots are usually the same as for the other systems for in vitro cultivation (Terryn et al. 2006). They also depend on the species, the ecotype, the explant, the nutrient media, cultivation conditions, etc. (Guillon et al. 2006).

3.6 Protocols/Approaches to Medicinal Plant Conservation

- *The Convention on Biological Diversity* (CBD) was ratified in 1992 at the Rio Earth Summit. The 190 Parties have agreed to commit to protecting biodiversity, develop sustainably and engage in the equitable sharing of benefits from the use of genetic resources. The conservation of biodiversity is acknowledged as the cornerstone of sustainable development.
- The World Trade Organization's (WTO) Agreement on *Trade-Related Aspects of Intellectual Property Rights* (TRIPS), 1994, sets out how to deal with the commercial use of traditional knowledge and genetic material by those other than the communities or countries where these originate, especially when these are the subject of patent applications.
- The *UNCTAD BioTrade Initiative* (launched in 1996) promotes the sustainable use of goods and services derived from biodiversity, in support of the objectives of the CBD.
- The eight *Millennium Development Goals* (MDGs) were agreed by world leaders in 2000, providing an agenda for reducing poverty and improving lives through environmental sustainability by the target date of 2015. Any measures which enable the sustainable use of natural resources to improve livelihoods will contribute to the MDGs.
- The *Millennium Ecosystem Assessment* (MEA) assessed the consequences of ecosystem change on human well-being, gathering data from 2001 to 2005 and providing a scientific appraisal of the condition and trends in the world's ecosystems and the services they provide, as well as the scientific basis for action to conserve and use them sustainably.
- The *Doha Declaration* of 2001 aimed to ensure that the TRIPS agreement and the CBD support each other; 'allowing for the optimal use of the world's resources by the objective of sustainable development, seeking both to protect and preserve the environment and to enhance the means for doing so in a manner consistent with their respective needs and concerns at different levels of economic development'.
- In 2002 the CBD adopted the *Global Strategy for Plant Conservation* (GSPC), which specifies 16 outcomes orientated targets for delivery by 2010.
- The 2002 *World Summit on Sustainable Development* aimed to promote a global commitment to sustainable development, improving the lives of the world's poorest people as well as reversing the continued degradation of the global environment.
- The WHO launched their *Traditional Medicine Strategy* in 2002, discussing the role of traditional medicine in healthcare systems.
- In 2004, the *Addis Ababa Principles and Guidelines* to the CBD detailed 14 interdependent practical principles and operational guidelines that govern the uses of components of biodiversity to ensure the sustainability of such use.

- Also in 2004, a new paragraph was added to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (*CITES*) Resolution Conf.8.3 stating that the Conference of the Parties recognizes that implementation of CITES listing decisions should take into account potential impacts on the livelihoods of the poor.
- *The European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR)* is a collaborative programme including most European countries aimed at facilitating the long-term conservation and the increased utilization of plant genetic resources in Europe.
- Policy frameworks and legislation are informed by essential data such as the endangerment assessments made by the *Species Survival Commission (SSC)* of the IUCN, which produces a Red List of Threatened Species. Using a network of thousands of scientists the Red List provides taxonomic, conservation status and distribution information on globally evaluated species according to specific categories and criteria.
- *The International Plant Genetic Resources Institute (IPGRI)* is an independent international scientific organization that seeks to advance the conservation and use of plant genetic diversity for the well-being of present and future generations. It is one of 15 Future Harvest Centres supported by the Consultative Group on International Agricultural Research (CGIAR).

3.7 Priorities and Strategies for Future Action

Convention on Biological Diversity (CBD) states that the systematic approach of medicinal plant conservation plays a vital role in environmental management and development through traditional as well as scientific practices (CBD 2010a, b, 2011). On priority, the strategy must address the conservation of wild medicinal plants, threatened with extinction. The present scenario offers a unique opportunity for government representatives, scientists and NGOs to work in coordination, to devise mechanisms to promote the traditional healthcare system and engage rural inhabitants in conservation, cultivation, processing and marketing of raw material. Medicinal plants should be cultivated to save the forests and alpine regions and, at the same time, meet the rising demands of herbs to improve the livelihoods of the hill people (Kala 2005). It is well known that the cultivation of medicinal and aromatic herbs to be one such potential option. At this stage, a strategy for future need is to be developed. The legal protection of MAPs was established in 1936 by passing a law prohibiting or limiting the collection of flowers and herbs in certain regions.

The use of medicinal plants from forest territories is regulated by the Forest Law and is controlled by the Forest Office at the Ministry of Agriculture, Forestry and Agrarian Reform. The Ministry of Environment and Waters is in charge of the management and control of the national natural resources (forests excepted) in the protected territories. A different regime of protection has been imposed on 3.5% of the total area of the country, and 82% of the protected territory (nature reserves and national parks) received nature protection status corresponding to categories I and II defined by IUCN (2012b). An effective method of promoting conservation may be by encouraging the use of medicinal plants and thus increasing their perceived value. To enhance the conservation of medicinal plant resources in the ranch and mitigate their perceived decline, appropriate conservation measures, monitoring and harvesting strategies by the community are strongly recommended.

The importance of the medicinal plant's sector can be gauged from the fact that herbal medicines serve the healthcare needs of about 80% of the world's population. According to the World Health Organization (WHO), the goal of 'Health for All' cannot be achieved without herbal medicines. While the demand for herbal medicines is growing in developing countries, there are indications that consumers in developed countries are becoming disillusioned with modern healthcare and are seeking alternatives. This has renewed interest by the multinational pharmaceutical industry in bio-effective international agreements on conservation of biodiversity has resulted in 'slaughter harvesting' of medicinal plants and massive depletion of biodiversity (GBIF 2014). This trend does not augur well for the sustainable use of medicinal plants resources. One of the immediate tasks for conserving medicinal plants diversity is to effectively implement the provisions on conservation and sustainable use of biodiversity (Biological Diversity Act 2002) and the Patents (Amendment) Act, 2005. The available flexibility under TRIPS provisions should be utilized fully for protecting the pool of our plant genetic resources and traditional knowledge effectively. Capacity building is another important area which would be a major source for harnessing the potential of medicinal plants sector. Perhaps the greatest challenge in making trade a positive force for development is ensuring that the benefits accelerate development in the poorest countries and for the poorest people. The root cause of many health problems in developing countries is poverty. Therefore, a national policy on utilising medicinal plants for herbal medicines need to be developed soon which should ensure that all herbal medicines in the market are safe, effective, of good quality, reasonably priced and are prescribed and utilized rationally. It is equally important that the interests of the growers are well protected by the supply of modern technologies, services and credit supplies and above all a good marketing system. The national policy should have effective provisions for ensuring equitable benefit sharing for all stakeholders. This would go a long way in fulfilling traditional healthcare needs and ensuring the conservation and sustained utilisation of medicinal plant resources of the country. Still, strategic actions based on research on issues identified above will be needed to realize the vision. However, the fact that all medicinal plants are not amenable for cultivation should not be ignored. Hence, conservation and cultivation must go together with prioritization for the development of the medicinal plant's sector as a whole. To harness the potential of this sector, we should have an economic outlook, realistic policy and effective planning strategy. Since available evidence is inadequate to fully capture the complex issues of this sector, three is a dire need to undertake in-depth socio-economic and policy research analysis to fill the gaps in understanding the dynamics of medicinal plants sector.

3.8 Conclusion

Conservation is the process of management of biosphere to obtain the greatest benefit for the present generation and maintaining the potential for the future. MAPs-based livelihood systems are often mediated by market demand and are a good source of employment and income generation to poor rural communities. Developing appropriate framework and technologies for the cultivation of MAPs is a critical factor to ensure a continuous and regular supply of medicinal plants for the pharmaceutical industry and to halt the degradation of the natural resource base. The participatory action research framework approach would be helpful to the farmers, traders, scientists and policy planners in the context of socio-economic development for sustainable livelihoods of the subsistence rural communities. With the increased realization that many wild MAP species are being overexploited, several agencies are recommending that wild species be brought into cultivation systems. Concerns about loss of medicinal plants, considered as material resources, relate to worries about healthcare, livelihood security and financial income. Now the major recommendations for future research are as follows: (1) as MAPs have vast potential, their cultivation should be promoted on barren, nonarable, and marginal lands under a participatory management action plan to improve the economy of deprived farmers; (2) strengthening indigenous techniques of MAP cultivation should be encouraged through promoting cost-effective and appropriate rural technologies such as polyhouse, nethouse, polypit, mulching and organic farming; (3) traditional healers, farmers and other stakeholders involved in medicinal plant sectors should be properly registered and officially recognized; (4) strict implementation of laws and rules should be enacted to secure community-based traditional knowledge and intellectual property rights and to ensure equitable benefits sharing among the stakeholders; (5) formulation of herbal products and promotion of value-added products of MAPs for possible market opportunities and better return to the farmers; (6) farmers need to be encouraged in the promotion, protection and uses of MAPs by providing incentives and training on the latest activities, development, and policies related to MAPs; and (7) regular backstopping should be promoted through meetings, exposure visits, and capacity building for long-term sustainability (Fig. 3.2). Conservation of MAPs resources is of global concern because we don't know what we are losing and what we will need in future.

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Chapter 4
Bioactive Secondary Metabolites of Medicinal and Aromatic Plants and Their Disease-Fighting Properties

Navneet Kaur and Tawseef Ahmed

4.1 Introduction

Medicinal and aromatic plants, i.e., MAPs may be defined as plants which contain compounds possessing medicinal and aromatic properties which may exert beneficial effect on human or animal body (Samarth et al. 2017). These plants are source of structurally variant bioactive compounds called secondary metabolites such as alkaloids, anthocyanins, chlorogenic acids, coumarins, essential oils, flavonoids, lignans, phenolic acids, resins, tannins, terpenoids, saponins, steroids, etc. These valuable secondary metabolites have been wisely utilized by mankind in numerous applications. In the form of plant extracts, isolated compounds, and chemically modified or structurally similar analogues, these bioactive secondary metabolites of MAPs have been used worldwide commercially in cosmetic and perfumery industry, food and flavor industry, and pharmaceutical and medicinal industry. In the past, phytochemical analysis of various species of MAPs have led to the discovery of many new bioactive secondary metabolites with immense therapeutic potential which makes them "*Chemical Goldmines*" of novel pharmaceutical products and applications (Padalia 2012).

Although the knowledge of healing properties of MAPs is ancient but due to advances in technology, development of analytical techniques for the characterization of compounds and recognition of traditional knowledge they are gaining immense popularity world over as the consumers are now getting more inclined towards natural and safe products. In modern medicine also, though the synthetic drugs have been majorly used to cure several ailments but these plants hold a

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significant status as they are used as raw material for the synthesis of many important drugs. Moreover, synthetic drugs are beyond the reach of millions of people living in remote and rural areas and where traditional medicine is still being used to cure deadly disease (Namdeo 2018).

Thus, MAPs due to their healing properties form the basis of many traditionally recognized systems of medicine such as Indian Ayurveda, Chinese Pharmacopoeia, Siddha, Unani, etc. Along with these properties, the plants are safe, effective, easily available, and low in cost (Namdeo 2018). As mentioned earlier, recent advancements in the technology have made possible the isolation of many new phytochemicals which offer huge opportunities for their use in healthcare. Hence, the chapter will help to understand the history of MAPs in traditional medicine and secondary metabolites present in MAPs and their functions in nature, getting a sneak peek into the biosynthesis of these secondary metabolites, the major type of secondary metabolites present in MAPs, and their role in preventing disease, and last but not the least the mode of action of these secondary metabolites attributing to their disease-fighting properties.

4.2 History of MAPs and Their Secondary Metabolites

The role of medicinal plants in healing mankind is long enough. Moreover, the proof of this partnership between man and medicinal plants is evident in the form of written records and documented monuments. The history of our ancestral beings is incomplete without use of medicinal plants for treating various disorders, and the knowledge they had has been passed through civilizations. The movement of humans also led to the discovery of new plants with curative action (Petrovska 2012; Inoue et al. 2019). Thus, Table 4.1 summarizes the major events in history of usage of medicinal and aromatic plants.

4.3 Secondary Metabolites in MAPs and Their Role in Nature

As mentioned earlier also, bioactive secondary metabolites are “chemical goldmines” as they are treasures of valuable chemicals which can be used for the production of various cosmetics, dyes, insecticides, medicines, perfumes, etc. (Thirumurugan et al. 2018). They originate as either the by-products or intermediates of biosynthetic pathway of primary metabolites. The basic structure of major classes of secondary metabolites, i.e., the alkaloids, phenylpropanoids, and terpenoids, is the result of carbon and nitrogen biosynthesis and similarly cyanogenic glycosides, polyketides, and quinones, while the other secondary metabolites like alkylamides and glucosinolates have been derived from fatty acid and sulfur metabolism (Bottger et al. 2018).

Table 4.1 Timeline of important events showing historical achievements of usage of MAPs till present

S.no.	Timeline	Event
1.	5000–3000 BC	Oldest medical records found on clay tablets by Sumerians at Nagpur
2.	3400 BC	Cultivation of MAPs such as cannabis and opium started in Egypt and Mesopotamia
3.	3400–3100 BC	Records of body preserved using medical plants found in cold mountainous Alps
4.	2900 BC	Medicine of Egypt started
5.	2800–1700 BC	Foundation of Traditional Chinese Medicine started
6.	1500 BC	Record of drugs found on *Elbers Papyrus* having 810 prescriptions including use of plants as inhalant, lotion, poultice, suppository, etc.
7.	460–377 BC	Hippocrates used 60 different kinds of MAPs for medicinal purposes
8.	AD 131–200	Galenus, a popular doctor in Roman era prepared different medicines using MAPs and termed them *galenical preparations*
9.	1493–1541	Chemical drugs were prepared using raw plants by Paracelsus
10.	16th century	World Bible of Medicine *De Materia Medica* containing plant preparations gained global recognition
11.	1783–1841	Morphine extraction from opium started and was used as analgesic
12.	1788–1877	Quinone was extracted from *Cinchona* bark to treat malaria
13.	Late 18th century	Role of spices as preservative and food flavoring was recognized New bioactive secondary metabolites were discovered and used in medicine to treat disease
14.	19th century	Alkaloids, glycosides, etheric oils, hormones, saponins, tannins, vitamins, etc. were discovered from MAPs using chemical isolation methods
15.	Early 20th century	Stabilization methods for long-lasting actions of fresh medicinal plants were discovered
16.	Present situation	All pharmacopoeias in the world such as PhEur 6, USP XXXI, and BP 2007 prescribe plant drugs for real medicinal value Homeopathic drugs containing herbal plants are in use Some countries allow the use of herbal drugs without any prescription

4.3.1 Role of Secondary Metabolites in Nature

The idea of secondary metabolites was first conceptualized by Albrecht Kossel in 1910 for which he was awarded Nobel Prize in Physiology/Medicine, but after around 30 years, Czapek deduced that these are the end products of nitrogen metabolism obtained by "secondary modifications" (Hussein and Anssary 2018). Secondary metabolites differ from primary metabolites as they do not participate in any basic process associated with plant growth such as development and reproduction. On the contrary, they play a major role in defense mechanism of plants and protect them against disease, predators, and environmental stresses (ultraviolet (UV) rays, oxidants, etc.). They also help in pollination and reproduction by producing essential oils which may produce attractive smells and anthocyanins which give attractive color. They are also helpful in preventing interspecies

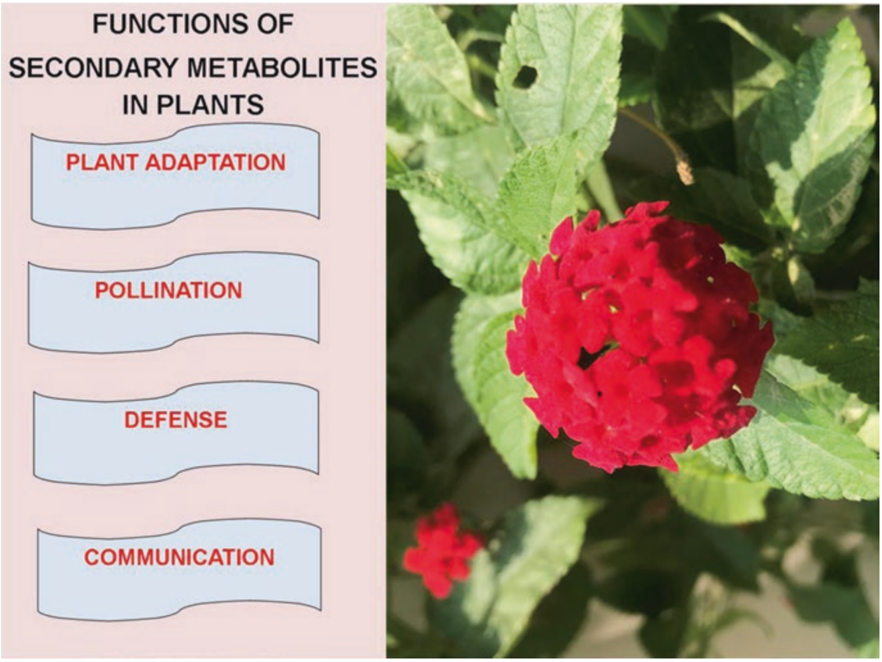


Fig. 4.1 Role of secondary metabolites in plants

competition (Zhao et al. 2015). Moreover, the number of plant bioactive secondary metabolites (greater than 200,000) is far more than primary metabolites, i.e., only 10,000. The major functions of bioactive secondary metabolites in plants (Fig. 4.1) are discussed below in detail:

(a) *Plant Adaptation*
The evolution of land plants started around 500 years ago, and this huge step to conquer land required the presence of distinctive secondary metabolites. To facilitate the development of plants on land, several problems were there to be addressed such as exposure to UV light, survival in water shortage, structural assistance to uphold them, and, last but not the least, protection against emerging herbivores and pathogens. To overcome all these shortcomings, new biosynthetic pathways came into being such as early land plants that started producing phenylpropanoids to protect against UV rays, followed by production of lignins in vascular plants to save them from wind and plant-eating organisms. Then with the arrival of insects on land, pteridophytes started forming cryogenic glucosides to protect against them (Bottger et al. 2018).

(b) *Pollination*
Pollination is the mechanism by which the plants propagate, and it is generally carried out in two means, biotic involving the use of animals, bats, birds, insects, etc. and abiotic involving use of environmental factors such as wind, water, etc. To achieve pollination, plants use various means to attract pollinators such as

colorful flowers, flavors, volatile scents, etc. However, the above attributes can also invite unwanted predators than desired pollinators. Thus, to overcome these difficulties, plants produce certain secondary metabolites such that irridoid glycosides are produced in floral nectar of *Catalpa speciose* (northern catalpa tree). These glycosides make the nectar toxic to predators such as ants, while it remains edible to potential pollinators, bumble bee, and moths. In addition, nectar also has antimicrobial properties due to the presence of secondary metabolites. Similarly, unripe fruits also consist of toxic metabolites or bitter principles to prevent premature eating by animals (Bottger et al. 2018).

Flavonols in flowers form nectar guides in the form of concentric circles which are thought to be helpful to insects in finding the site of pollen and nectar (Taiz et al. 2015).

(c) *Defensive Mechanisms*

Plants form the first trophic level in food energy pyramid, and hence every organism obtains their energy directly or indirectly from plants as food source. Thus, to survive against herbivores and to protect themselves from pests and microbial attack, they need to develop efficient defense mechanisms. Secondary metabolites play an important role in defending plants by making them toxic and unpalatable, for example, cyanogenic glucosides in plants result in formation of toxic hydrogen cyanide (HCN) in response to herbivore attack. These chemical substances help plants by different mechanisms (Mithofer and Maffei 2017):

- By reducing herbivore attack
- By killing insects upon ingestion
- By hindering herbivore development
- By lowering their digestive ability
- By decreasing their immunity to disease

Terpenes can either be poisonous or simply stops or repels the herbivores, insects, and mammals, e.g., pyrethroids in flowers of *Chrysanthemum* species are insecticidal in nature. Essential oils such as limonene and peppermint oil are insect repellents. Lignins not only provide physical strength to plants to resist attack by herbivores but also are practically indigestible to herbivores. Flavones and flavanols protect certain plants against UV radiation which causes damage to DNA, by absorbing it. Tannins being bitter in nature work as herbivore repellents. Even mammals such as apes, cattle, and deer do not eat plants with high tannin content. Saponins harm herbivores by interfering with sterol uptake in digestive system, or they may damage cell membrane once they are absorbed into bloodstream (Taiz et al. 2015).

(d) *Communication*

Plants communicate with other plants in order to warn other or nearby plants against threats, disease, injury, and environmental stresses such as wind, extreme weather conditions, etc. and even to remove inter- or intraspecies competition (also called allelopathy). Plants produce volatile secondary metabolites to perform above functions. These volatile compounds travel by air to nearby plant species and warn them against the danger and give them time to strengthen their immune system (Ueda et al. 2012). The effect of allelopathy, i.e., secondary metabolites released by one plant in order to affect another plant, can be harmful or beneficial. Derivatives of phenylpropanoids and benzoic acid have been found to show allelopathic activity. Similarly, caffeic acid and ferulic acid limit the germination efficiency and growth of various plants when present in Soil (Taiz et al. 2015).

4.4 Pathways for Biosynthesis of Secondary Metabolites in MAPs

Primary and secondary metabolites in plants share a common biosynthetic pathway. Secondary metabolites basically absorb and store extra carbon and nitrogen produced during primary metabolism. However, under circumstances of need, the stored reservoirs in the form of secondary metabolites can be disintegrated and again utilized in primary metabolism. Hence there is effective and intricate balance between both primary and secondary metabolism (Thirumurugan et al. 2018). On the basis of biosynthetic pathways, there are mainly three types of secondary metabolites, i.e., terpenes, phenolic compounds, and nitrogen-containing compounds (Verma

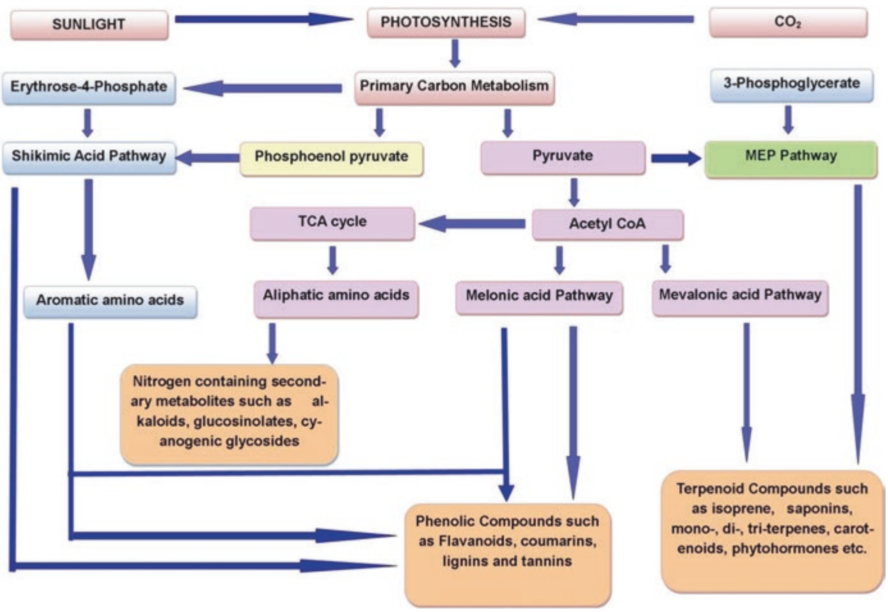


Fig. 4.2 Simplified sketch of biosynthetic pathway of secondary metabolites adopted by plants where CO_2 carbon dioxide, MFP methylerythritol-4-phosphate, TCA tricarboxylic acid pathway, CoA coenzyme A. (Adapted from: Verma and Shukla 2015)

and Shukla 2015). The biosynthetic pathways of all these are discussed below, and Fig. 4.2 gives simple description of biosynthetic pathways occurring in plants.

4.4.1 Biosynthetic Pathways for Terpenes

Terpenes are synthesized by two different pathways, cytosolic pathway or mevalonic acid pathway and chloroplast pathway or methylerythritol-4-phosphate pathway (MFP pathway). The cytosolic pathway is exclusive to eukaryotes and is responsible for the biosynthesis of triterpenes, sesquiterpenes, sterols, brassinosteroids, etc., while chloroplast pathway occurs in prokaryotes and plastids of eukaryotes and is involved in synthesis of hemiterpenes; mono-, di-, and tetra-terpenes; isoprene; carotenoids; phytohormones; etc. (Verma and Shukla 2015; Ahmed et al. 2017).

Mevalonic pathway begins with condensation of three molecules of acetyl-CoA which eventually results in formation of mevalonic acid, which further undergoes phosphorylation to give mevalonate-5-phosphate as product. This is lastly used to form isopentyl diphosphate (IDP) which is a precursor molecule for the biosynthesis of all terpenes (Ahmed et al. 2017).

In MEP pathway on the other hand, pyruvate and glyceraldehyde-3-phosphate react to form methyl erythritol-4-phosphate which in turn is used to form 4-hydroxy-3-methylbut-2-enyl diphosphate or HMBDP which finally results in the formation of either IDP or dimethyl-allyl-diphosphate (DMAPP or DMADP) (Ahmed et al. 2017).

4.4.2 Biosynthetic Pathway for Phenolics

The major pathways involved in biosynthesis of phenolic compounds are malonic and shikimic acid pathways. Both these pathways occur in prokaryotes and eukaryotes with an exception that malonic acid pathway do not occur in higher plants while shikimic acid pathway in animals. Shikimic acid pathway utilizes precursors from glycolysis and pentose phosphate pathway to form various aromatic amino acids frequent being phenyl alanine (Ahmed et al. 2017).

4.4.3 Biosynthetic Pathways for Nitrogen-Containing Compounds

Alkaloids, glucosinolates, and cyanogenic glycosides fall under the category of nitrogen-containing compounds (Verma and Shukla 2015). Biosynthesis of nitrogen-containing compounds is carried out by pyruvate and shikimic acid obtained from phosphoenolpyruvate and erythrose-4-phosphate. L-Amino acids such as aspartic acid, lysine, tryptophan, and tyrosine are basically involved in the biosynthesis of generally all major alkaloids with exception of purine alkaloids formed from xanthosine and pyrrolizidine alkaloids obtained from putrescine and spermidine (Ahmed et al. 2017).

4.5 Various Types of Secondary Metabolites in MAPs

Secondary metabolites of MAPs can be classified by many different ways on the basis of:

- *Chemical structure:* such as cyclic and acyclic compounds or those comprising sugar units
- *Composition:* nitrogen and non-nitrogen-containing compounds
- *Solubility* in water or organic solvents
- *Biosynthetic pathways:* such as terpenoids, phenolic fractions, and nitrogen-containing compounds

Thus, it is quite clear from the above discussion that there is no particular strategy to group natural products. Due to their vast differences in structure, functional ability, and biosynthesis, it is hectic to put them into simple classes or groups. However, scientific community worldwide divides these bioactive natural substances into five major classes, viz., alkaloids, enzyme co-factors, fatty acid derivatives and polyketides, non-protein fractions, and terpenoids and steroids (Brahmkshatriya and Brahmkshatriya 2013), hence keeping in mind the major classes the authors have tried to summarize the various types of secondary metabolites in detail:

4.5.1 Terpenoids/Terpenes

Terpenes or isoprenoids form 55% of total secondary metabolites in nature, and the basic unit of terpenes is isoprene (C_5) (Fig. 4.3) meaning all the terpenes are made of this single unit repeated in head to tail fashion. However, there are exceptions such as carotenoids, in which isoprene units are joined in tail to tail fashion and even terpenoids with carbon content not a multiple of 5 also exist (Brahmkshatriya and Brahmkshatriya 2013).

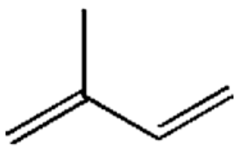


Fig. 4.3 Structure of isoprene

The general formula for terpenes is $(C_5H_8)_n$ where n is the number of carbon atoms present in the structure. Based on this formula and structural arrangement, terpenes can be classified as hemi-, mono-, sesqui-, di-, sester-, tri-, and tetraterpenoids. Mono-, di, sesqui-, and sester-terpenes are all joined in head to tail arrangement, while tri- and tetra-terpenes are linked in tail to tail arrangement. Usually all the terpenes are hydrocarbons, but those containing oxygen like alcohol, aldehydes, and ketones also are terpenes, but due to the presence of oxygen moiety, they are referred to as terpenoids (de las Heras et al. 2003).

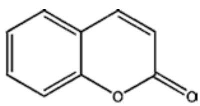
Terpenes are accountable for many functions in plants such as protection from predators, thermoregulation, signaling functions, etc., but the advantages are not limited to plants only as they are gathering the attention of researchers worldwide due to the numerous health benefits they offer (Kim et al. 2020). Besides having pleasant smell, terpenes are spicy to taste and offer array of medicinal benefits. Plants such as balm trees, carnation, caraway, cinnamon, citrus fruits, cloves, eucalyptus, ginger, lavender, lemongrass, lilies, peppermint, roses, rosemary, sage, thyme, violet, etc. exhibit terpenes in their blossoms, fruits, leaves, rhizomes, roots, seeds, and stems (Brahmkshatriya and Brahmkshatriya 2013). As reported by several workers, terpenes exhibit potent anti-inflammatory activity (de las Heras et al. 2003; Kim et al. 2020). Other pharmacological activities displayed by terpenes and terpenoids include antibacterial, anticancer activity, central nervous system (CNS)-protective activity, cardiovascular system (CVS)-protective activity, and antihyperglycemic agent, antioxidant, antiparasitic agent, and antiviral activity. They are also immunomodulatory in action. Essential oils are generally composed of mono- and sesquiterpenes, while balsans, resins, rubber, and waxes contain other terpenes. Some of the major terpenes found in plants are D-limonene, taxol, perillyl alcohol, carveol, uroterpenol, sobrerol, farnisol, geraniol, citronellol, puuphenone, cineol, barneol, etc. (Brahmkshatriya and Brahmkshatriya 2013).

4.5.2 Phenolic Compounds

Phenolic compounds are major secondary metabolites ubiquitous in plants. They may be defined as the compounds either linked to mono- or polysaccharides form bonds with one or more phenolic group or as derivatives of methyl or ethyl esters. They are mainly divided into two major classes—polyphenols (coumarins, flavonoids, lignans, anthocyanins) and phenolic acids (Minatel et al. 2017). The major categories of phenolic compounds are discussed below in detail:

(a) *Coumarins:* Vogel first isolated coumarin from Coumarou or tonka beans (*Dipteryx odorata*) in 1820 from where it has derived its name also. Coumarins are widely distributed in plants and belong to a class of benzopyrones. They can

Fig. 4.4 Simple structure of coumarin



either be naturally occurring or chemically synthesized by oxygen carrying heterocycle and a typical benzopyrone ring in the structure. Figure 4.4 gives a simple structure of coumarin (Stefanachi et al. 2018).

Coumarins can be classified as simple coumarins (e.g., coumarin, flaxidin, etc.), furanocoumarins (e.g., psoralen, marmalade, etc.), dihydrofuranocoumarins (e.g., anthogenol, rutaretin, etc.), pyronocoumarins (e.g., xanthylettin, inophyllum, etc.), phenylcoumarins (dispardiol B, disparinol D, etc.), and biocoumarins (e.g., dicoumarol) (Matos et al. 2015).

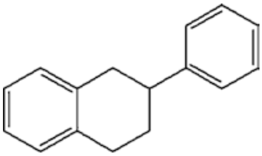
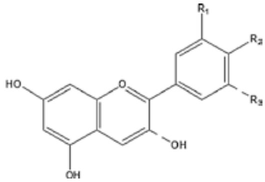
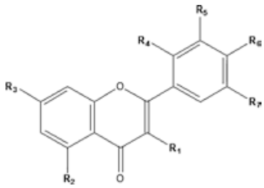
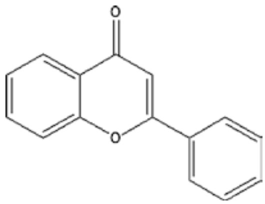
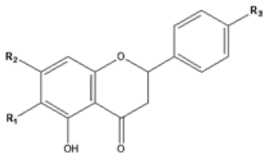
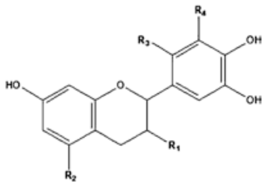
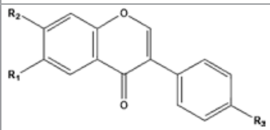
The major function of coumarins in plants is to save them against predators. They are also widely used in cosmetic and perfumery industry for a decade and also to enhance aroma in alcoholic and tobacco-based drinks. Coumarins offer potential medicinal benefits as they possess strong pharmacological action, low toxicity, lesser side effects, and better bioavailability. They also possess several pharmaceutical properties being an analgesic, anticancerous, anticoagulant, antidiabetic, anti-inflammatory, antioxidant, antimicrobial, and anti-neurodegenerative agent (Matos et al. 2015).

(b) *Flavonoids:* Flavonoids are an important class of secondary metabolites consisting of polyphenolic structure and widely distributed in plants. They play several crucial role in plant survival by protecting them against various biotic and abiotic factors such as UV light, draught, frost hardiness, extreme thermal and low temperature conditions, predators, microbes, etc. They also help in seed dispersal and are responsible for the attractive color and aroma of plants. Due to beneficial biochemical and antioxidant effects, they are widely used in health promoting, nutraceutical, pharmaceutical, and cosmetic industry (Panche et al. 2016). Structurally the flavonoids are composed of 15-C skeleton comprising two benzene rings attached to each other by heterocyclic pyrane ring. Further they are divided into several subclasses such as anthocyanins, flavonols, flavones, flavonones, flavan-3-ols, and isoflavones. The major difference between various classes is their level of oxidation and the fashion in which C-ring is substituted (Kumar and Pandey 2013). The major subclasses are discussed below in comprehensive manner and are given in Table 4.2 along with examples:

- *Anthocyanins*

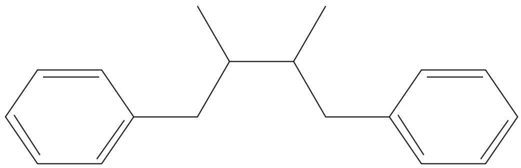
  The colorful anthocyanins belong to flavonoid family having oxygen atom with positive charge present at C-ring of basic flavonoid structure (flavylium ion). Anthocyanins not only impart color to flowers and fruits of plants but also play a crucial role in treating various human health issues. They have proven activity against diabetes, cancer, obesity, cardiovascular disease, inflammation, and microorganism-related infections. Anthocyanins exist as glycosides, while in aglycone form, they are termed as anthocyanidins. The major anthocyanidins present in plants are cyaniding, delphidin, peonidin, malvidin, petunidin, and pelargonidin (Khoo et al. 2017).

Table 4.2 Basic structure of flavonoid and its types along examples

	Flavonoid (basic structure)		
			
S.No.	Class of flavonoid	Basic structure	Examples
1.	Anthocyanin		Cyanidin-3-glucoside, delphidin-3-glucoside, malvidin-3-glucoside, etc.
2.	Flavanols		Quercetin, kaempferol, etc.
3.	Flavones		Apigenin, luteolin, chrysin, etc.
4.	Flavanones		Naringenin, hesperetin, neohesperetin, etc.
5.	Flavon-3-ols		Catechin, epicatechin, gallocatechin, etc.
6.	Isoflavones		Genistein, daidzein, glycitein, etc.

- *Flavonols*
Flavonols are also called 3-hydroxylavones and are white to yellow in color. They bear hydroxyl group at 3-C position; otherwise they closely resemble flavones in structure. The major bioactive compounds of this group include quercetin, kaempferol, and myricetin. They mainly occur in two forms, i.e., aglycon and glycosides. Flavonols offer many benefits to human health as they are potent antioxidants. They also show anticancer, anti-atherosclerosis, and neuroprotective activities (Zuiter et al. 2014) (Table 4.2).
- *Flavones*
Flavones are mainly found in leaves, flowers, and fruits as flavon-C-glucosides and are colorless to pale yellow in color. They share structural similarity to flavonols and differ only in lack of hydroxylation at 3-C-ring. The major sources of flavones include chamomile, celery, ginkgo biloba, parsley, and thyme, while chief flavone glycosides are apigenin and luteolin. Methoxylated flavones such as tangeretin, nobiletin, and sinensetin are mainly present in the skin of citrus fruits. Bioactive flavone compounds own several health-promoting properties such as anticancer, anti-inflammatory, antimicrobial, and antioxidant activities (Zuiter et al. 2014; Panche et al. 2016).
- *Flavanones*
Flavanones or dihydroflavones are colorless to yellow compounds mainly found in citrus species such as grapefruit (e.g., naringenin), lemons (e.g., eriodictyol), and oranges (e.g., hesperetin) and aromatic plants such as mint. The C-ring is saturated in case of flavanones which makes them different from flavones and flavonols. Flavanones can further be classified in two groups, viz., simple flavanones having hydroxyl, methoxy, methylenedioxy, and C-methyl or related groups and complex flavanones like benzylated, furano, prenylated, and pyranoflavanones showing distinct substitution behavior. Like other classes of flavonoids, they also possess various medicinal properties such as anticancer, anti-cholesteromic, antidiabetic, antihypertensive, anti-inflammatory, anti-lipidemic, and antioxidant activity (Zuiter et al. 2014; Panche et al. 2016).
- *Flavan-3-ols*
Flavan-3-ols or commonly known as flavanols or catechins are again colorless to yellow in color and composed of hydroxyl group at C-3 position of ring with absence of carbonyl group. They are abundantly present in apples, blueberries, bananas, cocoa, peaches, pears, and tea. Aromatic plants containing flavanols are mint, sage, rosemary, basil, and dill. The flavanols present in these fruits and plants include catechin, epicatechin, epicatechin-3-O-gallate, gallocatechin, procyanidin, theaflavin, etc. They possess several pharmaceutical properties such as anticancer, anti-inflammatory, anti-thrombotic, antioxidant, vasodilating, and cardioprotective activity (Zuiter et al. 2014).
- *Isoflavones*
Isoflavones structurally resemble estrogens and contain B-ring linked to C-3 position. On the basis of oxidation level of central pyran ring, isoflava-

Fig. 4.5 Basic structure of lignans



nones can be divided into subcategories like daidzein, genistein, formononetin, biochanin A, prunetin, 6-hydroxy daidzein, glycitein, orobol, and santal. They are mainly found in family Leguminosae, *Prunus* species, soybeans, and *Pueraria lobata* (Kudzu). The major health benefits of isoflavones include various activities, viz., antidiabetic, anti-estrogenic, anticancer, and anti-lipidemic, prevent osteoporosis, improve cardiovascular health, treat menopause symptoms, and inhibit platelet aggregation (Zuiter et al. 2014).

(c) *Lignans*

Lignans may be defined as bioactive phenolic compounds formed by linking two phenylpropanoid units (Fig. 4.5) and are non-nutritive and non-caloric in nature (Peterson et al. 2010; Zuiter et al. 2014). Lignans as a group was first described by Haworth in 1936 (Basu and Mukherjee 2018). They perform several functions in plants as in plant defense by acting as biocides and phytoalexins and thereby providing protection against predators and insects (Peterson et al. 2010). Lignans occur in nature either as free entities or bound to sugar moieties. The major lignan sources in nature include cereals, fruits and vegetables, legumes, and oil seeds. The major lignans found in plants are lariciresinol, isolariciresinol, lariciresinol sesquilignan, secoisolariciresinol, pinoresinol, matairesinol, 7-hydroxylariciresinol, syringaresinol, cyclolariciresinol, etc. (Peterson et al. 2010; Zuiter et al. 2014; Basu and Mukherjee 2018)

Lignans comprise estrogenic and anti-estrogenic activity which is responsible for several disease-fighting properties that lignans exhibit such as anticancer, antimicrobial, and cardiovascular protective activity (Peterson et al. 2010; Herrero et al. 2012; Zuiter et al. 2014).

(d) *Tannins*

Tannins or commonly called tannic acid is water-soluble phenolic compound formed by condensation of flavan derivatives or polymerization of quinone units (Basu and Mukherjee 2018). Tannins are widely distributed in medicinal and aromatic plants such as black cohosh, cinnamon, curry leaves, feverfew, and thyme (Ghosh 2015) (Fig. 4.6).

Tannins are categorized into two major subclasses, i.e., hydrolyzable tannins and non-hydrolyzable or condensed tannins (Fig. 4.6). The former are made up of hydrolyzable polyhydric alcohol esterified by gallic acid or hexahydroxydiphenic acid at hydroxyl group either partially or completely, e.g., gallotannins (geraniin, etc.) and ellagitannins (corilagin, etc.), while the latter are condensation products of phenolic compounds mainly catechin or epicatechin. Condensed tannins are also called *proanthocyanidins* (PAs) or *flavolans*, e.g., proanthocy-

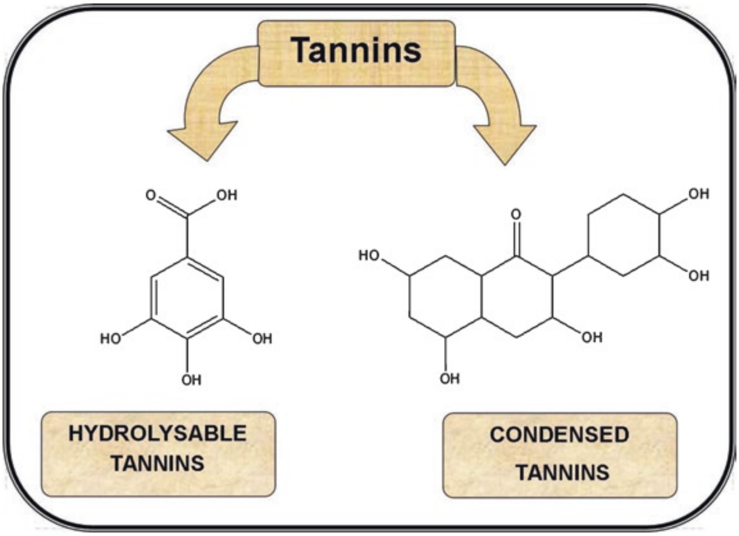


Fig. 4.6 Major types of tannins in nature and their basic structure

anidin B_1, proanthocyanidin B_2, etc. Condensed tannins are basically polymerized forms of flavan-3-ols and flavan-3,4-diols or consortium of two (Chung et al. 1998; Ghosh 2015; Ky et al. 2016).

Tannins are immense source of therapeutics and are used to treat allergies, acute dermatitis, burn injuries, cardiovascular disorders, cold sores, cough, diarrhea, dysentery, gastric ulcers, heavy metal-induced oxidative stress, inflammation, inhibits platelet aggregation, microbial infections, painful joints, prickly heat, snake bites, sore throat, tonsils, tumors, etc. (Ghosh 2015).

(e) *Phenolic acids*

Phenolic acids or phenolcarboxylic acids are class of phenolic compounds comprising a phenolic ring attached to an organic carboxylic acid. They perform vital functions in plants such as allelopathy, enzyme activity, nutrient uptake, photosynthesis, synthesis of proteins, and structural components. They can further be classified into two subclasses based on the C-units linked to the phenolic ring, hydroxybenzoic acids and hydroxycinnamic acids (Goleniowski et al. 2013).

Hydroxybenzoic acids are derivatives of benzoic acids such as gallic acid, vanillic acid, protocatechuic acid, salicylic acid, etc., while hydroxycinnamic acids are cinnamic acid derivatives, e.g., chlorogenic acid, caffeic acid, p-coumaric acid, etc. (Fig. 4.7).

Phenolic acids have tremendous therapeutic value as they act as antidiabetic, antimicrobial, antioxidant, anti-allergic, anticancer, anti-inflammatory, and antiviral agents (Goleniowski et al. 2013; Kumar and Goel 2019).

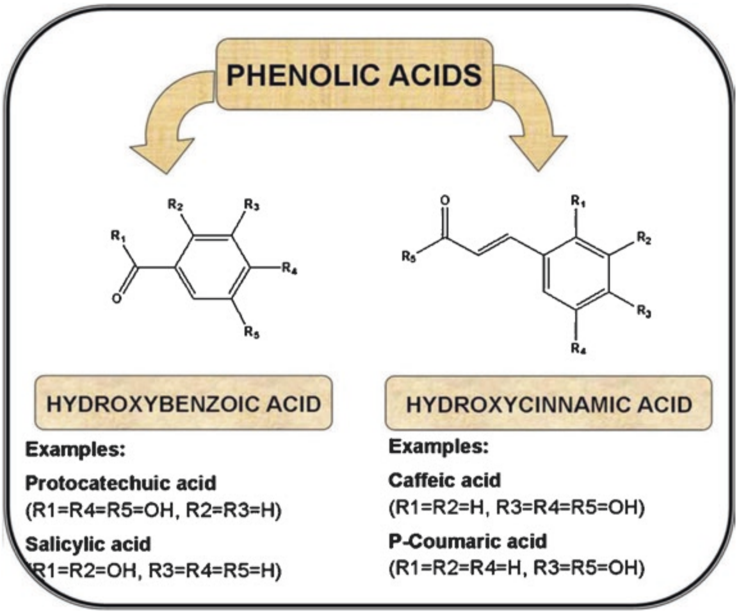


Fig. 4.7 Classes of phenolic acids along with basic structure and examples

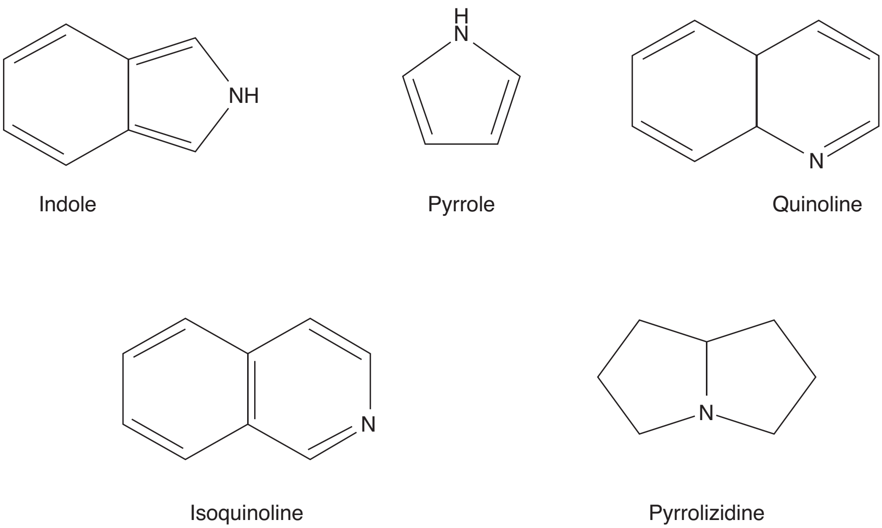


Fig. 4.8 Basic structures of some of the alkaloids based on structure

4.5.3 Nitrogen-Containing Compounds

(a) *Alkaloids*

Alkaloids (term coined by Wilhelm Meissner, German pharmacist) are bioactive plant secondary metabolites containing at least single nitrogen atom in heterocyclic ring structure (Eguchi et al. 2019). They are mainly amino acid derivatives. The classification of alkaloids is strenuous as they are sometimes classified on the:

- Basis of structure: indoles, quinolones, isoquinolines, pyrrolidines, tropane, and izidine alkaloids. The basic structure of alkaloids is given in Fig. 4.8.
- Basis of biological distribution or the family of plant species in which they occur such as opium alkaloids belonging to *Papaver somniferum* (opium poppy) (Kurek 2019).

Alkaloids give plants protection against herbivores and insects. They also show allelopathy and due to their toxic nature some even cause death in animals if ingested (Hussein and Ansary 2018).

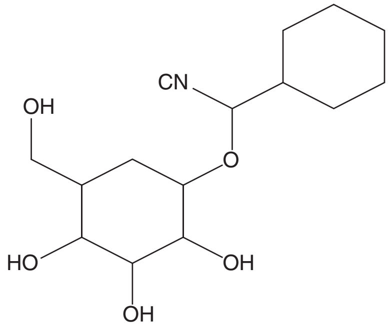
More than 10,000 alkaloids have been identified till date from about 300 different plant species. They have even gained acclaim due to their pharmaceutical and medical importance. They are widely used in narcotics. They exhibit various medicinal properties, viz., antibacterial, analgesic, anti-inflammatory, anticancer, anti-mitotic, anti-malarial, etc. (Zhao et al. 2015; Kurek 2019). They are also used as blood vessel constrictors, muscle relaxants, and sexual stimulants, to treat bradycardia and also to relieve discomfort due to hay fever, sinusitis, and common colds (Zhao et al. 2015; Kurek 2019). Some of the major alkaloids used today include atropine, caffeine, codeine, colchicine, ephedrine, nicotine, and morphine (Zhao et al. 2015; Ahmed et al. 2017).

(b) *Cyanogenic glycosides*

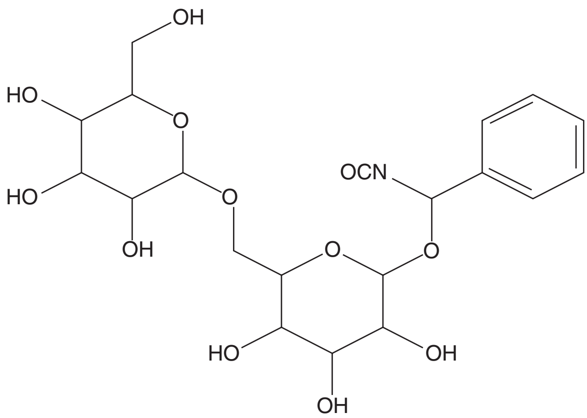
Cyanogenic glycosides or commonly called cyanoglycosides form major group of plant toxins known as cyanogens. The principle feature of this group is release of free hydrogen cyanide (HCN) by the process called cyanogenesis. These are α-hydroxynitriles, β-linked with mostly D-glucose, and result in production of aglycone and sugar moiety upon enzymatic hydrolysis by β-glucosidase (Vetter 2000). Upon crushing of plants, these N-containing compounds release poisonous gases such as HCN and hydrogen sulfide (H_2S) which form defense system of plants by giving protection against insects and herbivores. The important plant families consisting cyanogen glycosides are Compositae (linamarin), e.g., *Linum usitatissimum*; Poaceae (dhurrin), e.g., *Sorghum* species; and Rosaceae (amygdalin and prunasin), e.g., *Prunus* species (bitter almond and peaches) (Pagare et al. 2015).

Although cyanogenic glycosides are poisonous to both human beings and animals but few in vitro studies have suggested the beneficial role of prunasin and amygdalin in prevention of cancer by inhibiting DNA polymerase (Yarnell 2007). The structures of prunasin and amygdalin are given in Fig. 4.9.

Fig. 4.9 Important cyanogenic glycosides. (**a**) Prunasin, (**b**) amygdalin having anticancer activity



a) Prunasin



b) Amygdalin

(c) *Glucosinolates*

Glucosinolates are class of secondary metabolites containing sulfur and nitrogen groups, i.e., thiohydroximate-O-sulfonate group forming bonds with D-glucose along with alkyl, aralkyl, or indole side chain. They are generally found in species of Brassicaceae family (Barba et al. 2016).

Glucosinolates are stable and biologically inactive metabolites of plants; however on disruption of plant tissue by any means or external factors such as crushing of plant by animals, insects, predators, etc., they release enzyme myrosinase which upon hydrolysis of glucosinolate produce unstable aglycon metabolites like thiocyanate, nitriles, sulfates, and goitrins having therapeutic properties. Thiocyanates play a vital role as antitumor agents by inhibiting mitosis and further stimulating apoptosis in malignant cells (Johnson 2002; Bischoff 2016; Barbara et al. 2016). The important glucosinolates are progoitrin, sinigrin, glucobrassicin, and glucoraphanin (Bischoff 2016).

4.6 Pharmacological Potential and Mechanism of Action of Bioactive Secondary Metabolites

According to World Health Organization (WHO), 80% of the total world population still trusts traditional medicine for the treatment of various ailments. Bioactive secondary metabolites form the backbone of ancient medicine, and around 3.3 billion people in developing nations use MAPs to cure common disease on daily basis (Ahvazi et al. 2012). Research on the bioactive secondary metabolites has increased tremendously in the past 50 years due to their vast impact on pharmaceutical and healthcare industry. The compilation of extensive data on secondary metabolites is quite arduous; hence the authors have made an effort to assemble data on disease-fighting properties of secondary metabolites from previous 5 years (2015–2020).

4.6.1 Antimicrobial Activity

Antibiotic resistance has emerged as a threat on global basis due to ineffectiveness of antibiotic drugs to respond against infection-causing microorganisms. Moreover, with the prevalence and emergence of new multidrug-resistant strains, researchers have been prompted to identify new antimicrobial agents having strong efficacy and lesser side effects to treat pathogenic microorganisms (Barbieri et al. 2017; Manadhar et al. 2019).

With the popularization and new methods for identification of bioactive secondary metabolites of MAPs in the past few decades, the research has found a pace with hundreds of articles published on antimicrobial activity of these plants and their secondary metabolites against deadly human infections (Barbieri et al. 2017). Some of the studies involving the use of MAPs against human and food pathogens are listed in Table 4.3.

Some of the secondary metabolites exhibiting antimicrobial activity are carvacrol, eugenol, linalool, linalyl acetate, menthol, citral, allicin, apigenin, quercetin, epicatechin gallate, epigallocatechin gallate, 3-O-octanoyl-(+)-catechin, 2,4,2′-trihidroxy-5′-methylchalcone, cinnamaldehyde, berberine, hermane, verbascosides, coumarin, allyl isothiocyanate, thymol, citronellol, tea tree oil, geraniol, p-cymene, piperine, curcumin, terpenine-4-ol, etc. (Radulovic et al. 2013).

The antimicrobial potency of various secondary metabolites from MAPs can be attributed to several mechanisms including (Raduvolic et al. 2013):

- Disintegration of structure of membrane along with hampering of membrane functions
- Termination of synthesis of genetic material, i.e., DNA/RNA and its functions
- Disturbance of intermediary metabolism
- Interference with mechanism of quorum sensing, i.e., cell-cell communication
- Inhibition of major enzymes

Table 4.3 Recent in vitro studies involving medicinal and aromatic plants exhibiting antimicrobial activity against human and food pathogens (2015–2020)

S.No.	Plant used	Extract used	Tested bacterial strains	Tested fungal strains	References
1.	*Psidium guajava*, *Phyllanthus niruri*, *Ehretia microphylla*, *Piper betle*	Ethanolic	*Methicillin-resistant Staphylococcus aureus* and *vancomycin-resistant Enterococcus*	–	Valle Jr. et al. (2015)
2.	*Maytenus macrocarpa*, *Dracontium loretense*, *Tabebuia impetigenosa*, *Eucalyptus camaldulensis*, *Uncaria tomentosa*	Ethanolic	*Pseudomonas aeruginosa*	–	Ulloe-Urizar et al. (2015)
3.	*Lophostemon suaveolens*, *Corymbia intermedia*, *Syncarpia glomulifera*	Aqueous-ethanolic	*Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*	*Candida albicans*	Packer et al. (2015)
4.	*Nauclea pobeguinii*, *Aframomum danielli*, *Albizia lebbeck*, *Baillonella toxisperma*	Methanolic	*Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhii*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Staphylococcus saprophyticus*	*Trichophyton rubrum*, *Candida albicans*	Njimoh et al. (2015)
5.	*Artemisia indica*, *Medicago falcata*, *Tecoma stans*	Chloroform, butanol, ethyl acetate, and n-hexane	*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhii*, *Staphylococcus aureus*	*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Fusarium solani*	Javid et al. (2015)

(continued)

Table 4.3 (continued)

S.No.	Plant used	Extract used	Tested bacterial strains	Tested fungal strains	References
6.	*Gnaphalium polycaulon (L.) Pers.*	Methanolic	*Aeromonas hydrophila*, *Escherichia coli* *MTCC739*, *Flavobacterium* sp., *Pseudomonas aeruginosa* MTCC 424, *Salmonella typhimurium* and *Yersinia enterocolitica*, *Bacillus cereus* *MTCC430*, *Listeria monocytogenes*, *Staphylococcus aureus* MTCC 3381	*Aspergillus flavus*, *Aspergillus fumigatus* MTCC 343, *Aspergillus oryzae*, *Candida albicans* MTCC 227, and *Penicillium notatum*	Kaminidevi et al. (2015)
7.	*Teucrium marum*, *Pinus sylvestris*, *Thymus vulgaris*, *Salviae aetheroleum*, *Cinnamomum aromaticum*, *Hippophae rhamnoides*, *Lavandula angustifolia*, *Abies alba*, *Zingiber officinale*, *Anethum graveolens*, *Coriandrum sativum*, *Origanum vulgare*	Essential oils	*Escherichia coli ATCCR CRM-8739*	–	Sandru (2015)
8.	*Verbena officinalis*, *Majorana hortensis*, *Salvia officinalis*	Essential oils	*Bacillus megaterium*, *Clavibacter michiganensis*, *Bacillus mojavensis*, *E.coli*, *Xanthomonas campestris*, *Pseudomonas savastanoi*, *Pseudomonas syringae*	*Colletotrichum acutatum* and *Botrytis cinerea*	Elshafie et al. (2016)
9.	*Syzygium aromaticum* and *Cinnamomum zeylanicum*	Ethanolic	*Listeria monocytogenes*, *Staphylococcus epidermidis* and *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*	–	Singh (2018)

S.No.	Plant used	Extract used	Tested bacterial strains	Tested fungal strains	References
10.	*Veronica biloba*	Aqueous, dichloromethane, n-hexane, and ethyl acetate	*E.coli* and *S. aureus*	–	Hassan and Ullah (2019)
11.	*Cymbopogon flexuosus*, *Pogostemon cablin*, *Curcuma caesia*, *Cymbopogon winterianus*, *Clausena heptaphylla*, *Cinnamomum tamala*, *Psidium guajava*, *Ocimum sanctum*, *Cinnamomum camphora*, *Kaempferia galanga*	Essential oils	*Staphylococcus aureus (ATCC 11632)*, *Bacillus cereus (ATCC-10876)*, *Bacillus subtilis (ATCC-11774)*, *Salmonella typhimurium (ATCC-13311)*, and *Escherichia coli (ATCC-11229)*	*Aspergillus niger (ATCC-16885*, *Aspergillus fumigatus (ATCC-204305)*, *Saccharomyces cerevisiae (ATCC-9763) and Candida albicans (ATCC-66027)*	Munda et al. (2019)
12.	*Oxalis corniculata*, *Artemisia vulgaris*, *Cinnamomum tamala*, *Ageratina adenophora*	Methanolic	*E. coli*, *S. typhii*, *MDR S. typhii*, *MDR K. pneumoniae*, *Citrobacter koseri*	*Rhizopus* spp., *A. niger*, *A. flavus*, *C. albicans*	Manandhar et al. (2019)
13.	*Solanum mauritianum* and its bacterial endophytes	Methanol-chloroform	*E. coli*, *S. aureus*, *K. pneumonia*, *P. aeruginosa*	–	Uche-Okereafor et al. (2019)
14.	*Coix lacryma-jobi*	Essential oil	*E. coli*, *S. aureus*, and *B. subtilis*	–	Diningrat et al. (2020)

4.6.2 Anti-carcinogenic Activity

Cancer may be distinguished by uncontrolled growth and proliferation of abnormal cells. These malignant cells spread and destroy non-malignant or normal cells also (Abdullahi et al. 2018). Cancer affects around 3.5 billion people all over the world. It spreads to various vital organs and ultimately leads to death. Drugs currently used for the treatment of cancer are expensive and toxic and may cause harm to healthy cells. Hence search for novel anticancer agents with effective and safe properties led the scientific community to bioactive secondary metabolites of MAPs (Alabi et al. 2020).

MAPs contain various bioactive constituents which act against cancer such as allicin (*Allium sativum*); lactone and vermodalin (*Vernonia amygdalina*); lycopene (*Solanum lycopersicum*); vinblastine, vincristine, vindesine, and vinorelbine (*Catharanthus roseus*); chebulinic acid, ellagic acid, and tannic acid (*Terminalia chebula*); etc. (Abdullahi et al. 2018).

Recent study carried out by Promraksa and co-workers (2019) to check the potential of ethanolic extracts of three medicinal herbs, namely, *Cardiospermum halicacabum*, *Gomphrena celosioides* Mart., and *Scoparia dulcis* L., against cholangiocarcinoma (CCA) cell lines KKU-100 and KKU-213 was investigated. The research group deduced that out of all the extracts used, *S. dulcis* extract significantly increased BAX/Bcl-2 ratio along with permeability of cell membrane leading to inhibition of cancer cells.

Similarly different doses of essential oil (EO) containing α-thujone, 1,8-cineole, and camphor from aromatic plant *Salvia officinalis* were studied for antitumor activity against human hormone-dependent tumor cell lines, viz., LNCaP (prostate cancer), MCF 7 (breast cancer), and HeLa (cervical cancer), and it was identified that both oil and its components significantly reduced the viability of cancer cells after 2 days incubation. The authors suggested that the EO and its fractions induced apoptosis via P13K/AKT and MAPK/ERK pathways along with cell cycle arrest and by alleviating the levels of reactive oxygen and nitrogen species (Privitera et al. 2020).

4.6.3 Anti-cholestromic/Anti-lipidemic Activity

Hypercholesterolemia may be defined as elevated levels of cholesterol in the bloodstream than normal concentration. The major risk factors associated with high cholesterol are atherosclerosis, heart stroke, and myocardial infection. Hypercholesterolemia and hyperlipidemia are the terms which can be used interchangeably as both involve increased levels of blood lipids such as cholesterol, lipoproteins (aid in transport of cholesterol through blood plasma), and triglycerides. Thus, hypocholesteromic and hypolipidemic agents basically reduce the concentration of cholesterol and triglycerides in blood and protect against cardiovascular disease (Hurell et al. 2015).

Several MAPs along with their secondary metabolites exhibiting anti-cholesteromic activity are *Allium cepa* L. (S-alkyl cysteine sulfoxides), *Allium sativum* L. (allicin), *Commiphora mukul* L. (E-Z-guggulsterone), *Terminalia arjuna* (arjunine, arjunetin, arjunoside I, arjunoside II, triterpene-O-glycoside), *Phyllanthus niruri* L. (phloretin-2-O-xylosyl glucoside, theaflavin-3-O-gallate, myricetin, cyanidin, delphidin-3-O-sambubioside, protocatechuic acid, 8-5′-diferulic acid, lupenone), *Cicer arietinum* (biocharin A and formomonetin), *Glycine max* L. (isoflavones, genistein, daidzein), *Oryza sativa* L. (triterpene alcohols, tocopherols, oryzanol, campesterol, β-sitosterol), *Curcuma longa* L. (curcumin), *Trigonella foenum-graecum* (galactomannan), *Glycyrrhiza glabra* (glabridin, isoliquiritigenin), *Lagenaria siceraria* (triterpenoid cucurbitacins B, D, G, 22-deoxycucurbitacin), etc. (Saravanan and Ignacimuthu 2015; Kaur et al. 2017; Srivastava and Srivastava 2018).

Essential oils in aromatic plants of citrus family such as *Citrus limon* L. Burm (lemon), *Citrus sinensis* L. Osbeck (orange), *Citrus paradise* Macfayden (grapefruit), etc. contain many bioactive secondary metabolites such as limonene, α-terpineol, terpinolene, γ-terpinene, etc. All these metabolites exhibit anti-lipidemic, anti-atherogenic, and anti-cholesteromic activity (Lee et al. 2018). The potential of these compounds was tested in hypercholesteromic rabbits by Lee and co-workers (2018), and they suggested that the metabolites show this effect by upregulating peroxisome proliferator-activated receptor alpha (PPARα) and liver X-receptor beta (LXRβ).

Similarly latest in vivo study in hypercholesteromic rats was carried out by Harb and company (2019) to study the effect and mechanism of bioactive secondary metabolite eugenol commonly found in essential oil of *Eugenia caryophyllus* (clove) and in other MAPs such as *Ocimum minimum* and *Melissa officinalis* against high cholesterol and lipids. The researchers concluded that eugenol effectively reduced total cholesterol, low density lipoproteins (LDL), and anthrogenic index in high cholesterol diet-fed rats. It also lowered steatosis, hepatic inflammation, and hepatomegaly. Additionally, significant reduction was observed in marker enzymes associated with hepatic injury such as alanine amino transferase (ALT) and alkaline phosphatase (ALP) while alleviating antioxidant enzymes superoxide dismutase (SOD) and catalase. The mechanism of action was attributed to downregulation of transient receptor potential vanilloid (TRPV1) channels in the liver which induce the action of β-hydroxy β-methylglutaryl-CoA, i.e., HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis.

4.6.4 Antiviral Activity

Around 219 virus species have been identified to be infectious to human beings, and yellow fever virus was the first to be discovered in 1901. From then, new viral disease-causing species have been discovered at the rate 2–3/year, and the research regarding novel pathogenic viral strains has accelerated after the discovery of human immunodeficiency virus (HIV) in the 1980s. New viruses emerged in the last

Table 4.4 MAPs exhibiting antiviral action against major infectious viruses

S.No.	Virus type	Plant used	Extract/compound used	Reference
1.	Hepatitis B virus (HBV)	*Acacia mellifera*	Aqueous, ethyl acetate, n-butanol	Arbab et al. (2015)
2.	Herpes simplex virus-I (HSV-1)	*Artemisia kermanensis*, *Eucalyptus caesia*, *Rosmarinus officinalis*, *Satureja hortensis*, *Zataria multiflora*	Essential oils	Gavanjia et al. (2015)
3.	Measles virus	*Uvaria chamae* and *Xylopia aethiopica*	Methanol	Oluremi and Adeniji (2015)
4.	Influenza A-virus/ Influenza virus A/ WSN/33 (H1N1)	*Aristolochia bracteolata*, *Boscia senegalensis*, *Pittosporum viridiflorum*, and *Rapanea melanophloeos*	Aqueous, acetone, methanol, ethanol	Mehrbod et al. (2018)
5.	Echovirus	*Crinum jagus*, *Macaranga barteri*, and *Terminalia ivorensis*	Methanol and dicholoromethane	Ogbole et al. (2018)
6.	Human immunodeficiency virus (HIV)	*Achyranthes aspera* and *Rosa centifolia*	Methanol	Palshetkar et al. (2020)
7.	SARS-CoV-2 (Covid-19) In-silico study	*Silybum marianum*, *Withania somnifera*, *Tinospora cordifolia*, and *Aloe barbadensis*	Silybin, Withaferin-A, cordioside, catechin and quercetin	Pandit and Latha (2020)

few years including deadly strains of Arenaviridae, Bunyaviridae, Coronaviridae, Flaviviridae, Hepeviridae, Paramyxoviridae, and Togaviridae (Woolhouse et al. 2012; Parvez and Parveen 2017).

The major virus outbreaks that have led to millions of causalities in previous years (2015–2020) include avian influenza virus [A(H7N9), A(H5N6)], polio virus, Middle East respiratory syndrome coronavirus (MERS-CoV), dengue virus, chikungunya virus, yellow fever, Oropouche virus disease, hepatitis E, HIV, influenza A (H1N1), Nipah virus, Ebola virus, Zika virus (ZICV), hantavirus, and novel coronavirus (WHO 2020).

Secondary metabolites of different MAPs exert different mechanisms to inhibit the viruses such as some anti-HIV plants work by inhibiting viral proteases, viral reverse transcriptase (RT), and integrase. Baicalin, a secondary metabolite, interacts with chemokine receptors to inhibit the entry of HIV virus. Several plants of *Phyllanthus* genus such as *Phyllanthus amarus*, *Phyllanthus niruri*, and *Phyllanthus urinaria* inhibit hepatitis B virus (HBV) polymerase activity and mRNA transcription and overexpress annexin-7 (Anx7) gene. Phytochemicals such as silybin and oxymatrine show anti-HCV (hepatitis C virus) activity by significantly reducing the level of serum aspartate aminotransferase (AST) and α-glutamyl transpeptidase along with HCV clearance (Mukhtar et al. 2008; Kaur et al. 2020). Some of the recent studies on MAPs exhibiting antiviral properties are listed in Table 4.4.

4.6.5 Antidiabetic Activity

Diabetes mellitus (DM) or diabetes is a common metabolic disorder characterized by hyperglycemia (increase in blood glucose levels). Under chronic conditions, it results in severe damage to blood vessels, eyes, heart, kidneys, and nerves (Malviya et al. 2010). Around 25% of total world population is suffering from diabetes. It is generally of two types, type 1, or insulin-dependent diabetes, in which insufficient insulin is produced by the pancreas and type 2 or non-insulin-dependent diabetes, in which body becomes ineffective to use the insulin produced which further results in elevated level of glucose in the blood. Out of the two, type 2 diabetes is more prevalent around the world (Salehi et al. 2019).

Although large numbers of drugs are available to control this disease, they inherent several adverse effects such as reduction in efficiency of drug after long-term usage and sometimes toxicity. To overcome these problems, scientific community is continuously working for new herbal alternatives of these drugs having easy availability, low cost, and lesser side effects (Salehi et al. 2019).

There are about 509 plants belonging to 140 genera having antidiabetic potential. The major antidiabetic plants are *Ficus elastica*, *Ficus hispida*, *Artemisia afra*, *Solanum viarum*, *Terminalia arjuna*, *Euphorbia hirta*, *Allium cepa*, *Mangifera indica*, *Phyllanthus amarus*, *Phyllanthus niruri*, *Aloe vera*, *Boerhaavia diffusa*, *Camellia sinensis*, *Nelumbo nucifera*, *Ichnocarpus frutescens*, etc. These plants are either α-amylase inhibitors or α-glucosidase inhibitors (Kaur et al. 2017; Salehi et al. 2019; Srujana et al. 2020).

Several bioactive secondary metabolites of plants exhibiting antidiabetic activity include alkaloids like berberine, boldine, neferin, lupanine, piperine, oxymatrine, sanguinarine, etc.; flavonoids such as catechin, baicalin, diosmin, genistein, fisetin, luteolin, kaempferol, rutin, naringenin, quercetin, etc.; triterpenoids like betulin, betulinic acid, lupeol, etc.; and polysaccharides, viz., galactomannan and inulin along with other compounds such as revesterol, piceatannol, butein, curcumin, chlorogenic acid, embelin, erianin, garcinol, honokiol, etc. (Salehi et al. 2019).

4.7 Conclusion and Future Prospects

It is impossible to imagine human existence without plants. They not only provide food but also form the foundation of modern medicine. They are widely used for medicinal purposes today also due to their easy availability, low cost, and lesser side effects. Research on ethnopharmacology has led to many discoveries in new strains of medicinal and aromatic plants and their bioactive secondary metabolites. Currently the research is more focused on methods for isolation of these bioactive metabolites into purified forms and utilizing them as drugs to treat various disorders. Although there has been extensive research in this field, still a lot needs to be done regarding their mode of action in detail. Clinical trials involving purified secondary metabolites also need to be carried out to prove their safety and efficacy.

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Chapter 5
Potential Uses of Bioactive Compounds of Medicinal Plants and Their Mode of Action in Several Human Diseases

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5.1 Introduction

Since ancient times, traditional medicinal system including several hundred plant genera has been the most affordable and the easily approachable source of treatment in the primary health-care system. Plant-based medicines are mainly used as herbal preparations in the native systems of medicine in different parts of the world and are rich sources of potent and powerful drugs which are being used till date and modern chemistry has not been able to replace most of them (Ahmad et al. 1998). Medicinal plants have long been known as remedies for human disease and provide a new source of biologically active compounds as antimicrobial agents and also are great bioresources of food supplements, pharmaceuticals and drugs of traditional medicinal system, modern medicines, folk medicines, nutraceuticals, intermediates, and chemicals entitled for synthetic drugs (Das et al. 2010). Developing countries count for 80% of the world's population, and about 64% of the total population of the world relies on herbal medicines, i.e., 3.2 billion people (Chadwick and Marsh 2008). Some parts of India and China and the developed areas of Europe cultivate several medicinal plants on large scale to maintain the high demands of alternative natural drugs (Greenwell and Rahman 2015). A wide range of bioactive compounds have been identified in folk medicines that can be used to treat chronic as well as infectious diseases (Sasidharan et al. 2011). A vast array of natural product drugs discovered from medicinal plants including galantamine, arteether, and tiotropium have either been approved by the US Food and Drug Administration (FDA) or are

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in clinical trials. A large number of therapeutically important drugs based on plant origin raise hopes against various human diseases such as HIV/AIDS, Alzheimer's disease, malaria, and cancer, and these natural products continue to contribute to the development of clinically important drugs in widespread clinical use such as paclitaxel, vincristine, vinblastine, and topotecan (Guimarães et al. 2014).

Medicinal plants or their extracts have continuously been providing new and valuable leads in the treatment of various medical conditions including malaria, cardiovascular diseases, cancer, and neurological disorders. Traditional and ethnobotanical utilization of medicinal plants provides as a source of useful knowledge for the isolation of bioactive compounds including D-tubocurarine from *Chondrodendron tomentosum* (direct use as a therapeutic agent), diosgenin from *Dioscorea floribunda*, cocaine from *Erythroxylum coca* (the model drug for new synthetic drugs), and for the production of local anesthetics (Ramawat et al. 2009). The World Health Organization (WHO) has recognized herbal medicines as essential components for primary health care, and about 11% of the 252 drugs are obtained from medicinal plants (Taylor 2000). The safe use of medicinal plants in different ailments has great advantage in therapeutics besides being economical and effective and their easy availability. These advantages allow the extensive use of medicinal plants by the traditional medical practitioners in their day-to-day practice (Hosseinzadeh et al. 2015a, b).

5.2 Bioactive Compounds

Bioactive compounds (BAC) are the substances synthesized by plants as secondary metabolites (phytochemicals) having pharmacological or toxicological effects in humans and animals (Bernhoft 2010). These secondary metabolites include terpenoids, glycosides, alkaloids, flavonoids, and phenolic compounds. Terpenoids generally exhibit potential pharmaceutical properties such as anticancer, antimalarial, anti-inflammatory, antiviral, antibacterial, and inhibition of cholesterol synthesis. On the other hand, alkaloids have antispasmodic, antimalarial, diuretic, and analgesic activities. Phenols and flavonoids are reported for their antioxidant, anti-allergic, and antibacterial properties, while glycosides have antifungal and antibacterial properties. Saponins are found to have anti-inflammatory, antiviral, and plant defense activities (Wadood et al. 2013; Shakya 2016). Plant bioactive compounds also exhibit antioxidant activity, stimulation of the immune system, modulation of detoxification enzymes, decrease of platelet aggregation, and modulation of hormone metabolism. Recent studies suggest the role of BACs in reducing the risk of coronary heart disease by regulating the oxidation of low-density lipoprotein (LDL) cholesterol, lowering the synthesis of cholesterol, maintaining blood pressure and clotting, and enhancing arterial elasticity (Saxena et al. 2013).

5.3 Functions of Bioactive Compounds

5.3.1 Alkaloids

Alkaloids are nitrogen-containing compounds (usually heterocyclic) and have emerged as a chemical defense against microorganisms, viruses, herbivores, or against other plants. They are structurally the most diverse class of secondary metabolites and occur as simple structures to complex ones such as many neurotoxins (Fig. 5.1). Their immense pharmacological properties (especially in medicines and stimulants) induce interest in selected plant products containing alkaloids (Bandaranayake 2002). A number of distinct classes of approximately 12,000 different alkaloids have been identified in plants, and majority of these potential secondary metabolites have striking positive effects in different human medical conditions (Takos and Rook 2013). Alkaloids are structurally more diversified than any other class of secondary metabolites; therefore, they are not classified uniformly. Depending on the precursor and the final structure, alkaloids have been divided into three major classes including true alkaloids, that are derived from amino acids and contain nitrogen in the heterocyclic ring such as atropine and nicotine; protoalkaloids, derived from amino acids but nitrogen is not present in the heterocyclic ring, e.g., phenylethylamine-derived alkaloids; and pseudoalkaloids, not synthesized from amino acids but have nitrogen in the heterocyclic ring such as caffeine and solanidine (Singh 2017). Some commonly used alkaloids in medicines are aconitine, ajmaline, atropine, berberine, boldine, caffeine, cathine, cocaine, col-

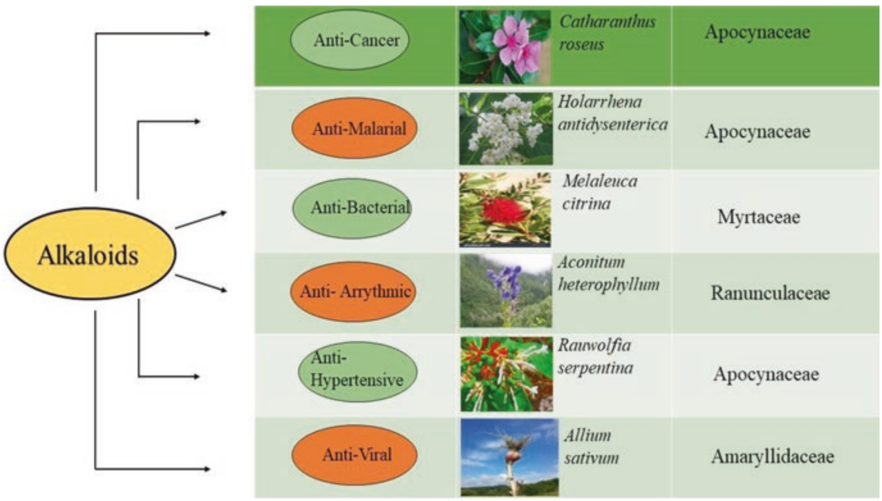

Fig. 5.1 Common properties of some important alkaloid-bearing medicinal plants

chicine, ephedrine, ergotamine, morphine, narceine, nicotine, papaverine, quinine, reserpine, taxol, vinblastine, vincristine, and many more (Schmeller and Wink 1998). Alkaloids possess several pharmacological activities such as antiarrhythmic effect (spareien, quinidine), anticancer actions (vincristine, vinblastine, dimeric indoles), antimalarial activity (quinine), and antihypertensive effects (many indole alkaloids) (Saxena et al. 2013).

5.3.1.1 Some Important Alkaloid-Bearing Plants and Their Medicinal Properties

Trigonella foenum-graecum, commonly known as fenugreek, is a traditional Chinese herb. The major alkaloid component of fenugreek, trigonelline (N-methylnicotinic acid, $C_2H_7NO_2$), has great pharmacological importance and is found beneficial in the prevention and treatment of diabetes and central nervous system diseases. Apart from these properties, trigonelline also has antimigraine, sedative, hypolipidemic, memory-improving, antibacterial, and antiviral properties. The mechanisms regulating these effects have been found to be connected with modulation of β cell regeneration, activity of glucose metabolism-related enzymes, insulin secretion, reactive oxygen species generation and scavenging, axonal extension, and neuron excitability. More findings are required to further explore the possible mechanism including trigonelline effects on antioxidant actions and transcriptional pathways. However, both in vivo and in vitro studies are required before its clinical use in the treatment of diabetes and other human disorders (Zhou et al. 2012).

Papaver somniferum, usually known as the opium poppy, is an annual herb belonging to the family Papaveraceae. It produces two main alkaloids, i.e., the narcotic analgesic morphinan alkaloid morphine and antimicrobial benzophenanthridine alkaloid sanguinarine (Huang and Kutchan 2000). Morphine is an isoquinoline alkaloid and used in the attenuation of moderately serious to extreme pain. Several studies have indicated that its analgesic activity is regulated by μ-opioid receptor (MOR), and it can also exhibit beneficial role in neuronal system injuries (Hussain et al. 2018).

Mahonia aquifolium (Berberidaceae), commonly known as Oregon grape, exhibits great pharmaceutical importance. It contains a variety of bioactive isoquinoline compounds such as berberine, jatrorrhizine, and a complex of bis(benzylisoquinoline) alkaloids (BBI). Berberine has been found to have antitumor activity in myelocytic leukemic HL60 cells, where it regulates the differentiation and apoptotic death. It also alters the reverse transcriptase activity of RNA tumor viruses (Vollekova et al. 2001).

Berberis vulgaris (barberry) also belonging to family Berberidaceae is a well-known plant having immense medicinal value. The roots, bark, leaves, and fruits of the plant have been used in traditional medicinal systems. The most studied constituents of the plant are its isoquinoline alkaloids, i.e., berberine, berbamine, and palmatine. However, some other important alkaloids, i.e., oxycontin, bervulcine, columbamine, and coptisine, have also been identified (Zarei et al. 2015). Berberine is one of the most studied protoberberine alkaloids. It has also been reported in many other plants in addition to *B. vulgaris* including *Coptis chinensis,* Ranunculaceae (*Coptis* or goldthread); *Hydrastis canadensis*, Ranunculaceae (goldenseal); *Arcangelisia flava*, Menispermaceae; *B. aquifolium* (Oregon grape), and *B. aristata* (tree turmeric) (Imanshahidi and Hosseinzadeh 2008). Recently, its antimicrobial activity against hepatitis C virus has been reported (Ghareeb et al. 2013). Several studies have confirmed that berberine could play role as anti-obesity and hypoglycemic agent as it increases insulin sensitivity and is capable of inhibiting alpha glucosidase and adipogenesis. It can also improve the function of liver enzymes by reducing the density of serum cholesterol and triglycerides; therefore, it can be suggested as hypolipidemic and hepatoprotectant (Zarei et al. 2015).

Taxus wallichiana (Himalayan yew), belonging to the family Taxaceae, is a temperate Himalayan forest tree of high medicinal value and ethnobotanical importance. Recently, it has received attention because of its alkaloid taxol, a potent anticancer drug. Taxol was found to have unique properties to prevent the growth of cancerous cells and is used in the treatment of ovarian and breast cancers. It was first isolated from the bark of *Taxus brevifolia*. However, taxol and related bioactive taxoids have been identified from several other species of the genus *Taxus. T. wallichiana* exhibits several medicinal properties other than its anticancer activity including anti-inflammatory, immunomodulatory, anticonvulsant, anticociceptive, antibacterial, antifungal, analgesic, antipyretic, antispasmodic, antiplatelet, and vasorelaxing effect (Juyal et al. 2014).

Catharanthus roseus (L.), an important medicinal plant, belongs to the family Apocynaceae. It is the most widely studied medicinal plant consisting of more than 100 monoterpenoid indole alkaloids (MIAs) (Gajalakshmi et al. 2013). It is used to treat several fatal diseases, and two major commercially important cytotoxic dimeric alkaloids, i.e., vincristine and vinblastine, are used in cancer chemotherapy (Naeem et al. 2017). Several hypertensive alkaloids such as ajmalicine, serpentine, and reserpine have also been reported in the roots of plant and can also be used in the treatment of platelet and platelet-associated disorders, malignancy, and in nonmalignant disorders (Idrees et al. 2011).

Rauwolfia serpentina, commonly known as sarpgandha, is an important medicinal plant of family Apocynaceae. It is commonly used in insomnia, hypertension, sexual aggression, and vertigo. A large number of important alkaloids have been identified in *R. serpentina*, i.e., ajmaline, ajmalimine, ajmalicine, deserpidine, indobine, indobinine, reserpine, reserpiline, serpentine, serpentinine, yohimbine, etc. (Kumari et al. 2013). However, reserpine, serpentine, and ajmaline have gained more interest having immense medicinal value. Reserpine is the most prominent alkaloid among all alkaloids and acts directly on the central nervous system, hence lowers blood pressure as compared to any other blood-pressure-reducing agents. Reserpine acts as a depressant on the central nervous system and peripheral nervous system by binding to catecholamine storage vesicles present in the nerve cells and regulates the antihypertensive actions. By depleting the transmitter substances from the adrenergic neurons, it interferes with the function of autonomic nervous system and possibly by activating the central parasympathetic nervous system (Bunkar 2017).

Strychnos nux-vomica (poison nut), belonging to the family Loganiaceae, is an evergreen tree native to India and Southeast Asia. Strychnine and brucine are two major alkaloids having pharmacological activities, i.e., relaxant in the stomach and bowel movements, promote blood circulation, and relieve pain. It is also a valuable remedy for chronic alcoholism and urinal incontinence (Patel et al. 2017). It has also been known to improve spinal reflexes and stimulates respiratory and sensory centers of the cerebral cortex (Akbar et al. 2010).

5.3.2 Terpenes

Terpenes are the polymers of isoprenoid units and one of the most extensive and varied compounds occurring in nature. They exhibit a wide range of biological and pharmacological activities and emit fragrances which allow them to be used in perfume preparation and cosmetics, aid in pollination, and act as insect repellents. There are seven classes of terpenes identified in nature on the basis of their attached isoprenoid units (IPUs) including monoterpenes (2 IPUs and 10 carbon atoms), sesquiterpenes (3 IPUs and 15 carbon atom), diterpenes (4 IPUs and 20 carbon atoms), sesterterpenes (5 IPUs and 25 carbon atoms), triterpenes (6 IPUs and 30 carbon atoms), tetraterpenes (8 IPUs and 40 carbon atom), and polyterpenes (several IPUs) (Kandi et al. 2015). Terpenes (also known as terpenoids) constitute the largest class of natural products having >55,000 known structurally diversified compounds. Terpenes raise interest in the clinical applications having broad range of the biological properties including antimicrobial, antifungal, antiviral, antihyperglycemic, cancer chemopreventive effects, analgesic, antiparasitic, and anti-inflammatory activities. The two most renowned terpene-based drugs are paclitaxel (anticancer) and artemisinin (antimalarial); however, other terpenes have also gained attention in pharmaceutical market, such as menthol. It is classified as a topical analgesic by the US FDA, and it composes topical formulation nonprescription analgesic or over-the-counter drug (OTC), as Salonpas® (5.7% menthol, 1.12% camphor, and 6.3% methyl salicylate). Terpenes are also found to have anticancer properties via acting on different stages of tumor development, i.e., induce the cell cycle arrest by inhibition of the early initiation and progression of tumorigenesis, tumor cell differentiation, apoptosis and the suppression of angiogenesis, invasion, and metastasis in the late stages through the regulation of various intracellular signaling pathways (Ansari and Akhtar 2019).

5.3.2.1 Some Important Terpenes Containing Medicinal Plants and Their Uses

Cannabis sativa (cannabis), the terpene and cannabinoid-rich resin of which, is the most valuable cannabis product with its various psychoactive and medicinal properties. The resin of different cannabis types has been identified to have more than 150 different terpenes and approximately 100 cannabinoids. *Cannabis* terpenes are mostly hydrocarbons (Booth and Bohlmann 2019). The most abundant terpene identified in *Cannabis* is myrcene, and it was found to protect the brain, heart, and skin tissues from inflammation and oxidative damage. It has also shown antinociceptive properties. A more predominant terpene of cannabis, i.e., β-caryophyllene, possesses significant anticancer properties, affecting the growth and proliferation of numerous cancer cells. However, β-caryophyllene has several other pharmaceutical properties including hepatoprotective, neuroprotective, nephroprotective, cardioprotective, gastroprotective, anti-inflammatory, and immunomodulatory actions (Nuutinen 2018).

Artemisia annua (sweet wormwood or quingho) is an important medicinal plant native to Asia and most probably China. The main component of *A. annua*, artemisinin ($C_{15}H_{22}O_{15}$), a sesquiterpene lactone containing a peroxide bridge, has been widely used for the treatment of malaria (Aftab et al. 2010). For the treatment of malaria, WHO recommends ACT (artemisinin-based combination therapy), considered as one of the most novel discoveries in the medicinal world (Zehra et al. 2020). Several other pharmaceutical properties of artemisinin are antibacterial, antifungal, antileishmanial, antioxidant, antitumor, and anti-inflammatory (Kim et al. 2015). Apart from all these properties, inhibitory activity of *A. annua* against some viruses including HSV1 has also been reported (Karamoddini et al. 2011). The bioactivity of artemisinin and its semisynthetic derivative artesunate has been scrutinized, and their inhibitory activity has been revealed against some viruses including cytomegalovirus and other members of the *Herpesviridae* family (i.e., Epstein-Barr virus and herpes simplex virus type 1), bovine viral diarrhea virus, hepatitis B virus, and hepatitis C virus (Efferth et al. 2008). Recently, it has been reported that artemisinin inhibited the replication of SARS-CoV-2 which causes the novel coronavirus COVID-19; however, the efficacy of artemisinin needs to be tested like other potential COVID-19 therapeutic agents such as hydroxychloroquine and remdesivir in well commanded and profusely powered clinical trials (Rolta et al. 2020).

Genus *Mentha* includes more than 25 species of mint, i.e., peppermint, spearmint, curled mint, American mint, Korean mint, wild mint, etc., constituting essential oil enriched with monoterpenes, i.e., menthol, menthone, carvone, and pulegone as major phytoconstituents. Mint exhibits innumerable medicinal and pharmacological properties that led to an enormous induction of research interest in this amazing herb (Shaikh et al. 2014). The essential oil of mint species usually consists oxygenated monoterpenes followed by sesquiterpene hydrocarbons.

Different species of the genus *Mentha* contain different terpene composition. *Mentha arvensis*, commonly known as corn mint or menthol mint, has the maximum share in global mint market among all the species of the genus *Mentha*, due to the highest content of menthol which has great pharmaceutical and medicinal value (Choudhary et al. 2020). Several research studies have confirmed its antiulcerogenic property (Tiwari 2016). The leaves of *M. arvensis* are used to treat fevers, hypertension, jaundice, nausea, diabetes, bronchitis, respiratory, and urinary tract infections (Akram et al. 2011).

Thymus vulgaris (thyme) synthesizes terpene alcohols and phenols which are known to have powerful antibacterial and antifungal properties (Cox-Georgian et al. 2019). Among all the terpenes present in thyme essential oil, thymol and carvacrol are the major phytochemicals having immense medicinal properties (Guesmi et al. 2018). *T. vulgaris* is commonly used in the treatment of many diseases especially in inflammation-related ailments, such as rheumatism, muscles swelling, insect bites, and pains. The essential oil of *T. vulgaris* is basically composed of monoterpenes, which act as antimicrobial, antispasmodic, antioxidative, medicinal drug, anti-tissue, and antibacterial activities. Several other therapeutic properties of thyme have been reported, including antiseptic, astringent, disinfectant, carminative, and treatment of several skin diseases (Hosseinzadeh et al. 2015a, b).

Azadirachta indica (neem) has medicinal properties known to Indians since time immemorial. Several different terpenoids including di- and triterpenoids have been reported in neem plant. These terpenoids exhibit antitumor, anti-inflammatory, immunostimulatory/immunosuppressive, antiviral, antiulcer, antimalarial, antibacterial, and antioxidant activities; however, several other potentially useful activities have also been reported, such as controlling the neoplastic growth, AIDS virus infected cells, hepatitis B virus, and malaria parasite (Dayanandan et al. 2000; Gupta and Chaphalkar 2016). *A. indica* contains four well-known limonoids, i.e., nimbinene, nimbinal, nimbandiol, and salannin; three diterpenoids including nimbidinol, ferruginol, and 6,7-dehydroferrugenol and recently two new terpenoids, i.e., limonoid morenolide and diterpene 17-hydroxy-sandaracopimar-8,15-dien-11-one, have been reported (Passos et al. 2019). However, azadirachtin, gedunin, and nimbolide are highly investigated due to their immense anticancer properties (Nagini 2014).

5.3.3 Glycosides

Glycosides comprise a large group of secondary metabolites, which are widely distributed in plants (Table 5.1). They are structurally diversified having significant medicinal potential and clinical scope as antidepressants. These compounds consist of an aglycone unit (genin) attached to the anomeric carbon of a sugar molecule (glycone) through a glycosidic linkage (Bartnik and Facey 2017). Several classes of glycosides have been identified such as alcoholic, anthraquinone, flavonoid, iridoid,

Table 5.1 Some important plant-derived glycosides and their medicinal properties

Plants	Family	Glycosides	Properties	Used in diseases	References
Stevia rebaudiana	Asteraceae	Steviol glycosides (stevioside, rebaudioside)	Non-mutagenic, and antimicrobial, and antifungal	Diabetes, obesity, and stomatal infections	Yadav and Guleria (2012)
Urginea maritima	Asparagaceae	Cardiac glycoside (proscillaridin)	Anticancer	Human tumor cell lines, prostate cancer	El-Seedi et al. (2013); He et al. (2018)
Digitalis purpurea	Plantaginaceae	Cardiac glycosides (digitoxin, digoxin)	Antitumor, anti-inflammatory, antimicrobial	Cardiovascular disease, pancreatic cancer, urothelial carcinoma, kidney cancer, etc.	Negi et al. (2012) Johan (2018)
Strophanthus gratus	Apocynaceae	Cyanogenetic glycosides, cardiac glycosides (ouabain)	Antimicrobial	Fever, metastatic prostate cancer	Henneh (2013)
Citrullus colocynthis	Cucurbitaceae	Flavon glucosides (isosaponarin, isovitexin) Cucurbitacin glucosides (2-O-β-D-glucopyranosylcucurbitacin, 2-O-β-D-glucopyranosylcucurbitacin)	Antioxidant,	Inflammation, cancer, constipation, bacterial infection, and diabetes	Delazar et al. (2006)
Lippia multiflora	Verbenaceae	Phenylethanoid glycosides (verbascoside, isoverbascoside, nuomioside A and isonuomioside A)	Antihypertension, antimalarial, antioxidant	Malaria, high blood pressure	Arthur et al. (2011)
Ipomea leptophylla	Convolvulaceae	Resin glycoside (jalapin, convolvulin)	Antimicrobial,	*Mycobacterium tuberculosis*	Barnes et al. (2003)
Plantago psyllium	Plantaginaceae	Phenylpropanoid glycosides (verbascoside and plantamajoside)	Anti-inflammatory, antibacterial, and diuretic	Constipation, diarrhea, and hyperlipidemia	Gonçalves and Romano (2016)
Calotropis procera	Asclepiadaceae	Flavonoid glycosides (quercetin, kaempferol, and isorhamnetin)	Antimicrobial, anticancer, antiangiogenic	Arthritis, cancer	Nenaah (2013); Al-Snafi (2015)

coumarin, cardiac, steviol, cyanogenic, chromone, phenolic, and saponins. There are a number of well-studied glycosides reported in plants including digoxin, hesperidin, rutin, catalpol, amygdalin, diosgenin, loganin, salicin, arbutin, lotaustralin, geniposidic acid, stevioside, rebaudioside, prunasin, sinigrin, sinalbin, antron, anthranol, aucubin, theviridoside, naringin, dhurrin, ginsenosides, etc. The validated pharmaceutical properties of glycosides are antidiabetic, anticancer, antithrombotic, analgesic, antifungal, antioxidant, and antiviral, among others (Khan et al. 2018a, b). Glycosides are found to cure irritant dry cough and urinary disorders and have sedative and relaxant effect on the heart and muscles under the prescribed doses (Visweswari et al. 2013). The cardiotonic steroids (cardiac glycosides) have long been known to treat heart-related disorders in folk medicinal system. A vast variety of plants especially belonging to Plantaginaceae, Apocynaceae, Asparagaceae, and Moraceae families possesses cardiac glycosides including *Antiaris toxicaria, Asclepias* sp., *Bowiea volubilis, Calotropis procera, Calotropis gigantea, Convallaria majalis, Digitalis purpurea, Drimia maritima, Kalanchoe daigremontiana, Nerium oleander*, *Thevetia peruviana*, *Strophanthus gratus* etc. (Patel 2016).

5.3.4 Flavonoids and Phenolic Acids

Flavonoids are highly diversified plant secondary metabolites and represent a broad family of more than 4000 phytochemicals. The four major classes are isoflavones, anthocyanins, 4-oxoflavonoids (flavones and flavonols), and flavan-3-ol derivatives (catechin and tannins). They possess various biological activities such as anti-inflammatory, anticancer, and antiviral antithrombotic, and cardioprotective properties and are consumed in the human diet on daily basis (Salvamani et al. 2014). Flavonoids (polyphenolic compounds) constitute one of the most characteristic classes of compounds in higher plants possessing 15 carbon atoms and 2 benzene rings joined by a linear 3-carbon chain. They are the small organic compounds and are normally absorbed by the human body for long period; thus, they might be one of the safest non-immunogenic drugs (Lee et al. 2007). Flavonoids recently gained consideration as requisite component of pharmaceutical, nutraceutical, medicinal, and cosmetic application. Flavonoids have functional hydroxyl groups which scavenge free radicals or chelate metal ions, thus exhibit antioxidant effects and help in the prevention of radical generation that damage the biological molecules leading to oxidative stress and many diseases (Tiwari and Husain 2017). Phenolic compounds are known to exert preventative actions against inflammation and allergies via antioxidant, infectious and degenerative diseases, neutralization/modulation mechanisms, and antimicrobial and protein/enzymes (Ozcan et al. 2014). Several medicinally important phenolic compound-containing plants are *Acacia catechu, Morus alba, Portulaca oleracea, Solanum nigrum, Curcuma longa, Zingiber officinale, Arctium lappa, Xanthium sibiricum, Cinnamomum cassia, Salvia miltiorrhiz*a, and many more (Cai et al. 2004).

5.4 Mode of Action

Proteins can function as enzymes, receptors, transcription factors, transporters, ion channels, and cytoskeletal proteins and are the major target molecules of cells. Plant secondary metabolites can form covalent bonds with proteins, peptides, and sometimes DNA through their reactive functional groups (such as aldehydes and SH-groups, epoxides, double bonds with enon configuration, triple bonds) and impair the protein function. Secondary metabolites can nevertheless be useful as multi-target drugs in diseases in which proteins are involved as they can attack a multitude of protein with their functional groups in a non-selective way. Herbal medicines usually contain phenolic compounds, which contain one or more hydroxyl groups, and can form many hydrogen bonds with electronegative atoms (O, N) in proteins and peptides. Positively charged amino groups of several amino acids such as lysine and arginine in proteins form ionic bonds with these negatively charged groups. The structural and functional flexibility of proteins can be impaired, if a secondary metabolite (polyphenol) forms several hydrogen and ionic bonds with a protein or with its binding or catalytic site. Transcription factors that regulate differential gene expression in an organism are also an important class of proteins and can also be modulated by secondary metabolites or phenolics which form covalent bonds, thus influence gene regulation indirectly. Many genes were found to be either up- or downregulated when transcriptome analysis have been conducted in cells or animals treated with an herbal drug or even a single compound. It has become apparent with the availability of RNA-Seq using next-generation sequencing (NGS) that several genes and the protein-mediated differentiation and epigenetics are also affected by secondary metabolites (Wink 2015).

Plant secondary metabolites have been reported to affect microbial cells in several different ways including disruption of membrane function and structure (including the efflux system), induction of coagulation of cytoplasmic constituents, interference with intermediary metabolism, interruption of DNA/RNA synthesis and functions, and alterations in normal cell communication (quorum sensing). Plants secondary metabolites primarily target cytoplasmic membrane and affect its structure, integrity, permeability, or functionality in different ways, such as some antifungal natural products alter the function of the major sterol of fungal cell membrane, i.e., ergosterol, which is involved in several processes such as maintenance of integrity and fluidity of membrane and regulation of enzymes necessary for the growth and division of cells. The most diversified group of plant bioactive compounds, i.e., flavonoids, exerts their inhibitory effect mainly on cytoplasmic membrane function and DNA synthesis and also affects RNA synthesis and proteins, but in a lesser content. The dehydratase activities of β-hydroxyl-acyl carrier protein and DNA gyrase were found to be inhibited by apigenin and quercetin together with some other flavonoids. It has further been reported that quercetin inhibits the ATPase activity of DNA gyrase enzyme via its binding to the GyrB subunit in *E. coli*. Along with this activity, quercetin has been considered as membrane active compound and also caused an increase in permeability of the inner membrane and a dissipation of the membrane potential (Radulovic et al. 2013).

A study on immunosuppressive and anti-inflammatory effect of terpenoids of *Azadirachta indica, Acacia catechu*, and *S. malabarica* showed inhibition of nitric acid (a fundamental signaling molecule) production from human peripheral mononuclear cells and also on the monocyte and granulocyte count from whole human blood (Gupta and Chaphalkar 2016).

Cardiac glycosides inhibit the Na^+-K^+-ATPase, building up Na^+ and K^+ gradients which are essential for transport activities and neuronal signaling, one of the most important molecular targets of animal cells. They exhibit positive inotropic, positive bathmotropic, weakly negative chronotropic, and dromotropic heart activity and slow down heart beat (Wink 2007; Van Wyk and Wink 2018).

Several agents cause oxidative DNA damage and increase the risk of cancer development. Plant-derived polyphenols are known to have strong antioxidant properties. They inhibit lipid peroxidation and peroxygenases, thus scavenging free radicals, i.e., superoxide, peroxyl, and hydroxyl. Glucosinolates have been reported to act against several carcinomas by modulating carcinogen metabolism. Isothiocyanate, a highly reactive derivative of glucosinolate, also regulates mitosis and stimulates apoptosis in human tumor cells in vivo and in vitro (Singh et al. 2003).

Hashemzaei and his research group tested the anticancer activity and the apoptosis-inducing ability of quercetin in both in vivo and in vitro conditions. They tested nine cancer cell lines including colon carcinoma, CT-26 cells, LNCaP cells, prostate adenocarcinoma, acute lymphoblastic leukemia MOLT-4 T-cells, human prostate PC3 cells, estrogen-receptor positive breast cancer, human myeloma U266B1 cells, ovarian cancer CHO cells, pheochromocytoma PC12 cells, and human lymphoid Raji cells. The experimental results proved quercetin significantly induces apoptosis in all tested cancer cell lines as compared to the control group. The in vivo experiment on mice MCF-7 tumor lines and mice bearing CT-26 tumors exhibited a significant decrease in tumor size and volume and also increased the survival period of tested animals. Another experiment has been conducted by the research team of Clifford to evaluate anticancer activity of quercetin on three established pancreatic cancer cell lines. They have tested the crosstalk between quercetin, microRNAs, and Notch (an important gene for signaling receptor encoding) signaling in the regulation of self-renewing cancer stem cell divisions, and results showed that quercetin can induce miR-200b-3p to mediate the mode of self-renewing divisions of the tested pancreatic cancer (Tungmunnithum et al. 2018).

Although a large number of plant secondary metabolites have been examined from medical plants, an innumerable count of natural compounds has still not been identified. However, functional evaluation of these natural products and profiling of several medicinal plants have not been completely performed.

5.5 Conclusion

To recapitulate, plant secondary metabolites are the potential candidates to treat several life- threatening human diseases. These natural bioactive compounds offer defense against various diseases with a wide array of defense properties (antimicrobial, antiviral, antifungal, anticancer, and many more) without harmful side effects and being cheap and affordable as compared to other treatments. The knowledge gained at the cellular and molecular levels could be useful in planning for future epidemiological diseases, several human cancer prevention trials, diabetes, several neurological disorders, etc. However, a large number of secondary metabolites have not been tested yet for their potential pharmaceutical and medicinal applications, and the mechanism of several known potential bioactive compounds is not clearly understood.

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Chapter 6
Understanding the Mechanistic Functioning of Bioactive Compounds in Medicinal Plants

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6.1 Introduction

Plants produce secondary metabolites to protect themselves against herbivores, parasitic plants, and microbes. Some of these secondary metabolites also act as signalling compounds. Over several ages, humans found a way to suitably use these secondary metabolites, so that the field of herbal medicine or phytomedicine emerged. According to the estimates made by the World Health Organization (WHO), plant-based pharmaceuticals are one of the most sought-after items for primary healthcare for 3.5–4.0 billion people across the world. A major chunk of these are traditional medicines involving plant extract and decoction which is often termed as "modern herbal medicine" (Srivastava et al. 2019).

Plant pharmaceuticals often interact with the main targets in cells like proteins, biomembranes, and nucleic acids (mainly DNA) which form the basis of their use in pharmaceuticals. Some secondary metabolites have been optimized for specific targets like alkaloids which act on neurotransmitter receptors, while others like phenolics and terpenoids have a more generalized action without a defined target. Mostly, these secondary metabolites interact by formation of hydrogen, hydrophobic, and ionic bonds, which interferes with the three-dimensional macromolecular structure imparting their bioactivity. The multitarget effect of many plant secondary metabolites justifies their use in overall health improvement. For several hundred medicinal plants all around the world, monographs have been published, where their therapeutic potentials have been assembled in an organized manner. Some such important monographs are *German Commission E* and the *European Pharmacopoeia* (Ph. Eur.) and the European Scientific Cooperative on Phytotherapy (ESCOP) and the WHO monographs (Wink 2015).

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This chapter discusses the general mode of action of different plant metabolites such as covalent interactions, non-covalent interactions, and interactions with biomembranes and antioxidant properties. Some important pharmaceuticals from the different categories of secondary metabolites, namely, phenolics (aspirin and quercetin), alkaloids and alkyl amines (quinine, lysergic acid dimethylamine or LSD, and *Vinca* alkaloids like vincristine), cardiac glycoside (digoxin), terpenoids (saponins), and atypical amino acids (myriocin) have been discussed in detail.

6.2 Role of Secondary Metabolites

Many secondary metabolites are used in pharmaceuticals as bioactive compounds with definite application. These secondary compounds may act on ion channels, neuroreceptors, enzymes which degrade neuroreceptors, acetylcholine receptor agonist, elements of extracellular matrix (ECM), etc. (Van Wyk and Wink 2015; Wink et al. 1998). Some of them may be potent hallucinogens like salvinorin from *Salvia divinorum* and cannabinoids from *Cannabis sativa* (Wink 2000). These bioactive compounds are very specific in their activity. Their structures are determined by "evolutionary molecular modelling" (analogous to chemical modelling) by evolution and selection for their function (Wink 2007; Wink and van Wyk 2010).

Phytomedicines are commonly available as extracts with water/alcohol or as distillate or essential oil. They are almost always a mixture of dozens or even hundreds of secondary metabolites often from a variety of structural groups. Most of the bioactivity of these phyto-extracts cannot be pinpointed to a single secondary metabolite (Chevallier 2001). They manifest due to the synergistic interactions of several secondary metabolites present in the mixture and help to treat a broad spectrum of diseases in place of a single disorder (Hellmann et al. 2010). These medicinal plants contain compounds like polyphenols, alkaloids, alkylamines, etc. and groups of secondary metabolites which have curative roles (Hänsel et al. 2007).

Understanding the mechanism of action of these secondary metabolites may help us modify them for improved effectiveness, reduced side effects, and use in other applications. While understanding the exact mode of action of every secondary metabolite individually would be tedious, Table 6.1 summarizes the mode of action of many useful secondary metabolites, along with their plant sources. Most of these activities may be studied under some broad categories like covalent interactions, non-covalent interactions, antioxidant properties, etc. which are discussed ahead.

6.2.1 Covalent Interactions

Plants constitute a storehouse of secondary metabolites, with various reactive functional groups attached to the skeletal framework (Wink and Schimmer 2010). They may be aldehydes, thiol groups, hydroxyl groups, triple bonds, etc. which interact

Table 6.1 Wide varieties of secondary metabolites and their diverse modes of action

Secondary metabolites	Source	Application	Mode of action	References
Adoniside	*Adonis vernalis*	Cardiotonic	Inhibits Na^+/K^+ pumps	Poliakov (1961)
Aescin	*Aesculus hippocastanum*	Anti-inflammatory	Endothelial NO synthesis, making endothelial cells more permeable to Ca^{2+}. Induces prostaglandin release	Berti et al. (1971)
Aesculetin	*Fraxinus rhynchophylla*	Anti-inflammatory	Blocks MAPK	Sarfraz et al. (2017)
Agrimophol	*Agrimonia eupatoria*	Anthelmintic	Inhibits DNA synthesis in tumor cells	Zhao et al. (2015)
Allyl isothiocyanate	*Brassica nigra*	Rubefacient	Kills bacteria by acting on its cell membrane	Lin et al. (2000)
Anabasine	*Anabasis aphylla*	Skeletal muscle relaxant	AChE inhibitor	Victor Kuete (2014)
Andrographolide	*Andrographis paniculata*	Treatment for bacillary dysentery	Kills bacteria by preventing DNA synthesis	Singha et al. (2003)
Anisodine	*Anisodus tanguticus*	Anticholinergic	Cyclooxygenase inhibitor blocks prostaglandin synthesis	Tian et al. (2015)
Anisodamine	*Anisodus tanguticus*	Anticholinergic	Blocks mAChR	Tian et al. (2015)
Arecoline	*Areca catechu*	Anthelmintic	nAChR/mAChR receptor agonist	Liu et al. (2016)
Asiaticoside	*Centella asiatica*	Vulnerary	Collagen synthesis, stimulates ECM accumulation	Fitri et al. (2018)
Atropine	*Atropa belladonna*	Anticholinergic	Inhibits mAChR	Wang et al. (2017a, 2017b)
Berberine	*Berberis vulgaris*	Treatment for bacillary dysentery	Inhibits choline acetyltransferase	Schmeller et al. (1997)
Bergenin	*Ardisia japonica*	Antitussive	Inhibits Interleukin 1β and TNF-α	De Oliveira et al. (2011)

(continued)

Table 6.1 (continued)

Secondary metabolites	Source	Application	Mode of action	References
Betulinic acid	*Betula alba*	Anticancerous	Induces apoptosis	Wang et al. (2017a, 2017b)
Bromelain	*Ananas comosus*	Anti-inflammatory, proteolytic	Cysteine protease	Van Wyk and Wink (2004)
Caffeine	*Coffea arabica*	Stimulant	Inhibits phosphodiesterase and adenosine receptors	Boswell-Smith et al. (2006)
Camphor	*Cinnamomum camphora*	Rubefacient	CNS stimulant	Matthews et al. (2009)
Camptothecin	*Camptotheca acuminata*	Anticancerous	Inhibitor of DNA topoisomerase	Van Wyk and Wink (2004)
Catechin	*Potentilla fragarioides*	Hemostatic	Alters apoptotic gene expression and NO synthase	Abbas and Wink (2009)
Cissampeline	*Cissampelos pareira*	Skeletal muscle relaxant	Cysteine protease	Smolarsky (1978)
Cocaine	*Erythroxylum coca*	Analgesic, stimulant	Inhibits Na^+ channels and reuptake of noradrenaline and dopamine	Van Wyk and Wink (2004)
Codeine	*Papaver somniferum*	Analgesic, antitussive	Interacts with opioid receptors	Bhandari et al. (2011)
Colchicine	*Colchicum autumnale*	Antitumor, antigout	Inhibits microtubule assembly	Van Wyk and Wink (2015)
Curcumin	*Curcuma longa*	Choleretic	PPARγ activation	Jacob et al. (2007)
Digitoxin	*Digitalis lanata*	Heart insufficiency	Inhibits Na^+/K^+ ATPase	Arispe et al. (2008)
Emetine	*Psychotria ipecacuanha*	Amoebicide, emetic	Protein biosynthesis inhibitor	Möller et al. (2006)
Ephedrine	*Ephedra* species	Sympathomimetics	Stimulate α- and β-adrenergic dopaminergic receptors	Limberger et al. (2013)

(continued)

Table 6.1 (continued)

Secondary metabolites	Source	Application	Mode of action	References
Galanthamine	*Galanthus woronowii*	Alzheimer treatment	Inhibits AChE	Van Wyk and Wink (2004)
Hyoscyamine	*Atropa belladonna*	Antagonist of mAChR	Antagonist of mAChR	Van Wyk and Wink (2015)
Irinotecan	*Camptotheca acuminata*	Anticancer, antitumor agent	Inhibits topoisomerase I	de Man et al. (2018)
Kawain	*Piper methysticum*	Tranquilizer	Interacts with Na^{+} and Ca^{+} ion channels	Walden et al. (1997)
Lobeline	*Lobelia inflate*	Smoking deterrent, respiratory stimulant	Inhibits ABC transporters	Ma and Wink (2008)
Morphine	*Papaver somniferum*	Analgesic	Agonist of endorphin receptors	Yadlapalli et al. (2017)
Nicotine	*Nicotiana tabacum*	CNS stimulant	nAChR agonist	Wink et al. (1998)
Ouabain	*Strophanthus gratus*	Cardiotonic	Na^{+}/K^{+}-ATPase inhibitor	Manunta et al. (2009)
Palmatine	*Coptis japonica*	Antidepressant	Regulates monoamine oxidase activity	Long et al. (2019)
Papain	*Carica papaya*	Cures digestive problems	Proteolysis	Van Wyk and Wink (2004)
Physostigmine	*Physostigma venenosum*	Alzheimer treatment	Inhibits AChE	Van Wyk and Wink (2015)
Pilocarpine	*Pilocarpus jaborandi*	Glaucoma treatment	mAChR agonist	Van Wyk and Wink (2015)
Podophyllotoxin	*Podophyllum peltatum*	Anticancer	Inhibitor of microtubule formation	Zhang et al. (2018)
Quinidine	*Cinchona pubescens*	Antiarrhythmic	Na^{+} channel inhibitor	Wink (2012)
Reserpine	*Rauvolfia serpentina*	Hypertonia treatment	Inhibits the uptake of noradrenalin into postsynaptic vesicles	Jerie (2007)

(continued)

Table 6.1 (continued)

Secondary metabolites	Source	Application	Mode of action	References
Rotenone	*Lonchocarpus nicou*	Piscicide	Inhibits mitochondrial respiratory chain	Wink and Schimmer (2010)
Salicin	*Salix alba*	Analgesic	Prostaglandin inhibitor	Maclagan (1876)
Sanguinarine	*Sanguinaria canadensis*	Dental plaque inhibitor	DNA intercalator	Hamoud et al. (2015)
Sparteine	*Cytisus scoparius*	Antiarrhythmic	Na^+ channel inhibitor	Van Wyk and Wink (2015)
Tetrahydrocannabinol (THC)	*Cannabis sativa*	Antiemetic, decreases ocular tension	Activates THC receptor	Tiwari et al. (2018)
Theobromine	*Theobroma cacao*	Diuretic, vasodilator	Inhibits phosphodiesterase and adenosine receptors	Martínez-Pinilla et al. (2015)
Tubocurarine	*Chondrodendron tomentosum*	Muscle relaxant	Inhibits nAChR	Sun and Wink (2014)

NO nitric oxide, *nAChR* nicotinic acetylcholine receptor, *mAChR* muscarinic acetylcholine receptor, *AChE* acetylcholine esterase, *THC* tetrahydrocannabinol, *ABC* ATP-binding cassette, *PPARγ* peroxisome proliferator-activated receptor gamma, *CNS* central nervous system, *ECM* extracellular matrix, *TNF-α* tumor necrosis factor-α, *MAPK* microtubule-associated protein kinase

with the covalent bonds of biological macromolecules like proteins and DNA (Fig. 6.1) (Wink 2008). Under physiological conditions, several random modifications may take place throughout the body, like aldehyde groups in secondary metabolites may interact with the amino or imino groups of amino acids, proteins, and DNA or with the thiol groups of proteins. Similarly, epoxy groups in these bioactive compounds may react with free amino acids, DNA bases, or thiol groups. Exocyclic methylene groups present in terpenes and phenylpropanoids, allicin in garlic, or groups like sesquiterpene lactones can interact with thiol groups in glutathione.

Proteins function as receptors, ion channels, transporters, enzymes, cytoskeletal structures, etc. inside the cell. On modification of their functional groups, they may lose their catalytic activity or structural role. Changes in these functional groups may prevent receptor ligand interactions and modify signalling pathways. Modifications like alkylation which affect the 3D structure of proteins may even prevent important protein-protein binding, catalytic activity, or turnover. Due to their varied mode of interaction, these secondary metabolites may non-specifically attack a number of proteins in a non-selective manner, resulting in multitarget drugs. These drugs are very useful indeed in curing several ailments and may even act on proteins that are still unknown to us as participants in relevant pathways (Wink 2015).

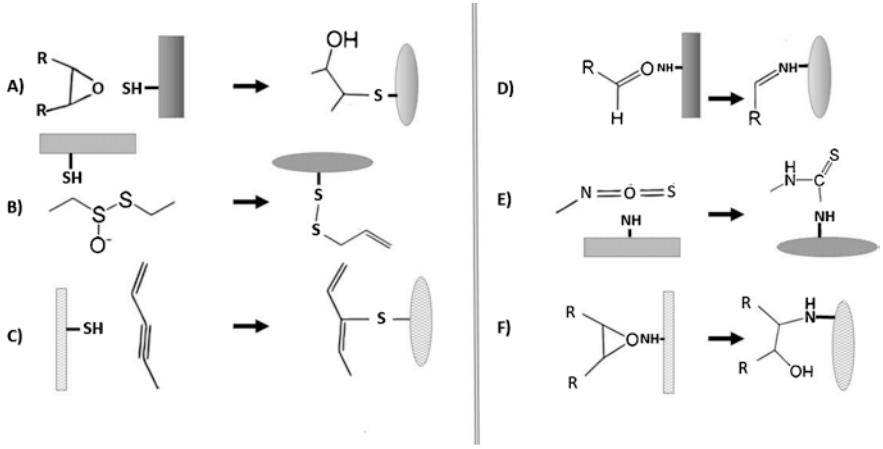

Fig. 6.1 Covalent interactions of secondary metabolites with biological macromolecules, like proteins, lead to a conformational change which also affects their biological function. (**a**–**c**) show interactions of some common functional groups with –SH group of proteins. It represents the interacting groups, namely, epoxide group in A, allicin in B, and thiophene in C, which lead to the formation of respective covalent bonded molecules. (**d**–**f**) show similar interactions of some functional groups with –NH group on proteins. Here, (**d**) shows NH interaction with an aldehyde, E represents –NH interaction with isothiocyanate, and F shows interaction with epoxide

DNA base modification, such as alkylation, may be brought about by secondary metabolites like aldehydes, epoxides, cycasin, aristolochic acids, furanocoumarins, etc. leading to mutations. Some commonly known secondary metabolites like pyrrolizidine alkaloids present in Boraginaceae and some Asteraceae are capable of alkylating DNA. Some others such as DNA-alkylating metabolites are aristolochic acids (*Aristolochia*), cycasin (Cycads), furanocoumarins (Apiaceae), and ptaquiloside (*Pteridium aquilinum*). Alkaloids like berberine and sanguinarine, and some furanocoumarins are planar in nature, which allows them to stack between DNA bases. Such intercalating secondary metabolites bring about frameshift mutations and may even affect the proto-oncogenes or tumor suppressor genes on long use, thus leading to cancer (Teuscher et al. 2012; Teuscher and Lindequist 2010). While sometimes used in traditional medicines, such mutagenic phytochemicals are no longer used in modern phytotherapy.

6.2.2 Non-Covalent Interactions

Cellular proteins have hydrophilic or charged patches which are subjected to modifications by reactive functional groups present in secondary metabolites like hydroxyl (OH) group, phenyl group, etc. These groups may form hydrogen bonds with the electronegative atoms like nitrogen or oxygen in proteins and peptides and modify their functions (Van Wyk and Wink 2004; Van Wyk and Wink 2015). At physiological pH, phenols dissociate to give negatively charged phenolic groups which interact with positively charged amino acids in proteins (Wink 2005). These interactions impair the proteins by modifying their structural and functional flexibility. Similar to the reactive secondary metabolites involved in covalent bonds, such hydrogen bond and ionic bond formations may also lead to a multitarget drug effect.

Phenolic compounds are well known for their pleiotropic effects on non-specific targets. Glycosylation of the phenolic secondary metabolites, which supplies a number of hydroxyl group, is a key reason for such non-specific interactions. Transcriptome analysis of cells or animals treated with different secondary metabolites like phenolics revealed that even transcription factors are modified by their interactions. As these factors control cascades of signals controlling gene expression, it has a huge impact on the up- or down-regulation of several genes (Pakalapati et al. 2009). It is expected that new technologies like next-generation sequencing (NGS) and RNA sequencing will help us to detect such secondary metabolite-mediated modifications on genes and on proteins that mediate differentiation and epigenetic regulations.

6.2.3 Interactions with Biomembranes

Cells of all living beings are surrounded by a semipermeable membrane that acts as a diffusion or permeation barrier between cytosol and its exterior, preventing the leakage of cellular metabolites into the surrounding and uncontrolled influx of external substances. This membrane houses a multitude of proteins like ion channels, specific receptors that mediate exterior signals, and transporters which allow specific molecules to pass in a specific direction. Proper functioning of these proteins and the non-porosity of the lipid bilayer of this membrane are vital for cell survival. There are many lipophilic secondary metabolites that have an affinity for these lipid bilayers, and they bind to the nonpolar inner core of these membranes (Wink 2015). Monoterpenes and sesquiterpenes are good examples of such secondary metabolites which can accumulate in these bilayers in very high concentration, often compromising its fluidity and enhancing permeability. This is the reason behind the antimicrobial and cytotoxic effect of bioactive compounds like essential oils (Van Wyk and Wink 2004; Van Wyk and Wink 2015).

Saponins can complex cholesterol in animal membranes, and monodesmodisic saponins with a single sugar group are also known to completely lyse such cells (Frenkel et al. 2014). Saponins can also interact with ergosterol in fungal bilayers and bring about similar outcome. Such interactions of secondary metabolites with biomembranes are non-specific (Herrmann and Wink 2011). However, they are very powerful and can occur at very low concentrations. Saponins may play a role in the uptake of polar secondary metabolite, aiding their interactions in a synergistic manner. Polyphenols in these essential oil extracts can affect the membrane proteins as well, disrupting their interactions with the lipid bilayer (Hamoud et al. 2015).

6.2.4 *Antioxidant Properties*

Many processes in our daily metabolism generate reactive oxygen species (ROS) like peroxide, superoxide, hydroxyl radicals, singlet oxygen species, etc. which may interact with macromolecules within the cell. An excess of such ROS may cause serious damage in our cell as seen in case of metabolic syndrome, diabetes, cardiovascular ailments, etc. (Van Wyk and Wink 2015). These ROS also have an impact on ageing process. Secondary metabolites with conjugated double bonds like phenolics and terpenoids and ascorbic acid (in association with glutathione) inhibit these ROS and prevent cell damage (Van Wyk and Wink 2004; Van Wyk and Wink 2015). A good way of studying such antioxidant properties of secondary metabolites is in *Caenorhabditis elegans* model. Algae rich in such phenolics and several other plant pharmaceuticals have potent antioxidant properties and anti-aging effects (Abbas and Wink 2009).

6.3 Understanding the Elaborate Mechanism of Functioning of Some Important Secondary Metabolites

Secondary metabolites may be broadly classified into phenolic compounds, alkaloids and alkyl amides, glycosides, terpenes, and some atypical amino acids. Some alkaloids have specific mode of action, as they interact with various extracellular receptors (mainly in CNS); action of others like phenolics and terpenes is more generalized. Several essential pharmaceuticals belonging to all these categories find their use in modern phytomedicines.

6.3.1 *Phenolics*

Polyphenols, with multiple OH groups, have a wide range of pharmacological properties like antioxidant, anti-inflammatory, sedating, wound healing, antimicrobial, and antiviral. This makes them invaluable in phytotherapy. While they have a generalized multitarget effect on our body, we will discuss the specific mode of action of two important phenolic pharmaceuticals, aspirin and quercetin.

6.3.1.1 Aspirin

The use of aspirin may be traced back thousands of years when this salicylate-containing plant secondary metabolite was used to relieve pain. Salicin, obtained from white willow, has been used way back in 1874 to work as an analgesic, anti-pyretic, and anti-inflammatory drug for treating rheumatic fever (Maclagan 1876).

Chemically, the drug "aspirin" is an acetylated form of salicylic acid that was first prepared by Felix Hoffman in 1897. Aspirin acts as a weak analgesic, compared to strong narcotic analgesics such as morphine. Its mechanism of action was also strikingly different both qualitatively and quantitatively from steroid drugs.

Piper and Vane (1969) was the first to propose a relationship between aspirin and prostaglandin while experimenting on anaphylactic processes. It was seen that during anaphylaxis, other than histamine and slow-reacting substance of anaphylaxis (SRS-A), prostaglandins like factors PG_2 and PGF_{2a} and a factor RCS (rabbit aorta contracting substance) were also released (Anggard and Samuelsson 1965). Aspirin blocked RCS release and reduced PG output by targeting prostaglandin synthesis. This pathway was evidently different from other steroid-based drugs like morphine (an analgesic), hydrocortisone (an anti-inflammatory drug), and mepyramine (an antihistamine) which have no effect on PG synthesis. It was shown by Smith and Willis (1971) that aspirin inhibited PG release from platelet samples and even oral administration of indomethacin was equally effective. This vital study proved that the effect of aspirin on PG synthesis inhibition was not restricted to species, tissue, or route of administration.

A membrane-bound dimeric protein of 72 kDa exhibits cyclooxygenase (COX) or prostaglandin-endoperoxide synthase (PGHS) activity, which works together to synthesize prostaglandin from arachidonic acid. While COX carries out the cyclization of arachidonic acid and addition of 15-hydroxy group to form prostaglandin G2 (PGG_2), a separate peroxidase reduces PGG_2 to its final form, prostaglandin H2 (PGH_2), as shown by (Vane and Botting 2003). Pure enzyme studies revealed that aspirin acetylated the COX enzyme, blocking PGG synthesis, while the peroxidase activity remained unperturbed. Aspirin specifically acetylates serine 530 residue, locating 70 amino acids from C-terminal end of this COX enzyme which leads to irreversible inhibition of this enzyme (Hemler et al. 1976; Roth and Majerus 1975; Smith 1986). This acetylation places a bulky substituent on serine 530 oxygen that inhibits the substrate (arachidonic acid) binding due to steric hindrance (de Witt et al. 1990).

COX genes are of the following distinct types:

I. *COX-1*: This mostly helps in expression of PGs which participates in physiological processes like platelet activation, kidney function, and stomach mucosa protection (Xie et al. 1991).
II. *COX-2*: This is induced by mitogens, growth factors, tumor promoters, and lipopolysaccharides and inhibited by glucocorticoids. This mostly leads to PGE_2 expression in inflammatory reactions.
III. *COX-3*: This is a variant of *COX-1*, which retains intron 1 and exists in humans in adenylated form. It is selectively inhibited by drugs like paracetamol and aspirin at low concentrations (Chandrasekharan et al. 1999).

Both COX-1 and COX-2 are inhibited by aspirin, which binds at serine 530 of COX-1 and serine 516 of COX-2. However, as the active site of COX-2 is slightly larger than that of COX-1, some arachidonic acid molecules may still squeeze past the steric blockage and get converted to the cyclized product (Vane et al. 1998). This difference in size of active sites has been exploited over the ages to develop COX-2-specific inhibitors like celecoxib, rofecoxib, and meloxicam which can function as anti-inflammatory drugs without damaging the stomach mucosal lining (Bombardier et al. 2000; Dequeker et al. 1998; Silverstein et al. 2000). Many other modified forms of this drug have been developed like nitroaspirin. Nitroaspirin liberates nitric oxide, which protects the stomach mucosa from hydrochloric acid damage (Fiorucci et al. 2003). While discovery of new anti-inflammatory drugs with fewer side effects is slowly replacing the use of aspirin, its use as a potent anti-thrombotic agent in treatment of heart ailments is unparallel.

6.3.1.2 Quercetin

Quercetin is a bioactive flavonoid extracted from fruits and vegetables which improves physical and mental performance and reduces infection risk (Davis et al. 2009). Quercetin helps in overall health improvement. While this secondary metabolite exhibits its anti-carcinogenic, antiviral, antioxidant, anti-inflammatory, and psychostimulant activity, it also acts as an effective inhibitor of lipid peroxidation and platelet aggregation and stimulates mitochondrial biogenesis (Aguirre et al. 2011). Chemically quercetin ($C_{15}H_{10}O_7$) is an aglycone, missing an attached sugar moiety, with the IUPAC (International Union of Pure and Applied Chemistry) nomenclature of 3, 3′, 4′, 5, 7-pentahydroxyflvanone or 3, 3′, 4′, 5, 7-pentahydroxy-2-phenylchromen-4-one (Li et al. 2016).

Quercetin finds its use as a strong anti-inflammatory drug with a long-lasting effect (Orsolic et al. 2004; Read 1995). This effect is established by regulating complex signalling pathways, affecting both mast cell stabilizing agent and gastrointestinal mucosal lining protective agent (Penissi et al. 2003). This bioactive secondary metabolite also exhibits an immunosuppressive effect on dendritic cell functions (Huang et al. 2010). Quercetin plays a role in inhibiting lipopolysaccharide (LPS)-induced signalling processes such as tumor necrosis factor-α (TNF-α) production in macrophages, Interleukin (IL)-8 production in lung cells, and TNF-α and IL-1 in microglial cells. Quercetin effect on such signalling mechanisms may be explained by the inhibition of Src- and Syk-mediated phosphorylation of phosphatidylinositol-3-kinase (PI3K) (a tyrosine kinase) at Tyr-85 which leads to its activation in response to an external signal (Endale et al. 2013). This subsequently inhibits the toll-like receptor 4 (TLR4)/myeloid differentiation primary response 88 (MyD88)/PI3K complex formation, hindering the downstream signalling cascade (Fig. 6.2).

Quercetin also inhibits inflammation producing COX and lipoxygenase (LOX) enzymes (Kim et al. 1998; Lee et al. 2010). Quercetin controls calcium flux and phosphorylated protein kinase C (PKC) signalling; this blocks high-affinity immunoglobulin IgE receptor FcƐRI-controlled release of pro-inflammatory molecules such as cytokines, tryptase, and histamine from human umbilical vein endothelial cells (HUVECs) (Kempuraj et al. 2005). Peroxide-induced inflammation of HUVECs may also be checked by quercetin by down-regulation of vascular cell adhesion molecule 1 (VCAM-1) and C5a receptor (CD80) expression (Yang et al. 2015).

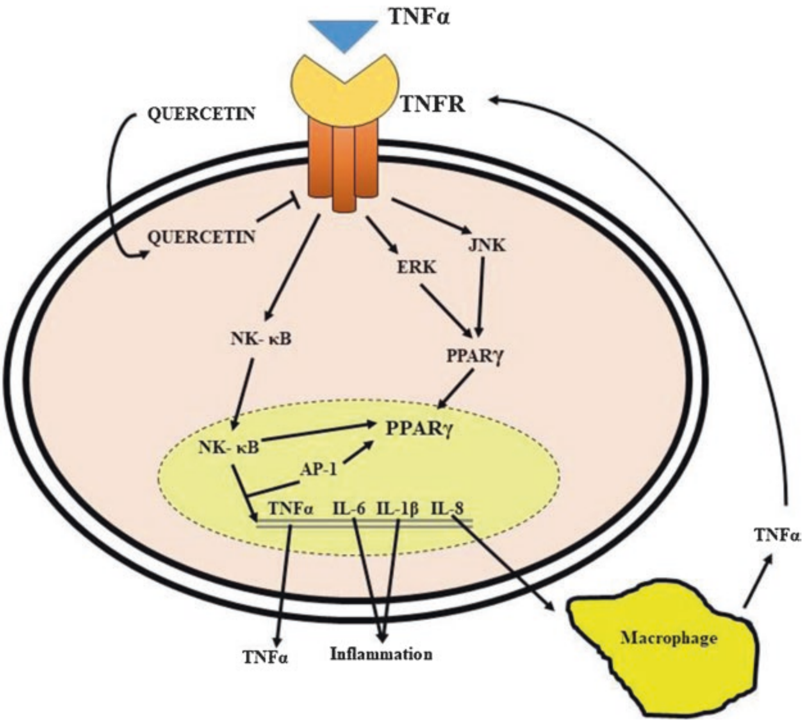

Fig. 6.2 Quercetin blocks the tumor necrosis factor-α (TNFα) receptor-mediated signalling which leads to inflammatory response. This secondary metabolite blocks the signal relay proteins like extracellular signal-related kinase (ERK), c-Jun NH_2-terminal kinase (JNK), and nuclear factor-κB (NF-κB) which help in inflammation. It also increases peroxisome proliferator-activated receptor c (PPARγ) activity, thus antagonizing NF-κB or activator protein-1 (AP-1)-mediated transcription of pro-inflammatory genes

Quercetin can affect the gene expression in T cells, which is mainly responsible for its immune-stimulant activity. This activity is brought about by two-faced regulations – stimulation of Th-1 (T-helper type 1)-derived cytokines and interferons (IFNs) and simultaneous down-regulation of Th-2 (T-helper type 2)-derived cytokine and IL-4 in normal peripheral blood mononuclear cells (PBMC) (Nair et al. 2002). Quercetin has an inhibitory impact on IL-12 signalling and prevents IL-12-induced phosphorylation of Janus kinase 2 (JAK2), tyrosine kinase (TYK2), and signal transducer and activator of transcription 3 and 4 (STAT3 and STAT4). This prevents IL-12-induced T-cell proliferation and differentiation (Muthian and Bright 2004).

Studies reveal that the huge impact of quercetin on several molecular targets may be due to its direct regulatory role over the extracellular regulated kinase 2 (Erk2) mitogen-activated protein (MAP) kinase signal pathway (Penissi et al. 2003). Quercetin targets several kinases, phosphatases, enzymes, and membrane proteins within the leukocytes to impart its immune effect, while it also down-regulation many inflammatory pathways in mast cells, basophils, etc., resulting in its anti-inflammatory effect. Some of the targets involved in this complex signalling web are still not studied effectively; further insight may help us to develop better therapeutic agents (Chirumbolo 2010).

6.3.2 Alkaloids and Alkyl Amines

These bioactive secondary metabolites are widely distributed in the plant kingdom, particularly in angiosperms. They may be true alkaloids with nitrogen-containing ring structure or pseudoalkaloids where nitrogen mainly occurs in the side chains. Alkaloids are mainly produced by the plants as a defense mechanism against herbivores and to some extent against bacteria, fungi, and viruses. These secondary metabolites usually target the neuroreceptors; they alter neuronal signal transduction and metabolize neurotransmitters or second messengers (Walstab et al. 2014). Some alkaloids may also intercalate between the nitrogenous DNA bases, leading to mutations. Pharmaceuticals derived from such bioactive secondary metabolites like *Vinca* alkaloids, paclitaxel, etc. are used in chemotherapeutics. Lipophilic alkaloids may affect the ABC transporters as well. Such combination therapy is often used against recalcitrant cancers.

6.3.2.1 Quinine

Quinine is a quinoline anti-malarial alkaloid extracted from the bark of cinchona tree. While quinine has been first isolated in 1820, cinchona extracts were in use for treatment of malaria since before 1633 (Foley and Tilley 1997). Quinine may also be used for its mild antipyretic and analgesic properties and in treatment of babesiosis. This secondary metabolite has a direct effect on muscle membrane and on sodium channels, which makes it a useful pharmaceutical for myotonia congenita and nocturnal leg cramps (Cechinel-Filho 2012).

Quinine is best known for its role as an anti-malarial drug against the malaria-causing pathogen *Plasmodium falciparum*. The mode of action of anti-malarial drugs like chloroquine has been established for decades. This drug is active only against the blood stages of the malarial parasite where it directly degrades hemoglobin. *Plasmodium falciparum* is unable to degrade the heme molecule of hemoglobin and in turn crystalizes it to overcome the toxicity. This crystal is a non-covalent coordination complex with the heme irons chelated to the carboxyl side chains of the adjacent heme molecules. This heme polymerizing ability of *P. falciparum* is essential for its survival. With the exact mode of action of quinine being less understood, it was expected to be similar to chloroquine. Hence, it was hypothesized to inhibit the hemozoin biocrystallization, leading to accumulation of cytotoxic heme which killed *P. falciparum* (Foley and Tilley 1997).

Recently, a Singapore-Karolinska collaboration attempted to unveil the mode of action of quinine using the latest technologies available like mass spectrometry-coupled cellular thermal shift assay (MS-CETSA) (Dziekan et al. 2019). Using the MS-CETSA, the effect of ligand binding on heat-mediated denaturation and melting of proteins was studied. It is based on the idea that interaction of ligand and protein stabilizes the complex. Comparing the number of stable complexes in compound-treated and compound-untreated cell lysates after subjecting them to heat stress, cellular targets for a drug may be excavated. Combining this assay with mass spectrometry helps to excavate the whole proteome for suitable drug targets.

The use of MS-CETSA on *P. falciparum*-infected red blood cells revealed that drugs like quinine and mefloquine target our purine nucleoside phosphorylase enzyme (PfPNP). This was validated against pyrimethamine (also known as Daraprim) whose target, dihydrofolate reductase, was initially well-characterized. It was also revealed that the drug resistance was not via mutation in target enzyme but due to modifications in transporter proteins which actively pumps mefloquine out of the cell. Mefloquine is a weak binder to PfPNP in comparison with quinine, while other anti-malarial drugs like chloroquine, primaquine, and lumefantrine do not bind to this target at all. This makes the mode of action of quinine strikingly different from that of earlier excavated chloroquine. X-ray structures of quinine and PfPNP have also been worked out. While other PNP targeting anti-malarial pharmaceuticals have been in use, quinine was not regarded as one of them. Thus, it may be hypothesized that quinine targets the purine salvage pathway of *P. falciparum* by inhibiting the human PNP and PfPNP, thus killing the parasite (Madrid et al. 2008). While quinine may also have some other modes of action, so far this model is considered to be the main one.

6.3.2.2 Lysergic Acid Dimethylamine (LSD)

Lysergic acid dimethylamine or LSD is a semi-synthetic derivative of lysergic acid, a secondary metabolite obtained from parasitic rye fungus, *Claviceps purpurea*. LSD consists of a tetracyclic ring indole system ($C_{20}H_{25}ON_3$). It was first synthesized by Albert Hofmann back in 1938 while searching for bioactive derivatives of lysergic acid. It was initially used in psychiatric research and psychotherapeutic treatments, as it altered neurotransmitter system (Passie et al. 2008). Currently, attempts are being made to treat Alzheimer dementia, migraine, and cluster headaches using LSD-based drugs.

Psychedelics like LSD acts on a member of family A-type G protein-coupled receptors (GPCRs) known as serotonin 5-hydroxytryptamine 2A receptor (5-HT2AR) (Fig. 6.3). As an agonist (or partial agonist), LSD stimulates these serotonin receptors that are widely distributed throughout the brain. These receptors are densely placed on apical dendrites of layer 5-cortical pyramids, reticular nucleus of the thalamus, locus coeruleus, amygdala, and ventral tegmental area and have the highest expression in the claustrum. While activation of 5-HT2A receptors of glutamatergic neurons does not generate action potentials, it makes these neurons more

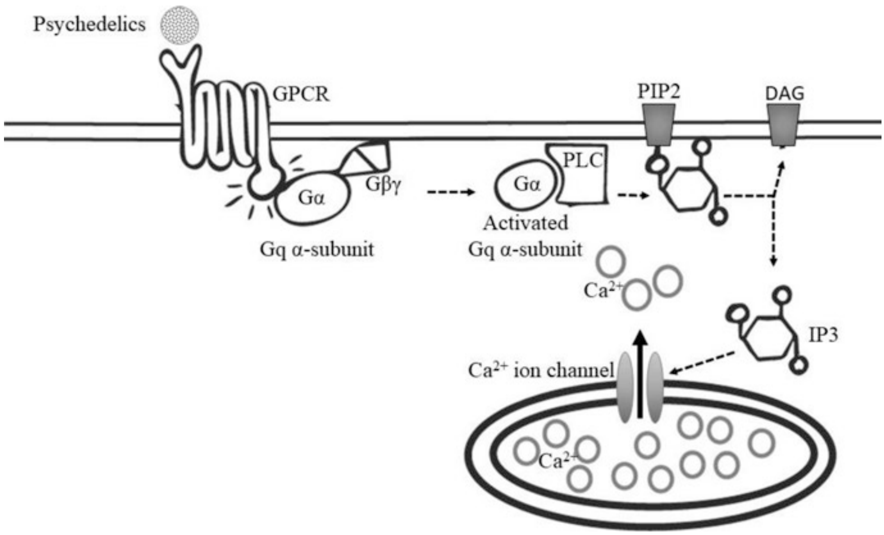

Fig. 6.3 Psychedelics like LSD acts as agonists for the family A-type G protein-coupled receptors (GPCRs). These receptors are widely distributed in the brain with some dense hotspots. Canonical signalling occurs by activation of Gαq on ligand binding. This activates phospholipase C (PLC), which in turn splits phosphoinositide 4,5 diphosphate (PIP2) into diacylglycerol (DAG) and inositol triphosphate (IP3). IP3 acts on intracellular calcium channels which lead to increase in cytosolic calcium concentration

excitable. LSD also suppresses raphe nucleus firing either directly or indirectly, via phenethylamines, which also culminates to cortical cell excitation. Such widespread 5-HT2A excitation in key brain regions has a marked effect on cognitive ability of the individual.

Martin and Nichols (2016) studied the effect of this psychoactive drug on rat brains and showed that LSD only excited the 5-HT2A receptors in less than 5% of the total brain neuronal population. Such excitatory neurons mainly belonged to the prefrontal cortex and claustrum. These neurons expressed the 5-HT2A receptors to a greater extent, making more sensitive to psychedelics. This resulted in differential response of specific brain regions to psychedelics, with strong excitation of a small trigger population. These activated trigger group neurons also recruit other cell types such as somatostatins and parvalbumin inhibitory gamma amino butyric acid (GABA)-regulated interneurons which leads to recurrent activity, destabilization of cortical network, perceptual and cognitive changes and visual imageries, characteristic of hallucinogens. This differential excitation of small subsets of excitatory and inhibitory neurons affects the coordination and communication between different brain regions.

It is suggested that the 5-HT2A receptor down-regulation in response to hallucinogens like LSD could help in stress-induced relapse. LSD also leads to structural changes in the brain by remodelling pyramidal cell dendrites and synaptic plasticity. It affects brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) expression patterns in the brain, which may culminate in the cognitive and behavioral changes characteristic of hallucinogens (Belouin and Henningfield 2018; Jalal 2018).

6.3.2.3 Vincristine and Vinblastine

Vincristine and vinblastine belong to the class of *Vinca* alkaloids, which were initially discovered from *Catharanthus roseus G. Don* (Kufe et al. 2003) back in the 1950s. This secondary metabolite has been utilized for its hypoglycemic activity and more importantly for its cytotoxic activity for decades. This anti-neoplastic secondary metabolite is currently used in treatment of various types of cancer like Hodgkin's disease, Kaposi's sarcoma, breast cancer, and testicular cancer. Vincristine differs from vinblastine only by a single methyl group which is exchanged to a formyl group. Some other *Vinca* alkaloids also approved for medicinal use include vinorelbine, vindesine, and vinflunine (Bennouna et al. 2008; Schutz et al. 2011).

The cytotoxic effect of this bioactive secondary metabolite is mainly brought about by its interactions with tubulin which blocks polymerization and crystalizes microtubules. Compromised microtubules cannot form mitotic spindles, and the cell remains arrested in metaphase stage leading to apoptosis (Himes 1991). In comparison to mitotic inhibitors like colchicine, podophyllotoxin, and guanosine-5′-triphosphate, *Vinca* alkaloids like vincristine and vinblastine have two distinct binding sites per mole of tubulin dimer (Correia and Lobert 2001; Downing 2000). There are high-affinity binding sites for *Vinca* alkaloids at the ends of each microtubule (Gregory and Smith 2000; Joel 1996). On interaction, these secondary metabolites inhibit new microtubule assembly and also shorten the already existing ones, producing a "kinetic cap" (Jordan et al. 1992). There is reduction in the rate of assembly and shortening of the microtubules, particularly at the ends of the mitotic spindle, arresting the cell in metaphase stage. This mitotic arrest may also occur at concentrations lower than that needed for microtubule disassembly (Toso et al. 1993). A concentration of 0.1–1.0 pmol/L is generally used to block endothelial proliferation; however, fibroblasts and lymphoid tumors require a higher concentration for effective results (Vacca et al. 1999). The combination therapy of anti-VEGF (anti-vascular endothelial growth factor) antibodies and vinblastine considerably increases the antitumor effect even on recalcitrant tumors.

Vincristine is known to have some immunosuppressant activity (Brade et al. 1981a, 1981b). The *Vinca* alkaloids mostly act on specific cell cycle phases. This secondary metabolite also induces an increase in intracellular cyclic adenosine monophosphate (cAMP) concentration and glutathione metabolism. Increased levels of cAMP stimulate polymerization of microtubules and redistributes myosin. cAMP also activated cAMP-dependent protein kinases which phosphorylates membrane proteins, myosin microtubules, and microtubule-associated proteins (MAP). *Vinca* alkaloids also inhibit calmodulin-dependent Ca^{2+} transport ATPase, cellular respiration, and nucleic acid biosynthesis (Moudi et al. 2013).

6.3.3 Glycosides

A category of saponins, carrying a 5/6 membered cardenolide or bufadienolide ring, is known as cardiac glycosides. Such cardenolides may be found among Apocynaceae, Brassicaceae, Celastraceae, Convallariaceae, Plantaginaceae and Ranunculaceae family plants like *Apocynum*, *Nerium*, *Euonymus*, *Convallaria*, *Kalanchoe*, *Helleborus*, etc. (Van Wyk and Wink 2015; Wink and Schimmer 2010). These cardiac glycosides mainly target some essential transporters in our body like sodium-potassium adenosine triphosphatase (Na^+/K^+ ATPase) pump. They generally have a strong neurotoxic effect, leading to cardiac and respiratory arrest. While previously it was mainly used as a poison, today this secondary metabolite finds its use in different pharmaceuticals related to heart health. It can slow down heartbeat and exhibit a positive inotropic effect. It modifies the degree of excitability of the heart and also regulates the dromotropic heart activity. Standardized secondary metabolite extracts from these plants are used in cardiac phytomedicine, one prominent example of which is digoxin (Wink 2015).

6.3.3.1 Digoxin

Digoxin is an important cardiac glycoside that was first isolated in 1930 from *Digitalis lanata* (Hollman 1996). Chemically, digoxin is a cardenolide glycoside similar to digitoxin with a beta hydroxylation at C-12. Digoxin is used in case of atrial fibrillation to control the ventricular rate. It may also be used in case of congestive heart failure with atrial fibrillation.

Digoxin specifically inhibits the Na^+/K^+ ATPase pump in the myocardium, which regulates the ionic balance and cardiac contractibility. This leads to an increased cytosolic sodium level, which affects the proper functioning of the sodium-calcium exchanger. Under normal conditions, three Na^+ ions are imported in exchange of one Ca^{+2} ion. Digoxin reverses this mechanism leading to an increased cytosolic Ca^{+2} level. Calcium being an important second messenger lengthens the cardiac action potential, thus decreasing the heart rate (Gheorghiade et al. 2004).

Increased calcium uptake in turn increases the amount of calcium stored in the sarcoplasmic reticulum. With every action potential, more calcium is released from sarcoplasmic reticulum, leading to stronger contractions without increasing energy expenditure. Digoxin also shows some neurohormonal effect, which may relate to the improved baroreceptor sensitivity in response to inhibition of sodium pump. This secondary metabolite also has a direct and indirect parasympathetic impart on atrioventricular (AV) node. Stimulation of vagus nerve slows down conduction via AV node, increasing the refractory period of myocytes. This gives the ventricles more duration to fill, before next contraction cycle. While the arrhythmia itself is not affected, the myocardial contractibility is improved to a great extent due to improved filling (Dart 2004).

To sum it up, digoxin increases the stroke volume and decreases the heart rate, which results in a net increase in blood pressure and tissue perfusion. It culminates in improved ventricular function curve, with optimized hydrodynamics. While there is an initial increase in action potential, eventually it decreases as the intracellular calcium levels rise leading to increased potassium conductance. The resting membrane potential becomes less negative, which makes the sinoatrial node more irritable. The conduction of stimulus increases in atria and is allowed down in the AV node. Electroencephalogram (EEG) reports reveal an increased PR interval and shortened QT interval as the AV node conduction is decreased. In addition, the T wave may be inverted, with ventricular tachycardia and fibrillation.

6.3.4 Terpenoids

Terpenes are secondary metabolites built on five carbon units. They may be subdivided into monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes, and polyterpenes (Dewick 2001). Terpenoid compounds are mainly lipophilic in nature enabling them to interact with biomembranes and membrane proteins. These secondary compounds increase the fluidity and permeability of the membrane and affect normal membrane transporter function, causing efflux of ions and metabolites, which may even lead to necrotic or apoptotic cell death (Wink 2007). Monoterpenes like *Mentha* extracts have been used as muscle relaxants in case of cramps and spasms. In general, terpenes show a non-specific cytotoxic activity against a diverse range of organisms like bacteria, fungi, insects, and even vertebrates; some may even affect membrane-enclosed viruses.

6.3.4.1 Saponins

Saponins are mainly glycosides of triterpenes or steroids. They may also include the group of cardiac glycosides and steroidal alkaloids. While steroid saponins are typical of monocots (Araliaceae, Fabaceae, Plantaginaceae, etc.), triterpene saponins are abundant in several dicot families (Poaceae, Primulaceae, Ranunculaceae, etc.), while gymnosperms generally lack this secondary metabolite (Wink and van Wyk 2010). Saponins have a range of biological functions like hemolytic, antimicrobial, insecticidal, and molluscicidal property. They are also used for the synthesis of steroidal drugs (like birth control pills) and cosmetic products (Sparg et al. 2004).

Saponins have a range of properties due to their extensive structural diversity, which includes bitter sweeteners, detergents, and emulsifying properties, in addition to the biological, medical, and pharmacological properties, such as hemolytic activity, antimicrobial, insecticidal and molluscicidal properties. Also noteworthy are the applications in pharmaceutical industries as raw material for the synthesis of steroidal drugs such as birth control, besides the intense use in the cosmetic industry.

Saponins are well-known for their ability to perturb cell membrane, which is essentially a lipid bilayer with hydrophobic membrane proteins embedded in it. The hemolytic effect of saponins, which ruptures the erythrocyte in circulation, is associated with its effect on cell membrane. The first model on mechanism of action of saponins on cell membrane was proposed by Glauert et al. (1962). This model suggests that when brought in contact, saponins interact with membrane cholesterol spontaneously. This complex then associates with a micelle, where the hydrophilic sugar chains are directed toward the centre of the complex, thus forming an aqueous pore. Such structures increase the membrane permeability to the hydrophilic ions and macromolecules leading to membrane disruption.

The recent models expand Glauert's hypothesis. It suggests that saponins are incorporated in the lipid bilayer through their steroidal nucleus. The lipophilic aglycones interact with the hydrophobic membrane sterols to form complex saponin; it also accumulates cholesterol in the matrix or membrane plates. As the final step of membrane perturbation mechanism, carbohydrates on the surface noticeably change the surface tension and curvature of the membrane. Such membrane curvature may lead to pore formation, lysis, or tubular protuberances which may eventually result in blistering via sterol extraction. As an alternative to this model, it is also suggested that the initial aglycone of saponins migrate to the areas of membrane sphingomyelin and complex cholesterol. Following this, there is similar complex formation which ultimately lead to membrane rupture in a dosage dependent manner (Barbosa 2014).

A steroid core has strong lipophilic attraction for erythrocyte membrane cholesterol. This explains the higher hemolytic activity of steroidal saponins over triterpenoidal saponins. This hemolytic activity is dosage-dependent and may be inhibited on addition of cholesterol in the middle of the reaction, confirming its mechanism of action through interaction with cholesterol (Barbosa 2014).

6.3.5 Atypical Amino Acid

While amino acids are mainly considered as primary metabolites, they may act as important secondary metabolites as well. These atypical amino acids often find their use in pharmaceuticals, as in case of myriocin.

6.3.5.1 Myriocin

Myriocin is a sphingolipid-like secondary metabolite which was initially known for its anti-fungal property. Its ability to disrupt sphingolipid homeostasis mainly results in its cytotoxic ability. Also known as thermozymocidin or ISP-1, myriocin is a secondary metabolite obtained from *Isaria* (*Cordyceps*) *sinclairii* (*Paecilomyces cicadae*, ATCC 24400). Pharmaceutical uses of myriocin make use of its antibiotic and immunosuppressive nature. However, its regular use is limited by its acute toxicity (Fujita et al. 1995). Myriocin, as an inhibitor of fumonisin B1-induced accumulation of free sphingoid bases, is used in the treatment of fumonisin-like diseases (Riley et al. 1999). *I. sinclairii* is also commercially exploited as a source of natural pigments (Cho et al. 2002). Today, a number of myriocin-like immunosuppressive analogues are in therapeutic use, e.g., FTY720 is a myriocin analogue used as anti-rejection medicines (Napoli 2000).

In the sphingolipid biosynthetic pathway, myriocin inhibits the rate-limiting enzyme, serine palmitoyltransferase (SPT) (Miyake et al. 1995). In this case, myriocin structurally mimics the natural product of SPT, sphinganine, which allows it to inhibit the enzyme. This inhibition leads to decreased free sphingoid bases, sphingosine-1-phosphate (S1P), and gradually reduced availability of complex sphingolipids, which are vital for normal cellular signalling and lipid bilayer functioning (Miyake et al. 1995). Intermediates of sphingolipid biosynthetic pathway are some of the major signalling molecules in the cell (such as sphingosine, sphinganine, ceramide, and sphingosine-1-phosphate) which controls cell proliferation, specialization, and apoptosis (Merrill Jr et al. 1997, 2001). These changes may lead to cell death, or altered signalling and immune regulation. Sphingolipids are also found in the lipid rafts and membrane microstructures, involved in receptor-mediated signalling. Thus, sphingolipids have a definite structural and signalling role, both of which regulate the development and responses of immune cells (Baumruker and Prieschl 2002).

Studies show that myriocin strongly inhibits proliferation of lymphocytes in mouse allogeneic mixed lymphocyte reaction (MLR), T-cell dependent antibody generation, and the synthesis of cytotoxic T-lymphocytes (CTL). It is about 10-100 times stronger than the clinically approved immunosuppressant cyclosporine A (Fujita et al. 1994). In the cytotoxic T-cell line (CTLL-2), myriocin inhibited growth by inducing apoptosis. Addition of sphingosine to the culture reversed its effect, confirming that myriocin targets sphingosine biosynthesis (Nakamura et al. 1996). IL-2-induced T-cell proliferation is also hindered by myriocin; however, production from alloantigen stimulated T cells is not affected (Fujita et al. 1994). Study by Johnson et al. (2004) showed that subacute levels of myriocin may significantly decrease the T-lymphocyte population in mice, particularly affecting the $CD4^+$ populations.

6.4 Conclusion and Future Perspectives

From the viewpoint of evolutionary pharmacology, secondary metabolites represent a class of bioactive compounds that show a diverse spectrum of activity in human cells, bacteria, fungi, viruses, and even parasites. Most of these secondary metabolites appearing in traditional medicine as complex mixtures of extracts are multitarget agents that non-specifically interact with several proteins, nucleic acids, and biomembranes. Some of these secondary metabolites act on the nervous system of animals, e.g., alkaloids like LSD, etc., and have mind-altering and hallucinogenic properties. Most of the activities of these multitarget pharmaceuticals can be correlated with simple biochemical interactions, where some of the interacting partners are still unknown. These non-specific groups of bioactive compounds often form a major part of rational medicine which may be used for maintenance of general good health as well as for treating diseases and infection.

Some secondary metabolite extracts interact synergistically, which potentiates their biological activities. This fascinating phenomenon needs to be further excavated and attracts more enthusiasts in the field of pharmacology. Herbal medicine cause a low-risk, low side effect, and cost-effective alternative compared to synthetic drugs. The use of new tools like genetic engineering, next-generation sequencing, and metabolomics to decipher the yet unknown interactomics of these herbal drugs will lead to better understanding of the mechanism of action of these herbal drugs. Authentication of the health benefits of these bioactive compounds may only be established through clinical trials, and implementation would rely on large-scale production, but understanding their proper mechanisms is a big step in the right direction.

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Chapter 7
Nutraceutical Potential of Herbs and Aromatic Plants of Himalayan Region

Smita Rana and Sofiya Anjum

7.1 Introduction

In recent years, a new diet health paradigm is evolving which places more emphasis on the positive aspects of diet (Das et al. 2012). The demand for foods with a positive impact on human health and wellness has exploded globally over the past two decades (Cencic and Chingwaru 2010). Nowadays, nutraceuticals are in high demand and are expected to increase the life expectancy and improve the quality of life of older adults (Bigliardi and Galati 2013). In the view of nutraceutical research in recent years, consumers have grown awareness about its usefulness and medicinal properties as a food supplement and received much attention that has led to its increased demand (Choudhary and Grover 2012; Cuellar-bermudez et al. 2015; Mishra et al. 2015a; Pandey et al. 2015; Patel et al. 2016; Tanna and Mishra 2018).

The term "nutraceutical" was coined from "nutrition" and "pharmaceutical" by Stephen DeFelice, founder and chairman of the Foundation for Innovation in Medicine (FIM), Cranford, New Jersey, in 1989. According to DeFelice, nutraceutical can be defined as "a food (or a part of food) that provides medical or health benefits, including the prevention and/or treatment of a disease" (Das et al. 2012; El Sohaimy 2012; Radhika et al. 2011). Nutraceuticals may range from genetically engineered foods to dietary supplements and herbal formulations and may even include processed products like cereals, soups, and beverages (Chaturvedi et al. 2011).

The principle, "Let food be thy medicine, and medicine be thy food," advocated by Hippocrates (460–377 BC), the well-recognized father of modern medicine, emphasized the association between nutrition and human health and

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conceptualized the relationship between the use of appropriate foods for health and their therapeutic benefits (Palthur et al. 2010).

7.2 Medicinal and Aromatic Plants in the Indian Himalayan Region

Medicinal and aromatic plants come up as an irreplaceable entity and are in high demand nowadays due to their significance as a raw material in the traditional healthcare system and modern pharmaceutical industries. The Indian Himalayan region covers approximately 59,1000 km^2 area, that is, 11% of the country's geographical area, and it contains more than 50% of the country's forest cover (Rawat and Satyakumar 2002; Saxena et al. 2001). Moreover, Himalaya is credited around the globe as a treasure of medicinal herbs and aromatic plant, accounting for about 30% of the endemic species reported in the Indian subcontinent (Nautiyal et al. 1998). Wide altitude range, different microclimatic conditions, and unique habitat type in the Himalayan region make the environment favorable for the growth and development of medicinal and aromatic plants (Dhar 2002; Kala et al. 2004; Sher and Alyemeni 2010). Approximately 44 gymnosperms, 600 pteridophytes, and 8000 angiosperms have been reported in the Indian Himalaya (Singh and Hajra 1996). Out of these, 1748 species are reported to have medicinal properties (Samant et al. 1998). Nearly 85% of medicines used in traditional healthcare system are being derived from these high-valued medicinal plants, and this helps in maintaining the livelihood of local people of the Himalayan region by providing essential day-to-day forest products for both food and medicine (Azaizeh et al. 2003; Diallo et al. 1999; Fransworth 1988; Kandari et al. 2012; Maikhuri et al. 1998; Phondani et al. 2014; Prasad and Bhattacharya 2003).

In India, more than 90% of raw materials of medicinal plants are harvested by industries for the production of various commercial products, including exports of these plants, and because of this, the population of medicinal and aromatic plants has come under threat (Dhar 2002). The huge demand for herbal drugs and the requirement of the products have led to the rapid depletion of several medicinal and aromatic plants from their natural habitat (Maikhuri et al. 1998; Nautiyal et al. 2001; Phondani et al. 2010). Along with industrial requirements, road connectivity, and infrastructure, a concept of the money-oriented market economy has been introduced. This has adversely affected the vegetation wealth of the Himalayan region. However, these developments have changed the indigenous socioeconomic pattern of people of higher altitude (Kala 1998). Since the majority of medicinal plant species of Indian Himalaya are endemic to the region (Chatterzee 1939), these are more vulnerable to extinction due to over-harvesting, urban development, climate change, the fulfillment of industrial needs, etc. In this chapter, we have discussed about the nutraceutical potential of few such medicinal plants of the Himalayan region having high nutritional profile but are on the verge of extinction (Srivastava et al. 2011).

7.3 Some Common Herbs Which Can Be Used as Nutraceuticals

7.3.1 Aconitum heterophyllum

7.3.1.1 Taxonomical Classification of *Aconitum heterophyllum* (Common Name – Atees)

Kingdom	Class	Order	Family	Genus	Species
Plantae	Magnoliopsida	Ranunculales	Ranunculaceae	*Aconitum*	*heterophyllum*

7.3.1.2 Vernacular Names of *Aconitum heterophyllum*

This plant is commonly known as *Ativisha*, *Shuklakanda*, *Aruna*, and *Vishada* in Sanskrit, as *Atees* in Urdu, as *Atis* and *Atvika* in Hindi, as *Atees* and *Atis* root in English, as *Ativasa* in Telugu, as *Ativishsa* in Kannada, as *Ataish* in Bengali, as *Ativakhani* in Gujarati, as *Ativish* in Marathi, as *Atividayam* in Malayalam, and as *Atis* in Punjabi (Konda et al. 2013; Paramanick et al. 2017).

7.3.1.3 Morphological Description and Distribution

Aconitum heterophyllum species are usually perennial or biennial herbs, often with stout leafy stems, bulbs, or creeping rhizomes. Leaves are mostly cauline, lobed, rarely divided, and dentate. Flowers are large, branched racemes, white, or purple. Around 300 species of *Aconitum* are found all over the world. About 24 species are found in India, mainly reported from Uttarakhand, Kashmir, Sikkim, and Nepal at an altitude between 2500 and 4000 m in the alpines and subalpine regions (Beigh et al. 2008). Generally, sandy loam and acidic soil are considered good for seed germination, survival, growth, and yield (Beigh et al. 2008).

7.3.1.4 Chemical Constituents

Aconitum plants contain many alkaloids and flavonoids. The phytochemical investigation carried out on *Aconitum heterophyllum* revealed the presence of active constituent such as atisine, atidine, 20 α-atisine, 20 β-atisine, hetratisine, heterophylline, heterophyllidine, heterophyllisine, hetisine, hetidine, hetisinone, and isoatisine. Two new nor-diterpenoid alkaloids, namely, 6-dehydroacetylsepaconitine and 13-hydroxylappaconitine, were also reported by Ukani et al. (1996) and Ahmad et al. (2008) (Fig. 7.1).

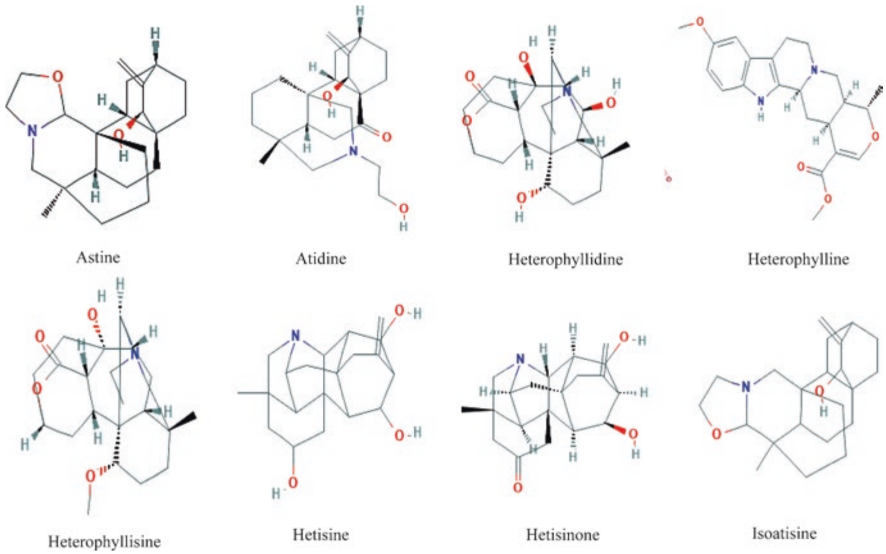


Fig. 7.1 Chemical structure of few bioactive compounds of *Aconitum heterophyllum*

7.3.1.5 Role on Traditional Medicine System

Aconitum heterophyllum is a medicinally significant plant and used as the main ingredient in many Ayurvedic medicines formulated by *The Ayurvedic Formulary of India*. It contains antidiarrheal activity and expectorant, diuretic, hepatoprotective, antipyretic and analgesic, antioxidant, alexipharmic, anodyne, anti-atrabilious, anti-flatulent, anti-periodic, anti-phlegmatic, and carminative properties used in the treatment of fever and rheumatism, nervous and digestive system disorders (Uniyal et al. 2002).

7.3.1.6 Pharmacological Approaches

Antidiarrheal Activity

Aconitum plant is reported as having antidiarrheal activity along with astringent and tonic properties (Prasad et al. 2012; Singh and Chaturvedi 1982). It is also used as the main ingredient in the antidiarrheal medicine Diarex VET (Mitra et al. 2001).

Anti-inflammatory Properties

According to Ayurvedic and ancient literature, the plant contains anti-inflammatory properties (Uniyal et al. 2002). Previous literature suggested that the plant possesses such chemical compounds which have potent anti-inflammatory effects through inhibiting prostaglandin pathways (Patwardhan and Hopper 1992). In another study suggested by Verma et al. (2014), *Aconitum* plant has the ability to inhibit sub-acute inflammation by interruption of arachidonic acid metabolism, and the anti-inflammatory effects were analogous to diclofenac sodium, a standard non-steroidal anti-inflammatory drug (Verma et al. 2014).

Antibacterial Activity

Crude alkaloid extracts from plant roots are reported to have moderate to high level of antibacterial activity against *Staphylococcus aureus*, *Bacillus bronchiseptica*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas fluorescens*, and *Xanthomonas campestris*, and the activity was found to be due to synergistic effects of several alkaloids (Sinam et al. 2014). Another study was done on the antibacterial activity of methanolic extract of the aerial parts of the plant against *Staphylococcus aureus* and *Bacillus subtilis* (Srivastava et al. 2011). The antifungal activity of the plant was reported against *Candida albicans*, *Aspergillus flavus*, *Aspergillus niger*, and *Alternaria solani* (Munir et al. 2014; Srivastava et al. 2011). New nor-diterpenoid alkaloids isolated from *Aconitum heterophyllum* along with few already known alkaloids were also reported for their antibacterial activity and were found to have potential against gram-negative (diarrhea-causing) bacteria *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* (Ahmad et al. 2008).

Antioxidant Activities

Several previous reports were suggested that plant parts possess antioxidant and free radical scavenging activities. The strong antioxidant potential of ethanolic and methanolic extracts of the plant was earlier described by Munir et al. (2014). DPPH free radical scavenging activity, nitric oxide radical scavenging activity, hydrogen peroxide scavenging activity, and ferrous reducing antioxidant power were also reported in the plant species (Ahmad et al. 2008; Konda et al. 2013; Prasad et al. 2012).

Immunomodulatory Activities

The immunomodulatory activity of the plant was tested on delayed-type hypersensitivity (DTH), humoral responses to sheep red blood cells, skin allograft rejection, and phagocytic activity of the reticuloendothelial system in mice and observed that the plant extract enhances the phagocytic function and inhibits the humoral component of the immune system (Atal et al. 1986).

7.3.2 *Nardostachys jatamansi*

7.3.2.1 Taxonomical Classification of *Nardostachys jatamansi* (Common Name – Jatamansi)

Kingdom	Class	Order	Family	Genus	Species
Plantae	Mangnoliopsida	Dipsacales	Valerianaceae	*Nardostachys*	*Jatamansi*

7.3.2.2 Vernacular Names of *Nardostachys jatamansi*

Nardostachys jatamansi is known for its various vernacular names in different geographical regions. It is known as *Jatamansi*, *Bhytajata*, and *Tapaswani* in Sanskrit; *Nard Indian* in French; *Musk-root*, *Indian spikenard*, and *Indian nard* in English; *Bhutijata* in Kashmiri; *Balchara* and *Jatamansi* in Hindi; *Jatamavshi* in Marathi; *Billilotan* in Punjabi; *Jatamanji* in Tamil, and *Jatamamsi* in Assamese and Bengali (Nakoti et al. 2017).

7.3.2.3 Morphological Description and Distribution

Nardostachys jatamansi is an erect perennial herb with 10–60-m-high stout, unbranched, or sparsely branched, woody, aromatic rhizome covered with reddish-brown thick fibers of remnant petioles of withered radical leaves with a single long taproot with rhizomes having a typical smell. Leaves are long, narrow, sessile, and oblong-ovate with 15–20 cm in length, whereas the root penetrates deep in the soil (Alam et al. 2016; Jadhav et al. 2009). Flowers are creamy-white to rosy or dark pink color in appearance arising in dense corymbose cymes and one, two, or five in number. The fruit is small, 4 mm long, covered with minute hairs. It has been described as a combination of three tastes – bitter, astringent, and sweet. The whole plant has a distinct lingering smell. The flowering season of jatamansi is usually from July to August, whereas the fruiting season is from September to October (Disket et al. 2012; Zalavadiya et al. 2013).

Nardostachys jatamansi is mostly grown between 3300 and 5000 m above sea level. The herb is found to be distributed in the subalpine to alpine areas of India, Pakistan, Nepal, Bhutan, Tibet, Southwest China, Myanmar, Yunan, and Afghanistan (Disket et al. 2012; Sharma 2017). In India, *Nardostachys jatamansi* is distributed in Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Arunachal Pradesh, Sikkim, and Assam. In China, it occurs in the Gansu, Sichuan, and Yunnan provinces and Xinjiang of Tibet. Its distribution in Nepal is throughout the Himalayan ranges at Dolpa, Humla, Jumla, Kalikot, north of Gorkha, Rasuwa, south Ganesh Himal, and Mustang (Dhiman and Bhattacharya 2020; Disket et al. 2012).

7.3.2.4 Chemical Constituents

The chemical composition of *Nardostachys jatamansi* is highly complex containing volatile essential oil and other biologically active compounds. And all its parts including its roots and rhizomes have significant and differing medicinal properties. The phytochemical investigation carried out on *Nardostachys jatamansi* revealed the presence of active constituents such as sesquiterpenes and coumarins. The roots of the herb contain valeranone or jatamansone as the principal sesquiterpene. The other sesquiterpenes include alpha-patchoulene, β-eudesmol, β-sitosterol, elemol, angelicin, jatamansin, jatamansinol, calarene, jatamansone, β-atchoulense, n-hexacosanyl arachidate, n-hexacosane, oroselol, valeranal, valeranone, seychelane, nardostachnol, nardostachone, beta-patchoulene, calarenol, n-hexacosanol, arachidate, n-hexacosanyl isolverate, nardol, norsechelanone, patchouli alcohol, seychellene, dihydrojatamansin, jatamansic acid, jatamansinone, oroselone, spirojatamol, and jatamol A and B (Fig. 7.2) (Alam et al. 2016; Nakoti et al. 2017; Purnima et al. 2015; Sahu et al. 2016; Singh et al. 2009, 2013; Zalavadiya et al. 2013). An alkaloid named actinidine has also been reported, and nardal has been reported to be an active component (Alam et al. 2016; Purnima et al. 2015; Sahu et al. 2016; Singh et al. 2013).

7.3.2.5 Role on Traditional Medicine System

Since the ancient times, the herb is known to have medicinal properties and is being popularly used in Indian Ayurveda, homeopathy, ethnomedicine, and Indian system of medicine to modern medicine industry as well as in Unani in ancient Greek and Arab and in ancient Egypt and Rome (Disket et al. 2012; Purnima et al. 2015). Its use has been well documented in several Ayurvedic classics such as *Sushruta Samhita*, *Nighantus Chikitsa Granthas*, and *Charak Samhita* (Dhiman and Bhattacharya 2020; Nakoti et al. 2017). The roots and rhizomes of the herb have various effects on doshas and are considered as tridoshashamak (as it is Vatashamak by snigdha; pittashamak by sheeta, tikta, kashaya, and madhur; kaphashamak by tikta and tikshna). Traditionally, *Nardostachys jatamansi* has been employed in the treatment of nervous system, digestive system, circulatory system, respiratory system, urinary system, reproductive system, and skin disorders (Jadhav et al. 2009). In addition to this, it has been considered to be effective in the treatment of headache, excitement states, menopausal symptoms, excessive flatulence, epilepsy, intestinal colic, nausea, stomachache, liver problems, jaundice, and kidney complaints and insomnia. The rhizomes of the herb are used to cure cough, fever, food poisoning, cholera, stomach disorders, intestinal worms, headaches, high-altitude sickness, leprosy, etc., whereas its oil has been reported to be useful in the treatment of atrial flutter and is also used in eye compounds and as poison antidotes. It also works as a memory enhancer (Alam et al. 2016; Dhiman and Bhattacharya 2020; Singh et al. 2009). In India, this herb is extensively used in more than 26 Ayurvedic and other herbal formulations including the anticonvulsant Ayush-56 (Dhiman and Bhattacharya 2020).

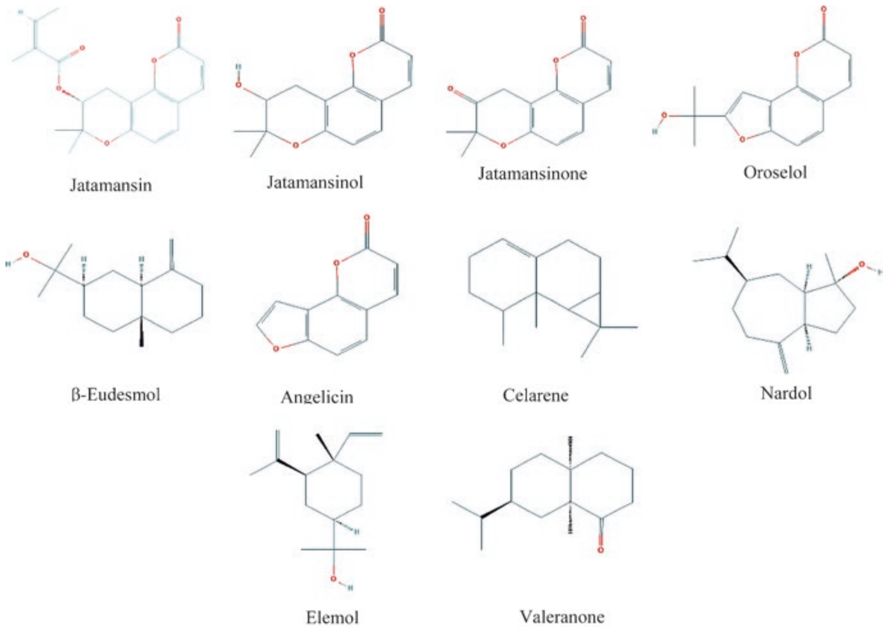


Fig. 7.2 Chemical structure of few bioactive compounds of *Nardostachys jatamansi*

7.3.2.6 Pharmacological Approaches

Antimicrobial Properties

Nardostachys jatamansi is known to have antimicrobial activity against the different bacterial strains, due to the presence of therapeutically important chemical constituents. The plant has been found to be active against a number of bacterial strains such as *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Micrococcus luteus* as well as several fungal strains such as *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus sulphureus*, *Fusarium oxysporum*, *Mucor fragilis*, and *Rhizopus stolonifer.* The herbal oil was also reported for its fungicidal activity (Alam et al. 2016; Disket et al. 2012; Purnima et al. 2015; Sahu et al. 2016; Sharma 2017). The antimicrobial activity of ethanol, ethyl acetate, and hexane extracts of *Nardostachys jatamansi* roots was studied by Sohail et al. (2007), in which the ethanol root extract exhibited maximum antimicrobial activity against all the tested bacteria and fungi (Nithya and Muthuraman 2016; Sohail et al. 2007).

Antioxidant Properties

All the extracts of *Nardostachys jatamansi* showed good antioxidant potential especially its roots and rhizomes, due to the presence of phenolics, flavonoids, and alkaloids (Kumari et al. 2010; Nithya and Muthuraman 2016; Sharma 2017).

The antioxidant activities of the methanol extract of *Nardostachys jatamansi* were found to contain only protocatechuic and syringic acids, which was analyzed by Pandey et al. (2013). Dugaheh et al. (2013) studied the antioxidant effect of *Nardostachys jatamansi*, which inhibited beta-carotene oxidation (Dugaheh et al. 2013). The antioxidative potential of the hydroalcoholic extract of *Nardostachys jatamansi* rhizomes was studied by Sharma and Singh (2012) that exhibited free radical scavenging against DPPH and superoxide anions (Nakoti et al. 2017; Parveen et al. 2011; Sharma and Singh 2012). Rasheed et al. (2010) investigated the aqueous root extract from *Nardostachys jatamansi* for its antioxidant activity, and the increased generation of TBARS and reduced GSH was restored to near-normal levels (Rasheed et al. 2010). The antioxidant effects of *Nardostachys jatamansi* tended to normalize augmented lipid peroxidation, nitrite, and superoxide dismutase activities and catalase level. Lyle et al. (2009) evaluated the antioxidant effect of hydro-ethanol extract (70%) of *Nardostachys jatamansi* that reversed the stress-induced elevation of lipid peroxidation and nitric oxide levels (Lyle et al. 2009; Nakoti et al. 2017; Nithya and Muthuraman 2016).

Hepatoprotective Activity

Ali et al. (2000) studied the pretreatment of rats with 50% ethanolic extract of *Nardostachys jatamansi* which shows significant hepatoprotective activity against thioacetamide-induced hepatotoxicity. Their results suggested good hepatoprotective activities of the root extract of jatamansi (Alam et al. 2016; Ali et al. 2000; Singh et al. 2009).

Antidiabetic Activity

The extract of jatamansi decreases glucose level significantly in both diabetic and non-diabetic rats as compared to respective controls; therefore, it has significant hypoglycemic activity (Mahesh et al. 2007). This was further investigated in two different studies that the different extracts of *Nardostachys jatamansi* exhibited significant antihyperglycemic activity in diabetic rats. The results showed that it has a significant antihyperglycemic effect in an experimental model of diabetes mellitus (Kumar et al. 2011; Mahesh et al. 2007).

Antiparkinson's Activity

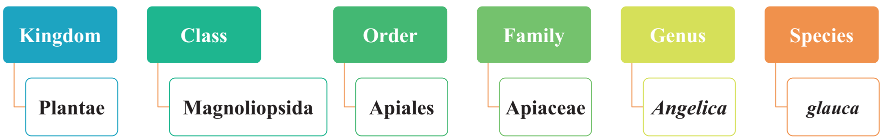
The root extract of *Nardostachys jatamansi* helps attenuate Parkinsonism. The activities of glutathione-dependent enzymes, catalase, and superoxide dismutase, which were reduced significantly by the infusion of 6-OHDA drug in the right striatum of rats, were restored by the extract of *Nardostachys jatamansi*. A significant decrease in the level of dopamine and its metabolites and an increase in the number of dopaminergic D2 receptors in the striatum were observed after 6-OHDA injection, and both were significantly recovered following *Nardostachys jatamansi* treatment (Ahmad et al. 2006; Alam et al. 2016).

Antiulcer Activity

The hydroalcoholic extract of *Nardostachys jatamansi* rhizomes possesses antiulcer activity mainly due to the modulation of defensive factors through the improvement of gastric cytoprotection and partly due to acid inhibition. The effect of the hydroalcoholic extract of *Nardostachys jatamansi* on pylorus ligation-induced ulcer in rats was studied by Rahman and Ahmed (2016). The drug at a dose of 500 mg/kg was found to significantly inhibit the formation of ulcers (Rahman and Ahmed 2016).

7.3.3 *Angelica glauca*

7.3.3.1 Taxonomical Classification of *Angelica glauca* (Common Name – Gandhrain)



7.3.3.2 Vernacular Names of *Angelica glauca*

Angelica glauca is commonly called *Choraa*, *Gandrayan*, and *Rikha Choraa* in Hindi, *Choraka Pullu* in Malayalam, *Gaddi Davanamu* in Telugu, *Chohore* and *Chorche* in Kashmir, and *Taskaraḥ* and *Ksemakaḥ* in Sanskrit (Butola and Vashistha 2013).

7.3.3.3 Morphological Description and Distribution

Angelica glauca is a perennial or biennial herb, which is 1–2 m tall in length, and its root is thick rhizomatous; its stem is hollow; its leaves are tripinnate, bipinnate, or unipennate; and its large pinna is lanceolate, ovate, or toothed. The inflorescence is a compound umbel with numerous rays. Flowers are white, yellow, or purple in color. Seeds are small in size and winged. Distribution of this plant is across Western Himalaya from Kashmir to Uttarakhand in forests' shade and alpine scrub between 2550 and 3800 m. It requires a cool and temperate climate with moist and porous soil with shade to grow. It can be utilized in the preparation of food as well as medicines (Butola and Badola 2004).

7.3.3.4 Chemical Constituents

The chemical composition of the root essential oil of *Angelica glauca* was studied by Rawat Mishra et al. (2018). They revealed the presence of active constituents such as α-pinene, camphene, sabinene, myrcene, α-phellandrene, p-cymene, β-phellandrene, limonene, cis-ocimene, trans-ocimene, γ-terpinene, linalool, borneol, terpinen-4-ol, carveol, citronellol, carvone, β-elemene, β-caryophyllene, allor-aromadendrene, germacrene-D, β-bisabolene, δ-cadinene, spathulenol, epi-α-cadinol, β-eudesmol, α-cadinol, (Z)-butylidene phthalide, (E)-butylidene phthalide, (Z)-ligustilide, and (E)-ligustilide (Fig. 7.3) (Purohit et al. 2015).

7.3.3.5 Role on Traditional Medicine System

Herbs and plants are being used in traditional medicines since ages and are widely used globally for the cure of numerous diseases. *Angelica glauca* is an important medicinal and aromatic plant species, which can be used as appetizer, stimulant, and expectorant and diaphoretic agent and can act as cardioactive, carminative, and cordial agent and can also be used as treatment for bilious

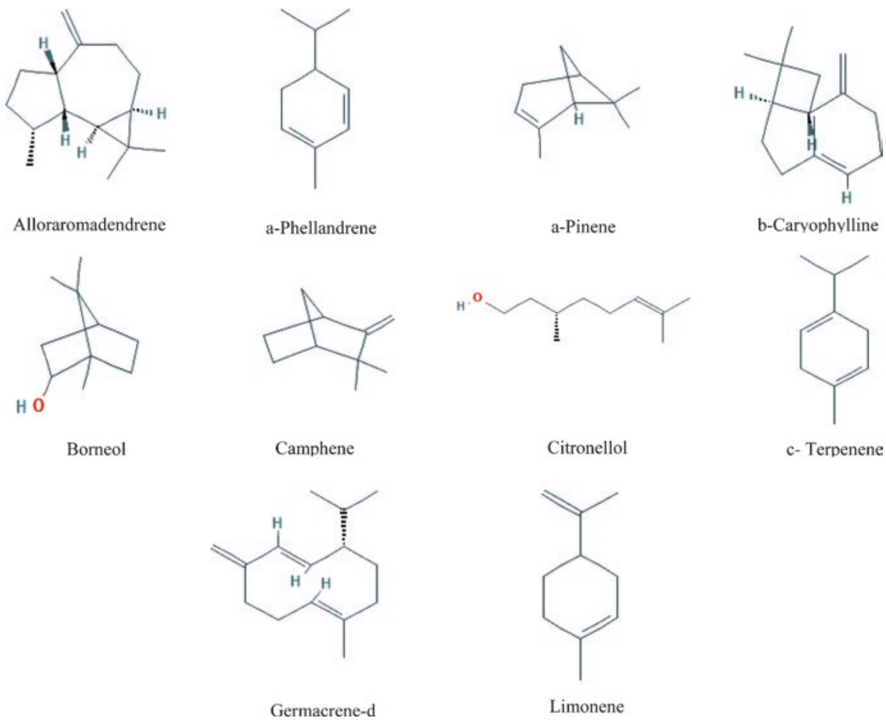


Fig. 7.3 Chemical structure of few bioactive compounds of *Angelica glauca*

complaints, digestive disorders, dyspepsia, lung disorders, menorrhagia, renal disorders, rheumatism, stomach difficulty, etc. (Agarwal 1986; Kirtikar and Basu 1983; Sharma et al. 1990).

7.3.3.6 Pharmacological Approaches

Antimicrobial Properties

The plant possesses high antimicrobial activity against bacterial strains like *Staphylococcus aureus*, *Bacillus subtilis*, *Pasteurella multocida*, and *Escherichia coli* and fungal strains like *Candida albicans*, *Aspergillus flavus*, *Fusarium solani*, and *Microsporum canis* (Irshad et al. 2011). Another study reported the antimicrobial activity of seven different extracts of the root tissues of *Angelica glauca* against *Klebsiella pneumoniae*, *Xanthomonas campestris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Lemna minor* (Zaman et al. 2018). Apart from the plant part, the oil of *Angelica glauca* also exhibits significant antimicrobial activity against different bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Pasteurella multocida*, and *Bacillus subtilis* and fungal strains *Microsporum canis*, *Fusarium solani*, *Candida albicans*, and *Aspergillus flavus* (Irshad et al. 2011). It might be possible that due to the presence of alkaloids and biologically important compounds, the plant possesses high antimicrobial activity (Ara and Nur 2009; Ramkumar et al. 2007).

Antioxidant Properties

The extracts of the plant can donate hydrogen and can act as a natural antioxidant. The methanolic, petroleum ether, chloroform, and aqueous extracts of *Angelica glauca* are potent in scavenging DPPH radicals (Arya and Mehta 2017). Irshad et al. (2011) reported that *Angelica glauca's* essential oil exhibited good DPPH radical scavenging and peroxidation inhibition activities (Irshad et al. 2011).

Phytotoxic Activity

Different extracts of *Angelica glauca* exhibited good phytotoxic potential against *Lemna minor*. Among the different solvents, butanol and n-hexane fractions showed maximum phytotoxic activity (Rauf et al. 2012; Zaman et al. 2018), and a good phytotoxic activity for crude methanolic extract, n-hexane, and acetate fractions was also reported (Grisi et al. 2013).

7.3.4 Hedychium spicatum

7.3.4.1 Taxonomical Classification of *Hedychium spicatum* (Common Name – Van-Haldi, Kapoor Kachri)

Kingdom	Class	Order	Family	Genus	Species
Plantae	Liliopsoda	Zingiberales	Zingiberaceae	*Hedychium*	*spicatum*

7.3.4.2 Vernacular Name of *Hedychium spicatum*

Hedychium spicatum is known for its various vernacular names in different geographical regions or systems. It is known as *Shati* in Sanskrit; *spiked ginger lily* in English; *Kapur- kachari* in Hindi; *Shati* and *Kachri* in Bengali; *Kapurkachari* and *Kapurkachali* in Gujarati; *Kapurakachari* in Marathi; *Kachur*, *ka-choor*, and *Sheduri* in Punjabi; *Poolankizangu* and *KichiliKizongu* in Tamil; *Gandhakachu-ralu* in Telugu; and *Gandhasunthi* in Oriya (Shantinath and Mandavkar 2015).

7.3.4.3 Morphological Description and Distribution

Hedychium spicatum is a tall perennial stout herb (up to 1.5 m tall) with fleshy aromatic rhizomes and thick straight stem with broadly lanceolate leaves. The bark is rough and reddish-brown with few deep-seated fibrous rootlets. Flowers are white in colour with orange-red base present in a dense terminal 15-25 cm long spike borne on a robust leafy stem. The fruit is a spheroid, three-valved capsule with orange-red lining, whereas the seeds are black with a red aril (Naithani 1984). Flowering of the plant occurs in July–August and seed formation occurs in September–October (Giri et al. 2010).

Hedychium spicatum occurs as a perennial herb in the Himalayas at an altitude of 800–3000 m above sea level (Rawat et al. 2018). The herb is native to Southeastern Asian countries with diverse habitats in temperate and subtropical areas ranging from Bhutan (Chettri et al. 2008), Nepal (Balami 2008; Kumar and Muller 1999), Thailand (Sirirugsa 1999; Sirirugsa and Larsen 1995), Japan, Pakistan, and China (Rawat et al. 2018).

In India, *Hedychium spicatum* is distributed throughout the subtropical Himalaya in Andhra Pradesh, Arunachal Pradesh, Darjeeling, Himachal Pradesh, Karnataka, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, and Sikkim (Arora and Mazumder 2017).

7.3.4.4 Chemical Constituents

The chemical composition of *Hedychium spicatum* is highly complex containing volatile essential oil. The rhizomes of the herbs contain essential oils along with starch, saccharides, organic acids, and resins which offer significant medicinal properties such as blood purification and treatments of bronchitis, indigestion, eye disease, and inflammations. 1,8-Cineole, terpinene, limonene, phellandrene, p-cymene, linalool, and α-terpineol are the major constituents of essential oils (Raina and Negi 2015). Along with essential oils, rhizomes are also chemically rich in sitosterol and its glucosides, furanoid diterpene-hedychenone and 7-hydroxyhedychenone (Giri et al. 2010). Other essential oil constituents include α-pinene, ß-pinene, terpinen-4-ol, sesquiterpenes, α-cadinol, ß-caryophyllene, ß-caryophyllene oxide, α-eudesmol, ß-eudesmol, 2-alkanones, camphor, linalyl acetate, β-terpineol, borneol, γ-cadinene, humulene, terpinolene, benzyl cinnamate, benzyl acetate, lindylacteate, γ-terpinene, methyl paracumarin acetate, cinnamic ethyl acetate, ethyl cinnamate, d-sabinene, drimane and labdane derivatives, spicatanoic acid, spicatanol and spicatanol methyl ether (Fig. 7.4) (Giri et al.

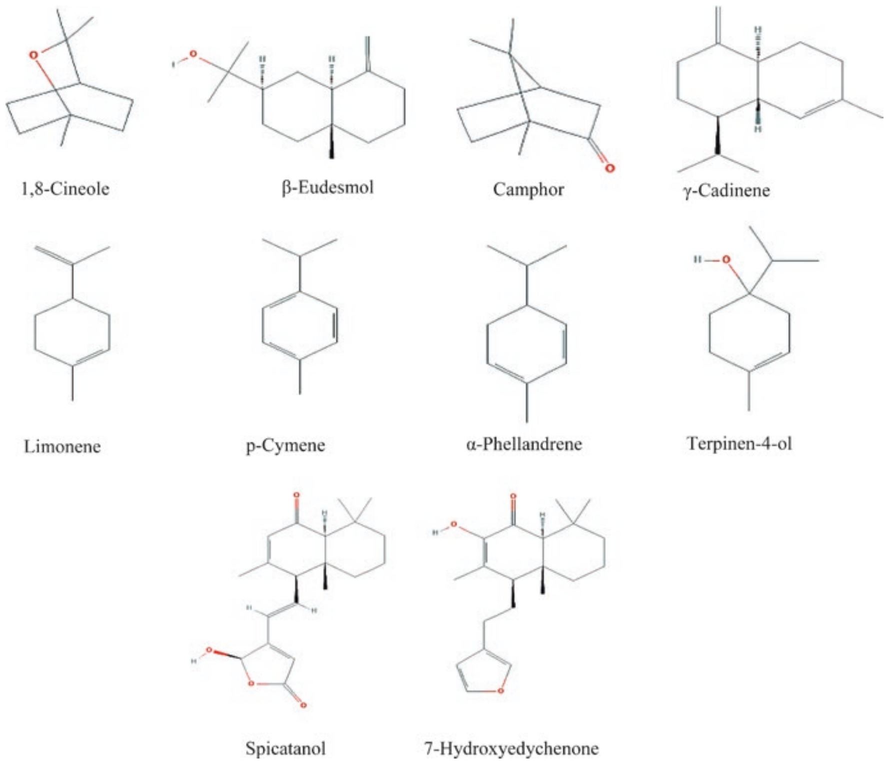


Fig. 7.4 Chemical structure of few bioactive compounds of *Hedychium spicatum*

2010; Rasool and Maqbool 2019; Rawat et al. 2019; Salkar et al. 2014; Verma and Padalia 2010), sesquiterpene -cadinene, sesquiterpene alcohols, sesquiterpene hydrocarbons, drim-8(12)-en-11-al, 11-nordermi-8-en-12-al, trans-5,5,8-alpha-trimethyldecal-2-one and γ- bicyclohomofarnesal (ambral), drim-8(12)-ene, 15,16-bisnorlabda- 8(17)-dien-14-al, diterpene 6-oxo-labda, 7,11,13-trien-16-oic acid lactone, 6-oxo-labda-7,11,13-trien-16-oic acid lactone, and ethyl-p-methoxy cinnamate (EPMC). EPMC is one of the important compounds isolated from the rhizomes of this plant. The essential oil obtained from the rhizome is reported to contain approximately 66% of this compound (Salkar et al. 2014; Shantinath and Mandavkar 2015).

7.3.4.5 Role on Traditional Medicine System

Since the ancient times, *Hedychium* species are used to treat a wide spectrum of diseases. Its roots, leaves, and rhizomes are also used as ingredients in several Ayurvedic preparations; in Tibetan medicine, traditional Chinese medicine, and the Unani medicinal system; and also in home remedies. The species is widely used and shared in 19.43% of 175 known herbal formulations in Tibetan medicines. In Indian medicines, the species finds a place in 233 formulations, and the therapeutic index of its uses is 10.3% (Rawat et al. 2018).

In Ayurvedic literature, *Hedychium spicatum* possess *Rasa* (tastes) such as *Katu* (pungent –stimulatory for digestion), *Tikta* (bitter – tends to be fairly dry), and *Kashaya* (astringent – effect on digestion); *Guna* (properties) such as *Laghu* (light – purification of body channels and development of vigor and joy of the body), and *Teekshna* (penetrating – fast in action and helps in evacuation of the body); *Veerya* (potency) such as *Ushna* (heating); and *Rogaghnata* (disease-curing properties) such as *Sandhishotha* (swelling), *Shoola* (pain), *Dantashoola* (toothache), *Mukhadurgandha* (halitosis), *Vrana* (wound healing), *Apatantraka* (apoplectic convulsions), *Amavata* (rheumatoid arthritis), *Aruchi* (tastelessness), *Agnimandhya* (poor digestion), *Adhamana* (flatulence), *Udarashoola* (colic pain), *Atisara* (diarrhea), *Arsha* (piles), *Raktavikara* (blood disorder), *Pratishyaya* (allergic rhinitis), *Kasa* (cough), *Shwasa* (respiratory disorder), and *Hikka* (hiccup). Also, in Tibetan medicine system, this species is widely used in many formulations such as PADMA-28, which was revived in Poland and now used against chronic inflammatory diseases and peripheral vascular occlusive disease and revealed promising results in intermittent claudication, atherosclerosis, and chronic active hepatitis (Rawat et al. 2018).

Traditionally, *Hedychium spicatum* is known to cure several diseases such as bad breath, bronchial asthma, blood diseases, hiccup and vomiting, asthma, body pain, inflammation, diarrhea, piles, high fever, liver problems and pain, indigestion, stomachache, rheumatoid arthritis, and snakebite and is used as laxative, expectorant, stimulant, tonic, and vasolidator (Jugran et al. 2011; Singh et al. 2018; Sravani and Paarakh 2011; Tavares et al. 2020).

7.3.4.6 Pharmacological Approaches

Antimicrobial Properties

Hedychium spicatum is known to have antibacterial and antifungal activity, thereby increasing its therapeutic importance. The rhizome extract has been found to be active against a number of bacterial strains such as *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella boydii*, *Shigella sonnei*, *Shigella flexneri*, *Vibrio cholera*, and *Pseudomonas aeruginosa* (Arora and Mazumder 2017; Arumugam et al. 2020; Bisht et al. 2006) as well as several fungal strains such as *Candida albicans*, *Rhizopus stolonifer*, *Trichoderma viride*, and *Trichoderma lignorum* (Bisht et al. 2006). The essential oil extracted from the flowers of *Hedychium spicatum* is also reported to be active against gram-negative bacteria *Borrelia burgdorferi* (Tavares et al. 2020), whereas the ethanolic fruit extract of *Hedychium spicatum* showed antibacterial and antifungal properties against *Salmonella* spp., *E. coli*, and various filamentous fungi (Bisht et al. 2006; Rasool and Maqbool 2019).

Antioxidant Properties

All the extracts of *Hedychium spicatum* showed good antioxidant potential especially roots and rhizomes, due to the presence of phenolics, flavonoids, alkaloids, and terpenoids. The rhizome essential oil exhibits moderate to good Fe^{2+}-chelating activity, reducing power, as well as DPPH radical scavenging profile (Choudhary et al. 2018; Joshi et al. 2008; Tavares et al. 2020). Choudhary et al. (2018) evaluated the antioxidant activity of methanolic, ethanolic, hydroethanolic, and aqueous rhizome extracts of *Hedychium spicatum* through ABTS, DPPH, and nitric oxide (NO) free radical scavenging assays which show that among all the extracts, methanolic extract has the most antioxidant potential (Choudhary et al. 2018).

Tranquilizing Action

The essential oil of the rhizomes of *Hedychium spicatum* was reported to possess mild tranquilizing action of short duration. It depressed conditioned avoidance response and rotarod performance and potentiated phenobarbitone hypnosis and morphine analgesia in rats (Chopra 1979; Shantinath and Mandavkar 2015).

Antidiabetic Activity

The essential oil obtained from the rhizomes of plants possesses significant antidiabetic activity. The main compound responsible for the antidiabetic activity was found to be 1,8-cineole. Oral doses of 0.3 ml/rat of *Hedychium spicatum* essential oil administered for 14 days reduced the blood glucose and urea level significantly as compared to the normal control (Kaur and Richa 2017).

Anthelmintic Activity

Sravani and Paarakh (2011) evaluated the anthelmintic activity of methanolic and aqueous rhizome extracts of *Hedychium spicatum* against adult Indian earthworms, *Pheretima posthuma*. The time taken for paralysis and death of individual worms was determined, and the results obtained were compared with that of standard drug, i.e., piperazine citrate. It was observed that the methanol extract of *Hedychium spicatum* produced dose-dependent anthelmintic activity which is quite comparable with the standard drug piperazine citrate, whereas the aqueous extract was not at all effective (Sravani and Paarakh 2011).

Cytotoxic Activity

The phytochemical investigation of the chloroform extract of the rhizomes of *Hedychium spicatum* led to the isolation of two new labdane-type diterpenes. All the isolates were tested for their cytotoxicity against Colo-205 (colon cancer), A-431 (skin cancer), MCF-7 (breast cancer), A-549 (lung cancer), HeLa (cervical cancer), HEP G2 (liver cancer), THP-1 (human acute monocytic leukemia), HL-60 (human promyelocytic leukemia), and A-375 (human malignant melanoma) cancerous cell lines. The two new labdane diterpenes exhibited good cytotoxic activity (Reddy et al. 2009a, 2009b). Moreover, the volatile compounds in the essential oil of the rhizomes rich in 1,8-cineol, eudesmol, cubenol, spathulenol, and α-cadinol also exhibited in vitro cytotoxic activities against human cancer cell lines, such as the lung (A-549), colon (DLD-1, SW620), breast (MCF-7, MDA-MB-231), head and neck (FaDu), and cervix (HeLa). Different essential oil samples exhibited different levels of cytotoxic properties (Mishra et al. 2015b).

7.3.5 *Bergenia ciliata*

7.3.5.1 Taxonomical Classification of *Bergenia ciliata* (Common Name – Pashanabheda)

Kingdom	Class	Order	Family	Genus	Species
Plantae	Magnoliopsida	Saxifragales	Saxifragaceae	*Bergenia*	*ciliate*

7.3.5.2 Vernacular Names of *Bergenia ciliata*

Bergenia ciliata is commonly known as *Amabhedaka* in Sanskrit; *Paashanbheda* in Hindi; *Patharkuchi* in Assamese; *Zahkm-e-Hayat* in Urdu; *Patharkuchi*, *Himasagara*, and *Patrankur* in Bengali; *Pashanbheda* and *Pakhanbheda* in Gujarati; *Alepgaya*, *Pahanbhedi*, *Hittaga*, *Pasanaberu*, and *Hittulaka* in Kannada; *Pashanbhed* and *Batweyaa* in Kashmiri; *Kallurvanchi*, *Kallurvanni*, and *Kallorvanchi* in Malayalam; *Pashanbheda* in Marathi; *Pasanbhedi* and *Pashanabheda* in Oriya; *Kachalu* and *Pashanbhed* in Punjabi; *Sirupilai* in Tamil; *Kondapindi* in Telugu; and *Rockfoil* in English (Khan and Kumar 2016).

7.3.5.3 Morphological Description and Distribution

The plant is generally known as Pashanabheda, or rockfoil, which itself depicts that this plant grows between rocks. *Bergenia* plants are perennial herbs up to 50 cm tall and are succulent. Rhizomes are woody, covered with leathery rounded leaf bases. Flowers are dense erect and in clusters in pink, purplish, or white color in terminal cymes. The fruit is a conical capsule having minute seeds (Hooker and Thomson 1858; Kumar and Tyagi 2013). This species is mainly reported in cold and temperate regions and distributed globally across Afghanistan, South Tibet, and Bhutan. In India, it is found in Uttarakhand, Meghalaya, West Bengal, Arunachal Pradesh, Sikkim at an altitude range between 900 and 3000 m (Hafidh et al. 2009; Handa 1997).

7.3.5.4 Chemical Constituents

Bergenin is the most abundant chemical constituent of the plant. Bergenin is also known as cuscutin, the C-glucoside of 4-O-methyl gallic acid, which is in colorless crystal form. Other major chemical constituents reported from *Bergenia ciliata* are (+) afzelechin, (+) catechin, quercetin-3-o-β-D-xylopyranoside, quercetin-3-o-α-L-arabinofuranoside, eriodictyol 7-o-β-D- glucopyranoside, arbutin, 6-o-p-hydroxybenzoylarbutin, 4-o-galloylbergenin, 11-o galloybergenin, p-hydroxybenzoic acid, protocatechuic acid, 6-o-protocatechuoylarbutin, 11-o-p-hydroxy-benzoyl bergenin, 11-o-protocatechuoyl bergenin, and 6-o-phydroxybenzoyl-parasorboside (Fig. 7.5) (Fuji et al. 1996; Kumar and Tyagi 2013; Kuzmin et al. 1985; Sticher et al. 1979).

7.3.5.5 Role on Traditional Medicine System

Bergenia species are reported as having the ability to dissolve gravel and stone in the kidney and urinary bladder; therefore, they are used for antiurolithiatic activities, thereby commonly calling them as Paashanbheda (i.e., Paashan, rockstone;

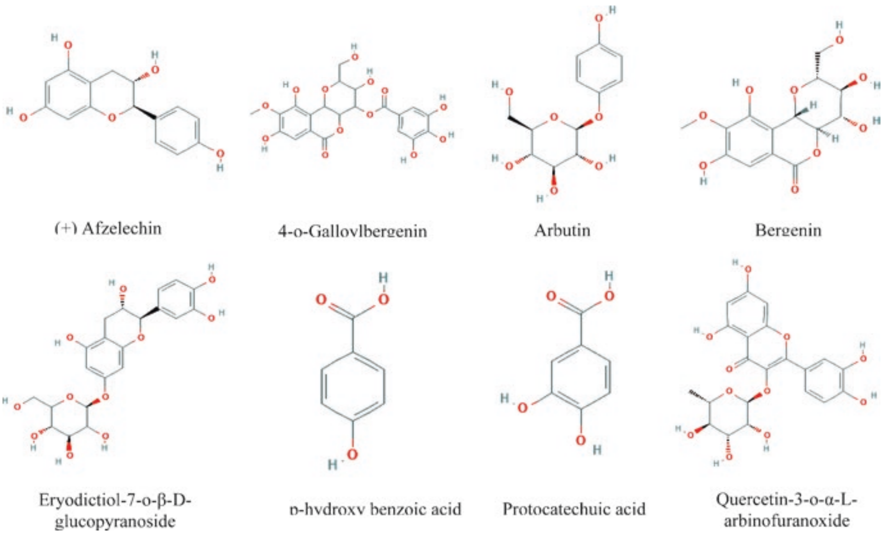


Fig. 7.5 Chemical structure of few bioactive compounds of *Bergenia ciliata*

bheda, piercing). The plant has been reported as having various other medicinal properties and has also been used in Ayurvedic medicine system for antibradykinin activity, diuretic activity, antilithic activity, antibacterial activity, antiviral activity, antipyretic activity, anti-inflammatory activity, and hepatoprotective activity (Kumar and Tyagi 2013).

7.3.5.6 Pharmacological Approaches

Antimicrobial Activity

The methanolic extract of *Bergenia ciliata* rhizomes exhibited a broad spectrum of activity against *Bacillus pumilus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, and *Vibrio cholera* (Sinha et al. 2001). *Bergenia* extracts are also reported as having antimicrobial activity against *Candida albicans*, the fungus that can cause life-threatening illnesses, particularly in immunocompromised patients (Zbikowska et al. 2017).

Anticarcinogenic Activity

Methanolic extracts of *Bergenia ciliata* contain cytotoxic effects and have apoptotic properties and play a significant role in the prevention and treatment of hepatocellular carcinoma. *Bergenia ciliata* decreases the levels of tumor markers: alanine aminotransferase, aspartate aminotransferase, alpha-fetoprotein, gamma-glutamyl transferase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and 5′-nucleotidase (Dar et al. 2019).

Antiurolithiatic Activity

Different leaf extracts of *Bergenia ciliata* exhibit antiurolithiatic activity against calcium oxalate and phosphate stones (Byahatti et al. 2010). In another study by Saha and Verma (2011), they reported that the hydroalcoholic extract of rhizomes of *Bergenia ciliata* when administered to rats with ethylene glycol induced lithiasis and reduced and prevented the growth of urinary stones (Saha and Verma 2011).

Hypoglycemic Activity

The different solvents, i.e., ethanol, hexane, ethyl acetate, and chloroform, and aqueous extracts of *Bergenia ciliata* have shown significant hypoglycemic activity (Islam et al. 2002). The hypoglycemic activity of different extracts of the plant species was also tested by determining blood sugar-lowering effect on streptozotocin (STZ)-induced rats (Basnet et al. 1994). Aswal et al. (1984) also reported the hypoglycemic activity in *Bergenia ciliata* ethanol-water (1:1) ratio extract through oral administration of rats, and it was observed that a dose of 25 mg/100 g actively lowers the sugar level of the experimental animals (Aswal et al. 1984).

Antioxidant Activities

Bergenia ciliata possesses significant antioxidant potential and contains various phytochemicals such as flavonoids, steroids, terpenoids, tannins, saponins, anthraquinones, and glycosides which might be responsible for the excellent antioxidant activity of the plant (Fischer et al. 2011; Santos et al. 2014). A study revealed by Zafar et al. (2019) showed the antioxidant potential of the phenolic compounds isolated from the rhizome of *Bergenia ciliata* against DPPH and ABTS free radical. In another work, the antioxidant activity of aqueous and methanolic extracts of *Bergenia ciliata* was tested, and both extracts are found to be active radical scavengers. The lipid peroxidation inhibition efficiency (TBARS assay) and the reducing power of both extracts exhibit excellent activity in preventing lipid peroxidation, and this might prevent oxidative damages to biomolecules. The capability of the extracts to defend DNA (pBR322) against UV-induced photolyzed oxidative damage was analyzed, and both extracts were able to protect DNA from oxidative damage (Rajkumar et al. 2010; Zafar et al. 2019).

7.3.6 *Rheum australe*

7.3.6.1 Taxonomical Classification of *Rheum australe* (Common Name – Himalayan Rhubarb)

Kingdom	Class	Order	Family	Genus	Species
Plantae	Magnoliopsida	Poygonales	Polygonaceae	*Rheum*	*australe*

7.3.6.2 Vernacular Names of *Rheum australe*

Rheum australe or Himalayan rhubarb is known for its various vernacular names in different geographical regions or systems. It is known as *Indian* or *Himalayan rhubarb* in English, *Gandhini* or *Revatchini* in Sanskrit, *Dolu* or *Ppita* in Hindi, *Pumbehakh* in Kashmiri, *Nattu-Ireval-Chini* in Tamil, *Natu-Revalchini* in Telugu, *Amlaparni* or *Pitamuuli* in Ayurvedic, *Revalchinikattai* in Siddha, and *Revandchini* in Unani system (Malik et al. 2016).

7.3.6.3 Morphological Description and Distribution

Rheum australe (Himalayan rhubarb) is a tall (1.5–3 m), robust, glabrous, leafy perennial herb with stout rhizomes. The leaves are thick, orbiculate, or broadly ovate with 5–7 basal veins, a cordate base, an entire margin, and sinuolate with an obtuse apex. The inflorescence is large, fastigiately branched, and densely papilliferous. The flowers are pedicellate and dark purple. The fruit is ovoid-ellipsoid, broadly ellipsoid, or ovoid-oblong in shape, large (0.5–1.5 cm), long, and purple, with wings more narrow than thick and notched at both ends. Flowering in the plant occurs in the months of July–August and fruiting from July–September (Anjen et al. 2003; Pandith et al. 2018; Rokaya et al. 2012a).

Rheum australe is a multipurpose, endemic, and endangered medicinal herb of North-Western Himalayas which grows in grassy or rocky slopes at higher altitudes and in forest margins at an altitude of 3200–5200 m. The plant species are distributed in the higher regions of Himalaya, covering the areas of Bhutan, China (southern Tibet or S. Xizang), India (Kashmir, Sikkim), Myanmar, Nepal, and Pakistan. In India, the herb is distributed in temperate and subtropical regions from Kashmir to Sikkim (Anjen et al. 2003; Bano et al. 2017; Pandith et al. 2018). In Himachal Pradesh, it has been reported to occur between 2800 and 4800 m in the districts of Shimla, Kangra, Chamba, Kullu, Kinnaur, Lahaul, and Spiti (Chauhan et al. 1992).

7.3.6.4 Chemical Constituents

A variety of constituents have been isolated from *Rheum australe* including essential oils, anthraquinones, organic acids, stilbenes, and many more. The essential oil obtained from the rhizome of *Rheum australe* has a characteristic odor due to the presence of eugenol and methyl heptyl ketone. Other major constituents obtained from the essential oil of rhizomes of *Rheum australe* include paeonol, α-copaene, δ-cadinene, methyl eugenol, and methyl stearate (Fig. 7.6) (Agarwal et al. 2001).

Anthraquinone derivatives that have been reported from *Rheum australe* include emodin, emodin-3 monomethyl ether (physcion), chrysophanol, aloe-emodin (9.10-dioxo anthracene), and rhein, which occur as free quinone, anthrone, or dianthrone glycosides, whereas the commonly reported stilbenes in *Rheum* include piceatannol and resveratrol. Other compounds that have been isolated from the herb include gallic acid, present as glucogallin, and catechin, tannins, cinnamic and rheinolic acids, volatile oils, starch, calcium oxalate, and anthrone C-glucosides in the form of 10-hydroxycascaroside C, 10-hydroxycascaroside D, 10R-chrysaloin 1-O-b-D-glucopyranoside, cascaroside C, cascaroside D, and cassialoin (Fig. 7.6) (Agarwal et al. 2001; Chauhan et al. 1992; Nazir et al. 2013; Qing-xia et al. 2013; Rokaya et al. 2012b).

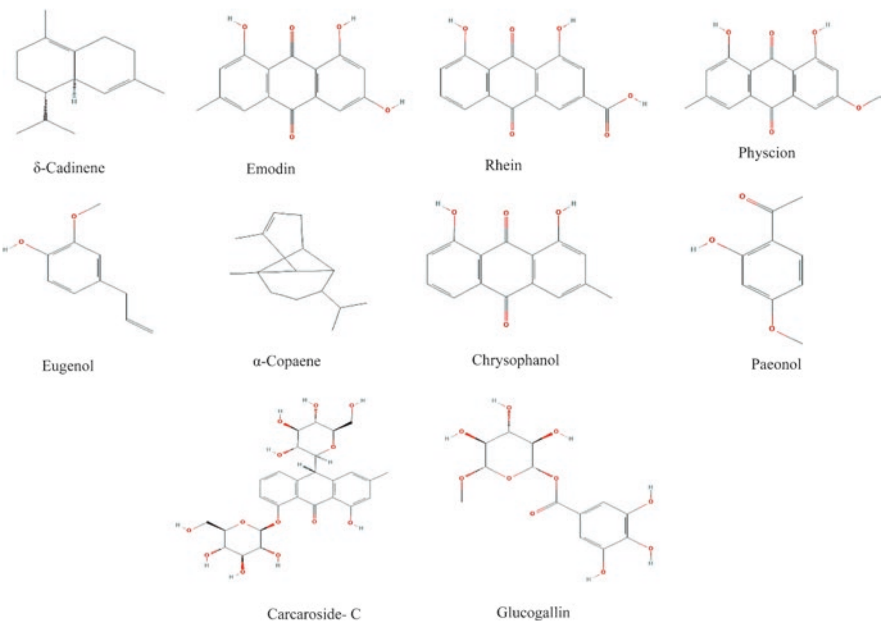


Fig. 7.6 Chemical structure of few bioactive compounds of *Rheum australe*

7.3.6.5 Role on Traditional Medicine System

Rheum australe is widely used in various traditional medicinal systems such as Unani, Ayurveda, and homeopathic, Chinese, and Tibetan systems (Agarwal et al. 2001; Bano et al. 2017; Chauhan et al. 1992; Malik et al. 2016; Nazir et al. 2013; Pandith et al. 2018; Qing-xia et al. 2013; Rokaya et al. 2012a, 2012b). Various ancient texts have mentioned the use of the herb for *udara roga* (stomach problems), *amala pitta* (gastritis), *yakrit vikar* (liver diseases), *rakta moksha* (blood purification), and *rakta pradhara* (menstrual problems) (Rokaya et al. 2012a). The roots of the herb are widely used in Ayurvedic and Chinese folk medicine (Bano et al. 2017). The root has anticholesterolemic, antiseptic, antispasmodic, antitumor, aperient, astringent, cholagogue, demulcent, diuretic, laxative, purgative, stomachic, and tonic properties. The root is used as local home remedy for stomach problems, cuts, burns, chronic constipation, diarrhea, liver and gall bladder complaints, wound, and muscular swelling, menstrual problems, hemorrhoids, tonsillitis, and mumps. Extracts from the roots, bark, and leaves of rhubarb have been used as a laxative since the ancient times and presently are widely used in various herbal preparations (Hou et al. 2015; Malik et al. 2016; Nazir et al. 2013; Rokaya et al. 2012a).

7.3.6.6 Pharmacological Approaches

Antimicrobial Properties

Rheum australe is known to have antibacterial and antifungal activity, thereby increasing its therapeutic importance. The ethanolic and benzene extracts of *R. australe* have been shown to display promising activity against *Helicobacter pylori*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Citrobacter freundii*, *Salmonella infantis*, *Salmonella typhimurium*, and *Streptomycin* (Hussain et al. 2010; Jiang et al. 2018; Pandith et al. 2018).

The different constituents isolated from *Rheum australe* rhizomes, i.e., rhein, physcion, aloe emodin, and chrysophanol, are reported to have antifungal activity against *Candida albicans*, *Candida tropicalis*, *Cryptococcus neoformans*, *Trichophyton mentagrophytes*, *Aspergillus fumigatus*, *Aspergillus niger*, and *Rhizopus oryzae* (Babu et al. 2003; Khan et al. 2012; Malik et al. 2016).

Antioxidant Properties

The methanolic and aqueous extract of the rhizomes of *Rheum australe* showed good antioxidant potential especially the roots and rhizomes, due to the presence of a high number of phenolic compounds displaying strong correlation with free radicals (DPPH and OH) scavenging efficacies, percentages of LPI, and $Fe3+$ reductions (Malik et al. 2016; Rajkumar et al. 2011a). The phenolic compounds that are responsible for the antioxidant properties include ß-resorcylic acid, daidzein-8-O-glucoside (puerarin), daidzein, (+)-taxifolin, and flavonols (Pandith et al. 2018; Rajkumar et al. 2011a).

Anticancer Activity

The methanolic and aqueous extract of the rhizomes of *Rheum australe* is found to exhibit extensive concentration-dependent cytotoxicity against human breast carcinoma (MDAMB-435S) and liver carcinoma (Hep3B) cell lines (Malik et al. 2016; Rajkumar et al. 2011a) as well as cause apoptosis of the breast carcinoma (MDAMB-435S), liver carcinoma (Hep3B), and human prostate (PC-3) cancer cell lines (Rajkumar et al. 2011b). Thus, *Rheum australe* can be used against tumors by reducing the neoplastic growth and malignancy that are often caused by oxidative stress (Rokaya et al. 2012a).

Antidiabetic Activity

The study on *Rheum australe*-derived crude extracts and isolated compounds (rhapontigenin, desoxyrhaponticin, desoxyrhapontigenin, chrysophanol-8-O-ß-d-glucopyranoside, and torachrysone-8-O-ß-d-glucopyranoside) against yeast and mammalian α-glucosidase shows the α-glucosidase inhibiting capacity of the compounds. Among them, the greatest inhibition of mammalian α-glucosidase was caused by chrysophanol-8-O-ß-d-glucopyranoside (Babu et al. 2004). In another experiment performed by Radhika et al. (2010), different enzymes (hexokinase, phosphoglucoisomerase, aldolase, glucose 6-phosphatase, and fructose-1,6-bisphosphatase) were analyzed in alloxan monohydrate-induced (150 mg/kg, i.p.) diabetic albino rats, in which the ethanolic extracts of the *Rheum australe* rhizome (250 mg/kg body weight) enhance the peripheral utilization of glucose, thereby possessing antidiabetic properties (Radhika et al. 2010). Li et al. (1997) also reported the hypoglycemic activity of ethanol extracts and the compound stilbene glycoside E obtained from *Rheum australe* rhizomes (Li et al. 1997; Rokaya et al. 2012a). Thus, due to the presence of such potent molecules, *Rheum australe* have possible implication for use in prevention and treatment of hyperglycemia-associated diabetes mellitus (Nazir et al. 2013; Pandith et al. 2018; Radhika et al. 2010).

Hepatoprotective Activity

The extract from the rhizomes of *Rheum australe* has shown significant hepatoprotective activity against CCl4-induced liver injury both in vitro and in vivo using 50 mg/kg, p.o. (peroral) dose of silymarin as a standard (Nazir et al. 2013). The ethanolic extract of *Rheum australe* could prevent and treat hyperlipidemia and fatty liver in rabbits via reducing blood lipids. It was found that the administration of rhubarb extracts to rabbits could decrease total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) in serum, increase high-density lipoprotein cholesterol (HDL-C) in serum, reduce liver fatty degeneration, and protect liver cell function (Qing-xia et al. 2013). The hepatoprotective effect of aqueous extract of *R. australe* against paracetamol-induced liver damage in albino rats also confirms the hepatoprotective activity of *Rheum australe* (Malik et al. 2016; Rokaya et al. 2012a).

Nephroprotective Activity

The renal effects of water-soluble (W-S) and water-insoluble (W-INS) portions of the alcoholic extract of Rheum australe rhizome against cadmium chloride-, mercuric chloride-, potassium dichromate-, and gentamicin-induced nephrotoxicity in rats by monitoring the levels of urea nitrogen and creatinine in serum. W-S fraction has a nephroprotective effect on all the proximal tubule segments (S1, S2, and S3) of the kidney possibly through antioxidant action of the tannins present in the fraction (Malik et al. 2016), whereas W-INS fraction has a nephroprotective effect on S2 segment only when the nephrotoxicity was induced by cadmium chloride and mercuric chloride (Alam et al. 2005; Malik et al. 2016; Rokaya et al. 2012a).

7.4 Conclusion

This chapter comprehensively validates the broad-spectrum information about the pharmacology, traditional, and nutraceutical application along with the scientifically claimed medicinal uses of few Himalayan herbs and its bioactive constituents. These are the native plants of the Himalayas which are being used by the native people since the millennia. Traditionally, these plants are being used for the treatment of asthma, bronchitis, leprosy, tuberculosis, wounds, ulcers, inflammation, diarrhea, and dysentery, burning sensation, giddiness, stomach problems, cuts, burns, chronic constipation, menstrual problems, and diabetes. These plants can have potential application in pharmaceutical industry exploring the full therapeutic potential of various parts of the plant in order to establish it as a standard drug which can be used to cure various diseases signifying its nutritional as well as pharmaceutical importance.

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Chapter 8
Phytochemistry, Pharmacology and Toxicity of Medicinal Plants

Lubna Tariq, Basharat Ahmad Bhat, Syed Suhail Hamdani, and Rakeeb Ahmad Mir

8.1 Introduction

Medicinal plants are known as the primary source of nourishment and natural metabolites for sound health maintenance (Mazzei et al. 2018). These are also called natural substances with a large number of pharmacological activities (Mishra et al. 2018b; Salehi et al. 2020) that are indispensable. After its invention, medicinal plants and herbal medicines have been used to treat different forms of acute and chronic diseases (Mishra et al. 2018a, b; Oladeji et al. 2020; Salehi et al. 2018b). Phytochemicals are bioactive, naturally present chemical compounds found in plants that provide health benefits for humans beyond those related to macronutrients and micronutrients (Hasler and Blumberg 1999; Septembre-Malaterre et al. 2018). These are the compounds which are responsible for the plant's flavor, aroma, and color and also contribute to the protection of plant from diseases and damage. Generally, these chemicals protect cells of plants from various environmental factors like drought, pollution, stress, pathogenic attack, and UV exposure (Gibson et al. 1998; Mathai 2000). More than 4000 phytochemicals have been characterized based on their physical and chemical characteristics and protective functions (Chae 2016; Meagher and Thomson 1999). Accumulation of phytochemicals occurs in different parts of plants such as stem, leaves, roots, fruits, flowers, or seeds (Chanda and Ramachandra 2019; Costa et al. 1999). Dietary phytochemicals in plants are found within the legumes, nuts, vegetables, seeds, fruits, herbs, spices, and fungi (Mathai 2000; Nahar et al. 2019). Some common sources include tomatoes, garlic, carrots, broccoli, grapes, cabbage, cherries, raspberries, strawberries, legumes, soy

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food, and whole wheat bread (Moorachian 2000). Phytochemicals are known as secondary metabolites as they have many bioactive properties like antimicrobial effect, antioxidant activity, immune system stimulation, hormone metabolism modulation, and anticancer property, a decrease of platelet aggregation, and modulation of detoxification enzymes. It is well-known that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Rao 2003), such as fatty acids, saponins, tannins, alkaloids, cardiac glycosides, and terpenoids (Iboroma et al. 2018; Melnyk et al. 2018; Shah et al. 2014). Medicinal plants have their unique characteristic properties against various diseases. Some of the phytochemicals in medicinal plants and their roles are illustrated in Table 8.1.

The physiologic properties of relatively few phytochemicals are well understood, and a lot of medicinal plants are under investigation to unravel their possible role in preventing or treating cancer and heart disease (Mathai 2000; Nahar et al. 2019). Phytochemicals have also been promoted for the prevention and treatment of diabetes, high blood pressure, and muscular degeneration (Chae 2016).

Table 8.1 Bioactive phytochemicals in medicinal plants

S. No	Class	Pharmacological activities	Characteristics	Uses	References
1.	Phenols	Antimicrobial, antiseptic, antitumor, anti-inflammatory,	Hydroxyl group directly attached to an aromatic ring, weakly acidic	Disinfection	Okwu (2005); Urquiaga and Leighton (2000)
2.	Tannins	Soothing relief, regenerates skin, anti-inflammatory, diuretics	Unpleasant taste, tans leather	In the production of leather and ink; in treating varicose ulcers, wounds, burns, and hemorrhoids, frostbite	Okwu and Okwu (2004)
3.	Flavonoids	Antioxidant, anti-carcinogens, antimicrobial, antitumor	Water-soluble, super antioxidant, and free radical scavenger	In prevention of oxidative cell damage, allergies, free radicals, microbes	Kandaswami et al. (1991); Manikandan et al. (2006)
4.	Alkaloids	Analgesic, anti-spasmodic, bactericidal effect	Bitter taste, colorless, nitrogen-containing bases, crystalline or liquid at room temperature	Raw material for the synthesis of useful drugs	Harbon (1998); Okwu and Okwu (2004); Stary (1991)
5.	Saponins	Expectorant, cough suppressant, hemolytic activity	Bitter taste, foaming property, hemolytic effect on red blood cells	Emulsifying agent	Okwu (2005); Oludare and Bamidele (2015)

8.2 Types of Phytochemicals

Depending upon the role in the metabolism of plants, phytochemicals are classified as primary or secondary. Common sugars, proteins, amino acids, pyrimidines and purines of nucleic acids, chlorophylls, etc. are classified under primary constituents, while the remaining chemical compounds like flavonoids, alkaloids, terpenes, plant steroids, lignans, saponins, phenolics, curcumin, and glucosides are classified as secondary constituents (Hahn 1998) (Table 8.2).

8.2.1 Phenolics

These are the largest category of a hydroxyl group (-OH) containing a class of phytochemicals that are ubiquitously distributed in plants (Walton et al. 2003). The three important dietary phenolics include phenolic acids, flavonoids, and polyphenols. The simplest natural class of this group is phenol (C_6H_5OH), whose hydroxyl group (-OH) is directly bonded to an aromatic hydrocarbon group.

Table 8.2 Common medicinal plants, part used, pharmacological action, and disease cured

S. No.	Plant	Part used	Action	Disease cured	References
01	*Zingiber officinale*	Rhizome	Carminative, anthelmintic emollient Laxative, expectorant	Anorexia, asthma, inflammations, vomiting flatulence, cough, nausea	Sofowora (1993)
02	*Carica papaya L.*	Fruits, leaves, latex	Appetizer, antifungal, anthelmintic, febrifuge, anti-inflammatory	Anorexia, fever, skin diseases intestinal worms, inflammations	Gill (1992)
03	*Allium sativum L.*	Bulbs	Expectorant, anti-inflammatory, antibacterial	Asthma, cough, bronchitis, nasal instillation	Gill (1992)
04	*Azadirachta indica*	Seed, bark, flowers, leaves	Anthelmintic, astringent, antiseptic, febrifuge, insecticidal, expectorant	Intestinal worms, skin diseases, malarial fever	Ade-Serrano (1982); Iwu (1993)
05	*Bridelia ferruginea Benth*	Root, stem, bark, leaves	Diuretic and hypoglycemic activity	Feverish pains, diabetes mellitus	Iwu (1993)
06	*Cymbopogon citratus*	Whole plant	Antiseptic, stomachic insecticide against mosquitoes, antifebrile, or as a deodorant	Vomiting, cold, eczema, fever	Gill (1992); Sofowora (1993)

Numerous beneficial properties to humans are exhibited by these phenolic compounds, and its antioxidant property plays an important role in the protection against free radicals like ROS. These compounds are important for the plants' color, growth, and reproduction and also act like defense mechanisms against parasites, predators, and pathogens (Báidez et al. 2007). Studies have shown that phenolic compounds exhibit potent antioxidant, anti-carcinogenic/anti-mutagenic, antibacterial, anti-atherosclerotic, anti-inflammatory, and antiviral activities to a lesser or greater extent (Han et al. 2007; Owen et al. 2000; Veeriah et al. 2006) (21–24), e.g., apples possess certain phenolic compounds which are linked to inhibiting the in vitro colon cancer (Veeriah et al. 2006). Several tested medicinal herbs and dietary plants have reported hundreds of natural phenolic compounds, especially phenolic acids, stilbenes, quinines, flavonoids, tannins, coumarins, curcuminoids, lignans, and other phenolic mixtures.

8.2.2 Phenolic Acids

Phenolic acids are a significant class of phenolic compounds that occur widely in the plant kingdom (Cai et al. 2004). Predominant phenolic acids include hydroxyl benzoic acids (e.g., protocatechuic acid, gallic acid, vanillic acid, syringic acid, and p-hydroxybenzoic acid) and hydroxycinnamic acids (e.g., p-coumaric acid, ferulic acid, chlorogenic acid, caffeic acid, and sinapic acid) (Cai et al. 2006). In medicinal herbs, gallic acid is widely present such as in *Cassia auriculata*, *Barringtonia racemosa*, *Cornus officinalis*, *Punica granatum*, *Polygonum aviculare*, *Rheum officinale*, *Rhus chinensis*, *Terminalia chebula*, and *Sanguisorba officinalis* as well as in dietary spices, for example, clove and thyme (Cai et al. 2003, 2006; Shan et al. 2005; Surveswaran et al. 2007). Hydroxybenzoic acids are also widely distributed in dietary plants and medicinal herbs. Plants like *Feronia elephantum*, *Paeonia lactiflora*, and *Dolichos biflorus* contain hydroxybenzoic acid; vanillic acid is found in plants like *Picrorhiza scrophulariiflora*, *Foeniculum vulgare*, and *Ipomoea turpethum*, while sugarcane straw and *Ceratostigma willmottianum* have syringic acid (Sampietro and Vattuone 2006; Stagos et al. 2006). The water-soluble salvianolic acid B is the major phenolic acid extracted from *Radix salviae miltiorrhizae*. This has been used for thousand years in China as a common clinically herbal medicine in terms of antioxidant agent. Phenolic acids play a key role in the reduction of lipid levels and blood cholesterol, in enhancing bile secretion, and in antimicrobial activity against strains of some bacteria like *Staphylococcus aureus* (Gryglewski et al. 1987). Phenolic acids also possess some diverse bioactivities such as anti-inflammatory, anti-ulcer, anti-spasmodic, anti-depressant, cytotoxic, and antitumor (Ghasemzadeh et al. 2010).

8.2.3 Flavonoids

Flavonoids are a class of compounds extensively present in nature. Concerns over their substantial profitable biologically active benefits, including antiviral/antibacterial, cardioprotective, anti-inflammatory, antidiabetic, anti-aging, and anticancer, have been received for a long time and have been well supported by various research findings (Krych and Gebicka 2013; Ragab et al. 2014; Tian et al. 2014). These compounds usually have the basic skeleton of the structure of phenyl benzopyrone (C6-C3-C6) composed of two aromatic rings (A and B rings) bound by three carbons normally in an oxygenated central pyran ring, or C ring (Cai et al. 2004). These polyphenolic compounds are widely distributed in nature. Flavonoids have been reported to play a major role in effective ancient medical therapies, and their use has continued to this day. In vascular plants, flavonoids are ubiquitous and exist as glucosides, glycones, and methylated derivatives. Recent attention has been paid to flavonoids due to their diverse pharmacological and biological activity. Flavonoid compounds have been reported to exercise several biological properties including anti-microbial, anti-inflammatory, cytotoxic, and antitumor activity, but the best described property of almost any group of flavonoids is their ability to function as strong antioxidants. Various forms of flavonoids are present in almost all dietary plants, such as fruits and vegetables, and previous reports indicate that flavonoids are the largest class of phenolics in the herbs and dietary spices tested (Cai et al. 2004; Huang and Xing 2007; Shah et al. 2014; Surveswaran et al. 2007). Different categories of flavonoids from medicinal herbs and dietary plants include hesperetin, naringenin, taxifolin, quercetin, luteolin, baicalein, apigenin, kaempferol, chrysin, catechin, myricetin, taxifolin, morin, galangin, glycitein, eriodictyol, catechin, epigallocatechin, silymarin, and epicatechin. Glycosides such as vitexin, apigetrin, and baicalin are mainly found in Labiatae, Asteraceae, inflorescences of *Chrysanthemum morifolium*, aerial parts of *Artemisia annua*, and roots of *Scutellaria baicalensis.* Flavonoids constitute a wide range of substances that play an important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids, and DNA (Atmani et al. 2009).

8.2.4 Tannins

Tannins are a high molecular weight heterogeneous group of polyphenolic compounds. They have capacity to develop reversible and irreversible complexes with mostly proteins, alkaloids, minerals, polysaccharides (cellulose, pectin, hemicellulose, etc.), nucleic acids, etc. (Licitra et al. 1996; Mueller-Harvey and McAllan 1992; Schofield et al. 2001). These compounds are classified into two classes: condensed tannins (proanthocyanidins) and hydrolysable tannins (gallo- and ellagitannins. Among culinary plants and medicinal herbs, tannins are a wide class of polyphenolics. Oligomeric proanthocyanidins, widely found in pine bark and skin and grape seed, are deemed to be the most effective antioxidants and are commonly used in the health care and treatment of cancer (Huh et al. 2004). Among 126 medicinal herbs in India, 10 have high levels of hydrolyzable tannins (Surveswaran et al. 2007). In *Euphorbia hirta*, *Rhus succedanea*, and *Glycyrrhiza glabra*, some gallotannins were found. Several ellagitannins such as corilagin and casuarictin have been extracted from fruits like *Chebula* and *P. granatum* peels. *Areca catechu* and *Camellia sinensis* also contain tannins named leucoanthocyanidins and proanthocyanidins, respectively. Many plant species synthesize both condensed and hydrolyzable tannins, e.g., *S. officinalis*, *P. granatum*, *Acacia catechu*, *Rosa chinensis*, etc. (Cai et al. 2004; Schofield et al. 2001; Surveswaran et al. 2007). The tannin-containing plant extracts are being used as astringents against diuretics, diarrhea, duodenal, and stomach tumors (De Bruyne et al. 1999), as well as antioxidant, anti-inflammatory, homeostatic, and antiseptic drugs in Asia (Japan and China) (Dolara et al. 2005).

8.2.5 Alkaloids

Alkaloid term is derived from the word "alkaline," and these are thus natural products with heterocyclic nitrogen atoms. The nature of the alkaloids is basic and is naturally synthesized in plants, animals, fungi, and bacteria. These are often optically active but are usually colorless and bitter. Quinine, one among the alkaloids, is the bitterest substance (Mishra 1989). Alkaloids feature one of the most effective and important therapeutic plant substances (Okwu 2005). Alkaloids are important for plant protection and survival because they ensure their safety against insects, microorganisms via antibacterial and antifungal activity, and herbivores (feeding deterrents), as well as other plants by means of allelopathically active chemicals (Molyneux et al. 1996). Some examples of alkaloids are morphine, nicotine, codeine (*Papaver somniferum*), reserpine (*Rauwolfia vomitoria*), cocaine, and quinine (*Cinchona succirubra*). The presence of alkaloids within plants gives plant some specific characteristics with respect to their medicinal values such as therapeutic potential including antiarrhythmic effects (quinidine, spareien), antihypertensive effects (indole alkaloids), anticancer action (dimeric vincristine, indoles, vinblastine), and anti-malarial activity (quinine). These are just a few examples which illustrate the tremendous importance of this plant constituent group (Wink et al. 1998). Synthesis of alkaloids is the characteristic of all organs of a plant. Pure, isolated plant alkaloids and related synthetic derivatives are used as essential therapeutic agents for their anti-spasmodic, analgesic, and bactericidal effects (Stary 1991). When administered to animals, they demonstrate marked physiological activity (Okwu and Okwu 2004). Besides, alkaloids are also poisonous to humans, and others have drastic physiological processes, which is why they are commonly used in medicine to produce medicines. Quinine extracted from the cinchona tree bark (*C. succirubra*) is exceedingly valuable for the treatment of malaria (Rao et al. 1978).

8.2.6 Saponins

Saponins are glycosides of both triterpenes and steroids distinguished by their bitter or astringent flavor, foaming properties, hemolytic effects on red blood cells, and binding properties to cholesterol (Okwu 2005). Saponins have been shown to have beneficial (lowering cholesterol) and deleterious (cytotoxic and intestinal epithelium permeabilization) effects and to exhibit biological activity dependent on structure. It is also used in medicine as an expectorant and an emulsifying agent (Harbon 1998).

8.3 Pharmacological Activities

According to the World Health Organization, over 80% of the world's population, or 4.3 billion people, rely upon traditional plant-based systems of medicine to provide them with primary health care (Bharti et al. 2018). Medicinal plants play a vital role in the development of new drugs. Many plant-based new drugs, including deserpidine, lectinan, plaunotol, Z-guggulsterone, nabilone, rescinnamine, E-guggulsterone, reserpine, teniposide, vinblastine, etoposide, vincristine, ginkgolides, and artemisinin that are extracted from higher plants, were introduced in the US drug market between 1950 and 1990. From 1991 to 1995, further 2% of the new drugs were introduced such as irinotecan, topotecan, paciltaxel, gomishin, etc. Several pharmacological activities including the treatment of cancer, immunemodulation, nervous system activation, antipyretic, analgesic, hepatoprotection, antidiabetic nature, etc. have been possessed by plants and their products (González-Stuart et al. 2014). Scientists have even started correlating phytochemical constituents of a plant with its pharmacological activity as well as the botanical properties of plants with their pharmacological activity (Heinrich et al. 2020; Nahar et al. 2019). Medicinal plants are being used as treatments for human diseases, as they contain pharmaceutical benefit components. The issue of microbial resistance is growing, and the prospects for potential use of antimicrobial drugs are still unclear (Lewis 2017). Therefore, plants have been a reliable source of natural products for the preservation of human health for a long time, with increasingly extensive natural therapy studies. Studies have revealed methanol extracts from bark and roots from several plants like *Sida cordifolia*, *Acacia nilotica*, *Tinospora cordifolia*, *Ziziphus mauritiana*, and *Withania somnifera* possess substantial antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Xanthomonas axonopodis*, and *Pseudomonas fluorescens* and antifungal activity against *Drechslera turcica*, *Fusarium verticillioides* (Harit et al. 2013), and *Aspergillus flavus*. Among these leaf extracts, the highest antibacterial activity against *B. subtilis* is shown by *S. cordifolia* and *A. nilotica*. Bark and leaf extracts of *A. nilotica* show important antifungal activity against *Z. mauritiana* and *A. flavus*, while *T. cordifolia* posses significant antifungal activity against *D. turcica*.

Studies done on aqueous and ethanol extracts of *A. paniculata* for investigating antibacterial activity against nine species of bacteria named *Shigella sonnei*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Escherichia coli*, *Bordetella pertussis*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* showed positive results only for *B. pertussis* and *L. pneumophila* (Xu et al. 2006). *A. paniculata* show anti-diarrheal activity against *E. coli*-associated diarrhea (Gupta et al. 1990, 1993). Methanol extracts from *Murraya koenig* leaf possess antibacterial activity against *E. coli* and *S. typhi* (Singh and Sedha 2018). Aqueous and ethanol extract of *Garcinia kola*, *Xylopia aethiopica*, and *Cajanus cajan* were tested on *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas aeruginosa*. All the results were positive for antibacterial activity, and ethanol extract was found to be more effective (Ezeifeka et al. 2004).

8.3.1 Anticancer

Cancer is the third leading health issue in both developing and developed countries, and one of the world"'s leading causes of death. About 12.5% of the population died due to cancer, according to the WHO in 2004. Chemotherapy, radiotherapy, immunotherapy procedures, and surgery actually induce several adverse effects on non-target cells/tissues. This fuels the need for alternative cancer treatments and therapies (Akindele et al. 2015; Veerakumar et al. 2016). Numerous cancer research studies are going on using traditional medicinal plants in an attempt to discover new therapeutic agents lacking the toxic side effects associated with the current chemotherapeutic agents (Hartwell 1982). Herbal plants/medicines have been shown to cause less side effects in cancer cure over the past decades and have been well-accepted worldwide at (Akerele et al. 1991; Hartwell 1982). Natural active components such as phenol and flavonoids that exist in medicinal plants protect toward harmful effects within biological systems. They were tested for their antitumor, proapoptotic, and anti-angiogenic effects (Carocho and CFR Ferreira 2013; Ghasemzadeh and Ghasemzadeh 2011).

Historically, secondary plant metabolite derives constituents of anticancer such as vinblastine, vincristine, camptothecin, flavopiridol, podophyllotoxin, silvestrol, etc. that are used worldwide (Batra & Sharma, 2013). *Cedrus deodara* contains a standardized lignin AP9-cd that is known to show the cytotoxicity in various cell lines of human cancer. Saponins dasyscyphin C isolated from leaves of *Eclipta prostrata* and gymnemagenol isolated from *Gymnema sylvestre* have shown anticancer activity after being tested on HeLa cells. *Catharanthus roseus*, vinca alkaloids, vincristine, and vinblastine were the first drugs to progress into clinical use for cancer treatment. These are mainly used for treating a range of cancers, including lymphomas, leukemias, breast cancer, advanced testicular cancer, lung cancers, and Kaposi's sarcoma, in conjunction with other cancer chemotherapeutic medications. Besides roscovitine, a synthetic agent derived from olomoucine, a natural product, extracted from *Raphanus sativus*, also shows anticancer activity (Cragg et al. 2011; Meijer and Raymond 2003). Silymarin has also prevented skin carcinogenesis caused by benzoyl peroxide due to its chemoprotective action (Agarwal et al. 1994; Kohno et al. 2002; Lahiri-Chatterjee et al. 1999).

8.3.2 Antidiabetic

Diabetes mellitus (DM) is a severe, acute, and complicated metabolic disorder with various etiologies with significant, both acute and chronic, consequences (Soumya and Srilatha 2011). It is estimated that this disease affects 25 percent of the world population (Arumugam et al. 2013). The quest for newer antidiabetic drugs from the natural source continues (Wadkar et al. 2008) due to the many drawbacks associated with the use of current synthetic antidiabetic drugs. Natural products, especially of plant origin, are the key quarry for the discovery of potential lead candidates and play an indispensable role in future drug development programs (Salehi et al. 2018a; Sharifi-Rad et al. 2018a, b). In addition, many plants provide a rich source of bioactive chemicals free from unwanted side effects and have effective pharmacological activities (Abdolshahi et al. 2018; Mishra et al. 2018a, b; Sharifi-Rad et al. 2018a, b; Singab et al. 2014). Most plants were considered as fundamental source of potent antidiabetic drugs for centuries. Across developing countries in particular, medicinal plants are used to treat diabetes, in order to alleviate the pressure on the population of the cost of traditional medicines (Bahmani et al. 2014). Nowadays it is advised to treat diseases like diabetes using medicinal plants (Kooti et al. 2015), since these plants incorporate different phytoconstituents such as flavonoids, saponins, terpenoids, carotenoids, glycosides, and alkaloids that may have antidiabetic activity (Afrisham et al. 2015). Ríos et al. (Ríos et al. 2015) identified medicinal plants such as bitter melon, aloe, caper, cinnamon, banaba, cocoa, fenugreek, coffee, garlic, gymnema, guava, nettle, soybean, green and black tea, sage, turmeric, yerba mate, and walnut used as antidiabetic agents for the treatment of diabetes and its comorbidity. The mechanisms of natural products, with attention to compounds of high interest such as *Artemisia* with 13 species, fukugetin, *Ficus* with 18 species, *Terminalia* with 11 species, *Euphorbia* with 10 species, and Solanum with 12 species, are some of the genera with a large number of antidiabetic species. Studies have shown that in rabbits hyperglycemia caused via oral administration of glucose was significantly cured by water extracts of *A. paniculata* (Borhanuddin et al. 1994). Pterostilbene and marsupin (important phenolic constituents of the *Pterocarpus marsupium* heartwood) significantly decreased blood glucose levels in streptozotocin-induced diabetic rats, and the results were comparable to metformin (Rizvi et al. 1995). Piperine is a naturally occurring alkaloid in fruit of *Piper* species. With metformin, it has bioenhancing effects in reducing blood glucose levels (Atal et al. 2016; Rizvi et al. 1995). *Camellia sinensis* is also known for significantly reducing the blood glucose level in streptozotocin-induced diabetic rats (Al-Attar and Zari 2010). *Phyllanthus amarus* is also found to have hypoglycemic effects (Adedapo and Ofuegbe 2014).

8.3.3 Antipyretic Activity

Medicinal plants possess this pharmacological activity in a number of plants. Plants used as an antipyretic agent help by lowering body temperature from an elevated state to prevent or reduce fever. An antipyretic is a form of medication that prevents or decreases fever by rising body temperature from an elevated state. Antipyretic activity of polyherbal formulation consisting of *Adhathoda vasica*, *Moringa oleifera*, and *Andrographis paniculata* named JU-RU-01 was assessed in Wistar rats having Brewer's yeast-induced pyrexia (Chandra et al. 2010). In rat models the methanol extract of *Rhynchosia cana* and *Nelumbo nucifera* also exhibited antipyretic influence (Mukherjee et al. 1996; Vimala et al. 1997). Chloroform and alcoholic extracts of the leaves of *Hygrophila spinosa* demonstrated potent antipyretic and anti-inflammatory activity in a dose-dependent manner (Patra et al. 2009).

8.3.4 Anti-Allergic Activity

The allergy refers to our immune systems' overreaction in response to body interaction with the kind of foreign substances. This is exacerbated since the body typically treats these foreign substances as harmless and no reaction occurs in non-allergic individuals. Together with prescription medications, herbal medicines are also in demand because of their reduced side effects as anti-allergens. *Vitex negundo* ethanol extract has been found to inhibit the immunologically mediated degranulation of mast cells effectively than that of the 40/80 compounds (Nair et al. 1995). Alcoholic extract of the *Andrographis paniculata* and *Nyctanthes arbor-tristis* plants, hexane-soluble extract of the *Cedrus deodara* wood, and aqueous extract of the *Albizia lebbeck* bark were found to have important anti-allergic activity when examined in rats of experimental anaphylaxis and mast cell degranulation models (Al Rashid et al. 2019).

8.3.5 Antioxidant

Oxygen is a highly reactive atom that can become a part of potentially destructive molecules commonly referred to as free radicals such as reactive oxygen species (ROS). We significantly exceed the ability of the endogenous cellular antioxidant defense system when ROS is present at certain levels and thus case oxidative stress. The resulting damage to the cells and organs will cause disease processes and/or accelerate them. Several degenerative conditions such as coronary heart disease, atherosclerosis, cancer, and aging involve oxidative stress (Finkel and Holbrook 2000; Song et al. 2010; Valko et al. 2007). The free radicals will invade the body's healthy cells, causing them to lose their function and structure. Antioxidants are able to regulate or deactivate free radicals before damaging cells. Antioxidants are necessary to maintain optimum cellular and systemic health (Halliwell 1994). Natural antioxidants are not commonly used today, because of their small sources and high price. In the food industry, synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxyltoluene are commonly used. There is, however, the consensus that synthetic antioxidants should be substituted with natural antioxidants because certain synthetic antioxidants have demonstrated health hazards and toxicity, most prominently carcinogenic effects (Ito et al. 1986; Safer and Al-Nughamish 1999). The best outcomes in health and nutrition can be obtained not only through the intake of high antioxidant quality fruits and vegetables but also from medicinal plants and herbs (Jastrzebski et al. 2007). Numerous studies have indicated that some medicinal plants have more powerful antioxidant activity than vegetables and fruits, and phenolic compounds have been a major contributor to these plants' antioxidant activity (Cai et al. 2004; Dragland et al. 2003). Some plants having the highest antioxidant activity are *Eriobotrya japonica*, *Dioscorea bulbifera*, *Ephedra sinica*, and *Tussilago farfara*, while some plants possess higher radical scavenging activity rather than an antioxidant activity, e.g., *Arctium lappa* and *Fritillaria verticillata.* It has been proved by various studies the total phenolic content and antioxidant capacity of a medicinal plant are linearly correlated with each other, but exceptions are also there, e.g., *Perilla frutescens*, displaying relatively high antioxidant potential but not having comparable phenolic concentration (Singleton and Rossi 1965).

8.4 Toxicity of Medicinal Plants

The use of plants for therapeutic purposes is becoming ever more popular as they are thought to be effective and free of side effects. However, the justification for using medicinal plants has largely rested on long-term clinical observation with little or no scientific data about their effectiveness and safety (Zhu et al. 2002). Medicinal herbs are used as a medicine based on the ancient folk use perpetuated over several generations. A thorough scientific investigation of these plants is imperative with the rise in the use of herbal medicines, based on the need to validate their folkloric usage (Abdullahi 2011). Herbs should be safe, but there have been reports of many insecure and fatal side effects (Izzo 2004; Whitton et al. 2003). Phytotherapeutic products are often considered less toxic, mistakenly because they are "natural" (Gesler 1992). However, those products contain biologically active principles that could lead to adverse effects (Bent and Ko 2004). Following exposure to the highly toxic material, an adverse effect is characterized as an irregular, unexpected, or harmful change. The serious effects of toxicity could be allergic reactions, the abnormal weight of organs and body, altered levels, and activities of enzymes (Duffus et al. 2009), while in some cases death could be the most serious effect. Thus, the same methods used for new synthetic drugs must submit all "natural" products used in therapeutics to the efficacy and safety tests (Talalay and Talalay 2001). Toxicology studies are important before determination of any appropriate dosage of drug. Clearly, the lungs are essential for all airborne compounds, while the significant site for absorption is rarely the skin (Deshpande 2002). These are also important in explaining drug toxicity profiles.

Toxicity depends not only upon the substance's dosage but also upon the substance's toxic properties. In the evaluation of therapeutic dose in pharmacology and herbalism, the relationship between these two factors is significant. The critical preclinical necessary information includes a 2-week toxicity testing in sensitive species (usually rodents) plus toxicokinetics which should allow the no-observed level of adverse effect (NOAEL) to be determined. In general, the NOAEL is based on studies of animal toxicity. For setting exposure limits, the NOAEL is essential. The acceptable daily intake (ADI), for example, is based on the NOAEL. This is a measure used to assess the appropriate intake for food additives and pollutants such as pesticides and veterinary drug residues and thus to assess the level of protection in food (Chae 2016; Renwick 1990). Different toxicity levels which are checked are mentioned as under:

8.4.1 Acute Toxicity

Acute toxicity is characterized as the toxic effects produced by single drug exposure by any route over a short period. Animal acute toxicity trials are deemed appropriate for any medication intended for human use. The main aim of acute toxicity studies is to identify a single dose that causes major adverse effects or life-threatening toxicity, often involving an estimate of the minimum dose that causes lethality. This is the only type of study in the pharmaceutical drug development where lethality or life-threatening toxicity is an endpoint as documented in current regulatory guidelines (Attah et al. 2019a; b). Different routes may be used to assess the toxicity of a compound in animals, but two most commonly used modes of animal studies are through intraperitoneal infusion or oral route (Poole and Leslie 1989). The acute study offers guidelines for the selection of doses for the sub-acute and chronic low-dose study, which might be more clinically important (Hasumura et al. 2004; Janbaz et al. 2002).

8.4.2 Sub-Acute Toxicity

Repeated doses of the drugs are given in sublethal amounts for duration of 14-21 days in sub-acute toxicity studies. Sub-acute studies of toxicity are being used to evaluate the effect of the drug on blood biochemical and hematological parameters and to determine histopathological changes (Baki et al. 2007).

8.4.3 Chronic Toxicity

For chronic toxicity research, to evaluate the carcinogenic and mutagenic potential of drug, medication is given at various doses over duration of 90 days to over a year. The criteria used in chronic toxicity studies are the same as those used in sub-acute studies. Multiple-dose trials are required to ensure natural products are safe. Clinical observations of acute assays, on the other hand, are valuable tools for defining the doses to be tested in multiple-dose experiments, together with pharmacological studies in humans and animals (Alvarez et al. 2004; Tagliati et al. 2008).

8.5 Importance of Different Parameters in Toxicity Studies

A summary of ethno-medicinal uses, toxicity study, and its safety level of different medicinal plants is given in Table 8.3.

8.5.1 Gross Behavior Assessment

The assessment of gross behavior generally in mice can be evaluated using the Morpurgo model (Morpurgo 1971). CNS depression (hypoactivity, exitus, passivity, ataxia, relaxation narcosis, ptosis), ANS effect (hyperactivity, exophthalmia, stereotypy, irritability), and CNS stimulus parameters (Straub tail, analgesia, convulsions tremors) and other parameters are specific parameters measured for gross behavior studies.

8.5.2 Body Weight

Changes in body weight are markers of adverse side effects, since surviving animals cannot lose more than 10% of the initial body weight (Teo et al. 2003). The hematopoietic system is one of the most responsive targets for toxic compounds and an important index of human and animal physiological and pathological status (Mukinda and Syce 2007). The different hematological parameters investigated in this study are valuable indices for evaluating plant extract toxicity in animals (Yakubu et al. 2008).

Table 8.3 List of medicinal plants, their family, ethno-medicinal uses, toxicity study, and its safety level

S. No.	Plant (family)	Pharmacological activity	Toxicity study	Result	References
1.	*Acacia karroo* Hayne (Fabaceae)	Gum is an important food source	Acute, sub-acute	Toxic	Adedapo et al. (2008)
2.	*Acmela brasiliensis* DC (Asteraceae)	Respiratory infections and pain	Acute, sub-acute	Less toxic	Burger et al. (2005)
3.	*Aconitum napellus* Linn. (Ranunculaceae)	Pain, coldness, vertigo, and general fatigue	Chronic	Safe	Wada et al. (2006)
4.	*Aframomum melegueta (Roscoe)* K. Schum. (Zingiberaceae)	Stomachache, diarrhea, and snakebite	Sub-chronic	Toxic (liver)	Ilic et al. (2010)
5.	*Artemisia afra (Jacq. Ex. Willd)* (Asteraceae)	Cough, colds, sore throat, heart burns, hemorrhoids, fever, malaria, asthma, and diabetes mellitus	Acute, chronic	Safe	Mukinda and Syce (2007)
6.	*Asparagus pubescens* Bak (Liliaceae)	Used as remedy for liver and kidney disorders	Acute	Safe	Nwafor et al. (2004); Taziebou et al. (2007)
7.	*Camellia sinensis (L.) Kuntze* (Theaceae)	Antioxidant, anti-allergic, antiangiogenic, anti-inflammatory, and hypolipidemic	Sub chronic	Safe up to 1.25%	Takami et al. (2008)
8.	*Calendula officinalis* L. (Asteraceae)	Anti-inflammatory, wound healing and antiviral	Acute, sub-acute	Safe	Silva et al. (2007)
9.	*Carica papaya* L. (Caricaceae)	Anti-fertility	Acute, sub-chronic	Safe	Lohiya et al. (2006)
10.	*Cassytha filiformis* R.Br. (Lauraceae)	Diabetes mellitus, ulcer, and cough	Sub-chronic	Safe	Babayi et al. (2007)
11.	*Centaurium erythraea (L.) Rafn.* (Gentianaceae)	Sedative, antipyretic, asthma, jaundice, intestinal parasitic infestation, rheumatism, wounds and sores, blood pressure, edema, and digestive disorders	Acute, sub-chronic	Safe	Tahraoui et al. (2010)
12.	*Euphorbia hirta* L. (Euphorbiaceae)	Inflammation, respiratory tract, and asthma	Repeated (14days)	Toxic	Adedapo et al. (2005)
13.	*Ficus exasperata (Vahl)* (Moraceae)	Stimulant, ring worm, and chest complications	Repeated (3 days)	Leaves were toxic while stems were safe	Irene and Iheanacho (2007)
14.	*Helicteres isora L.* (Sterculiaceae)	Diabetes mellitus, colic, gastropathy, diarrhea, and dysentery	Acute, repeated (28 days)	Safe	Kumar et al. (2007)

(continued)

Table 8.3 (continued)

S. No.	Plant (family)	Pharmacological activity	Toxicity study	Result	References
15.	*Ipomoea batatas* L. (Convolvulaceae)	Isolated compound ipomeamarone	Acute	Toxic for liver	Pandey (2008)
16.	*Salvia scutellarioides* Kunth (Lamiaceae)	Antihypertensive and diuretic properties	Acute, sub-acute	Safe	Ramírez et al. (2007)
17.	*Sida cordifolia* *L.* (Malvaceae)	Stomatitis of asthma and nasal congestion	Acute	Toxic at high dose	Franco et al. (2005)
18.	*Smilax kraussiana* (Liliaceae)	Inflammation	Acute	Safe up to 0.24 g/kg	Assam et al. (2010)
19.	*Polyalthia longifolia (Sonn.) Thw* (Annonacea)	Treatment of fever, skin disease, diabetes, hypertension	Acute	Safe	Chanda et al. (2012)
20.	*Ficus exasperata (Vahl)* (Moraceae)	Chest pain, eye troubles, and stomach pains and to arrest bleeding	Acute	Safe	Bafor and Igbinuwen (2009)
21.	*Garcinia hanburyi* Hook. f. (Guttiferae)	Cytotoxic and anticancer activity	Chronic	Safe	Qi et al. (2008)
22.	*Datura stramonium* L. (Solanaceae)	Asthma, gastric pain, anti-inflammatory, stimulation of central nervous system, and skin infection	Chronic	safe	Gidado et al. (2007)
23.	*Cucurbita maxima* Duch. (Cucurbitaceae)	Stomach pain, anti-inflammatory, and antipyretic	Acute, sub-acute	Safe	Cruz et al. (2006)
24.	*Cassia fistula* L. (Caesalpiniaceae)	Mild, pleasant purgative action, antifungal, antiviral, menstrual disorders, and fever	Acute, sub-chronic	Less toxic	Akanmu et al. (2004)
25.	*Zingiber zerumbet* Smith. (Zingiberaceae)	Anticancer and cytotoxic activity	Acute	Toxic at high dose	Ibrahim et al. (2010)
26.	*Bridelia ferruginea* *Benth* (Euphorbiaceae)	Diabetes	Acute, sub-chronic	Safe	Bakoma et al. (2013)
27.	*Vernonia amygdalina* Del (Compositae)	Antimalaria, anticancer, antimicrobial, as laxative herbs, and anthelmintic	Sub-acute	Safe	St Augustines (2009)
28.	*Tanacetum vulgare* L. (Asteraceae/ Compositae)	Menstrual irregularities, anthelmintic, carminative, antispasmodic, stimulant, and tonic properties	Acute, chronic	Safe	Lahlou et al. (2008)
29.	*Pongamia pinnata* (L.) Merr. (Papilionaceae)	Anticonvulsant, hypotensive effects, bronchitis, chronic fever, whooping cough, and skin diseases	Sub-acute	Safe	Baki et al. (2007)

(continued)

Table 8.3 (continued)

S. No.	Plant (family)	Pharmacological activity	Toxicity study	Result	References
30.	*Pothomorphe umbellata* L. Miq. (Piperaceae)	Liver and inflammation disorders	Acute, sub-chronic	Safe	Barros et al. (2005)

8.5.3 Organ Weight

Changes in organ weight have long been recognized as a sensitive measure of chemically induced changes in tissues, and in toxicological studies, a comparison of organ weights between control and treated groups was historically used to predict the toxic effect of a test substance (Nisha et al. 2009; Pfeiffer 1968). Organ weight is a swelling, atrophy, or hypertrophy index (Amresh et al. 2008). Body weight and internal organs such as the heart, liver, thymus glands kidney, spleen, etc. are toxicity indices that are simple and sensitive after exposure to toxic substance (Teo et al. 2003). Toxicity data are important to predict the safety of medicinal products before use (McNamara 1975).

8.5.4 Serum Biochemical Importance

When ingested, the body interacts with a herbal product in an attempt to get rid of any harmful toxins, especially if the body is unable to convert the foreign substance into cellular components. These insults are usually expressed by changes in enzyme levels and other components of the cells. The commonly involved enzymes are glutamate-oxaloacetate transaminase (AST/GOT), pyruvate transaminase (ALT / GPT), and alkaline phosphatase (ALP). Components such as urea and uric acid are also critical resources for toxicity (Maxwelll et al. 2007).

8.6 Conclusion and Future Prospectus

Medicinal herbs are considered the most basic and effective therapeutic approach since time immemorial and play a major role in the advancement of primary health care. All the pharmacological properties of the plants are mostly associated with the presence of secondary metabolites. According to the WHO, nearly 25% of current pharmaceutical products are derived plants. Many more are synthetic analogs based on plant-isolated prototype compounds. In India, approximately 70% of modern medicines are extracted from natural products. In the coming future, the basic uses of plants in medicine will continue as a source of bioactive and as basic raw material for the food, pharmacology, cosmetics, and perfume industries. It is extremely difficult to design or identify a phytochemical with a high specificity and functionality to act on a biological system. This is largely due to the presence of a large number of phytochemicals with similar chemical structures and physiological reaction complexities. While herbal drugs are considered to be the safest and most harmless therapeutic device, recent adverse effects reported from herbal use have significantly undermined its claims for protection or effectiveness, and also, most herbal plants are not well cited or documented. Herbal drug toxicological testing should promote and explain their validity and health. However, despite the number of phytochemicals isolated so far, nature still has a lot more in reserve. A lot more of these phytochemicals can be detected with the developments in synthetic biology and the development of more advanced isolation and analytical techniques. To further encourage research in this area, the proper use of technical innovations and a clear understanding of the formalized language and of traditional medicine also become a requirement.

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Chapter 9
Amaranthus caudatus L. as a Potential Bioresource for Nutrition and Health

Asiya Ramzan and Reiaz Ul Rehman

9.1 Introduction

The plants satisfy all our basic needs, i.e., food and shelter and clothes, and also our accessory needs like they provide medicines, fiber, fuels, and other valuable products. The backbone of traditional medicine is medicine of plant origin (Das et al. 2010). These medicinal plants possess secondary metabolites which act as phytoprotectants and can cope up many environmental stresses. Besides being useful for plants itself, they have been seen to be useful for humans also as they show strong antioxidant activity and anti-inflammatory effects and have capacity to work against bacteria and also virus (Kazemi et al. 2018). This antibacterial activity is due to the presence of extracts containing chemicals (Marcus et al. 2019). The secondary metabolites present in plants like flavonoids, terpenoids, tannins, and phlobatanins owe them antimicrobial activity (Gaur et al. 2018). The continuous exposure of these organisms to pollution, smoke, drugs, tobacco, chemicals, and UV radiations lead to increased ROS levels. Thus like plants, animals are also subjected to the oxidative stress as a result of imbalance created between prooxidants and antioxidants. This stress is the main contributor to cardiovascular disease, hypertension, diabetes mellitus, aging, neurodegenerative diseases, and Alzheimer's and Parkinson's diseases in humans.

In order to maintain their health, humans might combat this stress to maintain the redox homeostasis by consuming plant products as different components present in these diets prevent the body from various diseases. Ascorbic acid, the water-soluble vitamin, detoxifies harmful reactions and has many beneficial effects on the humans as it has the capability of preventing cancer and cardiovascular diseases (Dragsted et al. 1993). Ascorbic acid has strong antioxidant activity (Aqil

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et al. 2006). Tocopherols are methylated, fat-soluble phenols that prevent the membranes from free radicals and also maintain the activity of vitamin A. Tannins possess strong lipid peroxidation (LPO) inhibition activity. Plants that possess tannins have medicinal importance as they act as astringents and are used to cure intestinal ailments like diarrhea and dysentery (Dharmananda 2003), intestinal bleeding and heavy menstrual flow (He et al. 2003), and ulcerated tissue and reduce inflammation (Akinpelu and Onakoya 2006). Carotenoids have strong antioxidant activity and decrease the possibility of cataract, heart diseases, and certain cancers. It has been reported that these carotenoids protect from sunburns, photo allergy, and also skin cancers (Lee et al. 2000). Squalene is a tri-terpene that has antioxidant, anticancer, hypocholesterolic, and antitumor activity and also bad cholesterol-reducing activity (Newmark 1999; Rao et al. 1998; Owen et al. 2000; Shin et al. 2004).

Betalain pigments are of great importance as they are restricted to few plant species especially the order Caryophyllales and are gaining attention of researchers because they are used as food coloring agents and have antioxidant and radical quenching properties in response to oxidative stress-related disorders, anticancer, antiviral, and antiparasitic properties (Cai et al. 2005). The sterols have the capability of reducing blood serum cholesterol level. These secondary metabolites terminate the chain reactions caused due to lipid peroxidation, thus protecting the humans from reactive species such as radicals, epoxides, and peroxides that would otherwise cause harm to DNA, RNA, proteins, and membranes of the cell and even cell organization. The phenols inhibit the oxidative enzymes such as lipoxygenases and cyclooxygenases involved in inflammatory reactions (Sadik et al. 2003; OLeary et al. 2004). The antioxidant activity of phenols is due to the hydrogen donating capability (Frankel and Meyer 2000). The radical scavenging activity of flavonoids is due to the ortho-dihydroxy structures in ring B and 2, 3-double bond in conjugation with the 4-oxo function in ring C and due to the presence of hydroxyl groups in unsaturated ring C (Heijnen et al. 2002; Cai et al. 2006). The plants thus offer a wide array of natural compounds which offer various medicinal properties to humans. Due to the pharmacological properties, these natural compounds attract the researchers to elucidate more information about medicinal plants that would bring about advancements in pharmacology. Thus, it is anticipated that these plants will be used for the treatment of various diseases in man, and in this context, the current review focuses on the important benefits of *Amaranthus caudatus* L.

9.2 Distribution

Although the *Amaranthus* species have proven itself to be food for the future, its cultivation and distribution in the world is little. Even this crop has failed to enlist itself in FAO statistics, and till now amaranth production data is not available; the main countries where *Amaranthus* is cultivated on large scale are Ecuador, Peru, Bolivia, Mexico, Guatemala, and Argentina. In the USA, the Rodale foundation and the Rodale research center funded the projects associated with the amaranth production, its cultivation, etc. Research centers were later opened in the countries like India, Indonesia, Peru, Thailand, and Nigeria for its propagation. In Europe it is cultivated in Austria, Czech, and Slovak Republic, Italy, Spain, and Poland (Valcárcel-Yamani and Caetano da Silva Lannes 2012). In Asia it is cultivated in Bhutan, China, India, Malaysia, Nepal, Pakistan, Papua New Guinea, and Russian Federation. In India the *Amaranthus* have been grouped in two sections (a) Blitopsis species are used as vegetables, and in India *A. tricolor*, *A. polygonoides*, *A. dubius*, and *A. blitum* species are found. (b) Grain species are cultivated for grain purposes, and in India *A. spinosus*, *A. cruentus*, and *A. caudatus* are found. In India, different research institutes especially Tamil Nadu Agricultural University, Coimbatore-3, have developed improved varieties like CO.1*(A. tricolor*), CO.2 (*A. tricolor*), CO.3 (*A. tristis*), and CO.4 (*A. hypochondriacus*). Pusa Lal Chaulai (*A. tricolor*), PusaKirti (*A. blitum*), and PusaKiran (*A. tricolor*) varieties are grown in Andhra Pradesh, Delhi, Gujrat, Haryana, Tamil Nadu, Delhi, Behar, etc. Sirukeerai (*A. polygonoides*) is traditionally grown in Tamil Nadu. *A. tricolor* is most cultivated species in India especially in Tamil Nadu and Kerala. *A. blitum* and *A. tristis* are grown in South India. The grain varieties like SKNA 21(GA-3) in 2008 having yield 12.58q/ha, RMA-4 in 2008 having yield 13.9 q/h, and RMA-7 in 2008 with yield 14.66 have been recommended for areas Gujrat and Jharkand, Rajasthan, Orissa and Jharkhand, and Delhi, Haryana, and Maharashtra, respectively (ICAR National Bureau of Plant Genetic Resources 2013–2014). In Kashmir, some weedy cultivars of amaranthus are used as vegetables. Indians traditionally grow these plants in small plots close to their houses. In Kashmir amaranthus species are not cultivated on large scale. *Amaranthus caudatus* is grown as ornamental plant in kitchen gardens, recreational parks, and Mughal gardens and also in hospital parks (skims), and only weedy species are used as vegetables. The species like *Amaranthus caudatus* and *Amaranthus hybridus* are occasionally grown in hilly areas of Jammu and Kashmir (Dar and Khuroo 2013).

9.3 Food Crisis and Alternatives

Although the total eukaryotic plant species present on earth are 298,000, humans use a limited number of plants for completing their nutritional requirement. Jaramillo and Baena (2002) reported that only 30 crops are used for human diet of which 95% is fulfilled by rice, wheat, and maize. During the past few decades, due to modernization and urbanization and globalization, the number of crop species have been decreased to 150 crops from 10,000 plant species for food and fodder purposes, and it has been estimated that three-fourth of genetic agrobiodiversity has been lost during the past 50 years, and this loss is continuously occurring (EC 2007). Out of these 150 crops, 12 crops fulfill 80% of energy through diet and provide 60%proteins. Out of these 12, the main species are rice, wheat, maize, and potato (FAO 2005). During the last few decades, people have become health conscious because of diet-related factors associated with the cause of diseases like celiac disease, obesity, hyperlipidemia, and diabetes; therefore research is being conducted on those foods or food items that not only provide energy but also maintain our health – functional foods (Hernández-Ledesma et al. 2011). These functional foods include pseudocereals – underutilized crops – but have potential of improving health and also have good nutritional values. Pseudocereals as the name indicates are not true cereals as cereals are monocotyledons while as pseudocereals are dicotyledons (Alvarez-Jubete et al. 2010: Schoenlechner et al. 2008). They possess grain-like seeds and have composition and function like that of true cereals. These pseudocereals mainly consist of quinoa, buckwheat, and amaranthus. *Chenopodium quinoa* belongs to family Chenopodiaceae and includes 250 species which constitute both cultivated and weeds (Abugoch 2009; Valencia-Chamorro 2003). Buckwheat originated from Southwest China, belonging to the family Polygonaceae. It is predominately found in China, Japan, and North America but is also found in Tibet, Australia, India, Bhutan, and many other countries (Kreft and Germ 2008).

Amaranthus belongs to family Amaranthaceae, order Caryophyllales, and genus *Amaranthus* (Sauer 1967: Berghofer and Schoenlechner 2002). *Amaranthus* genus comprises 70 species that are categorized into three subgenera (Mosyakin and Robertson 2003). Most of these 70 species are weeds distributed throughout the world, but their cultivation is difficult after soil is disturbed or when seed is exposed to light (Berghofer and Schoenlechner 2002; Mlakar et al. 2009). Depending on their cultivation and utilization by humans, they are categorized into two main groups: vegetable amaranth and grain amaranth. Species like *A. blitum*, *A. viridis*, and *A. tricolor* L. are used as vegetables as their leaves have high nutritive value. The three important species for crop production are *Amaranthus caudatus* (Peru), *Amaranthus hypochondriacus* (Mexico), and *Amaranthus cruentus* (Guatemala) (Bressani 2003; Sauer 1967). Although there are 60 to 70 species with 4000 to 6000 different varieties of amaranth, the most important amaranth species are *A. cruentus*, *A. hypochondriacus*, and *A. caudatus*. *Amaranthus caudatus*, *A. hypochondriacus*, and *A. cruentus* have awaken a great interest during the last few decades as agricultural crops in many parts of the world, because of their very high nutritional value of their seeds and leaves possessing the agronomical potential. The amaranthus especially *Amaranthus caudatus* plant can be also called as multipurpose crops, as its different parts are used for different purposes like leaves used as vegetables, stem, and seeds used as animal feed and dietary fiber for fattened pigs, rabbits, lambs, and broilers (Andrasofszky et al. 1998; Zraly et al. 2006). The amaranth seed oil is used as nutraceutical resource from Ecuadorian flora (Bruni et al. 2002) and whole plant used for ornamental purpose as it contains beautiful, non-fading, attractive inflorescence coloration (Breene 1991; Mlakar et al. 2010). The grains and leaves of amaranth are used as a food for human beings as well as for animals (Martirosyan 2001). Amaranthus leaves are an excellent source of protein (Kadoshnikov et al. 2005) (Table 9.1).

Table 9.1 Comparison of main chemical components present in leaves of amaranth and spinach

Main nutrients	Amaranthus mg/100gm	Spinach mg/100gm
Protein	3.5	3.2
Fat	0.5	0.3
Carbohydrates	6.5	4.3
Ascorbic acid	80	51
Calcium	267	93
Iron	3.9	3.1

9.4 Ethnobotany of *Amaranthus*

The word amaranth is derived from Greek *Amereino* meaning "immortal or non-withering." The Hindi name for amaranth, *Ramdana*, means "God's own grain." Its other name, *Rajgeera*, means "the king's grain." The Pueblo Indians, the Mayans, Aztecs, the Greeks, and Romans believe that this grain has been sent from heaven. It is estimated that more than 80% of population rely on plants bearing grains, but grain production during the last 2–3 decades has decreased per capita (Pimentel and Pimentel 2008). In order to satisfy our basic needs, we should use those forgotten grainy plants which ancient people used to grow since centuries – the plants that can grow easily in almost all the environmental conditions and has high nutritional values and their production value is high. Amaranthus is one of those ancient crops. Amaranth was eaten by hunter-gatherers in both North and South America before the domestication of agriculture (Sauer 1967). It is known since pre-Columbian times. Inca and Aztecs used to grow these plants on large scale. The ancient amaranth was an everlasting plant used by the ancient Greeks for garlands of deity statues and in death rituals (Gunther 1959). It was known by the name The *Huauhtli* (Wimmer 2003). For Aztecs the ethnobotany and history of huauhtli is interesting because of economic, social, and religious importance during Aztec Empire (Sauer 1950, 1967; Early 1992), and about 20,000 tones were sent in annual tribute to Aztec emperor Montezuma from 17 different provinces. Amaranth was interwoven with legend and ritual. On religious days, seeds were crushed and then mixed with human blood or honey, and this material was given a specific shape like snake, bird, mountain, god, etc. This specific shaped recipe was eaten in temples during religious gathering or in houses during family gathering. Human sacrifices for making this recipe shocked spinach conquistadors; they eradicated its use as the crop fields were put to ash and also people associated with the cultivation of these crops were killed (Sauer 1950; Cole 1979).

Its production was now restricted to Americans. Mexicans also used these in their diet and continued to grow them. In Europe species like *Amaranthus caudatus*, *Amaranthus hypochondriacus*, and *Amaranthus cruentus* were cultivated in the sixteenth century after Spaniards conquered the New World (Sauer 1950, 1967) and then it diffused throughout the world; in spite of the efforts of Spanish conquistadors, they could not eradicate this crop permanently. Strabo (1959–1961) stated that these plants were named after village *Amarantus* in Euboea in Ancient Greece. Pliny the Elder used the Latin word *Amarantus* (Pliny The Elder 1951). As per Tournefort (1694), its name came from Alpha private (ἀ)—meaning "not" and *marceo* meaning "to fade," i.e., nonfading. Sprague (1928) stated that both amaranthus and Amarantus were used in the sixteenth and seventeenth centuries. Linnaeus retained the spelling amaranthus (Sprague 1928). In the eighteenth century, amaranthus was a minor grain plant in Europe and Russia, and in the nineteenth century, it reached Asia and Africa. It is still used in Himalayan region of Asia. Down town (1973) reported that *Amaranthus edulis* contains lysine in high concentrations which arose interest among researchers especially in countries like Germany, Austria, Hungary, Italy, and Slovenia (Berghofer and Schoenlechner 2002). Amaranthus is considered to be one of the ten cultivated species in the twenty-first century (Midler 2003). It is also used as coloring agent (Pasko et al. 2011). Plants are used by man for betterment of his life from the existence of this earth. Ethnobotany has its importance as a lot of information has been gathered by the ancient people related to the use of plants as shelter, as food, as clothing, and as medicine (Arshad et al. 2013). Later this traditional knowledge was piled up, and then their properties were tested in the laboratories, research institutions, and big companies. Therefore ethnic knowledge regarding plants is important, and the same plants are used for different purposes in different localities. Therefore traditional knowledge is important. Some traditional uses of plant *Amaranthus caudatus* is given as follows.

South Americans use *Amaranthus* as medicine and as dye. Ethiopians use roots as laxative and seed extracts for treating eye disease and for eradicating tapeworms. Also these plant parts are used in amoebiasis, jaundice, and kidney diseases (Haile et al. 2008; Sushila and Seema 2003). It has also potential to cure diseases like kidney stones, leprosy, fever, and piles (Vanila et al. 2008). Khare reported that it acts as a blood purifier, diuretic, and astringent (Khare 2007). In India *Amaranthus caudatus* has also been traditionally used to work against kidney stones, leprosy, fever, and piles (Vanila et al. 2008). Tea is made from the leaves that lessens lung ailments (Anonymous 1988). Leaves are used as abortifacient in South Africa (Watt 1962; Yusuf et al. 1994). It is sold as an ornamental in Europe and North America. Indians use *Amaranthus caudatus* on sores and also as diuretic (Agong 2006). Seed is used as a food in western Ethiopia, and Ari people in South Omo use stems in their food (Bink and Belay 2006). *Amaranthus caudatus* was traditionally used for the treatment of the eye disease (Yoganarasimhan 2000).

9.5 Antioxidant Activity of *Amaranthus caudatus*

Prakash et al. used various leaf extracts to view the in vitro antioxidant activity, and it was concluded that *Amaranthus caudatus* possesses good carotenoid and ascorbic acid content in leaves (Prakash et al. 2009). Leaves of *Telfairia occidentalis* and *Amaranthus caudatus* were screened for bioactive components like total phenolics, flavonoids, condensed tannins, saponins, and alkaloids (Enujiugha et al. 2014). The results clearly depicted that ethanolic extracts showed good activity in *Amaranthus caudatus* leaves. The flowers, leaves, and seeds of *Amaranthus caudatus* were analyzed, and the most predominant groups were tannins, flavonoids, and phenolic acids. These compounds accredit the plant with the radical scavenging and antioxidant activities (Hyeon-Ju et al. 2015; Olajire and Azeez 2011). The presence of the different compounds in *Amaranthus caudatus* makes it a potential plant species for studying the antioxidant potential.

The flavonoid content in *Amaranthus caudatus* has been shown 28.19–42.84 mg/g (Nyonje et al. 2014) and 69.67 mg/ g (Olajire and Azeez 2011). In another study, the values reported for flavonoid content ranged between 0.38 and 0.83 mg/100 g (Akubugwo et al. 2008).

Our previous studies were carried out to check the in vitro antioxidative potential of *Amaranthus caudatus* grown in Kashmir region at two different altitudinal sites. The ethanolic leaf extracts were prepared, and antioxidant activity was evaluated at three different growing stages, viz., vegetative stage (Stage I), pre-flowering stage (Stage II), and post-flowering stage (Stage III), and at two different altitudinal sites, viz., Site 1, situated at an altitude of 1581amsl, and Site 2, situated at an altitude of 1620 amsl. It was observed that with the increase in the altitude, the total reducing power increased, i.e., total reducing power was high at Site 2 compared to Site 1. After comparing three stages, it was observed that total reducing power was higher in case of Stage I. However, the total reducing power decreased at Stage II and then exhibiting an increase in Stage III. This again indicated that seasonal effect is clearly playing an important role as the less activity of total reducing power was observed in Stage II. The study also showed that seasonal effect clearly played an important role in antioxidant activity. The expression of compounds in plant parts is influenced by the different environmental conditions (Ramzan et al. 2018).

9.6 Nutritional and Medicinal Importance of Amaranthus

Heat and drought diseases and pest resistance have made amaranthus cultivation more interesting (Barba de la et al. 2009). *Amaranthus* spp. has a potential of acting as global resource by providing grains and leafy vegetables (Saunders and Becker 1984). Amaranthus grain can yield 42% protein, which is far better compared to dried, milled fraction of other commonly used grains (Saunders 1985). Amaranthus contain protein more than wheat and maize (Pedersen et al. 1990). The *Amaranthus* spp. can be used for feeding hens and pigs (Pisarikova et al. 2005). Amaranths consist of more essential amino acids as compared to egg protein (Drzewiecki et al. 2003). Amount of crude proteins are higher than spinach (Segura-Nieto et al. 1994). Dans and Lupton (1988) reported that amaranth lowered the serum cholesterol levels and stated that dietary amaranth can lower the blood cholesterol (Dans and Lupton 1988). The oil also contains squalene, precursor of cholesterol, which is used in the cosmetics and as a penetrant and lubricant (Becker 1994). Amaranthus extracts have antidiabetic, anti-hyperlipidemic, and anti-cholesterolemic activity (Sangameswaran and Jayakar 2008; Girija et al. 2011). The consumers buy amaranth or its products as they are gluten-free (Brenner et al. 2000). Amaranthus grain is rich in micro elements like iron (72–174 ppm), calcium (1300–2850 pm), magnesium (2300–3360 ppm), riboflavin (23 mg/100 g flour), and ascorbic acid (4.5 mg/100gm flour) (Becker et al. 1981). Amaranthus can be used in making bread, cakes, cookies, and muffins (Bejosano and Corke 1998; Berghofer and Schoenlechner 2002; Mlakar et al. 2009). Protein content in amaranthus is very high, the protein efficiency ratio is almost equal to that of casein, and it is digestible up to 90% (Venskutonis and Kraujalis 2013), and it can be can changed by extrusion, roasting, and popping (Muyonga et al. 2014). Further high content of unsaturated fatty acids, vitamins, and minerals makes it popular (Mburu et al. 2012).

The earlier reports regarding the phytochemicals present in *Amaranthus caudatus* L. reveal its medicinal importance as leaf extract is used as antinociceptive and antipyretic (Kumar et al. 2010a). The plant has potential to cure amoebiasis, jaundice, kidney stones, leprosy, and piles (Sushila and Seema 2003; Vanila et al. 2008); atherosclerosis (Kabiri et al. 2010); and eye diseases (Yoganarasimhan 2000) and also possesses hepatoprotective, antihelmintic ((Kumar et al. 2010b), and antidepressant activity (Kumar et al. 2015). Ethnobotanists have witnessed the use of *Amaranthus caudatus* as abortifacient (Yusuf et al. 1994); blood purifier, diuretic, astringent, and vermifuge (Khare 2007). Methanolic extracts of three species of amaranthus has shown anti-hyperlipidemic activity (Girija et al. 2011). Andrea et al. reported that the consumption of extruded amaranth (*Amaranthus caudatus* L.) results in cholesterol-lowering activity in case of hypercholesterolemic rabbits (Plate and Areas 2002). Two varieties of *Amaranthus caudatus* L. seeds were assessed for the inhibition of α-amylase and in vitro antioxidative activities, and it was concluded that compounds like phenols, squalene, and α-amylase were present which bestow antidiabetic and lipid-lowering activity to plant seed (Lakshman 2011; Repo et al. 2009).

Further, the amaranth oil has been reported to decrease the amount of cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C) significantly and contribute to an increase in the concentration of poly-unsaturated fatty acids, particularly long-chain omega-3 fatty acids in patients suffering from hypertension and coronary heart diseases (Pogojeva et al. 2006). *Amaranthus caudatus* contains flavonoids, anthocyanins, carotenoids, vitamin C, folic acid, high amount of methionine, lysine, and unsaturated fatty acids (Quershi et al. 1996). Rastrelli et al. reported the presence of saponins, rutin, tannins, essential amino acids vitamins, folic acid, calcium, carotenoids, etc. in *Amaranthus caudatus* leaves. These chemical constituents scavenge free radicals and thus have a strong antioxidant effect (Rastrelli et al. 1998). Changes in polyphenol content have been scrutinized during the different four growth phases in *Amaranthus caudatus*; phase I represented intensive stem growth, phase III was associated with the formation of the flowers and pollination, and phases III and IV represented milky and full ripened seeds (Alena et al. 2015). Phytochemical and antiradical activities were evaluated by Nyonje (Nyonje et al. 2014) in amaranth varieties before and after flowering. Merna and Jeyanthi evaluated the in vitro antioxidant activity of aqueous extracts of *Amaranthus caudatus* leaves and proved that *Amaranthus caudatus* is a promising source of naturally occurring potential antioxidants (Merina and Jeyanthi 2015).

Amaranthus caudatus has been screened for antioxidant potential, and it was concluded that they possess good antioxidant properties (Inglett et al. 2015). Vitamins like β-carotene, ascorbic acid, riboflavin, and α-tocopherol have been reported to be present in *Amaranthus caudatus* (Adewale et al. 2013). Prakash et al. reported the presence of high contents of ascorbic acid, i.e., 38.08/100 mg in fresh leaves of *Amaranthus caudatus* (Prakash et al. 2009). Amaranthus seeds contain α-tocopherol (26.37–34.81 mg/kg), β-tocopherol (33.18–43.86 mg/kg), γ-tocopherol (1.42–1.81 mg/kg), and δ-tocopherol (39.36–48.79 mg/kg) (Bruni et al. 2002). Prakash et al. (2009) used various extracts to view the in vitro antioxidant activity, and it was concluded that *Amaranthus caudatus* possesses good carotenoid and ascorbic acid content in leaves (Prakash et al. 2009). Leaves of *Telfairia occidentalis* and *Amaranthus caudatus* were screened for bioactive components like total phenolics, flavonoids, condensed tannins, saponins, and alkaloids (Enujiugha et al. 2014). The flowers, leaves, and seeds of *Amaranthus caudatus* were analyzed, and the most predominant groups were tannins, flavonoids, and phenolic acids. These compounds accredit the plant with the radical scavenging and antioxidant activities (Hyeonju et al. 2015; Olajire and Azeez 2011). *Amaranthus caudatus* leaves also contain tannins and phlobatanins (Maiyo et al. 2010). Tannin inhibits nitric oxide (NO) production, superoxide generation, and other free radicals during stress conditions (Hyeon-Ju, 2015).

Amaranthus seeds possess good squalene content (Czaplicki et al. 2011). The seeds also contain a high content of sterols like stigmasterol and campesterol. Takasuto et al. reported the presence of phytosterols like sitostanol, stigmastadienol, cycloartenol, stigmasterol, citrostadienol, 24-methylene cholesterol, and δ-5-avenasterol (Takatsuto et al. 1999). Cai et al. (2005) reported the presence of betalains in leaves, seedlings, and inflorescence of *Amaranthus caudatus*, possessing strong antioxidant activity (Cai et al. 2005). Lectin, amaranthin is present in the seeds of amaranthus (Bianco-Colomas and Hugues 1990) and has specificity for the Thomsen-Friedenreich antigen, thus acting as a specific carcinoma marker. Leaves of *Amaranthus caudatus* contain amaranthin 5-o {2-*O*-(beta-D-glucopyranosyluronic acid) beta-D glucopyranoside} and its epimer isoamaranthin that showed strong action against aphids (Dias et al. 2015). Plants contain proteins that protect them from pathogens and are called as pathogenesis-related proteins (Klaus et al. 1997). These proteins are grouped into five families, and the most studied group is chitinase which inhibits the fungal growth by degrading the chitin present in fungal hyphae (Collinge et al. 2019). *Amaranthus caudatus* seeds also contain such two antifungal peptides Ac-AMP and Ac-AMP2 (Broekaert et al. 1992). These peptides also act against gram-positive bacteria. Ac-AMP is

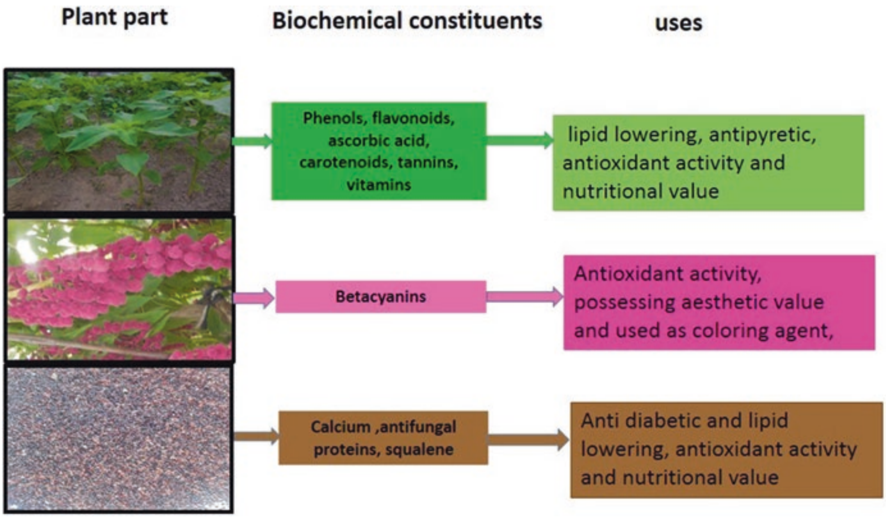

Fig. 9.1 Different plant parts and their biologically active components along with their uses

present in the young seedlings and protects the young plant from the harmful pathogens (Fig. 9.1).

9.7 Future Prospects

Being agronomically important, such crop should be given keen attention especially toward its cultivation, conservation, and sustainable development (Ray and Roy 2008). Teutonico and Knorr stated that the main thing regarding research and development is to modify the nutritional and functional quality so that new products are created in market Teutonico and Knorr (1985). *Amaranthus caudatus* is found in different regions of world including Jammu and Kashmir, but the crop is included among underutilized crops, and there is a need to conserve this germplasm and study more about its nutraceutical and medicinal aspects.

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Chapter 10
Antidiabetic Property of *Aloe vera* (*Aloe barbadensis*) and Bitter Melon (*Momordica charantia*)

Junaid Ahmad Malik, Shazia Iqbal, Joy Biswas, Umair Riaz, and Sumitra Datta

10.1 Introduction

Use of native ethnobotanical medicine is an integral part of Indian tradition particularly for the diabetes mellitus treatment since ages (Patil et al. 2011). Diabetes mellitus is a metabolic dysfunction which is characterized by hyperglycemia and anomalous metabolism of proteins, fats, and carbohydrates that initiate complete or comparative insufficiency of insulin caused by the malfunction of beta (β) cell of islets of Langerhans or by impaired insulin intake in the peripheral tissues and also by insulin resistance. There are two types of diabetes mellitus: insulin-dependent type 1 and insulin-dependent type 2 diabetes mellitus. Majorly 90% of all sort of diabetes is initiated by type 2 diabetes mellitus (ADA 2012; Larejani and Zahedi 2001; Li et al. 2012; Naveen and Baskaran 2017). Worldwide nearly 1.5 million individuals lose their lives annually to this disease, and it is estimated to affect 592 million people by 2035 (Chinsembu 2019; Mahwish et al. 2017; Pahlavani et al. 2019). More than 61 million Indians are diabetic which makes India the "capital of diabetes." Treatment of diabetes with synthetic drugs has shown side effects and is costly. According to the World Health Organization

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(WHO), 80% people prefer ethnobotanical medication globally (Bindu and Narendhirakannan 2018; Chaturvedi 2012; Modak et al. 2007; Viswanathan and Rao 2013).

Diabetes mellitus (DM) is a metabolic disorder affecting about 25% of the people of developed as well as the developing countries with acute and chronic consequences (Arumugam et al. 2013; Soumya and Srilatha 2011). In diabetes the body cells cannot properly metabolize sugar because of deficient action or lack of a peptide hormone insulin. The main reason for non-metabolization of the sugar is less insulin production by the pancreas or its non-utilization by the body which initiates the breakdown of body's own fats and glycogen to scuffle the sugar content (Buowari 2013; Folorunso and Oguntibeju 2013) which in turn may cause severe harm and malfunction of many organs (Salsali and Nathan 2006) with symptoms like polyurea, blurred vision, and weight loss (Sperling et al. 2014).

From a long time, the plants have been preferred as the important sources of potential antidiabetic medicines, particularly for diabetes (Arumugam et al. 2013). At present, the many medicinal plants are vastly preferred and recommended for the use in therapeutic purposes of some common ailments and diseases (Kooti et al. 2015) owing to the fact that these plants contain some important phytoconstituents like alkaloids, flavonoids, carotenoids, etc. These components are known to upsurge and increase the secreting ability and performance of pancreatic tissues by either increasing insulin secretion or by diminishing the intestinal capability to absorb the glucose (Arumugam et al. 2013). Some important traditional medicinal plants and their mode of action are depicted in Table 10.1.

10.2 Antidiabetic Drugs and Their Side Effects

The antidiabetic and hypoglycemic drugs act through different mechanisms. Sulfonylureas have a hypoglycemic effect as it stimulates islets of Langerhans of pancreas to secrete more insulin (DeFronzo 1999; Inzucchi 2002). The biguanides drugs work by replenishing the sensitivity of outlying and peripheral tissues to insulin thru uplifting insulin-stimulated uptake (Lebovitz 1997). The α-glucosidase inhibitors obstruct the pathway of intestinal carbohydrate breakdown by inhibiting some enzymes responsible for it (DeFronzo 1999; Lebovitz 1997). Another hypoglycemic drug group, thiazolidinediones (TZDs), works by increasing the sensitivity of muscular and adipose tissues to insulin (DeFronzo 1999; Koski 2004). This class of drugs also helps in the reduction of glucose production by the liver. Non-sulfonylureas secretagogues and repaglinides are also known to be beneficial in reducing the blood glucose by somewhat same mechanism as used by sulfonylureas. Non-sulfonylureas secretagogues and repaglinides improve the insulin secretion from pancreatic β cells, however binding to different β-cell receptors (Koski 2004).

Table 10.1 Some antidiabetic medicinal plant species

S. no.	Scientific name	Family	Plant part	Activity/hypoglycemic mechanism	Ref.
1.	*Urtica dioica*	Urticaceae	Leaves	Stimulation of pancreatic beta cells	Das et al. (2009)
2.	*Carthamus tinctorius*	Compositae	Flower	Rich source of quercetin and kaempferol which have antioxidant and hypoglycemic properties	Asgary et al. (2012)
3.	*Swertia punicea*	Gentianaceae	Whole plant	Improves insulin resistance	Tian et al. (2010)
4.	*Syzygium cumini*	Myrtaceae	Seeds	Inhibits insulinase action in the liver and kidney	Kumar et al. (2013)
5.	*Mangifera indica*	Anacardiaceae	Leaves	Reduces the intestinal glucose absorption rate	Aderibigbe et al. (2001)
6.	*Momordica charantia*	Cucurbitaceae	Fruit	Inhibition of protein tyrosine phosphatase 1B (PTP1B), activation of AMPK, increase of glucose transporter type 4 (GLUT4) expression	Joseph and Jini (2013)
			Fruit pulp, seed, leaves, and whole plant	Inhibits the action of glucose-6-phosphatase and fructose-1, 6-biphosphatase in the liver	Miura et al. (2001)
7.	*Trigonella foenum-graecum*	Fabaceae	Leaves	Its saponins have the hypoglycemic effects	Vats et al. (2002)
8.	*Allium sativum*	Amaryllidaceae	Bulb	Enhances insulin secretion and insulin sensitivity	Ashraf et al. (2011)
			Seed	Reduces the diabetic complications like renal dysfunction	Mostofa et al. (2007)
9.	*Aloe barbadensis*	Asphodelaceae	Leaf	Stimulates the pancreatic cells for more insulin secretion	Noor et al. (2008)
10.	*Citrullus colocynthis*	Cucurbitaceae	Fruit	Decreases the activities of glucose-6 phosphatase and fructose 1, 6-bisphosphatase and increases the activity of liver hexokinase	Shi et al. (2014)

Even though the synthetic and man-made hypoglycemic medicines are the chief ways aimed at controlling the blood glucose and diabetes, they are not fully able to monitor its complications besides having prominent side effects (Rao et al. 2010). Hence determining the substitute medicinal families of antidiabetic agents is the need of the hour.

10.2.1 Aloe vera *(*Aloe barbadensis*)*

10.2.1.1 Aloe Vera and Its Antidiabetic Properties

Aloe vera is a species of *Aloe* that is known and famous particularly for its medicinal properties. The *Aloe vera* name comes from the Arabic word *Alloeh* meaning "shining bitter substance," while *Vera* in Latin means "true." The Greek scientists 2000 years ago regarded *Aloe vera* as the universal panacea. It is the plant of immortality for Egyptians (Itrat and Zarnigar 2013). Aloe can be found in Africa, Australia, Central America, India, Mexico, the Caribbean, the Pacific Rim countries, and South America. There are above 550 species of aloe that are grown around the world. However, only two species are grown today commercially, with *Aloe arborescens* Miller and *Aloe barbadensis* Miller being the most popular (Wynn 2005). The *Aloe vera* plant today is used in dermatology for numerous purposes (Itrat and Zarnigar 2013). Aloe plant leaves grow in rosette pattern and originate from the base. Length of mature plants can go about 2 and a half inches to 4 feet and average height being around 28–36 inches. At maturity, they may weigh up to 3 pounds. Number of leaves is usually 12–16 on each plant. Each leaf is composed of three layers: the outer thick layer has protective function, and it is of 15–20 cells called as rind. It also produces proteins and carbohydrates. The middle layer of latex is the bitter yellow sap. It comprises glycosides and anthraquinones. An inner clear gel that comprises of 99% water and rest is made of amino acids, glucomannans, sterols, lipids, and vitamins (Bozzi et al. 2007; Nandal and Bhardwaj 2012). The plants can be harvested every 6–8 weeks by removing 3–4 leaves per plant.

10.2.1.2 Active Constituents of *Aloe vera*

Water is the major constituent of *Aloe vera* gel, and it comprises of about 98% of leaf matter (Pandey and Singh 2016). The soluble solids are 0.56% with some seasonal fluctuation, and total solid content of *Aloe vera* gel is 0.66%. *Aloe* gel consists of phenolic compounds (2%), lipids (5%), proteins (7%), minerals (16%), sugars (17%), and polysaccharides (53%) on dry matter basis (Fig. 10.1**)**. *Aloe vera* comprehends 200 active constituents potentially: amino acids, enzymes, vitamins, minerals, lignin, sugars, salicylic acids, and saponins which are accountable for the *Aloe* multifunctional activity (Sharrif and Verma 2011; Sahu et al. 2013). *Aloe vera* gel is reported to be the significant wound healer also as compared to the other chemicals like iodine. This property is as an outcome of keratinocyte multiplication stimulated by the glycoprotein fraction G1G1M1D12, which leads to reepithelialization and wound healing. Apart from the wound healing activity, *Aloe barbadensis* is also well reported for its therapeutic uses in other clinical diseases like chronic obstructive pulmonary disease (COPD), heart disease, and cardiomyopathy as well as other bacterial infections (Atik et al. 2019).

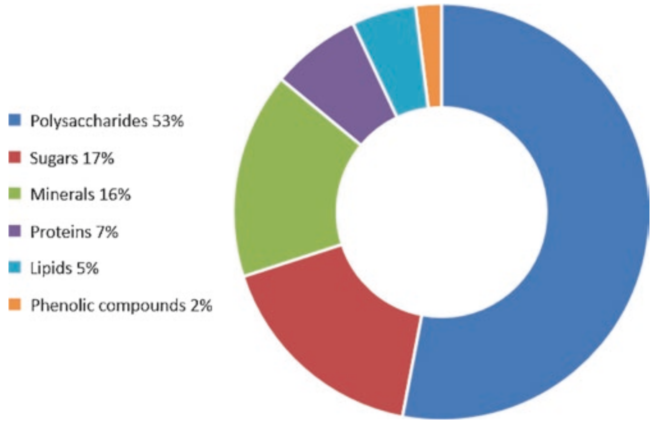

Fig. 10.1 Chemical composition of *Aloe vera* gel

10.2.1.3 Antidiabetic Activity

Among several plants, *Aloe vera* was a strong candidate for the treatment of diabetes with the hypoglycemic trace elements Cr, Zn, and Mn, as it has been used for treating diabetes in Arab and other developing countries and has potential insulin action (Mohamed 2011). Multiple mechanisms have reported antidiabetic effects of *Aloe vera* plant parts especially its gel, but its antihyperglycemic effects are controversial. Mechanism includes that alloxan acts as a cytotoxic agent on the insulin-secreting β cells of pancreas and effectively induces diabetes mellitus in a wide variety of animal models which share many features with that of human type (Manjunath et al. 2016). The influence of pseudoprototinosaponin AIII as well as prototinosaponins AIII on the uptake of glucose and release of insulin states that hypoglycemic properties exist because of the activities on hepatic gluconeogenesis or glycogenolysis (Bnouham et al. 2006). Frequent quantities of *Aloe vera* doses in diabetic mice have shown the hypoglycemic influence by stimulating the synthesis of insulin in the beta cells of pancreas (Modak et al. 2007).

In recent years the researchers have been exploring the potential of *Aloe vera* to help people with the hypoglycemic effects and keep their blood glucose level in check. During 2016, a group of researchers, while reviewing a number of investigations on the use of *Aloe vera* as an antidiabetic agent, found that there are some important constituents which could prove useful for the people with higher glucose levels in their blood as well as for the prediabetics. The frequent supplements of *Aloe vera* juice are known to benefit the diabetic patients as well as have the following benefits:

- Lowers fasting blood glucose levels and reduces weight as well as the body fat.
- The use of *Aloe vera* is known to reduce the levels of HbA1c which in turn helps to lower the blood glucose levels in diabetic patients.

Several clinical (in human) and pre-clinical (in animal) research trials showed that *Aloe vera* gel preparations in diverse forms (e.g., juice or as constituents in bread, etc.) lowered the blood glucose effects. Oral administration of *Aloe vera* gel that is alcohol-insoluble residue extract, in a study on streptozotocin induced diabetic rats, significantly decreased the fasting total cholesterol (TC), free fatty acids (FFA), blood glucose (FBG), triglycerides (TG), hepatic transaminases, and phospholipids. In addition, it also increased the plasma insulin levels significantly. *Aloe* gel extract treatment increased low-density lipoprotein cholesterol (LDLc) levels, and plasma levels of high-density lipoprotein cholesterol (HDLc) decreased to normal levels in the streptozotocin-induced rats (Kar and Bera 2018; Rajasekaran et al. 2006). It was stated that the antioxidant mechanism could be the mechanism for the decrease in glucose effect because in the kidneys of streptozotocin-induced diabetic rats, it reduced peroxidation levels and in the brains of streptozotocin-induced mice, oxidative damage is attenuated (Jones 2005).

Also in streptozotocin-induced rats, *Aloe vera* leaf extract is effective in lowering hyperglycemia (Rajasekaran et al. 2004; Noor et al. 2008). Another study reported the prevention of hyperglycemia in alloxane-treated rabbits by *Aloe vera* extract (Akinmoladun and Akinloye 2007). The treatment with *Aloe vera* juice filtrate was also confirmed to have led to considerable improvements in diabetic rats serum glucose compared with nondiabetic control (Mohamed et al. 2009).

Aloe vera leaf extract has a favorable effect in retreating hyperglycemia induced by alloxan in rats. *A. vera* leaf extract treatment at doses of 200 mg/kg and 400 mg/kg decreased the elevated blood glucose levels in diabetic rats which was comparable to 50 mg/kg of metformin with no statistically significant difference ($P < 0.0001$) (Manjunath et al. 2016). By increasing glucose metabolism, the hypoglycemic effect of the *Aloe vera* leaf extract was attained. It was also hypothesized that an antioxidant mechanism may explain the glucose decreased effect of *Aloe vera*, because the blood loss in the brains of streptozotocin-induced mice and the reduced peroxidation of the kidneys of streptozotocin-induced diabetic rats were attenuated (Boudreau and Beland 2006). The data of a clinical experiment on the antidiabetic activity of *Aloe vera* juice by Yongchaiyudha et al. (1996) depicts the antidiabetic property of *Aloe vera* gel as the considerable decrease was seen in the patients treated daily with one tablespoonful of *Aloe* juice twice a day for 42 days consecutively. The blood glucose level of the patients treated with aloe juice was reported to be considerably reduced from 250.36 mg/dL at day 1 to 233.83 mg/dL at day 7 and as such continued to decrease during all the treatment time. The blood glucose level was reported to be 141.92 mg/dL on day 42.

The antidiabetic effect of *Aloe vera* leaf pulp extract in vitro and in vivo is compared to glimepiride in adult male albino rats. In vitro study isolated islets of pancreas from adult female albino rats were used. Both glimepiride (10 mg/kg, p.o.) and *Aloe* extract (10 ml/kg, p.o.) pointedly significantly increased serum insulin and decreased serum glucose levels comparative to control diabetic rats. *Aloe* treatment increased serum levels of blood glutathione (GSH) significantly, while superoxide dismutase (SOD) and malondialdehyde (MDA) were decreased significantly as compared to diabetic rats. *Aloe* effect was improved compared to glimepiride. In the vitro study, both stimulated and basal insulin secretions increased significantly from pancreatic-isolated islets by both glimepiride (10 lmol/l) and *Aloe* (10 ll/l) compared to control (Abo-Youssef and Messiha 2013). The research has shown that extracts of *Aloe vera* gel can not only normalize blood glucose and serum insulin but also decrease levels of triglycerides, cholesterol, and free fatty acids in the liver, urine, and streptozotocin-induced kidney diabetes rats (Rajasekaran et al. 2006).

*10.2.2 Bitter Melon (***Momordica charantia***)*

10.2.2.1 Bitter Melon and Its Antidiabetic Properties

Plants are remarkable natural reserves of phytochemicals and significant drugs (Patil et al. 2011). *Momordica charantia* (bitter gourd), affiliated to Cucurbitaceae family is a perennial climber effective against hypoglycemic effects of type 2 diabetes mellitus (Bhushan et al. 2010; Mahwish et al. 2017; Peter et al. 2019; Taylor 2002). Hypoglycemic phytoactive compounds are usually alkaloids, glycosides, polypeptides, and sterols. Such compounds derived from *M. charantia* are classified into distinct groups: cucurbitane-type triterpenoids, cucurbitane-type triterpene glycoside, flavonoids, oleanane-type triterpene, polypeptide p-insulin, phenolic compounds, and saponins. Among them vicine (glycoalkaloid with pyrimidine nucleoside), polypeptide p-insulin (plant insulin), and charantin (stigmastadienol glucosides and sitosterol) are the most important that enhance sugar levels in blood by glycogen synthesis and glucose uptake in the liver, facilitate insulin release from beta cells of pancreas, and promote repair and growth of insulin-secreting beta cells, muscles, and adipocytes. These have also been found effective in treating diabetic patients suffering from glycoconjugate and heparin sulfate-related kidney problems (Alam et al. 2015; Chaturvedi 2012; Joseph and Raj 2010; Joseph and Jini 2013; Janmajoy et al. 2019; Pahlavani et al. 2019; Raman and Lau 1996; Tan et al. 2008).

10.2.2.2 Antidiabetic Activity

The hypoglycemic effects in *M. charantia* are supported by numerous researches based on a cellular assay, animal modeling, and clinical trial (Czompa et al. 2017; Efird et al. 2014; Gushiken et al. 2016; Xu et al. 2015). The following section discusses about the reported antidiabetic mechanisms of *M. charantia.* Active phytochemicals manipulate glucose and lipid metabolisms through several proposed mechanisms to maintain standard blood glucose and lipid levels in the body and prevent pathophysiological diabetes disorders (Chaturvedi 2012). These bioactive formulations have been supported by various physiological, pharmacological, and biochemical studies (Bhushan et al. 2010; Mahwish et al. 2017; Taylor 2002). The plausible antidiabetic mechanisms reported in *M. charantia* are HMP pathway key enzyme stimulation, gluconeogenic enzymes suppression, skeletal muscle

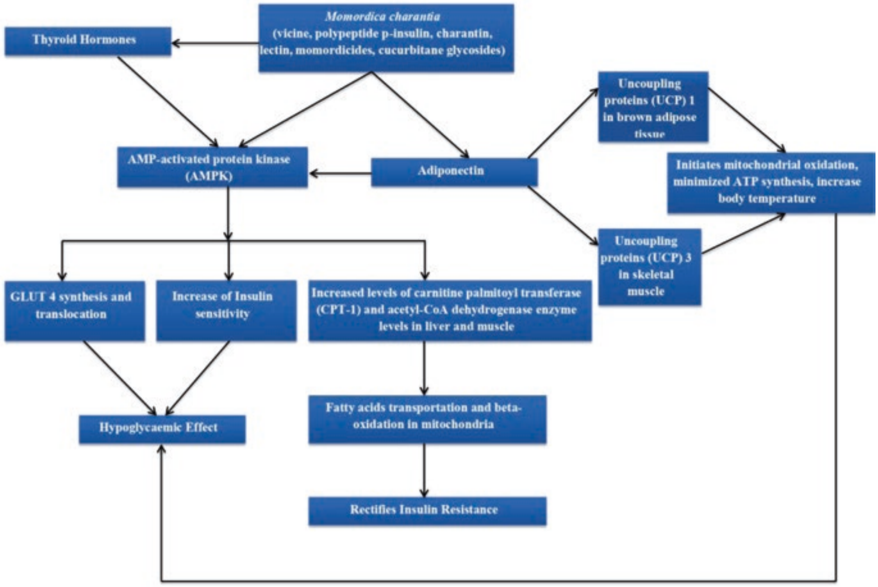

Fig. 10.2 Probable schematic antidiabetic mechanism of action in *Momordica charantia*

stimulation, peripheral glucose utilization, intestinal glucose uptake inhibition, islet β-cell restoration, and adipocyte differentiation inhibition (Bindu and Narendhirakannan 2018). The schematic representation of the mechanisms is shown in Fig. 10.2.

Vicine, polypeptide p-insulin, charantin, and glycosides have been found to prevent gluconeogenesis and stimulate glucose oxidation through the pentose phosphate pathway or shunt pathway which increases glycogen levels in the liver. They increase the peripheral insulin sensitivity by repairing impaired β cells and accelerate insulin secretion. Insulin sensitivity is also increased by other mechanisms such as activation of AMP-activated protein kinase (AMPK) by momordicides, cucurbitane glycosides and their aglycones, lectin linking of the two insulin receptors, inhibition of skeletal muscle protein tyrosine phosphatase 1B, increase in the translocation and number of GLUT4 receptors, and, lastly, the enhancement of phosphorylation rate of insulin receptor substrate. *M. charantia* regulates the glucose uptake into the jejunum brush border vesicles and stimulates the glucose uptake into muscle cells, working along similar lines with insulin (Ahmed et al. 2004; Chaturvedi and Akala 2003; Chaturvedi 2012; Janmajoy et al. 2019; Klomann et al. 2010; Ojuka et al. 2002; Winder and Hardie 1999). Apparently, it prevents intestinal absorption of di- and monosaccharides by inhibiting enzymes like disaccharidase and glucosidase, respectively, and stimulates adipose tissue to release adiponectin which enhances AMPK activities in the cell. It has found that Na^+/K^+-ATPase-dependent intestinal glucose absorption which is significantly higher in diabetic patients is reduced by intake of *M. charantia*. Glucose and lipid absorption is also impaired by suppressing the activities of intestinal sucrase, maltase, and pancreatic lipase (Chaturvedi 2012). AMPK is an insulinotropic metabolic switch that promotes GLUT4 synthesis and its translocation which regulates glucose uptake. *M. charantia* has been found to enhance adiponectin and thyroxine which activate AMPK. Subsequently, AMPK transports of fatty acids in mitochondria for oxidation while activating 3-hydroxy-3methylglutaryl-coenzyme reductase in the liver and there upon inhibiting cholesterol synthesis. Adiponectin on the other hand facilitates mitochondrial oxidation without generating ATP and thus maintains the normal blood glucose level in the body. A study by Matheka et al. (2011) on glucose reduction in healthy rats after administration of *Momordica charantia* envisaged the glucose level of 2.93 ± 0.12 on day 28 from the base level of 3.20 ± 0.18 mmol/L on day 1.

Apart from increasing glucose influx, *M. charantia* also facilitates glucose oxidation by enhancing the activities of hepatic hexokinase, glucokinase, and phosphofructokinase. However, it inhibits lipogenesis by downregulating lipogenic gene expression in the adipocytes (Chaturvedi 2012; Nhiem et al. 2010; Sridhar et al. 2008; Tan et al. 2008). Furthermore, fatty acid transportation and beta (β) oxidation in mitochondria increase by the development of acyl-CoA dehydrogenase and carnitine palmitoyltransferase (CPT) enzymes levels in the liver and muscle (Chaturvedi 2012; Ma et al. 2017; Pahlavani et al. 2019). The increased production of cytokine signaling-3, CPT-1, Akt, and c-Jun N-terminal kinase (JNK) expression at both mRNA and protein levels in the liver on the influence of AMPK restores insulin resistance (Bruce et al. 2009; Chan et al. 2005; Chaturvedi 2012; Pahlavani et al. 2019). In addition, AMPK promotes the uncoupling proteins (UCP) 1 and 3 in brown adipose tissue and skeletal muscle protein, respectively, in mitochondria and initiates fuel oxidation without ATP synthesis by electron-transporting proteins. The energy produced in this process is mostly utilized to maintain body temperature (Alam et al. 2015; Chaturvedi 2012; Janmajoy et al. 2019; Mahmoud et al. 2017; Nhiem et al. 2010; Pahlavani et al. 2019; Sridhar et al. 2008; Tan et al. 2008).

Momordica charantia plant is having numerous diverse chemical components which have the capability to act either singly or in combination with the other product for the hypoglycemic effects. Saponin or charantin are the active chemicals with a direct glucose-lowering properties as well as having the insulin like effects. The research shows that consumption of a daily half cup of the *M. charantia* concoction twice a day is adequate to regulate the glucose levels and maintaining the cellular metabolism in the diabetic patients, hence, is a best alternative herbal therapy. Furthermore, for the people with type 2 diabetes, there is no detriment or risk factor for using *M. charantia* extract alone.

10.3 Future Research and Conclusion

In this chapter, we discussed and reread the use of two traditional medicinal plants *Aloe barbadensis* and *Momordica charantia* for the treatment of diabetes. The antidiabetic consequences of both the debated plants stand accredited to the phytochemical combinations or single constituents of the plant extracts. Alkaloids, phenolic acids, flavonoids, glycosides, and saponins are the main elements with antidiabetic properties. Various machineries have been brought forth by the researchers with regard to the phytochemicals and their antidiabetic regulations, for example, glucose regulation and fat metabolism, insulin secretion, β-cell stimulation, NF-*k*B-inducing kinase (NIK) pathway, etc. Further research and developments in the utilization of traditional treatments have considerably powered the drug progression of innovative effects intended for diabetes. Consequently, further effectual scientific examinations are necessary for supplementary authentication. Alternatively, the main antidiabetic principles of medicinal plants should be prioritized and explored to ensure the better outcomes.

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Chapter 11
Ethnobotanical Properties and Traditional Uses of Medicinal Plant *Abutilon theophrasti* Medik

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11.1 Introduction

The traditional herbal medicine possesses significant importance globally; hence in the recent years, the interest in herbal medicine has gradually increased (Farnsworth and Soejarto 1991). Particularly, the herbal medicinal plants are often been used for ecological, cultural and historical reasons. This is due to their better compatibility

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(Kamboj 2000), continued availability (Bhattarai 1989) and high acceptance (Ghimire et al. 2012) as used by the tribal people for the relief of many acute diseases. As a result, the herbal medicine all over the world has been revalued by extensive researches based on their therapeutic principles. However, till this date only about 5% of the total medicinal plant species have been thoroughly investigated (Kumar et al. 2010; Patwardhan et al. 2004) regarding their safety and efficacy of ethnomedicinal uses. As herbal medicines possess recognizable therapeutic effects (Bailey and Day 1989) as well as some toxic side effects (Keen et al. 1994), the ethnomedicinal properties of the traditional herbal plants are being screened for their active pharmacological effects.

Malvaceae is a multicultural family, and *Abutilon* is one of the important genera of this family. The genus *Abutilon*, originating from China (Holt and Boose 2000), contains approximately 160 species worldwide, primarily in tropical and subtropical areas. *Abutilon* species are cultivated for medicinal and ornamental purposes as well as a fibre source. Valuable fibres are drawn from different species of *Abutilon*, e.g. *Abutilon indium*, *Abutilon polyandrum* and *Abutilon theophrasti*. The fibres obtained from these plants are used for making ropes cordage's, jute dyes, drugs, rugs, wrapping cloth, tissue papers, coarse cloth, cigarette paper, rubber, tyre, fabrics, shoe polishes, etc. Different parts like leaves, stem, roots and fruit body are used to treat various diseases like ulcers, gonorrhoea cough, otitis media, gout, etc. Among all species of this genus, *Abutilon theophrasti* is least investigated.

Abutilon theophrasti Medik (velvetleaf) has become a problem weed in many countries in Europe (Weber and Gut 2005). This species is highly competitive, while it is primarily competing for light with the crop plant (Lindquist et al. 1998; Warwick and Black 1988). If *Abutilon theophrasti* and the crop emerge simultaneously, it can surpass crop growth, which ultimately results in reduced yield due to interference with crop light interception (Sattin et al. 1992). However, yield losses vary largely depending on the crop type infested, weed density and environmental conditions (McDonald et al. 2004; Schweizer and Bridge 1982). Moreover, its importance has increased over time, partly because it is relatively tolerant to many herbicides (Sattin et al. 1992).

11.1.1 Nomenclature

Mitch (1991) reports the nomenclatural history of *Abutilon theophrasti*. Carolus Linnaeus classified this species as *Sida abutilon*. Its genus companions included such plants as *Sida spinosa* L. Friedrich Casimir Medicus, director of the garden at Mannheim, published a volume in which he rearranged the Malvaceae, placing *Sida abutilon* in the genus *Abutilon* with the specific epithet theophrasti. Joseph Gaertner reclassified the plant as *Abutilon avicennae*, but scientific precedence held sway, and Medicus's name was restored.

11.1.1.1 Taxonomic Tree

- Domain: Eukaryota
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Malvales
- Family: Malvaceae
- Genus: *Abutilon*
- Species: *theophrasti*

11.1.1.2 International Common Names

1. *English*: China jute; Chinese lantern; Indian mallow; pie-marker; velvetleaf
2. *Spanish*: Malva banca; Malva de terciopelo; Malva grand; Yute de la China
3. *French*: jute de Chine
4. *Chinese*: Ching-ma

11.1.1.3 Local Common Names

1. *Germany*: Chinesisch jute; Chinesischer Hanf; Lindenblattrige Schonmalve; Samtpappel
2. *Italy*: Cencio molle; Iuta cinese
3. *Japan*: Bouma; Ichibi
4. *USA*: Butter-print; buttonweed

11.1.1.4 EPPO Code

ABUTH (*Abutilon theophrasti*)

11.2 Description (*Abutilon theophrasti*)

Abutilon theophrasti (Fig. 11.1) is an annual herb which reproduces only by its seeds. It has typical seed pods containing up to 40 large, hard black seeds (Warwick and Black 1988). This species produces many seeds (up to 8000 seeds per plant), which can remain viable for decades in the soil contributing to its long-term success (Spencer 1984).

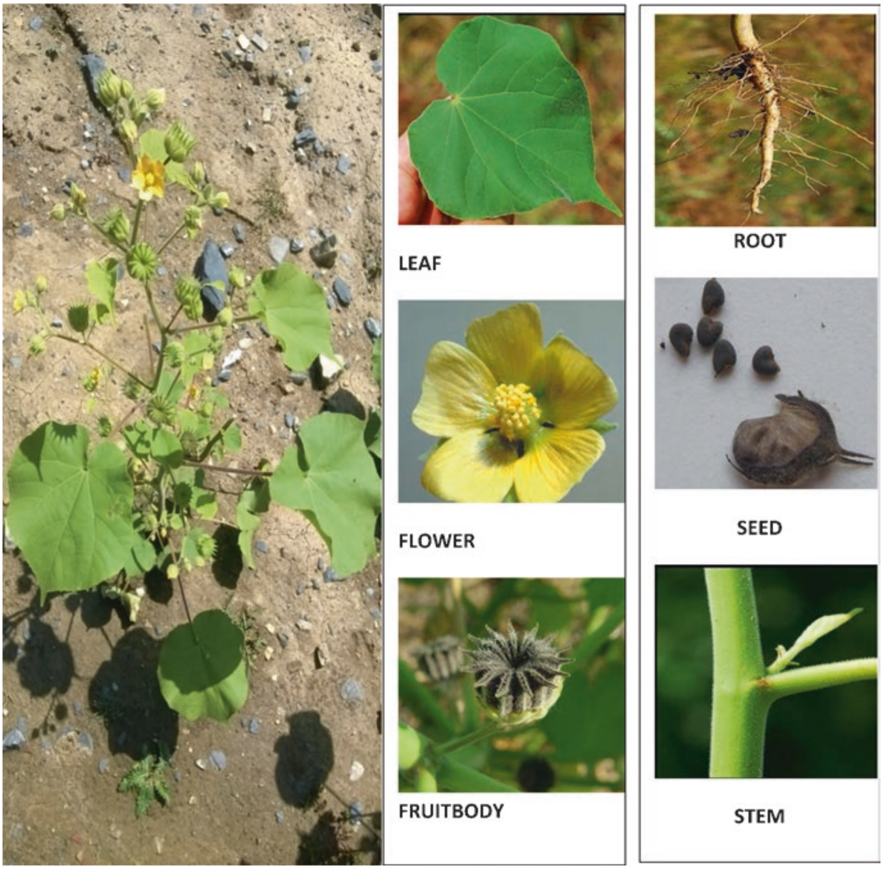

Fig. 11.1 *Abutilon theophrasti* (velvetleaf)

Its stem is erect up to 5 meters tall. Stem is branched in the upper part with smooth and velvety hairs; leaves are petiolate long heart shaped and alternate in position; width of leaf is seen 7–20 cm; flowers are seen either single or in clusters and arise from the axial of leaf; colour of flower is yellow to yellow orange; fruit body is cup shaped with 1.3–2.5 cm length and 2.5 cm width; and seeds are purplish brown, kidney shaped with 1 mm thick and 2–3 mm long (Warwick and Black 1988). Velvetleaf is hexaploidy species with 2n:6×:42 chromosome (Warwick and Black 1986). Tap root is slender along with many small branches.

11.2.1 Growth and Development

Velvetleaf is an annual herb. Seeds are protected by hard seed coat which prevents the seed from ingestion by poultry and most livestock. High light level provides the plant high photosynthetic rate with different transpiration rate when compared with the same plant under low light levels. Yun and Taylor (1986) studied the adaptive effect of leaf thickness on two important processes (photosynthesis and transpiration) under high and low light levels; high carbon dioxide concentration revealed the increase dry matter present in velvetleaf. Patterson (1979) showed that increasing CO_2 concentration over 350 ppm (vol/vol) to 600 and 1000 ppm increased the net assimilation rate. Studies carried out by Breuninger et al. (2012) showed that when field-grown velvetleaf was subjected to extra light level (1300 pmol m^{-2} s^{-2}) and to 1000 cm^3 m^{-3} of CO_2, it responded to added CO_2 four times as much as to an added increment of irradiance; meanwhile Garbutt and Bazzaz (1987) evaluated that different concentrations of carbon dioxide which vary from 300 to 900 μL L^{-1} did not affect the time of flowering and number of abortions in fruit body; however, there was reduction in seed, flower and capsule number, and also seed weight was seen decreased. House studies on velvetleaf demonstrated that cool temperature imposes the drastic effects on the growth of velvetleaf (Flint et al. 1983). Plants grown in cold regions (17/13 °C and 26/17 °C) are seen to possess decreased heights, low dry weight and small leaf area compared to the plants grown in higher temperatures (26/21 °C and 32/23 °C); this decreased growth is due to decreased net assimilation rate, photosynthetic rate and stomatal conductance. Young seedlings of velvetleaf instantly develop the taproot followed by lateral roots. Lateral roots develop after 1 or 2 days after the emergence of taproot.

11.2.2 Distribution

Abutilon theophrasti (Fig. 11.1) is widely seen in China; only Tibetan plateau shows the scarcity of the plant. It is cultivated as well in various nations like north-eastern China, India, Japan, North America and Vietnam. The plant is native to Asian tropical region. It was naturalized in the Mediterranean area and the United States. In the east part of Canada, this plant starts flowering in September to October and produces flowers on the axillary branches until the first frost (Warwick and Black 1988). In Jammu and Kashmir (India), flowering starts from August to late October; Hassan et al. 2016a, 2016b reported *Abutilon theophrasti* in Qazigund and J&K (latitude 33.56/longitude 75.20).

11.2.3 Reproduction

Flowers are the organ part for the reproduction in velvetleaf; fertilization takes places when flower opens and seeds mature after 17–22 days after pollination (Winter 1960). Self-compatibility is seen in velvetleaf; along this plant is autogamous (Garbutt and Bazzaz 1987; Winter 1960). Average number of seed produced per capsule is 35–45. Weight of per individual seed is known to be 4–6 mg (Warwick and Black 1986). According to Winter (1960), early season-produced flowers meet with abortion. Velvetleaf seeds are dry and hard, and seed coat is impermeable to water; this results in primary dormancy classically known as hard seededness (Egley and Chandler 1978; Lueschen and Andersen 1980; Horowitz and Taylorson 1984; Warwick and Black 1986). Commonly new harvested velvetleaf seeds are seen to possess seed dormancy; after a period of time (1 year), seeds lose their dormancy and become water-permeable. Seeds which are stored under high relative dampness became more permeable with great rate (Steinbauer and Grigsby 1959). Velvetleaf seeds are known for long viability; they can stand up to 50 years when stored dry.

Abutilon theophrasti is a self-compatible, autogamous species. As pollen of *Abutilon theophrasti* is released by the anthers before or in immediate conjunction with flower opening, pollination would have occurred before stigmas could be exposed to pollen from another flower (Andersen 1988). Approximately 3% of seeds produced in field conditions could originate from outcrossing, in occasionally found buds with a stigma protruding from otherwise tightly closed petals (Andersen 1988).

11.2.4 Natural Enemies

A wilt pathogen, *Verticillium dahliae*, causes necrosis to leaves of *Abutilon theophrasti* and reduces seed production. Infections of *Abutilon theophrasti* by the pathogen have been reported in Illinois (Kirkpatrick and Harrison 1979), Wisconsin (Sickinger et al. 1987; Sickinger and Harvey 1980), Iowa (Hartzler 1996) and Indiana (Wiley et al. 1985) in the United States.

Gibb (1991) studied five insect species, *Heliothis zea [Helicoverpa zea]*, *H. virescens*, *Liorhyssus hyalinus*, *Niesthrea louisianica* and *Althaeus folkertsi*, attacking *Abutilon theophrasti* seeds, and noted that these species had a significant negative impact on the number of viable *Abutilon theophrasti* seeds produced in Indiana, USA. *N. louisianica* reduced the number of viable seeds of *Abutilon theophrasti* by 17.5% and 15.5% in two places in Missouri (Kremer and Spencer 1989).

Colletotrichum coccodes has been investigated as a possible mycoherbicide (Fernando et al. 1996; Gotlieb et al. 1987). The pathogen causes more serious damage to *Abutilon theophrasti* in competitive conditions with soya beans than in monoculture (Ditommaso et al. 1996).

Infection by *Phomopsis longicolla* was first reported in Illinois, USA, in 2000 (Li et al. 2001). The pathogen causes reddish-brown lesions on the lower stem and upper root area of *Abutilon theophrasti* plants growing in soya bean fields. Turnip mosaic potyvirus also caused severe mosaic symptoms to *Abutilon theophrasti* in Piedmont, Northwest Italy (Guglielmone et al. 2000). *Abutilon theophrasti* can be a host of a parasitic plant, *Cuscuta pentagona*, only when roots of *Abutilon theophrasti* are colonized by mycorrhizal fungi (*Glomus intraradices*) (Sanders et al. 1993).

McKeen and Thorpe (1973) found that *Verticillium nigrescens* cause wilt on velvetleaf in Ontario and Wisconsin. *Verticillium dahlia* has been now suggested as natural agent for velvetleaf eradication (Green and Wiley 1987). Wiley and Williams 1985 have provided a list of fungi on velvetleaf:

- *Alternaria abutilonis*
- *Cercospora abutilonis*
- *Cladosporium herbarum*
- *Letotrichum malvarum*
- *Macrophomina phaseolina*
- *Phymatotrichum omnivorum*

Velvetleaf has been also seen as host for soybean pathogens:

- *Phomopsis sojae*
- *Colletotrichum dematium*
- *Colletotrichum gloeosporioides* (Hepperly et al. 1980)

11.2.5 Impact on Other Crops

Abutilon theophrasti causes severe crop loss in maize, soya bean and cotton. In soya bean, 72% crop loss can be caused by infestation of *Abutilon theophrasti* (Sterling and Putnam 1987), while 70% crop loss has been recorded in maize (Campbell and Hartwig 1982). In the United States, the estimated cost for control of *Abutilon theophrasti* was $343 million in 1982 (Spencer 1984).

11.2.6 Medicinal Value

Abutilon theophrasti is widely used in Chinese traditional medicine. Properties like expectorant, diuretic, analgesic, anti-inflammatory, anthelmintic, demulcent, aphrodisiac and emollient are attributed to this plant:

- Blotter and Calis. *The Indian Medicinal plants Delhi* (2000) *vol. 2, 433* reports that plant is used to treat the following:

    1. Rheumatic pains
    2. Arthrosis
    3. Bruises
    4. Sprains
    5. Dysentery
    6. Otitis media
    7. Tinnitus
    8. Deafness

- The powdered seeds are demulcent, diuretic, emollient laxative and stomachic (Duke and Ayensu 1985).

- Seeds contain fatty acids and mucilage; hence they are used to treat constipation in Chinese traditional medicine (CTM) (Pârvu 2005).

11.2.7 Phytochemistry

As we know that *Abutilon theophrasti* has been least investigated, still literature provides various reports on the phytochemistry. Generally, compounds like glucuronic acid, glucose, fructose, xylose, amino acid sequence of cytochrome $C_{(6),}$ mucilage, inositol and naphthoquinones have been isolated. Different parts of the present plant possess different chemical composition.

11.2.7.1 Flower

Research carried out by Matławska and Sikorska (2005) confirmed the following compounds in the flower of *Abutilon theophrasti*:

1. Kaempferol 3-0-β (6-D coumaroyl)-glucopyranoside
2. Myricetin 3-0-β-glucopyranoside
3. Quercetin 3-0-β-glucopyranoside
4. Quercetin 3-0-α-rhamnopyranosyl (1-6)-β-glucopyranoside
5. Kaempferol 3-0-α-rhamnopyranosyl (1-6)-β-glucopyranoside
6. Kaempferol 3-0-glucopyranoside
7. Quercetin 7-0-β glucoside

11.2.7.2 Leaves

Kiyamova et al. (2012) isolated the four compounds from the leaves of *Abutilon theophrasti*:

1. $C_{26}H_{53}OH$ (White crystals with similar physiochemical and spectral properties as hexacosanol)
2. $C_{29}H_{59}OH$ (White crystals with similar composition and spectral properties to non a cosanol)
3. $C_{29}H_{50}O$ (White needle-like crystals and mass spectra which corresponds to sitosterol)
4. $C_{29}H_{48}O$ (White needle-like crystals with IR spectra identical to stigmasterol)

11.2.7.3 Seed Coat

Paszkowski and Kremer (1988) gave tentative list of compounds from the seed coat:

1. Delphinidin
2. Cyanidin
3. Quercetin
4. Myricetin
5. (+) – Catechin
6. –Epicatechin

- *Seed oil*
  Studies carried out by Dinu et al. (2010) revealed the presence of the following classes of biologically active compounds:

- Oil.
- Sterols.
- Free and glycosylated triterpenes.
- Sugars and mixed polysaccharides (mucilages).
- Oleanolic acid.
- A triterpenoid sapogenin with biological activity was also identified in the seed.

Tian et al. (2017) quantified nine chemical constituents from the five parts (root, stem, leaves, seeds, exocarp) of *Abutilon theophrasti*:

- Gallic acid
- Protocatechuic acid
- Tannin
- Catechin
- Vanillic acid
- Caffeic acid
- Ferulic acid
- Rutin
- Quercetin
- Syriacusin

Mamadalieva et al. (2014) isolated two compounds from the aerial part of *Abutilon theophrasti* which were new to the genus:

- (6S,9R)–Roseoside
- (6S,9S)–Roseoside

Sun (1996) isolated white crystalline cholesterol and purified it from the non-saponifiable component of seed oil of *Abutilon theophrasti.* Physicochemical properties and spectral data confirmed it to be cholesterol.

Benner and Bazzaz (1985) reported the response of seeds of the annual *Abutilon theophrasti* (Malvaceae) to the timing of nutrient availability.

Carmody et al. (1945) observed that buttonweed seeds (*Abutilon theophrasti)* contain 15–17% oil, which consists of about 58% linoleic acid.

11.2.8 Allelopathic Effect

Allelopathy is a phenomenon in which plants release some chemicals into their environment, which results in the declined growth of other plants. According to Ashrafi et al. (2007) allelopathy is a marvel ecological connection between living communities which can be imparted for the control of unwanted biota. Chemicals which are produced by the plants with allelopathic attributions possess many other ecological roles like protection of plants and adjustment of soil biotype that are having effect on soil humiliation and fertility.

Abutilon theophrasti is a plant with same property (allelopathy). Its high allelopathic potential is due to inhibition of germination and growth of competitive plants, it for this it reaches to uppermost position. Allelopathic potential of *Abutilon theophrasti* is long before known, and less attention has been paid for its interactions with other plants (Gressel and Holm 1964).

Balah and Nassar (2011) studied the allelopathic potential of *Abutilon theophrasti*, against several weeds including *Portulaca oleracea*, *Corchorus olitorius*, *Echinochloa crus-galli* and *Arabidopsis thaliana* seedlings' growth and germination. *Abutilon theophrasti* extracts were seen to impose allelopathic effect because of high toxicity with increase in concentration. The higher concentration of aqueous extracts was found to display complete inhibition of *Portulaca oleracea* seed germination by 100%, at 10 g DW100 ml^{-1} compared to its control.

Konstantinovic et al. (2013) studied the allelopathic effects of *Xanthium strumarium* and *Abutilon theophrasti* and found that plant showed allelopathic effect, but the effect was not as much potent as control.

Elmore (1980) found various amino acids from *Abutilon theophrasti* (velvetleaf) seeds with high content; detection of toxic amino compound and unusual amino acid was zero. It was assumed that amino acids present are responsible for the allelopathic effects (Table 11.1).

11.2.9 Biological Activities

- Hassan et al. (2019) evaluated the anthelmintic activities of velvetleaf (*Abutilon theophrasti*) against L_3 and eggs of *Haemonchus contortus* and found that the said plant is having the efficacy to inhibit the helminths.
- Hassan et al. (2018) studied the antibacterial activities of *Abutilon theophrasti* and concluded that crude root extracts have potential to get against various bacterial strains like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Proteus vulgaris*, *Klebsiella pneumonia* and *Escherichia coli.*
- Tian et al. (2017) studied the phenol extracts of *Abutilon theophrasti* against various bacterial strains and found potential efficacy against them and also suggested that the said plant can be useful in agro and pharmaceutical industries.

Table 11.1 Free amino acid content in *Abutilon theophrasti*

Amino acid	Concentration (μmol/g dry weight)	Relative content (%)
Glutamine + glutamic acid	6.4	19.4
Ammonia	5.6	17.0
Aspartic acid	5.1	15.5
Arginine	5.1	15.5
Asparagine	4.5	13.6
Alanine	1.4	4.2
Phenylalanine	0.7	2.1
Histidine	0.7	2.1
Valine	0.6	1.8
Serine	0.5	1.6
Threonine	0.4	1.2
Lysine	0.4	1.2
Glycine	0.4	1.2
Isoleucine	0.3	0.9
Leucine	0.3	0.9
γ-Aminobutyric acid	0.3	0.9
Tyrosine	0.2	0.6
Ethanolamine	0.1	0.3
Proline	T	–
Cystine	T	–
Methionine	T	–
Ornithine	T	–

- Hassan et al. (2016a, 2016b) studied the anthelmintic activity of different parts of *Abutilon theophrasti* and revealed that methanolic root extracts were showing potent activity.
- Hassan et al. (2016a, 2016b) studied the antifungal activities of *Abutilon theophrasti* and concluded that *Abutilon theophrasti* possesses phytoconstituents with antifungal attribution.
- Mamadalieva et al. (2014) elevated the bioactivity of the aerial parts of *Abutilon theophrasti* and concluded that this plant has significant in vitro antioxidant and cytotoxic and anti-inflammatory activities.
- Michael et al. (1991) showed atrazine resistance in a velvetleaf (*Abutilon theophrasti*) biotype due to enhanced glutathione S-transferase activity.
- Paszkowski and Kremer (1988) studied the biological activity of aqueous extracts of velvetleaf (*Abutilon theophrasti*) seed coat and their flavonoid components against three plant species and five soil fungi. Germination was inhibited with complete inhibition of radical growth, the effect on five elected fungi was compromising and studies further revealed that flavonoid compounds significantly inhibited germination and radical growth.
- Gressel and Holm (1964) studied the nature of inhibition by *Abutilon theophrasti* and found the seeds of *Abutilon theophrasti* were inhibitory to tomato

germinating in sterile and non-sterile soil in the laboratory. The inhibition was carried out under conditions which kept light out along this water and minerals were as factors in the competition.
- Kremer (1986) analysed the diffusion zones from agar plates and seed leachates of *Abutilon theophrasti* Medic and reported the presence of phenolic compounds in the seeds.

11.3 Hybrids

While going through literature, no such information was found that confirm hybridization between *Abutilon theophrasti* and other plant species.

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Chapter 12
Phytochemistry, Pharmacology, and Toxicity of an Epiphytic Medicinal Shrub *Viscum album* L. (White Berry Mistletoe)

Mahak Majeed and Reiaz Ul Rehman

12.1 Introduction

Viscum album L., popularly known as European mistletoe is an evergreen, hemi-parasitic, perennial, globular-shaped medicinal shrub that belongs from the Loranthaceae family and grows on stems and branches of various species of woody trees dispersed in Europe, Africa, and Asia (Anthony 1995; Jurin et al. 1993; Zuber 2008). The plant is hosted by more than 200 species of trees and shrubs found in subtropical and temperate regions of Himalayas at an altitudes ranging from 900 to 2100 m (Suheel et al. 2018; medicinal plants of Nepal 1970). As a hemiparasitic globular shrub, it has low potential to carry out photosynthesis due to presence of lesser amounts of functional chlorophyll-protein complexes (Catal and Carus 2011; Turquet and Salle 1996). Thus it uses haustorial attachment with its host plants most noticeably *Salix* spp., *Populus* spp., walnut spp., *Acer* spp., *Malus* spp., *Crataegus* spp., *Prunus* spp., *Sorbus* spp., *Abies* spp., and *Pinus* spp. for deriving water and nutrient minerals and then utilizes its own oblongated, green, and leathery leaves for synthesis of carbohydrates using sunlight (Barney et al. 2007; Osadebe and Uzochukwu 2006).

Moreover, *V. album* absorbs specific soil minerals. This specificity of mineral absorption shown by a plant is strictly correlated with the type of host plant that eventually results in differential accumulation of soil minerals in tissues of plant (Ramm 2006). Botanical extracts of mistletoe were prepared in many European regions and are commercially available under the brand names as Lektinol™, Isorel, Eurixor®, Helixor®, Iscador®, ABNOBAViscum, Cephalektin, Plenesol, Vysorel, etc., which offer safe and less toxic alternative strategy for cancer treatment (Chernyshov et al. 2007; Frazer 1920; Hajto et al. 2011; Taiga 2013; Twardziok

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2015; Vicas et al. 2012). In Central Europe, mistletoe extracts, namely, (Iscador, Isorel, Eurixor, Plenesol, Vysorel, Lektinol, Helixor, etc.) are used in anticancer therapy as subcutaneous and intravenous injections with main aim to enhance immune system, improve patient's health quality, and reduce ADRs caused by conventional anticancer therapies (Beuth et al. 2008; Eisenbraun et al. 2011).

12.2 Phytochemistry of Mistletoe

European and American mistletoe are two important verities of *V. album* which show marked similarity in their protein constituents mainly in lectins (agglutinin and viscumin), alkaloids, and polysaccharides but with different medicinal properties. Lectins are double-chain proteins, structurally similar to ricin and abrin causing agglutination of cells and inhibit protein synthesis (Franz 1986). Activity and amount of lectins produced along with diverse spectrum of chemicals obtained from *V. album* including flavonoids, viscotoxins, terpenoids alkaloids, lecithins, saponins, acetylcholine derivatives, histamine, thionins, cardenolides, vitamin C, amines, caffeic acid, and resins may show fluctuations with type of host tree species, harvesting season, and the processing method (Bussing and Schietzel 1999; Jolanta and Przemysław 2016). With gradual improvement of analytical techniques, multitude class of phytochemicals, viz., tannins, glycosides, flavonoids, coumarins, proteins, emodin, terpenoids, phenols, and reducing sugars have been analyzed in methanolic extract of *V. album* alkaloids (Ihegboro and Ebuehi 2012; Umoh et al. 2011). Further, research conducted on *V. album* shows it contains special class of peptides called thionins with multi-disulfide bonds that have been shown to elicit cytotoxic and antitumor effects (Guzmán-Rodríguez et al. 2015; Larsson 2004; Poojary and Belagali 2005; Segneanu et al. 2015). Various kinds of thionins investigated from mistletoe are viscothionin A1, viscothionin A2, viscothionin A3, viscothionin B, viscothionin C1, viscothionin D, viscothionin E, and viscothionin P1 (Guzmán-Rodríguez et al. 2015; Larsson 2004), while glycoprotein-mistletoe lectin I, II, and III, protein-viscotoxin; polysaccharides-galacturonan, arabinogalactan, and alkaloids have been reported as active phytoconstituents (Loeper 1999).

Phytochemical analysis of *V. album* has also revealed presence of many classes of secondary metabolites, namely, cyclitols, phenylpropanoids, triterpenes, phytosterols, cyclic peptides, and flavonoids (Bussing 2004). 4,5,4′-trihydroxy-3,3′-iminodibenzoic acid and 4,5,4′,5′-tetrahydroxy-3,3′-iminodibenzoic acid are two novel aminoalkaloids that have been recently isolated and characterized from mistletoe (Amer and Fossen 2012). Phytochemical analysis indicated berries to be rich source of polysaccharides that are composed of rhamnogalacturonans, arabinogalactans, and lesser amounts of xyloglucans (Edlund et al. 2000). Further studies undertaken on phytochemical examination of *V. album* leaf extracts have demonstrated diverse range of phytoconstituents such as tannins, flavonoids, catechols and alkaloids. The specificity of chemical diversity of plant parts is dependent on specificity of host plant shown by a *V. album* that later determines its medicinal potential as well (Uwemedimo et al. 2011) (Table 12.1). Research has shown variation of season, and host plants significantly affect the phenolic content and antioxidant capacity of *V. album* extract (ÖnayUçar et al. 2006; Vicas et al. 2009) (Table 12.1).

12.3 Ethnomedicinal Uses of Mistletoe

Over ages, there is well-documented use of mistletoe in curing various health problems such as hypertension, nervous tension, infertility, cancer, diabetes, dermatitis, menopause, headache, and asthma (Grossarth and Ziegler 2007; Obatomi et al. 1994). Traditionally, *V. album* is used for prevention of aging, inflammation, headaches, epilepsy, tumor, plague, fertility problems, and as poison antidote (Chernyshov et al. 2007; Frazer 1920; Taiga 2013). In traditional practice, decoctions are made from whole plant by grinding and mixing with common salt to be used as laxative. However, in wound healing, plant is dried and burnt, and then ash is used on affected parts. Warm paste of the plant is used in cure for rheumatism and arthritis. Another preparation made from dried plant called poultice has been used for treatment of bone fractures. Many studies have also indicated that this plant is also best in treating migraine, epilepsy, abscess, and joint pain (Bhat et al. 2012; Gairola et al. 2014; Kapahi et al. 1993; Khan et al. 2004; Rather and Baba 2015). Various reports have documented prevalent traditional uses of mistletoe in many parts of the world. Extract obtained from *V. album* was given as treatment to diabetics in West Indies (Peters 1957). *Viscum album* tea is highly valued among Nigerians for its hypoglycemic and hypotensive potential which enables its use in treatment of strokes and to improve cardiovascular functions of body (Choudhary et al. 2010; Deeni and Sadiq 2002). Over a long use of 80 years, extracts of *V. album* have yielded positive results as complementary therapies for melanoma (Kienle et al. 2011; Kirsch 2007; Thies et al. 2008). About 73% people in Europe mostly from German nations have relied on mistletoe extracts as complementary alternative medicine in treatment of various cancers (Eustachi et al. 2009; Laengler et al. 2008; Molassiotis et al. 2005).

12.4 Pharmacological Roles of Mistletoe

Many studies suggested that mistletoe exhibits prompt antimicrobial, proapoptotic, and immunomodulatory effects (ÖnayUçar et al. 2006). Reports have also confirmed cardiotonic, hypoglycemic, and immunomodulating activities of mistletoe (Adeeyo et al. 2013; Antony et al. 2000; Eno et al. 2004; Gray and Flatt 1999; Hajto et al. 2011; Kuttan et al. 1990; Marvibaigi et al. 2014; Twardziok 2015; Valentiner et al. 2002). European countries have succeeded in commercial preparations of mistletoe extracts under the names (Iscador, Isorel, Eurixor, Plenesol, Vysorel, Lektinol, Helixor, etc.) as a complementary medicine in cancer treatment (Chernyshov et al.

Table 12.1 Phytochemical screening of *Viscum album* L. extracts from different host plants

Metabolite	Host plant	Compound isolated	Plant part	Extraction solvent	References
Proteins	*Quercus robur*, *Malus domestica*, *Pinus longifolia*, *Abies alba*	Lectin, viscotoxins	Stem and branches	Ethanol	Chaitrali et al. (2016)
Alkaloids	Hybrid poplar	4,5,4′-trihydroxy-3,3′-iminodibenzoic acid 4,5,4′,5′-tetrahydroxy-3,30-iminodibenzoic acid	Stem and branches	Methanol	Amer and Fossen (2012)
Flavonoids	*Taxinus pennsylvanica* *Sorbus aucuparia* *Populus nigra* *Tilia cordata*	Taxifolin	Berries	Methanol ans H_2O	Pietrzak et al. (2017)
	Malus domestica *Populus nigra* *Sorbus aucuparia* *Populus nigra*	Quercetin Kaempferol Apigenin Luteolin Rhamnazin			
	Populus nigra *Taxinus pennsylvanica* *M. domestica* *Sorbus aucuparia* *Tilia cordata*	3-O-Methylquercetin			
	Populus nigra *Taxinus pennsylvanica* *M. domestica* *Sorbus aucuparia*	Sakuranetin			
	Populus nigra *Taxinus pennsylvanica* *Malus domestica* *Sorbus aucuparia* *Tilia cordata*				
Phenyl-propanoids	*Armeniaca vulgaris*	Syringing, coniferin, Kalopanaxin D	Whole plant	Ethanol	Orhan et al. (2002)
Phytosterols	*Malus domestica*	Campesterol, stigmasterol γ-Sitosterol β-Sitosterol α-Tocopherol β-Amyrin Lupeol Olean 1,2-en-3-ylacetate Lupenyl acetate	Leaves and berries	Ethanol and H_2O	Daliborca et al. (2016)

(continued)

Table 12.1 (continued)

Metabolite	Host plant	Compound isolated	Plant part	Extraction solvent	References
Terpenoid	*Malus domestica*	β-Amyrin Lupeol Olean 1,2-en-3-ylacetate Lupenyl acetate	Leaves and berries	Ethanol and H_2O	Daliborca et al. (2016)
Fatty acid	*Malus domestica*	Oleic acid, cis-11-eicosenoic acid	Leaves and berries	Ethanol and H_2O	Daliborca et al. (2016)

2007; Frazer 1920; Hajto et al. 2011; Taiga 2013; Twardziok 2015; Vicas et al. 2012). Traditional healers use various pharmacological components of mistletoe for many ailments like tannins which due to their ability of binding with proteins have been used in cure of diarrhea and bleeding caused by cuts. Retention of nitrogen due to anabolic steroids helped in treatment of protein degenerative diseases like osteoporosis (Adachi and Takayanagi 2008). Besides various components of *V. album*, phenolic components being ubiquitous and indispensable plant secondary compounds have also been investigated especially for their antagonistic effects in cancer as well as for diverse biological roles as antiviral, antioxidant, and anti-inflammatory agents (Batra and Sharma 2013; Rosa et al. 2016). These significant biological activities of phenolics are partly explained by their free radical scavenging and metal chelating activities (Carbonaro et al. 1996; Okuda et al. 1992; Rice-Evans et al. 1996) (Fig. 12.1).

12.5 Antioxidant Activity of Mistletoe

It has been demonstrated that mistletoe contains antioxidant phytochemicals such as flavonoids quercetin and quercetin methyl ethers (Haas and Bauer 2003). Quercetin and flavonol are known to possess strong antioxidant activity (Materska 2008). Studies conducted by Senguel et al. have revealed that *V. album* had highest antioxidant activity than other medicinal plants (Sengul et al. 2009). Digallic acid and o-coumaric acid are phenolic acids which in free or glycosylated forms also show antioxidant activity (Luczkiewicz et al. 2001). They have ability to form stable resonance phenoxy radicals which explains their strong antioxidant activity (Graf 1992). Studies have showed that alcoholic extract of *V. album* had greater antioxidant activity that vitamin E. The strong antioxidant activity is due to presence of hydroxyl groups in the flavonoid structures (Trifunschi et al. 2016). Vicas et al. (2012) investigated the antioxidant capacities of *V. album* of leaves and stem wherein they concluded the highest antioxidant activity was obtained in ethanolic extracts. Further the differences in antioxidant potentials of stem and leaves can be attributed to many factors like season, climate, and temperature which determine the accumulation of antioxidant components in plant tissues.

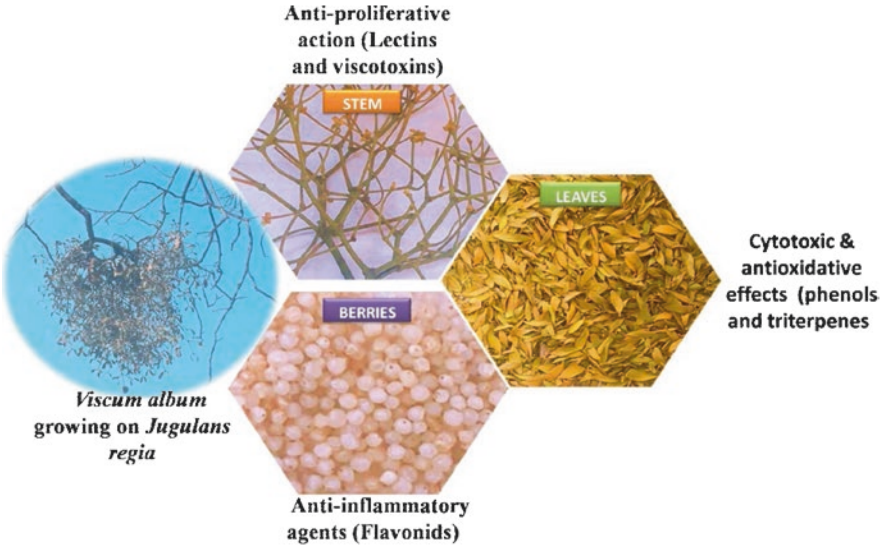

Fig. 12.1 Pharmacological properties of active components in different parts of *Viscum album* hosted by *Juglans regia L*

12.6 Antimicrobial Activity of Mistletoe

Viscum album extract was analyzed for its constituent molecules followed by its screening for antimicrobial activity using five gram-negative bacterial strains. The results showed active phytochemicals of plant are responsible for antibacterial activity assay. The crude extract was observed to show considerable zone of inhibition against *Pseudomonas aeruginosa* which confirms this plant has effective antimicrobial potential against some resistant forms of bacteria (Kusi et al. 2015). The leaves, stem, and fruits of *V. album* are known to be good sources of active phytoconstituents such as alkaloids, flavonoids, saponins, steroids, glycosides, phenols, etc. These active plant molecules are further explored for their roles as antimicrobial agents by disc diffusion methods. Butanol, ethyl acetate, aqueous, as well as crude extracts showed plausible effects against both gram-positive and gram-negative species (Shah et al. 2017). Other studies also reported this plant as potent source of natural antibiotics which was further demonstrated by extraction of stems and leaves using various organic solvents. Among all organic extracts of plant, ethyl acetate and methanolic crude extracts showed promising antimicrobial activities against gram-positive and gram-negative bacteria. Also crude extracts were also effective against gram-negative than gram-positive bacteria (Muhammad et al. 2011).

12.7 Hypoglycemic Properties of Mistletoe

There is report of increased attention gained by African mistletoe for its use to treat diabetes mellitus in folk medicine (Ibatomi et al. 1994). Hypoglycemic activity of *V. album* aqueous extract was investigated on allonized male Wistar rats. After administering extract, the blood glucose levels were found to be decreased using glucose oxidase test. ELISA indicated the increased serum insulin levels (Mohammad et al. 2011). In other studies on experimental rats, both aqueous and alcoholic leaf extracts of *V. album* showed hypoglycemic effects (Chikezie and Iheanacho 2014).

12.8 Anti-inflammatory Functions of Mistletoe

The anti-inflammatory role of *V. album* preparation was shown by its inhibitory action on cyclooxygenase-2 (cox-2) by decreasing the half-life of cox-2 mRNA (Saha et al. 2015). This results in decreased levels of prostaglandin E2 (PG) E2. Accumulation of PG E2 leads to pro tumoral condition and also rise in dendritic cell-mediated T-cell proliferation (Banerjee et al. 2006; Maddur et al. 2015; Nakanishi and Rosenberg 2013; Trinath et al. 2013). *Viscum album*-mediated inhibition of PG E2 levels explains its antitumoral function. Carrageenan-induced hind paw edema model was used to examine the immunomodulatory effect of *V. album* flavonoids. Administration of mistletoe preparation Isorel (100 mg/kg) reduced the tumor growth and induced increased tumor necrosis with inflammatory response, edema, and destruction of the malignant tissue (Zarkovic et al. 1997).

12.9 Immunomodulatory Activity of Mistletoe

Saponins, viscotoxins, and lectins are known to possess dual immunomodulatory and anti-tumor effects (Chahar et al. 2011; Delebinski et al. 2012; Harmsma et al. 2004; Seifert et al. 2008; Zarkovic et al. 2001). These active phytoconstituents of *V. album* are known for their immunomodulatory functions especially in elevating the responses of interferon gamma (IFN-γ) (Lyu and Park 2007). *Viscum album* have regulatory effect on dendritic cells which are known for their role in regulating immune responses (Mac Keon et al. 2015; Maddur et al. 2014; Maddur et al. 2015; Steinman and Banchereau 2007; Walsh and Mills 2013). *Viscum album* is known to increase the expression of co-stimulatory molecules on dendritic cells and antigen-presenting cells. There is also finding which suggests its stimulating effects on proliferation of CD4+ T cells and release of inflammatory cytokines such as IL-6 and

IL-8 (Elluru et al. 2006). Research also revealed that administration of mistletoe extract increased the innate immune responses such as respiratory burst and phagocytic cells (Harikrishnan et al. 2011). Natural killer (NK) cells are known as potential candidates in directing the tumor cells for destruction (Chink 1997). Mistletoe lectins are found for their immunomodulatory functions on lymphocytes, natural killer cells, and macrophages (Elluru et al. 2006). Furthermore, the subcutaneous administration of *V. album* has elucidated to increase the number of natural killer cells and activated lymphocytes including CD4+ T cells, B cells, and cytotoxic T cells (Bussing et al. 1999). Aqueous extract of *V. album* is known to enhance the phagocytic activity of neutrophils and reduced the adhesion properties of epithelial cells. Also extract induced the proliferation of various immune cells like T cells and NK Cells (Fidan et al. 2008). Reports suggested use of *V. album* in treating various immunological disorders. Studies have also mentioned that VA Qu Spez is strong preparation of *V. album* inactivating dendritic cells and increasing the response of Th1 cells (Chaitrali et al. 2016).

12.10 Anticancer Activity of Mistletoe

The extracts of *V. album* are used as anticancer therapy over a long time. Dual pharmacological activities of mistletoe extract which causes cytotoxic effect to tumor cells and immunomodulatory effect to host immune cells against malignant cells facilitates its use in anticancer human medicine. Mistletoe proteins called lectins ML I, II, and III are believed to be active constituents in extracts used as anticancer therapy. However, later studies confirmed that these are not only lectins in aqueous extract, but various other phytochemicals also exert pharmacological effects and increase the overall activity of extract such as flavonoids, oligosaccharides, alkaloids, lipids, phytosterols, and triterpenes (Eggenschwiler et al. 2007; Ganguly and Das 1994; Heiny and Beuth 1994). Research also revealed that an outstanding anticancer activity of mistletoe is due to presence of various active components such as peptide derivative called viscumamide, lectins, viscotoxins, alkaloids, and thionins (Poojary and Belagali 2005; Amer and Fossen 2012; Larsson 2004; Guzmán-Rodríguez et al. 2015). Furthermore, presence of flavonoids and phenols that are well-known for their strong antioxidant and anti-inflammatory action explains its use against cancer (Dubios 2011). Research has also elucidated triterpenes as principal active components of *V. album* (Jagers et al. 2009). Studies have concluded that triterpenes have antitumor properties and topical application of lipophilic extract of mistletoe prevented various carcinomas (Kunz et al. 2013). Other plant metabolites with antitumor potential are jasmonic acid and its precursors 12, oxo-phytodienoic acid (Dorka et al. 2009).

12.11 Cardiovascular (Antihypertensive Effects) of Mistletoe

Mistletoe extract has been known to contain strong cardiotonic components such as cardiac glycosides which exhibit an important role in regulation of Na+/K+-ATPase pumps (Katz 1985). Aqueous extract of *V. album* has been used in both traditional and official medicines in treatment of hypertension (Jolanta and Przemysław 2016). Transient antihypertensive properties of extract have been ascribed to compounds such as choline, acetylcholine, and γ amino butyric acid (Samuelsson 1959; Winterfield and Kronenthaler 1942). In studies conducted on animal models, aqueous extract of *V. album* was found to elicit diverse effects on central nervous system which resulted in sedative, antipsychotic, antiepileptic activities. All these activities involved the GABAergic and dopaminergic systems (Gupta et al. 2012).

12.12 Toxicological Studies of Mistletoe

The varied medicinal use of *V. album* is explained by binding of its important chemical components, lectins and viscotoxins with different parts of cell. Lectins and viscotoxins are known to cause cytotoxicity (Kienle et al. 2003). Lectins are cytotoxic plant glycoproteins causing agglutination of cells resulting in inhibition of protein synthesis. Lectins are formed of two chains: chain A inhibits protein synthesis, and chain B is involved in release of lymphokines from lymphocytes on activation of macrophages. However, both chains inhibit the biological activities of leukocytes and platelets (Franz 1986). The most noticeable toxicity contributing components of mistletoe are viscotoxins, phoratoxins, lectins, and toxic alkaloids. Alkaloids are known toxic cellular agents (Aniszewski 2007). Plant toxins are not reported to cause much toxicity to humans, but some reports have shown ingestion of berries may result in vomiting, hypertension, bloody stools, and nausea (Bussing 2004). Recently, research has shown that clinical use of recombinant mistletoe lectins can cause reversible hepatotoxicity to certain extent (Kienle et al. 2011). The 3D structural analysis of viscotoxins has revealed its important phosphate-binding site (Debreczeni et al. 2003). The phosphate-binding site and bipolar nature of viscotoxins are indispensable for displaying their cytotoxicity in disruption of cell membrane or loss of membrane integrity, thereby causing toxicity, necrosis, and apoptosis of a cell (Bussing 2000). The cell-killing potential of mistletoe extract is correlated with type of host tree instead of mistletoe lectin and viscotoxin content (Bussing et al. 1997). The cytotoxicity mediated by killer cells was found to be increased by newly isolated viscotoxins such as VTA1, VTA2, and VTA3 (Tabiasco et al. 2002).

12.13 Other Uses

Mistletoe has find use as a preferable cattle feed for farmers during winters. It is also consumed by herbivores as fodder during period of fodder scarcity and drought (Umucahlar et al. 2007).

12.14 Conclusion

Viscum album is an ideal medicinal epiphyte hosted by number of host trees due to its semiparasitic mode of nutrition. It has been seen that leaves and twigs of *V. album* show difference in phenolic and flavonoid content depending on type of host plant. Some studies also showed that mistletoes from different trees harvested from different seasons showed differences in the antioxidant activity between stem and leaves due to environmental factors such as climate and temperature which can significantly affect the accumulation of the antioxidant components in the plant tissue. Literature has showed well-documented and an extensive use of the various parts of this plant in treatment of many serious health problems including cancer. Thorough anticancerous properties reported from the plant are partly explained by its cytotoxic and immunomodulatory constituents most noticeably viscotoxins and lectins, respectively. Hemiparasitic nutrition and occurrence of this plant throughout the season affect the phytochemical profile which later determines its pharmacological attributes. Further research needs to be undertaken to explore the meagerly understood active phytoconstituents for examining their underlying effect in particular health disorder.

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Chapter 13
Saffron: A Therapeutic and Prophylactic Nutrition for Human Population

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13.1 Introduction

Saffron (*Crocus sativus* L.) a perennial herb belonging to Iris family Iridaceae is cultivated from the times immemorial. The primary illustration dates back from 1600 to 1700 BC that was established on a frieze of Minos Fort in Crete, unfolding records plucking saffron (Algrech 2001). Vavilov indicated that it originated in the Middle East, even though other authors proposed Central Asia as well as islands of Southwest Greece (Tammaro 1989), and from these areas, its spread was found in India, China and Middle East countries. The cultivation of saffron differs from one region to another depending on various factors like cultural practices and climatic and edaphic conditions of that particular area. The average water requirement of the spice varies from 400 to 600 mm/year. Saffron is economically much more viable spice since it fetches higher price, both at national and international market, and presents a strong added value. Besides economic importance, it also plays a significant role in environmental and social domain. The harvesting of crop mobilizes a large workers especially females. For production of 1 kg of saffron, 150,000–170,000 flowers are required and about 400 working hours (Mzabri et al. 2019). This chapter will describe the importance of saffron in therapeutic use, role of phytochemicals generally found in this spice and other uses that give it specific popularity particularly in the field of medical sciences where there are not many available reports authenticating the use of this spice.

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13.2 Genetic Origin

The information regarding saffron ancestors is not yet clear; however, botanical studies revealed that *C. sativus* is a mutant population of *C. cartwrightianus*. *Crocus sativus* and *Crocus cartwrightianus* are morphologically indistinguishable; *Crocus sativus* flowers are twice as broad as those of *Crocus cartwrightianus* (Gresta et al. 2008). Grilli et al. (2001) conducted RAPD (random amplification of polymorphic DNA) investigation to scan the putative predecessors of *Crocus sativus* and reported that *Crocus cartwrightianus* is the nearest related species to *Crocus sativus*, trailed by *Crocus thomasii*. AFLP investigation (enhanced DNA section length polymorphisms) affirmed that the quantitative and subjective DNA attributes of both *Crocus cartwrightianus* and *Crocus thomasii* species are compatible with those of *Crocus sativus* (Zubor et al. 2004).

13.2.1 Botanical Description

Saffron (*C. sativus* L) is one of the exceptional plants in the world which belongs to the family Iridaceae. There are around 80 members of this family worldwide (Hagh-Nazari and Keifi 2007). This herbaceous plant (Fig. 13.1a) attains a height of 10 and 25 cm which arises from its corm. The corm is of variable size and is somewhat subovoid in shape. It has a large foundation and is surrounded by various dense spathes. The apical buds of each mother corm produce one to two flower-bearing corms and numerous small corms (non-flower-bearing) from lateral buds (Grilli et al. 2001). There are two types of roots found in saffron, viz. fibrous roots which arise at the base of mother corm and contractile roots which are formed at the base of lateral buds (Zubor et al. 2004) (Fig. 13.1b). Each bud produces 5–11 leaves (Fig. 13.1c), which are very thin, attaining a size of 1.5–2.5 mm of dark green colour. The leaves are 20–60 cm long with a whitish band in the internal part and a rib outwardly.

Fig. 13.1 *Crocus sativus* plant morphology: (**a**) saffron plant; (**b**) types of roots in saffron; (**c**) saffron leaves; (**d**) saffron flower

At the beginning of autumn and towards the end of September, the flowers of saffron begin to appear which are purplish and composed of six petals which are joined at the cylindrical long tube that emerges from the upper portion of the ovary (Fig. 13.1c). The flowers at the exterior end are sheltered by whitish membranous bracts. The ovary is inferior from which a thin style, 9–10 cm long, arises which in turn consists of a single stigma that is composed of three stigmas of intense red colour which are the most important economical part of the plant (Molina et al. 2003).

13.3 Main Phytochemical Components of Saffron

Chemical analysis of saffron reported presence of about 150 volatile and non-volatile compounds. Until now less than 50 constituents have been recognized (Winterhalter and Straubinger 2000). The three major biologically active compounds are crocin, a carotenoid pigment accountable for the spice's yellow-orange colour; picrocrocin, which provides flavour and bitter taste for the saffron; and safranal, which produces aroma to saffron (Fig. 13.2).

Crocin ($C_{44}H_{64}O_{24}$) is a naturally occurring carotenoid which is easily soluble in water. Compared with other carotenoids, it has a broader application as a dye in foods and medicines, owing to its high solubility. Picrocrocin ($C_{16}H_{26}O_7$) is the principal element that makes bitter taste of saffron which can be solidified by hydrolysis (Moghaddasi 2010). Safranal ($C_{10}H_{14}O$) is responsible for the flavour and aroma of

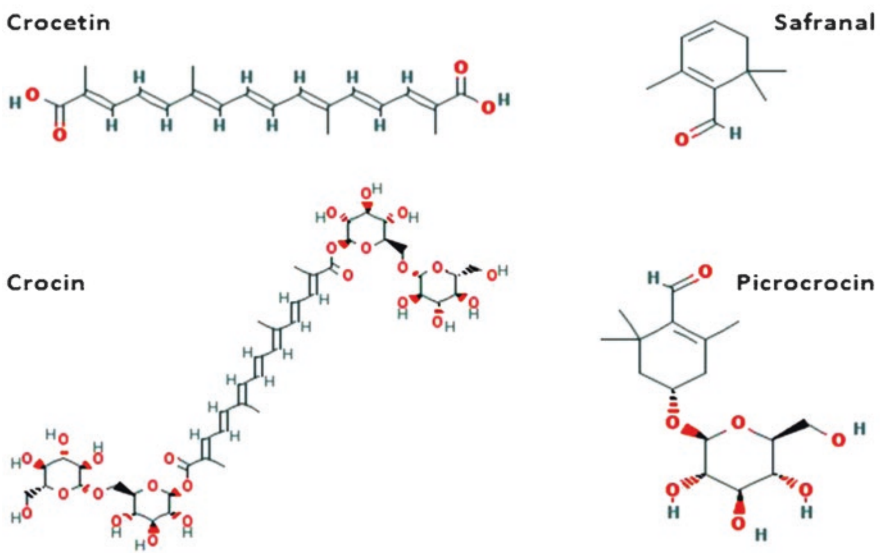

Fig. 13.2 The main biologically active compounds of saffron

the spice, and its concentration depends on the post-harvest handling of the saffron (Ait Oubahou 2009). Besides these three compounds, the major constituents in saffron are anthocyanins, flavonoids (such as kaempferol), vitamins, amino acids, proteins, starch, mineral matter and gums (Winterhalter and Straubinger 2000). It also contains many non-volatile active components many of which are carotenoids including zeaxanthin, lycopene and various α- and β-carotenes. There are 34 volatile components in saffron, which include terpenes, terpene alcohols and their esters (Liakopulou and Kyriakides 2002).

The three compounds (crocin, picrocrocin and safranal) determine the quality of saffron and provide the colour, taste and aroma of stigmas. The concentration of these compounds depends on the environment and cultural practices (Gresta et al. 2009). The post-harvest techniques like drying, extraction and stigma analysis determine the chemical composition of saffron samples (Kanakis et al. 2004; Tarantilis et al. 1995; Zareena et al. 2001). The quality of saffron is regulated by ISO 3632 standards which are directed at standardizing the classification of saffron globally and are updated every 3 years.

13.4 Nutritional Facts

Saffron is rich in vital minerals like zinc, magnesium, copper, calcium, potassium, iron, selenium and manganese. Besides these essential minerals, it also contains many important vitamins (Table 13.1).

13.5 Ethnomedical Importance of Saffron

Research's showed that saffron was used by Assyrians and Babylonians to treat dyspnoea, head problems, menstruation, delivery and painful urination. The earliest evidence of medicinal usage of saffron is in the Assurbanipal library, in inscriptions from the twelfth century BC (Mousavi and Bathaie 2011). It is used in Ayurveda to treat chronic illnesses such as asthma and arthritis. Also, it is used to treat colds and cough. Ayurvedic, saffron-containing drugs are used to cure acne and multiple skin disorders. Ancient texts have shown that the herb is useful as an aphrodisiac; therefore, it stimulates reproductive system. The stigmas of saffron are usually used as folk medicine as well as traditional medicine in India and China, respectively (Bhandari 2015). Saffron was a royal colourant in ancient Greece and was used as a perfume in saloons, courts, theatres and bathrooms. Besides herbal cure properties of crocus, many great personalities like Hippocrates (5–4th century BC), Erasistratus (2nd–3rd century BC), Diokles (3rd century BC) and Dioscorides (1st century AD) used this golden spice for therapeutic purposes like treating eye diseases (painful eye, corneal disease and cataract, purulent eye infection), earache, toothache, ulcers (skin, mouth, genitalia) and erysipelas; they believed that it has styptic and sedative features (Mousavi and Bathaie 2011).

Table 13.1 Saffron (*Crocus sativus*), nutritional value per 100 g

Principle	Nutrient value
Energy	310 Kcal
Carbohydrates	65.37 g
Protein	11.43 g
Total fat	5.85 g
Cholesterol	0 mg
Dietary fibre	3.9 g
Vitamins	
Folates	93 μg
Niacin	1.46 mg
Pyridoxine	1.010 mg
Riboflavin	0.267 mg
Vitamin A	530 IU
Vitamin C	80.8 mg
Electrolytes	
Sodium	Sodium 148 mg
Potassium	Potassium 1724 mg
Minerals	
Calcium	111 mg
Copper	0.328 mg
Iron	11.10 mg
Magnesium	264 mg
Manganese	28.408 mg
Phosphorus	252 mg
Selenium	5.6 μ g
Zinc	1.09 mg

13.6 Biological Activities of Saffron

The biochemical components of saffron particularly crocin have many therapeutic properties such as antitumour, antioxidant, anxiolytic, neuronal protective and anti-ischaemic and act as a protectant against various DNA damage activities (Mohajeri et al. 2010). These components are possible anticancer agents as well as hypolipidaemic and antioxidant agents, protect the brain against unnecessary oxidative stress, protect the ischaemic heart, help to mitigate neurological deficiencies, have important anti-proliferation effects on human prostate and colorectal cancer cells, have aphrodisiac function, are immunomodulatory, improve inhibition of Dalton's lymphoma, are helpful in diabetic neuropathy treatment, affect glucose uptake and insulin sensitivity and have many more activities. The sub-component of crocin i.e. crocetin, enhances alveolar oxygen transport and increases pulmonary oxygenation, obstructs skin tumour promotion and avoids oxidative damage, has cardiovascular protective effects, improves hypertension-mediated acetylcholine-induced vascular relaxation, acts as a powerful antitumour agent, inhibits angiogenesis mediated by the endothelial growth factor, etc. (Agnihotri and Aperito 2015).

Crocetin has several positive effects although some work to the contrary has shown that crocetin may be a teratogenic agent. Crocetin's high dose induced miscarriage which may be dangerous (Mohajeri et al. 2007). Crocin has a lot of biological activity; even so crocin (200 mg/kg, i.p.) has not shown anticonvulsants which means crocin is a good antidepressant (Ahmad et al. 2012). Crocin has shown numerous pharmacological effects on the nervous system, such as anti-anxiety function, therapeutic effects, aphrodisiac powers and thinking and performance improvement (Alavizadeh and Hosseinzadeh 2014). Saffron slightly reduces some haematological parameters like red blood cells, haemoglobin, haematocrits and platelets. It improved sodium, nitrogen urea in the blood, creatinine, etc. (Modaghegha et al. 2008).

13.7 Possible Mechanism of Action of Saffron and Its Ingredient in Disease Prevention

The stigma of the saffron is blend of different segments, and such constituents have therapeutic significance in the well-being of human beings occurring through different natural pathways. The accurate component by which saffron and its constituents exhibit the remedial job in disease anticipation is yet to be completely explained. Lab-based exploration has uncovered that saffron constituents through the action of different compounds are engaged with free radical detoxification. Additionally, these segments diminish the lipid peroxidation and upgrade the cell reinforcement status. The ethanolic fraction of stigma demonstrated the most noteworthy cancer prevention action that may be perceived because of the presence of different phenolics and flavonoids (Amin et al. 2014). Other test information advocates that saffron helps in the decrease of irritation through the hindrance of cyclooxygenase protein action. Saffron eases neuropathic torment by means of decrease of pro-inflammatory cytokines, cancer prevention agent and apoptotic pathways (Xu et al. 2009), and in vitro examination has uncovered that crocin has double inhibitory action against COX-1 and COX-2 enzymes (La Barre 1961). Saffron additionally shows a significant role in the tumour avoidance, and a few discoveries advocate that saffron and its constituents initiate apoptosis or change the proportion of bcl2/bax lastly restraining the turn of events and movement of tumour.

13.8 Pharmacological and Prophylactic Activities of Saffron

Saffron is known for a very huge number of potential uses and activities. The pharmacological properties of saffron components, like safranal, crocin and crocetin, are due to their chemical structure. The studies based on in vitro experiments showed the most important pharmaco-active properties of saffron that appeared in the Chemical Abstracts between 1925 and 1999, however, these old information were observational and their medical significance remains doubtful. Safranal was proposed as one of the gastrointestinal chemical function modifiers. It can stimulate appetite and prevent gastrointestinal atonia through this action (Giaccio 2004). Saffron has also shown therapeutic action on the female genitals. Safranal may be helpful in the treatment of respiratory, mostly chronic bronchitis, with respect to its major components. Owing to its wide dissemination to the lungs, safranal sedates coughing, functioning as an anaesthetic on the alveoli's vagal nerves (Lequerc 1973). Crocin has been suggested for painful relief of dysmenorrhoea because it may reduce uterine contractions. Picrocrocin tends to have a sedative effect on lumbar pressure and spasms (Frank 1961). However, crocetin has been linked to the most extraordinary results of saffron, as it is a material capable of raising oxygen transport speed and diffusivity, both in vivo and in vitro. This ability to carry oxygen makes crocetin a useful therapeutic candidate in different situations, such as atherosclerosis, alveolar hypoxia, haemorrhages, fermentation and cell reproduction. The detailed pharmacological and possible therapeutic applications of saffron are given under the following heads.

13.8.1 Antidepressant and Anxiolytic Properties

Aqueous and ethanol extracts of saffron stigmas have been found to have antidepressant effects in mice, primarily attributed to saffron and crocin acting by inhibiting dopamine, norepinephrine and serotonin intake (Karimi 2001). These results were further confirmed by Wang (2010) who demonstrated behavioural models that revealed antidepressant effect of aqueous saffron extract is attributable to crocetin. It has been shown by Pitsikas (2008) that crocin @ 50 mg/kg has a positive effect on mice behaviour while having anxiolytic effect in rats. Hosseinzadeh and Noraei (2009) showed that safranal specifically can bind to some subtypes of benzodiazepines (BZ1, BZ2 and BZ3).

In clinical trials, 20–30 mg of saffron extract was used for the treatment of mild to moderate depression and was compared with chemically synthesized molecules, such as fluoxetine and imipramine (Akhondzadeh 2005; Hosseinzadeh et al. 2012a; Noorbala 2005). The comparative evaluation showed that saffron was similarly successful in mild to moderate depression (antidepressant action) and epilepsy (anticonvulsant action) without inducing its side effects as chemically synthesized medicines. However, clinical trials have not been conducted on dose-related toxicity and adverse effects of saffron extract use as an alternative in the treatment of depression (Alavizadeh and Hosseinzadeh 2014). Hosseinzadeh et al. (2012a) studied saffron and its effect on recognition and spatial memory after chronic cerebral hypoperfusion in Wistar rats. Their study revealed that saffron extract and crocin improve spatial cognitive abilities following chronic cerebral hypoperfusion and the effect may be associated with these antioxidant compounds (Hosseinzadeh et al. 2012a). It has been reported that saffron and its components possess antidepressant and anxiolytic effects (Maes et al. 2011).

Depression is characterized by a reduction in key antioxidants such as vitamin E and zinc and increased oxidative stress and production of ROS (reactive oxygen species) (Hannestad et al. 2011). Increased amounts of inflammatory cytokines such as tumour necrosis factor-α (TNF-α), beta-IL-1 (IL-β) and interleukin-6 (IL-6) induce severe depressive disorder (Akhondzadeh et al. 2004). Saffron has antioxidant, anti-inflammatory, serotonergic, hypothalamic-pituitary-adrenal (HPA) axis-modulating and neuroprotective effects, making it a strong choice for depression treatment (Asdaq and Inamdar 2010a). The main saffron metabolites – crocin, crocetin and safranal – serve as strong antioxidants. In animal studies the components of saffron were effective in increasing the levels of antioxidant enzymes (Wang et al. 2012) and also inhibited the generation of reactive oxygen species resulting in protection against oxidative stress (Rios et al. 1998). Crocin inhibits the take-up of dopamine and norepinephrine. Safranal impacts serotonin intake (Modabbernia et al. 2012). Saffron has been shown to be successful in managing erectile dysfunction associated with fluoxetine in male patients (Kashani et al. 2013) as well as in the treatment of sexual dysfunction induced by fluoxetine including lubrication, arousal and pain (Akhondzadeh et al. 2010). Saffron's safety and efficacy has been assessed for the treatment of disorders such as anxiety, obsessive-compulsive disorder and Alzheimer's disease in mild to moderate form. It inhibits aggregation and deposition of amyloid β in human brain (Maggi et al. 2011).

13.8.2 Radical Scavenging and Antioxidant Activity of Saffron

Crocetin, crocin, safranal, phenolic and flavonoid compounds from dried *Crocus sativus* L. has important intracellular free radical scavenging behaviours and defend cells and tissues from oxidation (Amin et al. 2011). It is considered that free radical scavenging activity involves anti-ageing, anti-inflammatory, anti-carcinogenic, anti-depressant, anxiolytic, aphrodisiac and also memory-enhancing properties (Asdaq and Inamdar 2010a). The phenolic substance and movement of ethanolic concentrate of saffron was higher when contrasted with the fluid concentrate (Shen and Qian 2006). The water/methanol (50:50, v/v) concentrate of *C. sativus* marks of disgrace displayed great cell reinforcement properties. The concentrate restrained Abeta fibrillogenesis in human cerebrum. Saffron had defensive impact against hyperlipidaemic appearance in liver tissue. Liver tissue which might be because of abatement in the raised lipid levels and oxidative radicals during hyperlipidaemia with enlargement of cell reinforcement exercises (Hosseinzadeh and Sadeghnia 2005). The cardio-defensive impact of crocetin is accounted for to be identified with tweak of endogenous cancer prevention agent enzymatic exercises (Farahmand et al. 2013). Crocetin treatment was successful in diminishing the substance of lipid peroxidation (LPO). An expansion in GSHP-x (glutathione peroxidase) and SOD (superoxide dismutase) action in cardiovascular hypertrophy was additionally watched. Defensive impact of safranal, a monoterpene aldehyde, on various markers of oxidative harm in hippocampal tissue from ischaemic rodents has been accounted for (Rahmani et al. 2017). It could fill in as a powerful possibility to smother age-instigated harm by securing against oxidative pressure and expanding cell reinforcement safeguards (Baba et al. 2015).

The imbalance between the production of reactive oxygen species (ROS) and the level of antioxidants is closely linked to disease pathogenesis. Improving the level of antioxidants or decreasing the level of reactive species is maintained through the antioxidant properties of plants or their derivatives. Natural products or medicinal plant derivatives typically comprise specific components like flavonoids that display a crucial function as antioxidants and free scavenging operation radicals. Numerous in vivo and in vitro studies have shown that *Crocus sativus* has important antioxidant activity (Table 13.2) (Karimi et al. 2010).

Saffron antioxidant activity was observed in the stigma extract, and such studies showed role in reducing chlorophyll injury, lipid peroxidation and protein oxidation (Assimopoulou et al. 2005). Similarly, other findings have confirmed that saffron stigma contains superior antioxidant activity (Goli et al. 2012). Previous findings have shown that active and inactive constituents of saffron extract have high antioxidant activity (Asdaq and Inamdar 2010b) which has been shown in saffron petal extract (Makhlouf et al. 2011). Another study has shown that saffron component such as crocin has high antioxidant activity (Ochiai et al. 2004). Lebanon-based finding showed that saffron significantly reduced lipid peroxidation as well as increased superoxide dismutase activity compared to control group (Papandreou et al. 2006). Crocin, a saffron constituent, showed a role in lipid peroxidation inhibition and restored SOD activity (Sheng et al. 2006), and *Crocus sativus* stigmas contain more antioxidant activity than tomatoes and carrots.

13.9 Saffron's Effect on Hyperlipidaemia and Hypertension

In several human and animal studies, crocin has been reported to be an effective hypolipidaemic agent. Crocin decreased cholesterol levels in hyperlipidaemic rats by feeding excessive cholesterol for 2 months (He et al. 2005). In another research, crocin was found to have significant triglyceridemic and cholesterolemic reduction effects in coils with coronary artery disease (He et al. 2007). The study has verified that crocetin can lower serum, total cholesterol and malondialdehyde levels and prevent nitric oxide reduction in the serum of hyperlipidaemic-diet quails (Joshi et al. 2012). Sheng et al. (He et al. 2005) indicated in the elucidation of crocin's hypolipidaemic mechanism that crocin inhibited the absorption of dietary fat and cholesterol. They stated that this inhibition was very much correlated with fat hydrolysis. Moreover, the adjusted fat-balance approach showed that crocin improved excretion of faecal fat and cholesterol in rats, but had no influence on bile acid removal. Data from the in situ loop method and enzyme assay showed that crocin could not directly inhibit cholesterol absorption from rat jejunum, but could selectively block the pancreatic lipase activity as a competitive inhibitor. These find-

Table 13.2 Pharmacological activities of saffron and its constituents

Aim of study	Biological activity	Finding/outcome	References
Evaluation of antioxidant activity of stigma extract	Antioxidant	Saffron stigma showed antioxidant activity	Kanakis et al. (2004)
Measurement of antioxidant	Antioxidant	Antioxidant activity has been observed	Amin et al. (2014)
Effects of crocin and safranal on local inflammation	Anti-inflammatory	Study finding reported that crocin and safranal showed role in the suppression of inflammatory pain responses and decreased the number of neutrophils	Karimi (2001)
Anti-inflammatory effects of crocin	Anti-inflammatory	Crocin, a constituent, showed anti-inflammatory effects and modulates inflammatory processes	Gresta et al. (2008)
C. sativus extract tested against bacteria	Antibacterial	Strong activity of *C. sativus* extract against bacteria and fungi was noted	Wang (2010)
Evaluation of protective effects of extract against hepatotoxicity	Hepatoprotective	Finding demonstrated that petals ameliorate acute liver injury	Akhondzadeh (2005)
Effect of safranal against nephrotoxicity	Protective effect against nephrotoxicity	Safranal has a protective effect against nephrotoxicity	Maes et al. (2011)
Cardioprotective effect of saffron and safranal	Cardioprotective	Result revealed that myocardial injury preserved nearly normal tissue architecture with saffron or safranal pretreatment	Akhondzadeh et al. (2004)
Inhibitory action on AChE via saffron extract and its constituents	Inhibitory action on AChE	Result indicated that saffron extract showed moderate AChE inhibitory activity	Maggi et al. (2011)
Saffron, crocin and safranal effects on the blood levels of fasting glucose, HbA1c and liver/kidney function tests	Anti-hyperglycaemic	Result demonstrated that saffron extract, crocin and safranal significantly reduced the fasting blood glucose levels but significantly increased the blood insulin levels in the diabetic rats compared with the control diabetic rats	Hosseinzadeh and Sadeghnia (2005)
Effects of saffron and crocin on body weight, food intake and blood leptin levels	Anti-obesity and anorectic	Result concluded that saffron has anti-obesity and anorectic effects and lowered leptin levels	Baba et al. (2015)
Evaluation of anti-obesity effects of saffron and crocin	Anti-obesity	Result showed that saffron extract significantly decreased food consumption in obese rats. Furthermore, crocin showed a significant decrease on rate of body weight gain in rats	Karimi et al. (2010)

(continued)

Table 13.2 (continued)

Aim of study	Biological activity	Finding/outcome	References
Aphrodisiac activities of stigma and safranal and crocin	Aphrodisiac activities	The results revealed that saffron extract and its constituents show aphrodisiac activity	Assimopoulou et al. (2005)
Evaluation of anxiolytic and hypnotic effect of saffron extract, crocin and safranal	Anxiolytic properties	Results confirmed safranal at higher doses demonstrated anxiolytic effects whereas crocin did not show anxiolytic properties	Asdaq and Inamdar (2010b)
Effects of saffron petal extract on blood parameters and the immune system	Immuno-stimulatory	Saffron petal extract use causes an increase in antibody response	Ochiai et al. (2004)
Evaluation of anticonvulsant of safranal and crocin	Anticonvulsant	Finding confirmed that safranal reduced the seizure duration and delayed the onset of tonic convulsions	Sheng et al. (2006)

ings suggest that crocin has lipid-degrading properties by inhibiting pancreatic lipase, resulting in fat and cholesterol malabsorption (He et al. 2005).

Hypertension is the common cardiovascular disease and is a major public health disorder in both developed and developing countries. Saffron produces a crocetin chemical that reduces blood pressure (Imenshahidi et al. 2013a). Researchers study the effects of chronic exposure to saffron stigma aqueous extract on normotensive blood pressure and deoxycorticosterone acetate (DOCA)-salt-induced hypertensive rats (Milajerdi et al. 2015). They conclude that in chronic application, the aqueous saffron extract has an antihypertensive and normalizing effect on blood pressure (Milajerdi et al. 2015). Saffron extract and its components, mainly crocin and safranal, have some modulating characteristics of the blood pressure. Nonetheless, further research need to be performed to identify successful dosage and action mechanisms (Rahmani and Aldebasi 2016).

13.10 Antitumour Activity

It is well established that medicinal plants have a therapeutic function in the treatment of various diseases, including tumour (Nair et al. 1991). Saffron and its active constituents play a significant role in the prevention of tumour growth and progression (Table 13.2). Numerous studies focused on animal models in this regard indicated that saffron plays a part in the prevention of various forms of tumour. Oral administration of saffron extract reveals improved lifespan of sarcoma-180-, Ehrlich ascites carcinoma- and Dalton's lymphoma ascites tumour-bearing mice (Magesh et al. 2006). Later studies have shown that crocetin, a saffron element, intensively reversed the pathological modifications found in cancer animals (Feizzadeh et al. 2008), and other reports have suggested that saffron aqueous extract has inhibitory effects on the development of both transitional cell carcinoma and regular cell lines (Salomi et al. 1991a). Anti-proliferative effects of saffron extract and individual constituents such as crocin were tested on different malignant and non-malignant cell lines of the prostate cancer. Based on these results, crocin decreased proliferation of all malignant cells, whereas non-malignant cells were not affected at all (Aung et al. 2007a). Furthermore, another experiment to explore the chemopreventive influence of aqueous saffron was conducted. The findings of the studies showed that saffron ingestion prevented the development of skin papillomas and simultaneously decreased their size in animals (Wang et al. 1991). In addition, the function of saffron in inducing cytotoxic and apoptotic effects in the lung cancer cells was examined. Results of the study revealed that proliferation of these cells decreased in a dose- and time-dependent manner after saffron treatment (Sun et al. 2013). Crocin inhibited both the weight of the tumour and the size of xenografts in nude mice (Table 13.3). In addition to this, expression of Bcl-2 was inhibited and the expression of Bax was increased in xenografts (Bakshi et al. 2010).

13.11 Chemopreventive Role of Saffron

Crocetin is the primary metabolite in crocins. It inhibits invasiveness of breast cancer cells of MDA-MB-231 by downsetting the expression of metalloproteinase (Bakshi et al. 2010). Dimethylcrocetine, a saffron chemopreventive drug, blocked tumour development by blocking associations between DNA and proteins (Zhang et al. 2013). Saffron's chemopreventive function may be traced to numerous phytochemicals such as terpenes, flavonoids, anthocyanins and carotenoids present in stigma that are reported to have anti-inflammatory, radio-protective, anti-carcinogenic and antitumour properties. It also prevents both DNA and RNA synthesis in HeLa cells originating from a cervical epithelioid carcinoma (Tanaka et al. 2012). Initiation/promotion of skin tumours induced by 7,12-dimethylbenz[a]anthracene (DMBA) in mice is inhibited by the saffron extract (Gutheil et al. 2012). Saffron induced apoptosis in concentration-dependent mode in A549 cells. It can be regarded as a successful chemotherapy agent against lung cancer (Molnar et al. 2000). Alone the crocin and DNA vaccine have been used as antitumour agents in different treatments (Chryssanthi et al. 2011). The dose-dependent inhibition of gastric cancer was observed in rats treated with the aqueous extract of saffron (Nair et al. 1995) and against hepatic cancer by apoptosis induction, oxidative reduction, destruction of inflammatory markers and cell proliferation (Abdullaev and Frenkel 1992; Salomi et al. 1991b). The function of carotenoids in cancer prevention has been widely documented by controlling cancerous growth via multiple mechanisms such as control of cell development, enhancement of cell differentiation, metabolic modulation of carcinogens, immune modulation, stimulation of cell-to-cell gap junction contact, impediment of growth factor signalling

Table 13.3 Antitumour activity of saffron and its ingredients

Aim of study	Tumour/cell type	Finding	References
Antitumour activity of saffron	Sarcoma-180, Ehrlich ascites carcinoma, and Dalton's lymphoma	Study finding demonstrated that oral administration of extract increased the lifespan of S-180-, EAC-, DLA tumour-bearing mice	Imenshahidi et al. (2013a)
Cytotoxic effect of extract of saffron	Transitional cell carcinoma	Study concluded that saffron extract has inhibitory effects on the growth of both TCC and normal cell lines	Rahmani and Aldebasi (2016)
Anti-proliferative effects of *Crocus sativus* extract and crocin	Colorectal cancer	Data from this study demonstrated that *Crocus sativus* extract and crocin significantly inhibited the growth of colorectal cancer cells	Magesh et al. (2006)
Anti-proliferative effects	Hepatocarcinoma	Study finding revealed that telomerase activity of HepG2 cells decreases after treatment with crocin	Salomi et al. (1991a)
Anti-proliferative effects of saffron extract and crocin	Malignant and non-malignant prostate cancer cell	The study concluded that both saffron extract and crocin can inhibit cell proliferation and arrest cell cycle progression	Aung et al. (2007a)
Cytotoxic and apoptosis induction	Prostate cancer	Finding based on result demonstrated a prostate cancer cell line to be highly sensitive to safranal-mediated growth inhibition and apoptotic cell death	Sun et al. (2013)
Chemopreventive effect of aqueous saffron	Skin carcinoma	Study result concluded that saffron inhibits skin carcinoma in mice when treated early	Wang et al. (1991)
Potential of saffron to induce cytotoxic and apoptotic effects	Lung cancer cells	Finding demonstrated that proliferation of the A549 cells was decreased after treatment with saffron	Sun et al. (2013)
Designed to elucidate apoptosis induction by crocin	Human pancreatic cancer	Crocin induced apoptosis and G1-phase cell cycle arrest	Zhang et al. (2013)

pathways and activation of apoptosis and retinoid-based signalling (Samarghandian et al. 2011). Saffron is an important chemopreventive agent owing to its bioactive molecules, crocin, crocetin, picrocrocin and safranal (Fig. 13.2) being the main anti-carcinogenic, antimutagenic, and immunomodulatory components (Bathaie et al. 2013a; Khavari et al. 2015).

Crocin delayed and reduced papilloma formation on a two-stage carcinogenesis (Konoshima et al. 1991; Li et al. 2015). Therefore, it can be used as a potential candidate for a safe drug against skin cancer. Crocetin has a chemical preventive function against oesophageal cancer by inhibiting cell proliferation, preventing the development of the cell cycle between the step S and G2, causing programmed cell death by increasing the production of bax proapoptotic protein and inhibiting the movement of carcinoma cells (Chryssanthi 2007). As an anticancer agent, crocetin induces its chemopreventive action via two distinct pathways – suppression of cell proliferation and activation of apoptosis. Inhibition of cell proliferation is dependent on concentration, and time-dependent on apoptosis induction (Alavizadeh and Hosseinzadeh 2014). Crocetin's showed dose-dependent inhibitory impact on cell proliferation (Mousavi et al. 2009) and caspase-dependent pathway through improved expression of Bax protein signifying its possible use as a chemopreventive agent for treating breast cancer (Samarghandian et al. 2014). Safranal is considered a potential therapeutic agent as it inhibits the proliferation of malignant cell line by apoptosis and inhibits cell growth by inducing apoptosis in the neuroblastoma cell line (Samarghandian et al. 2015). It can effectively protect the susceptible aged brain against oxidative damage by increasing the levels of GSH, SOD and GST supplemented by reducing lipid peroxidation (Bajbouj et al. 2012), enlightening that crocetin, crocin and safranal can be considered as a potential chemotherapeutic drug or as a cancer chemotherapy sensitizer.

Saffron causes DNA disruption and apoptosis in colorectal cancer cell lines and thereby reduces the chance of new cancer formation (Aung et al. 2007b). Saffron or its active constituents in culture may reduce the proliferation of some human cancer cells. Crocin considerably inhibited the growth and proliferation of colorectal cancer cells (D'Alessandro et al. 2013). The major constituent of saffron (crocin) tempted apoptosis in prostate cancer which repressed cell proliferation and arrested progression in the cell cycle (Dhar et al. 2009). A related proliferation limitation has been shown in culture of breast cancer cells. The proliferation of MDAMB231 and MCF-7 cells was significantly inhibited by constituents of saffron, transcrocin-4, crocetin and saffron extract (Mousavi et al. 2009). There was a significant regression of growth in in vivo pancreatic tumours due to apoptosis induced by crocetin treatment, and cell proliferation was inhibited (Hoshyar et al. 2013). Crocin might induce apoptosis in cancer cells where apoptosis depended on caspase activation by dose- and time-dependent regulation of adenocarcinoma (Bathaie et al. 2013b; Mousavi et al. 2014). In a cell cycle analysis utilizing flow cytometry, apoptosis activation in the gastric cancer tissue was demonstrated due to the administration of higher doses of aqueous saffron extract (Nair et al. 1995). Angiogenesis plays a crucial role in the growth and metastasis of tumours.

The various extracts of crocus are known to decrease vascular endothelial growth factor receptor expression by serving as an effective chemotherapeutic agent in breast cancer treatment (Mohajeri et al. 2009). Saffron extract has been reported to be useful as a preventive or therapeutic agent against diabetes mellitus with potential as a diabetes medication (Kang et al. 2012). The extracts are known to improve glucose synthesis and phosphorylation of AMPK (AMP-activated protein kinase) and MAPK (mitogen-activated protein kinases) in skeletal muscle cells and also enhance insulin sensitivity via insulin-dependent and insulin-independent pathways (Rajaei et al. 2013). In alloxan-induced diabetic rats, the hypoglycaemic and anti-hyperglycaemic essence of the ethanol saffron extract and its function in the regeneration of damaged pancreas have been demonstrated (Mousavi et al. 2010). Hypoglycaemic and antioxidant effects of crocin may be helpful in treating diabetes (Hosseinzadeh et al. 2005).

The aqueous extract of saffron (*Crocus sativus* L.) showed preventive function in the oxidative injury caused by renal ischaemia-reperfusion (IR) in rats (Fadai et al. 2014). The aqueous extract may avoid metabolic syndrome caused by olanzapine and insulin resistance and raise blood glucose in schizophrenia patients (Imenshahidi et al. 2013b). Aqueous extracts resulted in a dose-dependent reduction in mean systolic blood pressure (MSBP), with antihypertensive and normalizing effect (Xu et al. 2005). Crocin inhibits hyperglycaemic atherosclerosis by reducing cholesterol (stuff), low-density lipoproteins and triglycerides in the blood with decreased lipoprotein production (Higashino et al. 2014). Crocetin employs antihypertensive and antithrombotic special effects due to reduced nitric oxide inactivation by reactive oxygen species (Yan et al. 2010) resulting in increased bioavailability of nitric oxides. The cardioprotective role of crocin in cardiac toxicity triggered by isoproterenol is attributed to the regulation of oxidative stress, thus preserving the cell's redox state (Maes et al. 2011). Crocetin is therefore believed to improve oxygen consumption and longevity in the entire body. The inflammatory cascades were blocked in anaesthetized rats, and the development of reactive oxygen species was impaired in the heart after haemorrhagic shock (Akhondzadeh et al. 2005). Crocin protects the brain from excessive oxidative stress and is a potential therapeutic candidate for global transient cerebral ischaemia (Akhondzadeh et al. 2005). Development in the spatial processing skills after persistent cerebral hypoperfusion with crocin and saffron extract has been noted. A 30 mg regular dosage of saffron as a possible neuroprotective agent dramatically decreased the level of mild to severe depression on a standard assessment scale relative to placebo (Hosseinzadeh et al. 2012b). This may be attributed to saffron's antioxidant and radical scavenging effects in neurological conditions including depression and Alzheimer's disease (Farokhnia et al. 2014).

13.12 Other Possible Applications

The constituents of saffron have often been ascribed to be very distinct from the applications described above. Histological analysis showed that saffron improved re-epithelialization substantially in burn wounds. While the precise saffron function remains unknown, anti-inflammatory and antioxidant activity may have led to the greater healing of wounds. Another research was performed on the immune modulatory effect of saffron alcoholic extract and its impact on selective upregulation of Th_2 and it was proved that in all animal models used, *C sativus* greatly modulates the immune reactivity (Bani 2011). The therapeutic role of saffron for therapy includes its soothing and inhibitory effect on histamine (H1) and muscarinic receptors. Besides it is known to have relaxant effect on tracheal smooth muscle leading to bronchodilatory effect and may be used for asthma symptom relief (Bayrami and Boskabadi 2012).

In addition, saffron has been suggested for therapy for sexual disorders (especially due to major depression problems) in both males and females and erectile dysfunction in males. It has been from both animal and human experiments that saffron has beneficial aphrodisiac effects (Modabbernia 2012). Treatment with selective serotonin reuptake inhibitors (SSRIs) on humans showed beneficial effects on their sexual function by administering saffron extract simultaneously. In addition, saffron appeared to improve the sexual arousal, lubrication and pain domains of fluoxetine-related sexual dysfunction in women (Kashani 2013).

13.13 Conclusion

Saffron is a potential medicinal and prophylactic agent with action varying from anticancer to cognitive enhancer. The bioactive compounds contained in saffron, in particular crocetin and crocin, have been identified to be promising candidates for the treatment and control of different diseases. However, to get accurate results, comprehensive clinical trials must be conducted. Future studies are required in order to determine optimum dosage appropriate to the disease and to choose the best practicable mode of administration. Scientific studies will concentrate on looking for certain natural compounds that may function synergistically with saffron or its compounds to treat cancer-like malignancies.

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Chapter 14
Phytochemistry, Pharmacology, and Pharmacokinetics of Phytoestrogens from Red Clover Extract: An Exhaustive Overview

Swati Chaturvedi, Nazneen Sultana, Mamunur Rashid, Zaiba Naseem, Sandeep Kumar Singh, and M. Wahajuddin

14.1 Introduction

Isoflavonoids gained popularity due to their resemblance with the endogenous estrogen agonists structurally as well as pharmacologically and the potential of being used as safe alternatives in estrogen replacement therapy. They are widely distributed in the leguminous and non-leguminous families of plant kingdom (Veitch 2013). The present chapter particularly deals with the phytoestrogenic isoflavones, that is, biochanin B also known as formononetin (FMN) and biochanin A (BCA). Chemically, the BCA is written as 5,7-dihydroxy-40-methoxyisoflavone and FMN is 7-hydroxy-4′-methoxyisoflavone. The chemical structure of FMN and BCA is represented in Fig. 14.1. Although both isoflavones are extracted at high amount from the same plant, but the precursor of both FMN and BCA is different, that is, liquiritigenin (7,40-dihydroxyflavanone) for FMN and naringenin for BCA (Fig. 14.2). In the last two decades, there has been an increasing interest in these compounds as beneficial biochemical and pharmacological properties have been reported for a number of isoflavonoids. Their basic chemical structure consists of benzene ring that is linked by a heterocyclic pyran or pyrone ring and a phenyl ring (Wang 2011). The majorly distributed isoflavonoids in soy are daidzein, genistein, and glycitein and their glycoside conjugates, including 7-O-glucosides, i.e., daidzin, genistin, and glycitin (Barnes et al. 1994). In red clover, the principal

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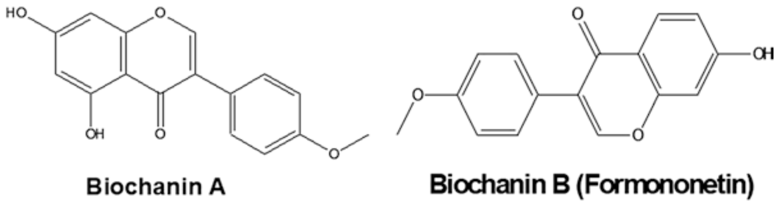

Fig. 14.1 Chemical structures of biochanins A and B

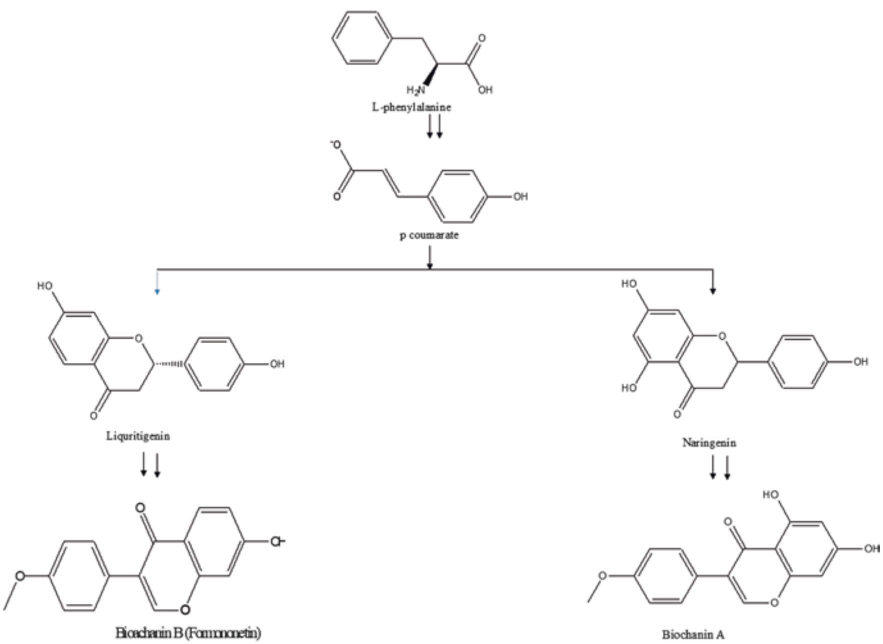

Fig. 14.2 Schematic representation of biosynthesis of BCA and FMN

isoflavonoids are FMN and BCA and their 7-O-glucosides, ononin and sissotrin (Raju et al. 2019).

Isoflavonoids are regarded as important nutraceuticals mainly due to their antioxidant effects, which give them a potential role in prevention of the various diseases associated with oxidative stress (Dixon and Pasinetti 2010). Understanding the basis of the health benefits derived from isoflavonoids requires a detailed knowledge on the absorption, distribution, metabolism, elimination (ADME), and bioavailability of these phytoestrogens. After ingestion, soy isoflavonoids are biotransformed in the intestinal tract, a process that is highly dependent on intestinal bacterial metabolism. Several major groups of colonic bacteria possess β-glucosidase activity, including *Lactobacillus* spp., *Bacteroides* spp., and *Bifidobacterium* spp. The respective isoflavone glucosides are hydrolyzed by both intestinal mucosal and bacterial β-glucosidases releasing the aglycones, which are then either absorbed directly or further metabolized by intestinal microflora in the large intestine into other metabolites, including equol (Setchell et al. 2001; Zhang et al. 2014b). Differences in the absorption rates between the glucosylated and aglycone forms of isoflavones were reported suggesting that not all isoflavonoids can be considered in the same form if present in different types of foods (Almeida et al. 2015). The absorption of these isoflavonoids would thus seem to be controlled by enzyme specificity and distribution. After initial absorption, FMN and BCA (aglycones) undergo extensive first-pass metabolism. The resulting glucuronide and sulfate conjugates could be transported through the systemic circulation to the tissue from where they can be excreted from the kidneys or secreted into bile and return to the intestine (Spencer and Crozier 2012). In the large intestine, the microbiota further degrade isoflavones; both daidzein and genistein can be further metabolized to secondary metabolites with significant interest in the potential health effects of equol, which is a reduced metabolite of daidzein. The plasma T_{max} of daidzein and genistein metabolites typically reached 6–8 h after isoflavonoid intake (Spencer and Crozier 2012).

Cytochrome P450 enzymes (P450s or CYPs) are involved in a wide variety of biotransformations including endogenous substrates (e.g., steroids, fatty acids, prostaglandins, leukotrienes) as well as exogenous compounds (xenobiotics, as drugs, environmental toxins, food preservatives) (De Montellano 2005). P450s are primarily responsible for most of drug metabolism reactions in tissues such as the liver and the gastrointestinal tract, brain, lung, kidney, and heart (Anzenbacher and Anzenbacherová 2001). Liver microsomal P450s take part in most of the reactions involving drug biotransformations and interaction of other drugs or bioactive compounds as isoflavonoids with these enzymes may significantly affect drug action and efficacy (Zanger and Schwab 2013). There were scattered reports available in the literature on the P450 induction or inhibition, however, often with extracts and with some P450 activities only – reviewed by Taneja et al. (2015).

14.2 Pharmacological Importance of FMN and BCA

Isoflavonoids gained popularity due to their resemblance with the endogenous estrogen agonists structurally as well as pharmacologically and the potential of being used as safe alternatives in estrogen replacement therapy. They have also been found to be neuroprotective and promote neuronal survival both in vivo and in vitro. They have been tried extensively for their therapeutic activity in so many diseases, and an abundance of publications is reported in the last decade.

Isoflavones have been found to cross the blood-brain barrier due to their highly lipophilic nature. This characteristic property of FMN and BCA has been applied by many researchers for their protective activity against neurodegenerative disorders. Both have been found to improve learning, logical thinking, and planning ability. Both FMN and BCA have been suggested to be a lead molecule for treating neurodegenerative disorders such as Alzheimer's and Parkinson's. It has been shown to provide neuroprotection against cerebral ischemia through modulation of concentration of antioxidants and inflammatory agents in the cells through Nrf2 signaling cascade (Guo et al. 2019). BCA and FMN also improve the cognitive neurobehavioral alteration through increasing the viable cells and ameliorating the degenerative cell count in cognitive deficit mice that further prove its potency towards treatment of Alzheimer's disease (Biradar et al. 2014). It has been reported to inhibit lipopolysaccharide (LPS)-induced activation of microglia and the production of TNF-α, nitric oxide, and superoxide in mesencephalic neuroglia and microglia-enriched culture (Wang et al. 2016). Furthermore, both the isoflavones had also been proved to be an antihyperglycemic molecule when tested in streptozotocin-induced diabetic rats. BCA successfully reduces the glucose and glycosylated hemoglobin levels in plasma of diabetic rats. It had also normalized the amount of plasma insulin and various enzymes involved in glucose metabolism (Harini et al. 2012). BCA also indirectly enhances the autoimmunity of host involved in protection against many fungi and bacteria by enhancing the retinoic acid receptor-related orphan receptors (ROR) α and γ that play the major role in IL-17 cascade pathway (Takahashi et al. 2017). BCA might also be a useful alternative estrogen therapy as suggested by Galal et al. for the management of renal and cutaneous changes observed in postmenopausal women (Galal et al. 2018). Despite of all the abovementioned therapeutic activity, BCA and FMN have also been proclaimed as an anticancer and anti-invasion by modulating the cell growth and migration of MDA-MB-231, MCF-7 (breast cancer cell line), and HUVEC cells (Zakłos-Szyda and Budryn 2020), hepatoprotective alone or in combination with any other hepatoprotective drug (Chaturvedi et al. 2018; Liu et al. 2016; Youssef et al. 2016) and renoprotective agent (Suliman et al. 2018) through different translational and molecular signaling cascades (Sarfraz et al. 2020).

These isoflavones possessing antioxidant activity lower the risk of certain cancers like breast cancer, prostate cancer, etc. They have also been used as expectorants in asthma (T. Li et al., 2018a) by inducing the vasorelaxation in thoracic aorta through regulating the PI3K/PTEN/Akt signaling pathway. These isoflavones have also been utilized as an alternative therapy to treat psoriasis, eczema and other skin conditions, help in reducing the blood pressure, in cardiac ischemia by inhibition of inflammasome pathway (D.-S. Wang et al., 2020) and lowering the cholesterol levels in blood. Recently, hepatoprotective activity of FMN has also been investigated against acetaminophen-induced hepatotoxicity by enhancing the Nrf2 binding (Jin et al. 2017).

Apart from innumerable human health benefits and pharmacological activity, these isoflavonoids could not be directly administered due to their low oral bioavailability resulting to less systemic exposure. This role is played by pharmacokinetics of all the compounds in which the compound is absorbed, distributed throughout the organs, and metabolized mainly by liver cytochromes (microsomal enzymes) and sometimes other enzymes present in different organs. Further, the chapter will be discussing the pharmacokinetics of both the isoflavones and their modulation by various cytochrome enzymes and membrane transporters. The multimechanistic role of BCA and FMN has been diagrammatically represented in Fig. 14.3 and Table 14.1.

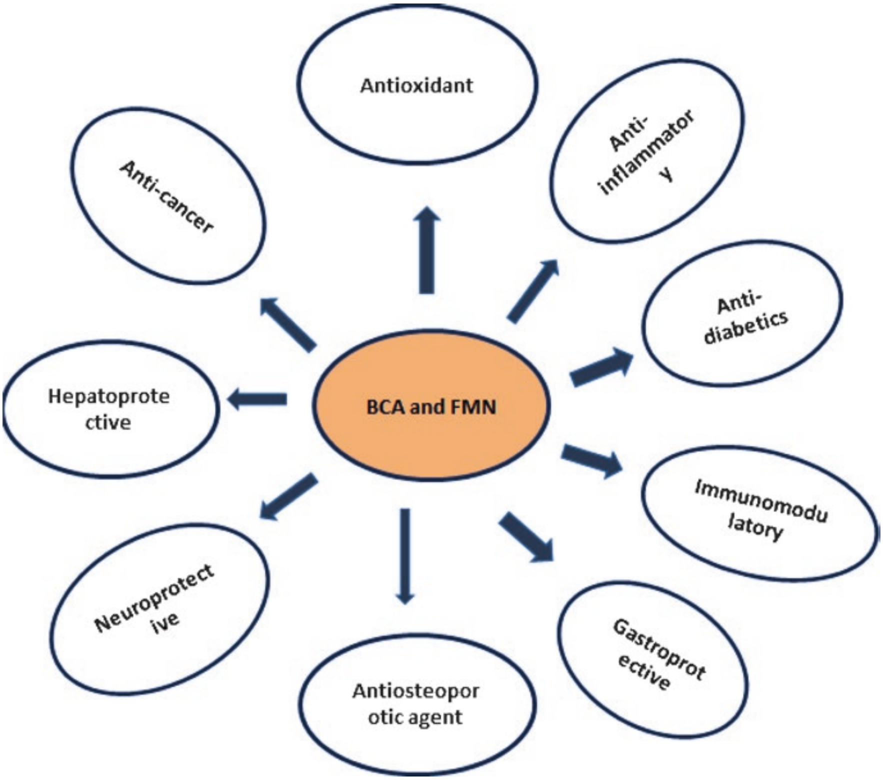

Fig. 14.3 Pharmacological activities of BCA and FMN (biochanin B)

14.3 Pharmacokinetics of FMN and BCA

The isoflavonoids exist as biologically inactive glucoconjugates in their natural form (Setchell et al. 2001). They could be only absorbed in their active aglycone form which is the result of a biotransformation reaction through an intestinal bacteria undergoing a deglycosylation pathway. They are inactive and could not be absorbed post administration in their natural form. There are literatures suggesting the conversion of inactive isoflavonoids into their active metabolite starts from the mouth itself (Allred et al. 2001). The pharmacokinetics and bioavailability information are assessed on the basis of the isoflavone's absorption, distribution, and metabolism and excretion data obtained from preclinical and clinical trials. Considering pharmacokinetics of FMN and BCA, they are reported to be metabolized into their demethoxylated isoforms, daidzein, and genistein. The schematic representation of metabolic pathway for both FMN and BCA is being detailed in Fig. 14.2. Many researchers have investigated about the pharmacokinetic characteristics of FMN and BCA under both in vitro and in vivo experimental conditions. Recently, pharmacokinetics, bioavailability, and permeation through Caco-2 cells of FMN were

Table 14.1 Various signaling pathways modulated by FMN and BCA under different pathological conditions

S. No.	Cell line\animal model	Disease associated	Signaling pathways	Dose/ concentration/ treatment exposure	Outcome	References
FMN and BCA						
1.	Male Sprague Dawley rats	Ritonavir-induced hepatotoxicity	Modulation of Nf-κB/pAkt molecules	50 mg/kg 14 days, oral	Exerts hepatoprotective effect through modulating the oxidative stress, inflammation, apoptosis, and reversing the tissue degeneration	Chaturvedi et al. (2018)
2.	ERα-positive breast cancer cells (T47D, MCF-7) Female mice (overiectomized)	Breast cancer	ERα-positive cell proliferation	2–6 μM, 48 h/2,4 mg/kg/ day, *i.p.*	Biochanin A promoted ERα-positive cell proliferation through mir-375 activation, and this mechanism is possibly involved in a mir-375 and ERα feedback loop	Chen et al. (2015)
3.	Mouse RAW 264.7 and 293T cells	Anti-inflammatory	Peroxisome proliferator-activated receptors (PPARs) modulation	10 μmol/l, 36 h	Ameliorate the cytokine secretion profile of lipopolysaccharide (LPS)-stimulated mouse RAW264.7 macrophages	Qiu et al. (2012)
4.	Male rats (Sprague Dawley)	Diabetic neuropathy	SIRT1 and NGF activator	10, 20, and 40 mg/ kg/day orally for 16 weeks	Continuous administration of FMNT-protected diabetic animals from hyperglycemia-induced neuronal damage by controlling hyperglycemia	Oza and Kulkarni (2020)
5	NSCLC cells	Non-small cell lung cancer	Suppression of EGFR-Akt-Mcl-1 axis	3 μM	Promoting ubiquitination-dependent Mcl-1 turnover might be an alternative strategy to enhance the anti-tumor efficacy of EGFR-TKI	Yu et al. (2020)

6	Male Sprague-Dawley rats, NRCMs	Myocardial ischemia/ reperfusion injury	ROS-TXNIP-NLRP3 pathway	10 mg/kg, 30 mg/kg 1 and 10 μM for 2 h	FMN notably attenuated cardiac dysfunction, infarct size, release of cardiac markers, and elevation of TNF-α, IL-1β, and IL-6. FN alleviated LPS plus nigericin-induced injury and ROS increase in NRCMs	Wang et al. (2020)
7	Saos-2 cell line-human osteosarcoma cells	Osteoporosis	Receptor activator of nuclear factor-κB ligand (RANKL)	1 μM	Regulates the expression of RANKL and OPG at the mRNA levels, as well as osteogenic differentiation markers: alkaline phosphatase (ALP), collagen type 1, and Runt-related transcription factor 2 (Runx2)	Zakłos-Szyda et al. (2020)
8	Ovariectomized (OVX) rats	Postmenopausal osteoporosis	OPG/RANKL ratio	*Cicer arietinum* extract (500 mg/kg)	Reverse TRAP5b and RANKL levels; it triggers upregulation of OPG, enhances the OPG/RANKL ratio, and modulates the bone and uterus alterations	Sayed and Elfiky (2018)
FMN						
9	HUVECs, MCF-7, BT474, and MDA-MB-231 cells	Breast cancer	Raf/MEK/ERK and PI3 K/Akt		Increase in the Akt phosphorylation Expression of Bcl-2 Proliferation induced Apoptosis inhibited	Chen et al. (2011)
10	T24 cell line	Bladder cancer	PTEN/Akt pathway	6.25–400 μM/24–72 h	Tumor growth inhibition Promoted apoptosis	Wu et al. (2017)
11	MCF-7 cell line	Bladder cancer	IGF/IGFR1/AKT pathway	30–100 μM/48 h	Inhibited the proliferation of MCF-7 cells Induced cell cycle arrest Downregulation of p-IGF-1 R, p-Akt, cyclin D1 protein expression, and cyclin D1 mRNA expression	Chen et al. (2011)

(continued)

Table 14.1 (continued)

S. No.	Cell line\animal model	Disease associated	Signaling pathways	Dose/ concentration/ treatment exposure	Outcome	References
12	ER-positive MCF-7 cells and T47D cell	Breast cancer	p38MAPK pathway	25–100 μM/48 h	Increased ratio of Bax/Bcl-2 Induced apoptosis on MCF-7 cells	Chen and Sun (2012)
13	ER-positive MCF-7 cells and T47D cell	Breast cancer	IGF-1R pathway	25–100 μM/48 h	Inhibited breast cancer growth	Chen et al. (2013)
14	MDA-MB-231 4TI	Breast cancer	PI3K/AKT pathway	2.5–40 μM/12 h	Inhibited breast cancer cell migration and invasion Reduced the expression of MMP-2 and MMP-9	Zhou et al. (2014)
15	HeLa cells	Cervical cancer	PI3K/AKT/ERK pathway	50 μM/24 h	Inhibited phosphorylation of AKT Induced apoptosis in dose-dependent manner	Jin et al. (2014)
16	HCT116 cell line	Colorectal cancer	p53 or Egr-1-related pathways	6.25–200 μM/24–72 h	Inhibit the growth of colon cancer Promoted apoptosis	Auyeung and Ko (2010)
17	SW1116 cell line HCT116 cell line	Colorectal cancer	PI3K/AKT/STAT3 pathway	20–200 μM/24 h	Tumor cells apoptosis promoted Reduced the expression of MMP-2 and MMP-9	Wang et al. (2018a)
18	RKO cell line	Colorectal cancer	ERK pathway TNF-α/NF-κB pathway	20–80 μM/48 h	Induced apoptosis Controlled cell growth	Huang et al. (2015)
19	Glioma C6 cell line	Glioma	Apoptosis signaling pathway	20–320 μM/48 h	Inhibited the growth of C6 cells in dose-dependent manner Showed synergy TMZ	Zhang et al. (2018)
20	U87MG cell line U251MG cell line T98G cell line	Glioblastoma	–	50–200 μM/48 h	Enhance the therapeutic efficacy of doxorubicin	Liu et al. (2015)

21	U266 cell line	Multiple myeloma	AKT pathway	5–60 μM/8 h	Suppress multiple myeloma	Park et al. (2018)
22	CNE1 cell line CNE2 cell line	Nasopharyngeal carcinoma	PI3K/AKT/ERK pathway	5–40 μM	Inhibited the proliferation Induced apoptosis	Qi et al. (2016)
23	A549 cell line NCI-H23 cell line	Non-small cell lung cancer	–	100–200 μM/48 h	Significantly inhibited the proliferation	Yang et al. (2014)
24	ES2 cell line OV90 cell line	Ovarian cancer	ERK1/2 pathway	20–40 μM/48 h	Significantly inhibited the proliferation	Park et al. (2018)
25	LNCaP cell line PC-3 cell line	Prostate cancer	ERK1/2 pathway	20–80 μM/24–72 h	Induced apoptosis	Ye et al. (2012)
26	PC-3 cell line DU-145 cell line	Prostate cancer	IGF/IGFR1 pathway	10–100 μM/48 h	Inhibited tumor growth	Li et al. (2014)
27	PC-3 cell line	Prostate cancer	IGF/IGFR1 pathway	25–100 μM/48 h	Induced apoptosis	Huang et al. (2014)
28	DU-145 cell line	Prostate cancer	IGF/IGFR1/AKT pathway	6.25–200 μM/48 h	Induced apoptosis	Liu et al. (2014)
29	PC-3 cell line	Prostate cancer	p38/Akt pathway	25–100 μM/48 h	Induced apoptosis	Zhang et al. (2014a)
30	MCF-7 cell-induced xenograft in Balb/c nude mice	Bladder cancer	IGF-1/IGFR-PI3K/AKT pathway	15, 30, or 60 mg/kg/day; 20 days	Decrease in the growth of tumor	Chen et al. (2011)
31	MDA-MB-231 cell-induced xenografts in Balb/c nude mice	Breast cancer	PI3K/AKT pathway and STAT3 pathway	100 mg/kg/day; 25 days;	Decrease in tumor growth Synergistic effect with sunitinib	Wu et al. (2015a)
32	MDA-MB-231-luc cell-induced xenografts in Balb/c nude mice	Breast cancer	PI3K/AKT pathway	10 or 20 mg/kg/day; 35days (once every 2 days)	Decrease in tumor growth Increase in the overall survival Decrease in lung metastasis	Zhou et al. (2014)

(continued)

Table 14.1 (continued)

S. No.	Cell line\animal model	Disease associated	Signaling pathways	Dose/ concentration/ treatment exposure	Outcome	References
33	HCT-116 cell-induced xenografts in Balb/c nu/nu mice	Colon cancer		20 mg/kg/day; 2 weeks	Decrease in the growth of tumor	Auyeung et al. (2012)
34	Human multiple myeloma U266 xenograft in Balb/c nude mice	Breast cancer	PI3K/AKT pathway	20 and 50 mg/kg/ day; 25 days	Decrease in the growth of tumor	Chen and Sun (2012)
35	PC-3 cell-induced prostate xenograft in nude mice	Prostate cancer	IGF/IGFR1 pathway	60 mg/kg/day; 20 days	Decrease in the growth of tumor	Li et al. (2014)
36	RKO tumor-bearing Balb/c nude mice	Colorectal cancer	IL6, TNF-α, NF-κB pathway	5, 10, or 20 mg/kg/ day; 14 days	Decrease in the growth of tumor	Huang et al. (2015)
37	nude BALB/c mice	Cervical cancer	PI3K/AKT/ERK pathway	20 and 40 mg/kg; 10 days	Suppressed the tumor growth	Jin et al. (2014)
BCA						
38	A549 cell line	Lung cancer	VEGF/VEGFR2 signaling pathway	100 μmol/L; 72 h	Inhibited tumor growth	Lai et al. (2020)
39	T47D, MCF-7	Breast cancer	ERα-mediated signaling pathway	24 h, 48 h	Promoted cell proliferation	Chen et al. (2015)
40	Panc1 and AsPC-1	Pancreatic cancer	Akt and MAPK signaling pathways	40–100 μmol/L; 72 h	Inhibited cell growth and exhibited cell survival	Bhardwaj et al. (2014)
41	A549 and 95D cells	Lung cancer	Bcl-2 and caspase-3 pathways	50, 100, 200, and 400 μmol/L; 24, 48, and 72 h	Inhibited cell proliferation Induces cell cycle arrest and induced apoptosis	Li et al. (2018b)
42	LNCaP cells and DU145 cells	Prostate cancer	Intrinsic (receptor-mediated) and extrinsic pathways	20–100 μM; 48 h	Induced apoptosis and inhibited tumor growth	Szliszka et al. (2013)

43	SK-Mel-28 melanoma cell	Malignant melanoma	NF-κB and MAPK signaling pathways	0–100 μM; 48 h and 72 h	Inhibited cell migration and invasion Induced apoptosis	Xiao et al. (2017)
44	A427:AML-193 co-culture cells	Lung cancer	–	5, 20, and 40 μM; 24h	Induced epithelial mesenchymal transition	Wang et al. (2018b)
45	HepG2, Huh-7 cells, and HCC cell lines	Liver cancer	Mitochondrial apoptosis signaling pathway	0.5–50 μM; 72h	Enhanced anti-proliferative Enhanced pro-apoptotic Inhibited cell growth	Youssef et al. (2016)
46	F98, U87, U251, and GL261 cells	Brain tumor	–	10, 50, 100 μmol/L; 4 days	Exhibited glioma cellular toxicity	Sehm et al. (2014)
47	FaDu cells	Head and neck cancer	P38, MAPK, NF-κB, and Akt signaling pathways	25, 50, and 100 μM; 24 h and 48 h	Reduced apoptosis Inhibited wound healing migration and proliferation of cells	Cho et al. (2017)
48	MG63 and U2OS cell line	Osteosarcoma	Mitochondrial pathway	0–80 μM; 48 h	Enhanced apoptotic rates Reduced migration and invasion ability	Zhao et al. (2018)
49	U-87 MG and T98 G	Glioblastoma	PI3K/AKT, Wnt, and Nf-κB signaling pathway	70 μM; 72 h	Enhanced inhibitory effects in combination with temozolomide	Desai et al. (2019)
50	MG63	Osteosarcoma	TP53 pathway	2–32 μM; 6 h, 12 h, 24 h, and 48 h	Suppressed the proliferation	Luo et al. (2019)
51	C6 and bEnd.3	Malignant gliomas	ERK/AKT/mTOR	5–100 μmol/L; 72 h	Inhibited factor-1α Inhibited vascular endothelial growth factor	Jain et al. (2015)
52	MG-63 and U2OS	Osteosarcoma	Intrinsic pathway	5, 10, 20, or 30 μg/mL; 24 h	Induced cell apoptosis and cell death	Hsu et al. (2018)
53	A549 cells	Anti-H5N1 influenza	AKT, ERK 1/2, and NF-κB pathway	40 μM; 48 h	Suppressed formation of ROS	Michaelis et al. (2014)
54	RBL-2H3 cells	IgE-mediated allergy	Akt and MAPK pathways	12.5, 25, and 50 μM; 2 h	Inhibited production of intracellular ROS Promoted phosphorylation	Chung et al. (2013)
55	BV2 microglial cells	Brain inflammation	MAPK pathway	1.25, 2.5, 5 μM; 36 h	Inhibited the LPS-induced phosphorylation	Wu et al. (2015b)

studied after oral and IV administration to rats at a dose of 20 mg/kg and 4 mg/kg. The investigators found that FMN showed absolute bioavailability of 21.8% after oral exposure with sufficient systemic exposure. Upon scrutinizing through Caco-2 cells, the efflux ratio of FMN was found to be within the range of 1.0–1.5, suggesting that there was no significant difference between permeability in either direction of bilayer, and transportation is basically across intestinal epithelial cells and was mainly through passive diffusion (Luo et al. 2018). The results from the latter mentioned research also manifest that FMN is largely absorbed from the large intestine of the gastrointestinal segment followed by the small intestine. This indicates the site-specific distribution of FMN and other related isoflavonoids (Luo et al. 2018). Similar pattern was also followed by BCA in another experiment performed by Jia and colleagues (Jia et al. 2004). The same was also justified by earlier studies through single-pass intestinal perfusion model (Liu et al. 2013). Zhang et al. have determined the pharmacokinetic behavior of FMN along with other isoflavones after oral administration of Buyang Huanwu Decoction to rats using tandem mass spectrometric technique (Zhang et al. 2011). Furthermore, upon oral administration of a cardio-cerebral vascular protective Chinese traditional medicine, Buyang Huanwu Tang, FMN was found to be at the second largest isoflavonoids which have the highest area under the curve concentration (Jia et al. 2004). A recent report has suggested the single pass intestinal perfusion technique to be the applicable agent for physiological based pharmacokinetic model to predict assess the human absorption of these aglycones (Liu et al. 2019).

Furthermore, FMN and BCA are also known to be metabolized by some demethylating enzymes of *Eubacterium limosum* (Hur and Rafii 2000). The bioconversion of FMN and BCA by glucosyl and malonyltransferase enzymes produces the most important bioactive isoflavones known as daidzein and genistein (Nielsen et al. 2000). Both FMN and BCA are extensively and majorly known to be metabolized in liver undergoing the glucuronidation and sulfation reaction. Moreover, inter-individual and genetic variability due to ethnic and seasonal differences in food phytoestrogen are also the paramount factors to affect the metabolism of these isoflavonoids (Křížová et al. 2019). Further, the metabolite daidzein gets converted to dihydrodaidzein followed by major metabolism in to desmethylangolensin and minorly to equol (reported in one-third of the human population). Moreover the bioactive molecule genistein also gets further metabolized in to dihydrogenistein followed by formation of p-ethylphenol. Microbial culture studies gave evidence of conversion of genistein into 5-hydroxyequol, but there is no investigation performed regarding this conversion at clinical level (Matthies et al. 2012). Enzymes like lactase-phlorizin hydrolase, enterocytic/microbial β-glucosidase also take part in the intestinal gut wall metabolism of these phytoestrogens. Despite the contribution of intestinal enzymes, their enzymatic conversion to their active metabolite also took place through hepatic phase I and phase II microsomal enzymes. The major pathway of metabolism is the demethylation at 4′methoxy group followed by hydroxylation by liver enzymes. The extent of metabolism through gut microsomes is far lesser than the liver microsomes. The rat liver microsomes could metabolize the BCA to genistein more rapidly as compared to the human liver microsomes (Křížová et al. 2019). Metabolism of isoflavonoids including BCA and FMN by phase II enzymes (uridine 5′-diphospho-glucuronosyl transferase and sulfotransferase) has also been reported instead of lower glucuronidation rate after oral administration of red clover extract. In some leguminous plants such as *Medicago truncatula*, FMN is known to get metabolized to a phytoalexin medicarpin, a pathogenic-resistant compound (Liu et al. 2017) and recently found to be an osteoprotective agent (Taneja et al. 2020). In vitro metabolic conversion of metabolite daidzein to 6-hydroxy daidzein by catechol-O-methyltransferase enzymes has been explored by one of the researchers. Same as other isoflavonoids, they are also reabsorbed again and undergo the enterohepatic circulation. Much like any other endogenous substance or xenobiotic, enterohepatic circulation and biliary elimination complicate the pharmacokinetic profiles of the majority of isoflavonoids. Extensive first-pass metabolism and biliary excretion are thought to be the causative agents for their lower bioavailability which is a major bottleneck in advancement of isoflavonoids as therapeutic agents. The metabolism of the isoflavones seems to be complicated and has not been studied extensively. However, the significant metabolites reported are daidzein and genistein in most of the cases. The newer metabolites need to be identified and evaluated for their biological activity and therapeutic efficacy if any.

These FMN and BCA are also present in the daily dietary intake of animals in high concentration. In earlier studies, the data indicates that unlike humans, these isoflavonoids are directly metabolized in to equol (Blair et al. 2003) followed by conversion to an inactive p-ethylphenol (Wocławek-Potocka et al. 2013). When investigated for the metabolism of FMN in sheeps, it is firstly demethylated to daidzein followed by further formation of equol through hydrogenation. The in vitro studies that described the incubation of FMN and BCA in the bovine rumen fluid showed that the half-lives were 4.3 h for FMN and 3.9 h for BCA (Křížová et al. 2019). The concentration of BCA and FMN was found to be only 0–9% of ingested BCA and 7–16% of FMN in the omasum of dairy cows pattern similar to sheep (Njåstad et al. 2014).

The lower and limited bioavailability of isoflavonoids could also be due to the extensive first-pass metabolism of isoflavonoids and their rapid elimination from the body. The maximum time to reach the maximum plasma concentration is within 4–8 h. In a study, the systemic levels of BCA after dietary intake were found to be minimal as compared to the administration of prepared herbal formulation. However, in some studies, it has been seen that exposure of BCA as a pure compound shows a level of saturation during absorption. The bioavailability of BCA when administered orally at a dose of 5 mg/kg is 2.6% and 1.2% at 50 mg/kg, suggesting the rapid absorption, extensive first-pass metabolism, and biliary elimination by the authors for its low bioavailability (Moon et al. 2006). There are also investigations revealing the higher bioavailability of BCA that could be the result of competitive inhibition and modulation of metabolic enzymes and transporters due to some other structurally similar compounds. This low bioavailability could be enhanced by administration of mixture of isoflavonoids that would also aid in increasing the therapeutic efficacy of isoflavonoids. However, there are reports that refuse the fact of accumulation of isoflavonoids upon long-term administration. The apparent isoflavone bioavailability in healthy children is found to be 30–40% higher relative to healthy adults. The differences in gender do not affect the bioavailability of isoflavonoids to much higher extent. The mode of excretion of these isoflavonoids is through urine and bile and sometimes through feces. The excretion of these isoflavonoids by urine is determined by the type of the dietary product consumed and its composition regarding isoflavones and also on the nature of the isoflavone. Foods rich in isoflavone glucosides have poor urinary isoflavone excretion. However, a few studies report that the rates of urinary isoflavone excretion are not affected significantly by the nature of the food. Daidzein excretion was found to be markedly lower in equol producers as compared to equol non-producers. The primary metabolites found are glucuronide conjugates (70–90% of the total urinary isoflavones), sulfate conjugates (10–25%), and aglycone forms (1–10%). A multiethnic population study reported that Japanese, Chinese, or Native Hawaiian ancestry women excreted more isoflavones in urine than Caucasian and Filipino women. Fecal elimination as mentioned above is a minor route of elimination. Only up to 4% of the dietary isoflavones are eliminated by this route. Aglycone of the isoflavones forms the principal (>80%) part of the fecal isoflavone content.

14.4 Interaction with Cytochrome P450 Enzymes

Cytochrome P450 enzymes are the metabolic enzymes constituting the hemeprotein involved in the enzymatic conversion of many drugs, steroids, carcinogens, xenobiotics, hormones, fat-soluble vitamins, and other chemicals into their inactive and active forms. These enzymes have been the responsible facet for number of reactions inside the body which indirectly implicates their role in drug-drug interaction, drug-herb interaction, side effects, adverse effects, and unwanted increase and decrease in plasma concentration of therapeutically active drugs that lead to their toxicity and less response towards the targeted disease. Furthermore, the herbal drugs are widely used throughout the world along with the prescribed allopathic medicines; hence, the maximum possibility of herb drug interactions occurs at this stage. Various IC50 values of both the isoflavonoids are mentioned in Tables 14.2 and 14.3. Moreover, FMN and BCA are among the extensively administered isoflavonoids owing to their therapeutic beneficial properties. Taking into consideration, their pharmacokinetic and pharmacodynamic interactions with other drugs are summarized in Table 14.4.

Zapletalova and colleagues have unveiled the isoflavonoids safety efficacy by systematically investigating the interaction potential of 12 isoflavonoids with nine isoforms of cytochromes (Kopečná-Zapletalová et al. 2017). He found the genistein and daidzein and the metabolites of BCA and FMN to be the most potent inhibitors of cytochrome enzymes that non-competitively inhibit the CYP2C9 and CYP3A4. CYP3A4 was also efficiently inhibited by the parent isoflavonoid BCA, but FMN

Table 14.2 In vitro and in vivo cytochrome enzymes inhibition profiling due to exposure of BCA

S. No.	Enzyme subtype	Test compound	Model	IC50 value	References
1	1A2	7-ethoxyresorufin O-deethylation	Cryopreserved human liver microsomes	>100 μmol/l, 38.57 μM	Arora et al. (2015), Kopečná-Zapletalová et al. (2017)
		Phenacetin O-deethylation	Human liver microsomes	24.98 μM	Arora et al. (2015)
			Rat liver microsomes	11.86 μM	Arora et al. (2015)
2	2A6	Coumarin 7-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
3	3A4/3A2	Testosterone 6b-hydroxylation	Cryopreserved human liver microsomes	65.11 ± 3.97 μmol/l	Kopečná-Zapletalová et al. (2017)
		Midazolam 10-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
		Nifedipine 4′-hydroxylation	Human liver microsomes	>100 μM	Arora et al. (2015)
			Rat liver microsomes	51.05 μM	Arora et al. (2015)
4	2B6	7-ethoxy-4-(trifluoromethyl) coumarin 7-deethylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
5	2C8	Paclitaxel 6-hydroxylation	Cryopreserved human liver microsomes	88.25 ± 5.46 μmol/l	Kopečná-Zapletalová et al. (2017)
6	2C9/2C11	Diclofenac 40-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
		Diclofenac 4-hydroxylation	Human liver microsomes	40.13 μM	Arora et al. (2015)
			Rat liver microsomes	>100 μM	Arora et al. (2015)
7	2C19	S-mephenytoin 40-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)

(continued)

Table 14.2 (continued)

S. No.	Enzyme subtype	Test compound	Model	IC50 value	References
8	2D6/2D4	Bufuralol 10-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
		Dextromethorphan O-demethylation	Human liver microsomes	>100 μM	Arora et al. (2015)
			Rat liver microsomes	>100 μM	Arora et al. (2015)
9	2E1	Chlorzoxazone 6-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l, 57.56 μM	Arora et al. (2015), Kopečná-Zapletalová et al. (2017)
			Rat liver microsomes	>100 μM	Arora et al. (2015)

inhibited the same only about 20–30% of the total concentration. Hence, he concluded the maximal possible pharmacokinetic interaction of other drugs with these isoflavonoids. Moreover, Arora et al. have predicted the in vivo potential of CYP metabolic interaction of FMN and BCA using human and rat liver microsomal data obtained from in vitro studies (Arora et al. 2015). In the aforementioned investigation, they concluded that both FMN and BCA showed concentration-dependent competitive inhibition of CYP1A2 activity in human and rat liver microsomes, respectively. CYP2D6 inhibition was also perceived by FMN as concluded by the researchers. The in vivo prediction data showed the significant level of inhibition of both the isoflavonoids at intestinal level but non-significant at the hepatic level. Thereby, they have suggested for the special care to be considered during the administration of these isoflavonoids along with any prescribed drug which is metabolized by the enzyme CYP1A2. In an another study, researchers have evinced the harmful effect of red clover extract containing FMN and BCA administered to breast cancer patients (Dunlap et al. 2017). They investigated the red clover effect on metabolic CYP enzymes using the non-malignant ER-negative breast epithelial cells (MCF-10A) and malignant ER-positive breast epithelial cancer cell line, and the quantification of methoxy estrogen metabolites was performed using LC-MS/MS technique. They found that there was no effect of red clover in MCF-10A cells, while the expression of CYP1A1 was downregulated in MCF-7 cell line. These data suggest that the isoflavonoid containing red clover extract has distinctive effect on both the cells. Therefore, it is necessary to avoid red clover extract and formulations composed of these isoflavonoids to the breast cancer patients.

In addition to the pharmacokinetic assessment, these CYP450 enzymes are also being targeted by isoflavonoids for therapeutic benefits. Taking an example, FMN has been supposed to be mechanistically involved in suppression of colorectal cancer by modulation of CYP 1A1 isoform of CYP450 (Zhang et al. 2019). Furthermore, BCA has also been reported to follow similar pattern as it is found to

Table 14.3 In vitro and in vivo cytochrome enzymes inhibition profiling due to exposure of FMN

S. No.	Enzyme subtype	Test compound	Model	IC50 value	References
1	1A2	7-ethoxyresorufin O-deethylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
		Phenacetin O-deethylation	Human liver microsomes	13.42 μM	Arora et al. (2015)
			Rat liver microsomes	38.57 μM	Arora et al. (2015)
2	2A6	Coumarin 7-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
3	3A4/3A2	Testosterone 6b-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
		Midazolam 10-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
		Nifedipine 4′-hydroxylation	Human liver microsomes	>50 μM	Arora et al. (2015)
			Rat liver microsomes	>50 μM	Arora et al. (2015)
4	2B6	7-ethoxy-4-(trifluoromethyl) coumarin 7-deethylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
5	2C8	Paclitaxel 6-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
6	2C9/2C11	Diclofenac 40-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
		Diclofenac 4-hydroxylation	Human liver microsomes	>50 μM	Arora et al. (2015)
			Rat liver microsomes	>50 μM	Arora et al. (2015)
7	2C19	S-mephenytoin 40-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
8	2D6/2D4	Bufuralol 10-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l	Kopečná-Zapletalová et al. (2017)
		Dextromethorphan O-demethylation	Human liver microsomes	24.83 μM	Arora et al. (2015)
			Rat liver microsomes	>50 μM	Arora et al. (2015)

(continued)

Table 14.3 (continued)

S. No.	Enzyme subtype	Test compound	Model	IC50 value	References
9	2E1	Chlorzoxazone 6-hydroxylation	Cryopreserved human liver microsomes	>100 μmol/l, >50 μM	Arora et al. (2015), Kopečná-Zapletalová et al. (2017)
			Rat liver microsomes	>50 μM	Arora et al. (2015)

be an anti-fibrotic agent against carbon tetrachloride-induced hepatotoxicity in rats (Breikaa et al. 2013a). The BCA acts through multimechanistic pathway among which one of the targeted molecules is the CYP450 enzymes (CYP4502E1 and CYP4501A1) in conjugation with pro-inflammatory and pro-fibrotic mediators (Breikaa et al. 2013b). Moreover, after searching the database, there are very few findings in the last 10 years that could be found reporting the interaction of both FMN and BCA with the microsomal enzyme cytochromes (CYP450).

Furthermore, despite inhibition metabolic enzymes, few investigations also reported the induction of some cytochrome enzymes by FMN listed in Table 14.4. This contradictory research needs more data to confirm the exact role of bioflavonoids upon CYP enzymes. Hence, still the studies are required to explore the further action of FMN and BCA upon various microsomal enzymes.

14.5 Interaction with ABC Transporters

The most widely distributed and largest integral protein family known is the ABC (ATP-binding cassette) transporter present in the body. Presence of two nucleotide-binding domains and two transmembrane domains is the characteristic feature of ABC transporter. Binding of any exogenous and endogenous ligand to the transporter leads to the conformational changes in the nuclear-binding domain that further alters the transmembrane domains of the receptor. These rearrangements of the domains outturn in to the modulation of internal cytosolic and nuclear molecular messengers for initiation of signaling pathways. Further, an untoward activation or inhibition in this feature of ABC transport could lead to various types of side effects or adverse events and drug toxicity. Moreover, the potential involvement of ABC transporters against the drug-drug, herb-drug, and herb-herb interaction had been already established by many researchers.

Furthermore, FMN and BCA are two major isoflavonoids found in red clover extract. They are also highly recommended for their anti-osteoporotic activity. Hence, it could be possible that they are being administered with other co-prescribed allopathic medicines that are ABC transporter activators or inhibitors or the substrates. Therefore, it is important to know the effect of FMN and BCA towards ABC

Table 14.4 Modulation of various transporters due to FMN and BCA

S. No.	Drug	Isoflavonoid	Targeted transporter	In vitro/in vivo model	Consequences	References
1.	Raloxifene	BCA	UGT substrates	Gunn rats deficient in UDP-glucuronosyltransferase (UGT) 1A, Eisai hyperbilirubinemic rats (EHBRs), which hereditarily lack multidrug resistance-associated protein (MRP) 2 and wild-type rats	Raloxifene concentration was absorbed twice than the wild type rats	Kosaka et al. (2011)
2.	–	FMN	P-gp and BCRP	HepG2 and wild-type C57BL/6 mice and nuclear factor E2-related factor-2 knockout (Nrf2−/−) C57BL/6 mice	Upregulated the P-gp and BCRP	Lou et al. (2019)
3	–	FMN, BCA	OATP	MDCKII cells	Aglycones inhibited the transporter with Ki value from 1 to 20 μM	Navrátilová et al. (2018)
4	–	FMN	Pgp	Molecular docking (computational model)	High binding affinity towards transporter and its inhibition	Wongrattanakamon et al. (2017)
5	–	FMN	CYP3A4, CYP2B6, CYP2E1, UGT1A, UGT1A6, SULT1A1, and SULT1A3 P-gp, MRP2, BCRP, MRP1, and MRP3	HepG2 cell line	FMN-induced CYP3A4, CYP2B6, UGT1A, P-gp, MRP1, MRP2, and MRP3 protein expression but inhibited the expression of CYP2E1, SULT1A1, and SULT1A3	Zhang et al. (2016)
6	–	BCA	OATP1B1	HEK293 cell line	The transporter expression was inhibited thus inhibiting bosentan-induced liver injury	Fan et al. (2020)

(continued)

Table 14.4 (continued)

S. No.	Drug	Isoflavonoid	Targeted transporter	In vitro/in vivo model	Consequences	References
7	Doxorubicin, temozolomide, and mitoxantrone	BCA	BCRP	BCRP-MDCKII cell	Inhibition of BCRP-mediated efflux increased the doxorubicin and temozolomide concentration while increased the concentration of mitoxantrone	Fan et al. (2019)
8	Doxorubicin	BCA	Pgp	Doxorubicin-resistant K562 cells	Inhibited the transporter	Dash and Konkimalla (2017)

transporters. There are literatures published regarding the modulation of ABC transporter by the administration of isoflavonoids (Wongrattanakamon et al. 2017) in which the authors have performed the molecular docking, pharmacophore modeling, and molecular dynamic simulation studies for an ABC transporter, P-gp (P-glycoprotein), of mouse origin with some of the mostly utilized bioflavonoids. They have discerned the certainty of plausible interaction of these isoflavonoids with other co-prescribed drugs as they have the potential to inhibit the P-gp efflux mechanism. Therefore, it is a major concern when the P-gp substrate drug is co-administered with these isoflavonoids which might lead to the intracellular increase in concentration of the substrate drug, thereby altering its therapeutic index and safety profile. In a research, FMN has been investigated as a nephroprotective agent against cisplatin-induced renal toxicity by altering the expression of organic cation transporter 2 (OCT2) and multidrug resistance-associated proteins (MRPs). FMN is reported to enhance the expression of MRP gene whilst alleviating the OCT2 expression, hence decreasing the intratubular accumulation of cisplatin in kidney resulting in to the reduced nephrotoxicity of the drug (Huang et al. 2017). In a recent investigation, a group of researchers evaluated the inhibitory effect of 99 major flavonoids upon BCRP (breast cancer resistance protein) under both in vitro and in vivo experimental conditions using BCRP-associated MDCK II cells and rat as an animal model (Fan et al. 2019). Their findings linked to molecular docking analysis along with structural activity relationship could further assist in predicting the potential risk in interaction between the isoflavonoids and other co-administered drugs.

Further, it has also been observed that BCA along with ciprofloxacin could also inhibit the efflux pump of methicillin-resistant *Staphylococcus aureus* in a synergistic manner (Zou et al. 2014). The concentration of ciprofloxacin was found to be significantly increased by 83% at 15 min after combining with BCA which was similar to the effect of positive control drug, reserpine. BCA was also examined as a P-gp inhibitor after being formulated into a solid dispersion. When investigated under in vitro experimental conditions, it remarkably augmented the cellular uptake of a P-gp substrate, rhodamine123 in NCI/ADR-RES cells by 2-3 folds. They have also examined the BCA for its inhibitory efficacy after oral and intravenous administration of BCA with diltiazem (a P-gp substrate) and its metabolite desacetyldiltiazem to rats. Upon pharmacokinetic analysis, the AUC (area under the curve) of desacetyldiltiazem was found to be threefold without affecting the concentration of diltiazem. Therefore, when BCA is developed into a new formulation as solid dispersion, its inhibitory potency is enhanced. Moreover, in a study, Singh and his colleagues have utilized BCA as a P-gp and CYP inhibitor to investigate its effect on bioavailability of an anticancer P-gp substrate tamoxifen and its metabolite. The concentration of tamoxifen and its metabolite was found to be decreased suggesting the low bioavailability of tamoxifen owing to its characteristic of being the P-gp substrate (Singh et al. 2012).

Furthermore, after searching the database, there are only few publications regarding the pharmacokinetic and pharmacodynamic interactions of both the isoflavonoids with either CYP450 microsomal enzymes or ABC transporters that have been summarized in the aforementioned paragraphs and their respective tables.

14.6 Conclusion

A large number of literatures have been published regarding the pharmacological importance of both the isoflavones. Newer drugs and conventional therapies involving the use of plants and/their constituents have been continuously scrutinized against many disorders. Most of the plant-derived compounds constituting flavonoids are polyphenolic in nature. A large number of papers have been published confirming the health-related benefits of dietary flavonoids. Large-scale clinical trials are required to be conducted in order to establish the potential usefulness of flavonoids in the treatment of various disease conditions. This requires the development of rapid and validated assays for the characterization and quantification of the phytol-constituents and their metabolites in biological matrices. Plant extracts though contain a mixture of chemical constituents which complicates the process of bioanalysis required in the drug development process. The present chapter highlights the pharmacokinetic interaction of FMN and BCA involving the microsomal CYP enzymes, multi-mechanistic membrane ABC transporters resulting in to the altered bioavailability of other co-administered drugs. They are increasingly being examined for their beneficial effect against many diseases. Apart from their therapeutic importance, large scale of research is required to investigate their interaction and binding capacity towards metabolic enzymes and ABC transporters at preclinical and clinical level. This necessitates to study the pharmacokinetic effect of these phytoestrogenic compounds in order to overcome the adverse events and synergizing the therapeutic potency of isoflavonoids.

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Conflict of Interest The authors declare that there are no conflicts of interest.

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Chapter 15
Roles of Phytometabolites in the Management of Obesity

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15.1 Introduction

Obesity is one of the major health problems and is increasing at an alarming rate in our society affecting over two-thirds of the population. In contrast to smoking, which has decreased due to improved public health awareness, overweight and obesity have steadily increased (Daniel 2008). According to the World Health Organization (WHO), a body mass index (BMI) greater than or equal to 25 is overweight, while a BMI greater than or equal to 30 is obesity (WHO 2000). Body mass index is an index of weight for height that is commonly used to classify overweight and obesity in adults.

Based on report from the World Health Organization, in the entire population, over 600 million are clinically obese, while about 1.9 billion adults are overweight up to the year 2014. Between the year 2013 and 2016, 18% of children within the ages 2–17 years were classified obese. The prevalence of obesity may vary in relation to sex, age, race, and Hispanic origin. Between 2003–2004 and 2013–2014, there were no significant changes in childhood obesity prevalence, but showed an increasing trend in adult (Ogden et al. 2015). The prevalence of obesity followed the same pattern among both men and women. Men aged 40–59 had a higher prevalence

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of obesity than men aged 20–39. Also, women aged 40–59 had a higher prevalence of obesity than women aged 20–39 (Hales et al. 2017).

There have been substantial increases in morbidity, premature mortality, impaired quality of life, and large healthcare costs in obese people (Fontaine et al. 2003; Haslam and James 2005; Kopelman 2000). There are comorbidities associated with obesity, but the major ones include type 2 diabetes, metabolic syndrome, hypertension, dyslipidemia, myocardial infarction, stroke, certain cancers, sleep apnea, and osteoarthritis (Flegal et al. 2007).

Currently, changes in human lifestyle and high-energy diet have contributed immensely to the increased incidence of obesity and even become a risk factor to the population of children (Klop et al. 2013; Kopelman 2000). Apart from aggressive dieting and exercising, some herbal products may aid in increasing metabolism or suppressing the appetite (Adesanya et al. 2019). Although several pharmacologic substances have been used as anti-obesity drugs, most of these substances have associated severe side effects, and hence natural products have been recommended in treating obesity especially in many Asian countries (Matson and Fallon 2012). Medicinal plants have gained importance as a possible basis of alternative and effective drugs with less or no side effects.

15.2 Common Terms Associated with the Explanation of Obesity

Obesity can medically be defined as a condition with excessive accumulation and storage of fats in the body resulting into reduce life expectancy and/or increased health problems (Mohamed et al. 2014). Obesity is not all about physical appearance alone but negatively affects almost all aspects of human health.

15.2.1 Assessment of Obesity

Body mass index (BMI) and waist-to-hip ratio (WHR) have been developed to identify people with the risk of severe health problems. The use of body weight as a means of assessment is not reliable; this is because it does not distinguish between fat and body mass (Mohamed et al. 2014). Body mass index is a measurement which correlates weight and height:

$$\mathrm{BMI} = \mathrm{Mass}(\mathrm{kg}) / \left[\mathrm{height}(\mathrm{m})\right]^2 .$$

Waist-to-hip ratio (WHR) can also be used for measurement of obesity and other serious health conditions. Abdominal obesity is defined as waist-hip ratio above 0.90 for males and above 0.85 for females according to the World Health Organization. The waist-to-hip ratio has been recommended to be a better predictor of cardiovascular diseases than waist circumference and body mass index (Brown et al. 1996). Waist distribution of fats can be assessed by calculating the waist/hip ratio.

$$\text{WHR} = \text{Waist circumference} / \text{hip circumference}.$$

15.2.2 Causes of Obesity

Imbalance between the energy intake and expenditure explains the etiologies of obesity at individual level (Patra et al. 2015). It is caused by altered lipid metabolic processes including lipogenesis and lipolysis (Pagliassotti et al. 1997). Obesity may also be caused in a limited number of cases by genetic factors, medical reasons, or psychiatric illness (Bleich et al. 2008). Societal factors can also cause obesity such as easy access and palatable diets, increased reliance on cars, and mechanized manufacturing (James 2008).

15.2.3 Comorbidities Associated with Obesity and Their Impact on Health

To some extent, the health effects of obesity are similar to those of cigarette smoking. This is because both cigarette smoking and obesity affect the cardiovascular, respiratory, digestive, neurologic, and reproductive systems among others. Both contribute to multiple diseases which include several major killer diseases such as heart diseases and cancer. Cigarette smoking is the greater killer and also the leading cause of preventable deaths both in the United States and the world as a whole (American Council on Science and Health 2003). Obesity is also a major contributor to preventable deaths; with good evidence, it was ranked second to cigarette smoking as a preventable cause of death and disease in the United States (Gilbert et al. 2008). In the United States, approximately 300,000 people die yearly because of diseases associated with obesity. Overweight subjects account for approximately 37% of the global burden of disease (Ng et al. 2014).

15.2.4 Conventional Preventive and Treatment Methods for the Management of Obesity

Since obesity is as a result of imbalance between the energy intake and expenditure, any treatment options and preventive measures to be used should also focus on how to restore balance of energy intake and energy expenditure (Sharma and Kanwar 2018). Losing weight can therefore reverse the harmful effects attributed to excess weight and may prevent obesity-related diseases such as diabetes mellitus, dyslipidemia, hypertension, and diastolic cardiac dysfunction (Klein et al. 2004).

The interventions involved in the management of obesity could be categorized into three classes:

- Changes in lifestyle such as energy-reduced diet and increased physical movement can be used to balance energy expenditure by increasing the usage of excess energy generated in the body. Exercises such as walking, swimming, cycling, and aerobics are very effective in the management of obesity and are therefore encouraged (Sharma and Kanwar 2018).
- Pharmacological interventions. There are two major classes of drugs that can be used for the treatment of obesity: orlistat and sibutramine. Orlistat reduces fat absorption through inhibition of pancreatic lipase, while sibutramine is an anorectic or appetite suppressant. Unfortunately, both drugs have adverse effects which include increased blood pressure, headache, dry mouth, insomnia, as well as constipation (Drew et al. 2007; Tziomalos et al. 2009). In 1997, the US FDA approved sibutramine drug as a treatment for obesity but was later withdrawn from the market in October 2010 because of its health risks associated with cardiovascular system and strokes (James et al. 2010; Kang and Park 2012).
- Surgical treatment. Bariatric surgery such as Roux-en-Υ bypass or gastric banding are much more effective in the management of obesity due to weight loss, comorbidity reduction, and enhanced survival (Kral and Naslund 2007; Sjostrom et al. 2007). However, they are reserved for the morbidly obese owing risks such as perioperative mortality, surgical implications, and the frequent need for reoperation (Field et al. 2009; Melnikova and Wages 2006).

15.3 Plants Are Promising Source of Anti-obesity Therapy

Various plants have been reported to possess anti-obesity activities through chemical extraction. Herbal plants with anti-obesity effects have active components that either cause decrease in body weight, body mass index, or waist circumference in humans. Crude extracts and isolated compounds of some medicinal plants are being used in the induction of weight loss and prevention of diet-induced obesity. Plants such as *Nigella sativa*, *Camellia sinensis* (green tea), and black Chinese tea have significant anti-obesity effects (Shirin et al. 2013). The long-term effectiveness of these medicinal plants need to be considered as well as the safety associated with their consumption. Long-term studies of these plants will elucidate their potential in the management of obesity; further studies on chemical extraction and isolation of plants metabolites are therefore a necessity. Figure 15.1 summarizes the activities of phytometabolites.

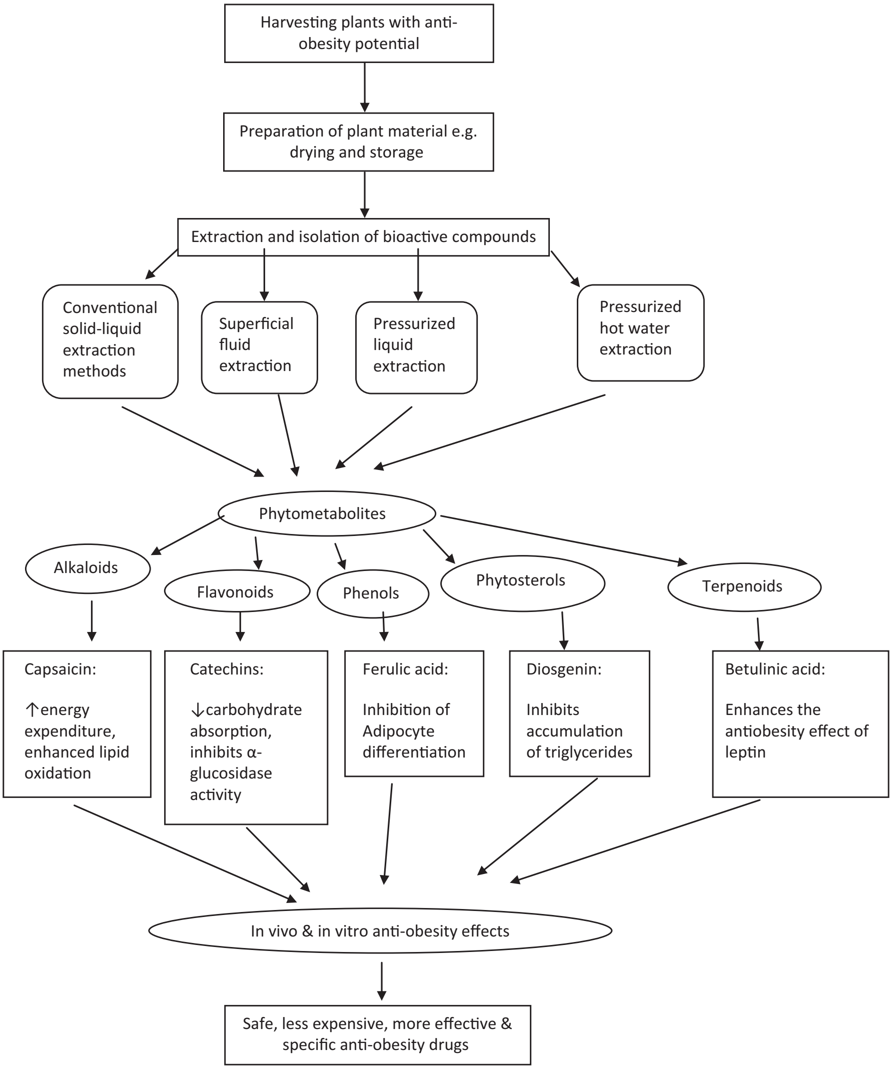


Fig. 15.1 Molecular activities of phytometabolites are crucial for the manufacture of anti-obesity drugs

15.4 Roles of Phytometabolites as Anti-obesity Resources

Ayurveda is an Indian ancient text that describes various natural treatments of obesity and hyperlipidemia (*Ayu* means life and *Veda* means knowledge). There are more than 50 individual dietary supplements and 125 proprietary products that already exist in the market for weight loss all over the world (Saper et al. 2004). A variety of plant products such as crude extracts and isolated pure natural products can interfere with diet-induced obesity and thus cause body weight reduction (Han et al. 2005).

There are several ways through which a phytomolecules can exert its anti-obesity effects. The possible mechanism of action of phytomolecules includes the following:

15.4.1 Increasing Energy Expenditure

Irregularities in energy homeostasis may be primarily associated with excess deposition of fats; this is caused by an imbalance between excessive food intake and increased energy expenditure (Aydin 2014). Increasing energy expenditure is necessary for effective weight management. Energy expenditure can categorically be classified into three groups: physical activity, obligatory energy expenditure, and adaptive thermogenesis (Sun et al. 2016). In most cases, the body weight is regulated by anti-obesity herbal products through obligatory energy expenditure. The presence of brown fats in the body enables the conversion of energy from food into heat energy. Found in the mitochondria of brown adipose tissue is a thermogenin (an uncoupling protein) that is used in the generation of heat energy. Therefore, the consumption of fat shredding foods that helps in burning of more calories during digestion will be one of the effective and reliable ways of controlling body weight. Foods capable of upregulating the activities of thermogenin (uncoupling protein 1) will be a better approach of achieving anti-obesity effect by increasing energy expenditure (Kajimura and Saito 2014).

15.4.2 Reducing Appetite

There are some bioactive phytochemicals derived from plants that have the ability of inducing satiety and as a result facilitate weight loss. Apart from enhancing the rate of metabolism, these active substances, known not specifically as nutrient compounds, also have the ability to suppress appetite (Yuliana et al. 2011). There is a complex interaction between neurological and hormonal signals that explains the biological mechanism of how appetite and satiety occur. Hormones derived from the guts such as GLP-1 (ghrelin) or neuronal system receptors such as TRPV1 are possible targets of expressing the effects of suppressants (Smeets and Westerterp-Plantenga 2009; Tominaga and Tominaga 2005; van Avesaat et al. 2016). *Camellia sinensis* (green tea) and *Capsicum annuum* have been reported to possess appetite-suppressing effects. The possible mode of action of *Camellia sinensis* is the inhibition of the catechol-O-methyltransferase (COMT) through catechins, an enzyme that deteriorates norepinephrine (Hursel et al. 2009). The bioactive compounds of chili pepper fruits are capsaicinoids, which are capable of increasing satiety based on studies conducted (Ludy and Mattes 2011; Westerterp-Plantenga et al. 2005; Yoshioka et al. 1999).

15.4.3 Stabilization or Modulation of Adipocyte Differentiation

Adipocyte plays a vital role in the regulation of energy. Increase in the size of adipocytes and its new formation from precursor cells leads to adipose tissue growth (Fig. 15.2). During adipocyte differentiation, committed preadipocytes undergo growth stoppage and subsequent terminal differentiation into adipocytes (Gregoire et al. 1998). Differentiation of adipocytes is accompanied by a dramatic change in the expression of adipocyte genes and is characterized mainly through the use of preadipose cell lineages (Mac Dougald and Lane 1995). Transcriptional factors, CCAAT/enhancer binding protein α (C/EBP-α), and peroxisome proliferator-activated receptor ϒ (PPAR- ϒ) are involved in growth arrest responsible for adipocyte differentiation and also activate adipocyte-specific genes (Niemela et al. 2008). These transcriptional factors act collectively in adipocyte differentiation by activating the expression of one another and likewise regulating the expression of other adipocyte-specific genes (Mandrup and Lane 1997). Studies showed that there are bioactive molecules that can potentially regulate adipogenic differentiation. For example, oxysterols are oxidized cholesterol metabolites commonly found in animal tissue and cholesterol-rich foods such as eggs, meats, and other dairy products (Galobart et al. 2002; Nielsen et al. 1995; Paniangvait et al. 1995; Pie et al. 1990). Certain oxysterols are capable of inhibiting expression of key adipogenic transcripts and adipogenic differentiation in different species (Moseti et al. 2016). Epigallocatechin gallate (EGCG) is the major polyphenolic catechin in green tea and has been reported to significantly reduce adipogenesis, fat tissue formation, and weight gain in various in vitro and in vivo studies (Bose et al. 2008; Chen et al. 2001; Klaus et al. 2005). Genistein is derived from soybean, a bioactive isoflavone that has been reported to inhibit adipogenesis and key adipogenic genes including PPAR-ϒ and C/EBP-α and promotes lipolysis (Harmon et al. 2002; Harmon and Harp 2001; Zhang et al. 2009). Plants such as grapes also contain polyphenolic

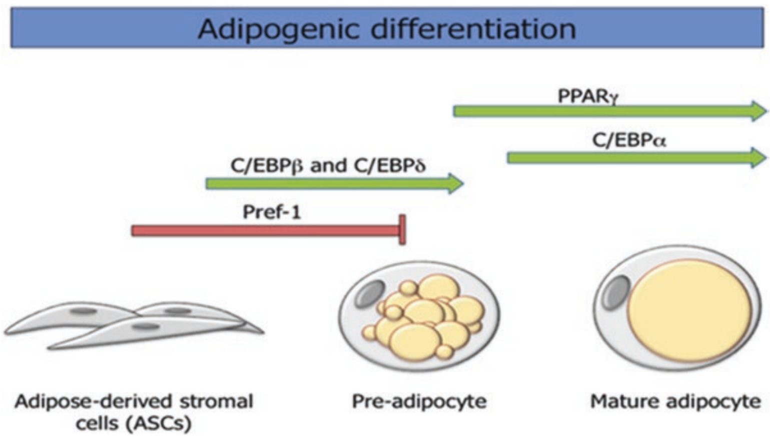


Fig. 15.2 Mechanisms involved in adipocyte differentiation

compound called resveratrol which has been reported to have the potential of modulating lipid metabolism and inhibiting the oxidation of low-density lipoproteins (Fremont 2000; Rayalam et al. 2008).

15.4.4 Inhibitory Activities of Lipase and α-Glucosidase

One of the promising approaches of treating obesity is the development of inhibitor that can interfere with absorption of fat. Inhibition of nutrient digestion and absorption is essential to facilitate energy intake reduction through gastrointestinal mechanisms (Foster-Schubert and Cummings 2006; Shi and Burn 2004). Triglycerides are most common lipids found in the body. They are hydrophobic in nature and binds to specialized proteins to form lipoproteins and provide the body with energy. Excess dietary lipid intake leads to deposition of excess calories, and as a result, inhibition of triglyceride (TG) will provide a new and effective approach of reducing the absorption of fat (Mukherjee 2003; Thomson et al. 1997). Pancreatic lipase (PL) is a water-soluble and major enzyme responsible for efficient digestion of triglycerides and hydrolysis of 50–70% of total dietary fats (Foster-Schubert and Cummings 2006). Inhibition of PL is one of the most widely studied mechanisms and strategies of treating obesity (Tsujita et al. 1989). Another factor for increased fat deposition is high consumption of carbohydrate because excess carbohydrates are converted into fats in the body (Mutai et al. 2015). Plants such as *Nigella sativa* and pistachio, psyllium fiber, and black Chinese tea showed to be significant weight loss agents and to reduce waist circumference with a mild reduction in blood sugar, triglycerides, and low-density lipoprotein levels (Datau et al. 2010; Kubota et al. 2011; Li et al. 2010; Pal et al. 2011).

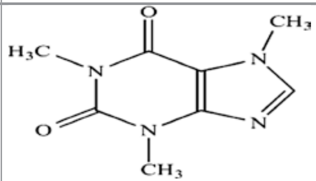
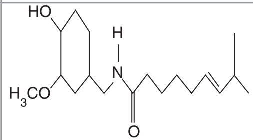
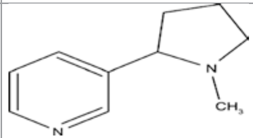
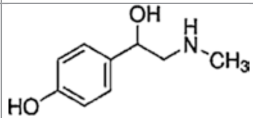
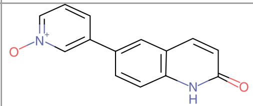
15.5 Promising Anti-obesity Phytometabolites

Chemical constituents found in plants can be classified into phenols, alkaloids, flavonoids, phytosterols, and terpenoids. Various compounds with anti-obesity potential have been summarized in Table 15.1.

15.6 Alkaloids

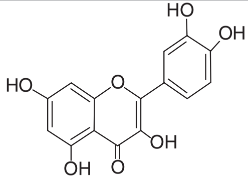
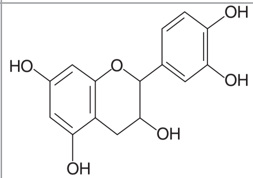
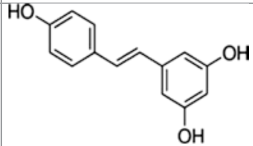
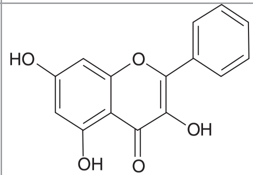
Alkaloids are secondary metabolites with a basic nitrogen atom comprising of approximately 12,000 natural products (Ziegler and Facchini 2008). Capsaicin is an alkaloid found in chili pepper (*Capsicum annuum*) which have 8% reduction in body weight and reduction in white adipose tissue (WAT) weight and adipocyte

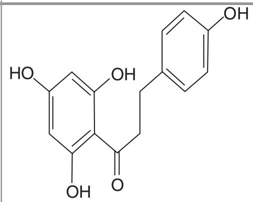
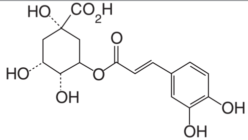
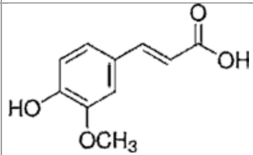
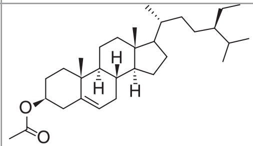
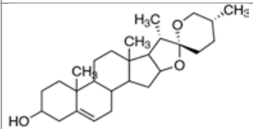
Table 15.1 Classes of phytochemical and their anti-obesity roles

Class of phytomolecules	Example	Structure	Plant source	Anti-obesity effect	References
Alkaloids	Caffeine		*Camellia sinensis*, *Coffea arabica*	Exert lipolytic and thermogenic actions	Hursel and Westerterp-Plantenga (2013) and Shimoda et al. (2006)
Alkaloids	Capsaicin		*Capsicum annuum*	Enhanced lipid oxidation and increased energy expenditure	Zheng et al. (2017)
Alkaloids	Nicotine		*Nicotiana tabacum* and *Capsicum annuum*	Nicotine increases metabolic rate and decreases food intake	Tucci (2010)
Alkaloids	p-synephrine		*Citrus aurantium* and *Citrus unshiu*	The active constituent *p*-synephrine increases metabolic rate, energy expenditure, and weight loss	Moro and Basile (2000) and Dragull et al. (2008)
Alkaloids	Halfordinol		*Aegle marmelos*	Anti-adipogenic activity and responsible for the decrease in adipocyte accumulation	Patra et al. (2015)

(continued)

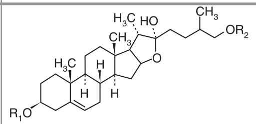
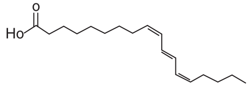
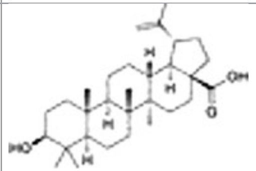
Table 15.1 (continued)

Class of phytomolecules	Example	Structure	Plant source	Anti-obesity effect	References
Flavonoids	Quercetin		*Coriandrum sativum*, *Brassica oleracea*, *Allium cepa*	Activates AMPK signal pathway in preadipocytes as a result of a decrease in in vitro adipogenesis	Ahn et al. (2008) and Choi et al. (2015)
Flavonoids	Catechins		*Camellia sinensis*, *Coffea*, and *Vitis vinifera*	Decreases the carbohydrate absorption because catechin inhibits α-glucosidase activity and small intestine micelle formation	Pan et al. (2016), Muramatsu et al. (1986) and Fischer-Posovszky et al. (2010)
Flavonoids	Resveratrol		*Arachis hypogaea*, *Vitis vinifera*, and *Cyanococcus*	Inhibits adipogenesis by reducing the transcriptional activity of PPARγ and enhances lipolysis	Zhao et al. (2017) and Zhang et al. (2012)
Flavonoids	Galangin		*Alpinia galangal*, *Helichrysum aureonitens*	Decreases accumulation of hepatic triglycerides, serum lipids, liver weight, and lipid peroxidation	Kumar and Alagawadi (2013)

Flavonoids	Phloretin-3′,5′-di-C-glucoside		*Cyclopia falcata* and *Cyclopia subternata*	Inhibit adipogenesis and intracellular triglyceride and downregulate peroxisome proliferator-activated receptor-2 (PPAR-2) expression and in vitro condition	Dudhia et al. (2013)
Phenols	Chlorogenic acid		*Glycine max* and *Coffea canephora*	Reduce body fat due to a reduction in the absorption of glucose	Corso et al. (2016)
Phenols	Ferulic acid		*Hordeum vulgare* and *Asparagus officinalis*	Hypolipidemic effect, dysregulated lipid profiles and inhibition of adipocyte differentiation	Mir et al. (2019) and Seo et al. (2015)
Phytosterol	Sitosterol		*Arachis hypogaea*, *Citrullus colocynth*, and *Bauhinia variegate*	Decreases cholesterol absorption by lowering the level of low-density lipoprotein cholesterol and inhibits pancreatic lipase	Gamboa-Gomez et al. (2015), Talabani and Tofiq (2012) and Patil et al. (2017)
Phytosterol	Diosgenin		*Trigonella foenum-graecum* and *Dioscorea villosa*	Inhibits accumulation of triglyceride and expression of lipogenic genes	Kumar and Bhandari (2015) and Naskar and Mishra (2017)

(continued)

Table 15.1 (continued)

Class of phytomolecules	Example	Structure	Plant source	Anti-obesity effect	References
Phytosterol	Protodioscin		*Trapa natans* and *Tribulus terrestris*	Significantly reduces blood levels of triglyceride, cholesterol, and low-density lipoprotein (LDL) and increases high-density lipoproteins (HDL)	Wang et al. (2010)
Terpenoid	Punicic acid		*Punica granatum*, *Momordica balsamina*, and *Trichosanthes bracteata*	Punicic acid binds and activates PPAR-α and γ, thus upregulating PPAR α and its responsive genes (Stearoyl-CoA desaturase-1, SCD1; carnitine palmitoyltransferase 1, Cpt-1; and acyl-coenzyme A dehydrogenase) as well as PPAR γ-responsive gene expression (CD36 and fatty acid binding protein 4, (FABP4)) in intra-abdominal white adipose tissue while suppressing expression of the inflammatory cytokine TNF-α and NF-κB activation	Adnyana et al. (2014) and Aruna et al. (2016)
Terpenoid	Betulinic acid		*Orthosiphon aristatus* and *Syzygium aromaticum*	Active constituent suppresses hypothalamic protein tyrosine phosphatase 1B and enhances the anti-obesity effect of leptin	Choi et al. (2013) and Silva et al. (2016)

sizes (Joo et al. 2010). Green tea (*Camellia sinensis*) has caffeine as parts of its bioactive ingredients and has been reported to cause 11.3–16.9% reduction in body weight and reduction in body fat index (Xu et al. 2015).

15.7 Flavonoids

Flavonoids are a general name used to describe secondary metabolites with 15-carbon skeleton consisting of more than 6500 molecules and have many health benefits. They can modulate neuronal function and are very effective in the prevention of age-related neurodegenerative diseases such as dementia and Parkinson's and Alzheimer's diseases (Macready et al. 2009; Prasain et al. 2010; Vauzour et al. 2008; Youdim and Joseph 2001). Flavonoids have a potential of preventing cardiovascular diseases which are attributed to their antioxidant properties (Majewska-Wierzbicka and Czeczot 2012). Flavonoids also have the ability to prevent cancer through their interaction with various genes and enzymes (Chahar et al. 2011). The fruits and leaves of *Morus alba* reduce fatty acid synthase, hepatic lipids, and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and increase carnitine palmitoyltransferase-1 and hepatic peroxisome PPAR-α (Birari et al. 2010). The leaves of *Morus alba* L. have flavonoids, salt of citric and malic acids, and anthocyanins (Mahmudur Rahman and Rahman 2018). Studies have reported that the administration of green tea catechins with caffeine is associated with reductions in BMI, body weight, and waist circumference (Phung et al. 2010).

15.8 Phenols

Phenols are naturally occurring and most widely distributed secondary metabolites of plants possessing one or more hydroxyl groups (Adebayo et al. 2018). Phenolic acids can be divided into many major classes which include benzoic acid and cinnamic acid. Derivatives of benzoic acid include gallic acid, whereas derivatives of cinnamic acid are coumaric, caffeic, and ferulic acid (Jin and Russell 2010). Chlorogenic acids have been reported to improve lipid metabolism in high-fat diet-induced obese mice and hence exhibit anti-obesity potential (Ae-Sim et al. 2010). Ferulic acids are found abundant in grain, fruits, and vegetables: rice bran, orange, tomatoes, carrot, broccoli, and banana (Molinar et al. 2009; Zhao and Moghadasian 2008). Studies demonstrated that ferulic acid suppresses obesity and obesity-related metabolic syndromes in high-fat diet-induced obese C57BL/6J mice (Weiwei et al. 2018).

15.9 Phytosterols

Phytosterols are found in plant cell membrane and are plant-derived lipid compounds that are similar in structure and functions to cholesterol (Osthund 2002). Vegetables oils and their products are the richest naturally occurring sources of phytosterols. They occur in free form of fatty acid/cinnamic acid or as glycosides (Raphael et al. 2015). The highest concentrations of phytosterols are found in unrefined plants oils such as olive oils, nuts, and vegetables (Osthund 2002). Sitosterol, campesterol, stanols, and diosgenin are phytosterols that are very effective for lowering the cholesterol level both in animals and humans based on studies (Swapna and Madhulika 2015). Sterols, stanols, campestanols, and diosgenin are important phytosterols found in fenugreek and wild yam and have been studied to significantly regulate metabolism: inhibiting cholesterol absorption (Gebhardt and Beck 1996; Plat and Mensink 2002; Rahman and Lowe 2006).

15.10 Terpenoids

Terpenoids (also known as isoprenoids) are natural products of plants which consist of more than 40,000 individual compounds of both primary and secondary metabolisms. Peroxisome proliferator-activated receptors (PPARs) are important targets for obesity, obesity-induced inflammation, as well as metabolic syndrome. PPARs are dietary lipid sensors that regulate lipid and carbohydrate metabolism (Evans et al. 2004). There are three subtypes of PPAR found in mammals which are α, δ, and ϒ (Kawada et al. 2008). In *Sapindus emarginatus* Vahl (Sapindaceae), its pericarp of flower contains triterpenoids, and its methanolic extract decreases body weight, BMI, blood glucose levels, total cholesterols, LDL-C, HDL-C, and triglycerides (Suneetha et al. 2013). In, *Nelumbo nucifera* Gaertn (Nelumbonaceae), its seed epicarp, leaves, seeds, and petals contain terpene, and its extract is effective in inhibiting preadipocyte differentiation and thus very crucial for management of obesity (Velusami et al. 2013). In *Orthosiphon aristatus* (Blume) Miq (Lamiaceae), its whole plant contains monoterpenes, diterpenes, and triterpenes. Its active constituent known as betulinic acid suppresses hypothalamic protein tyrosine phosphatases 1 B in mice and enhances the anti-obesity effect of leptin in obese rat (Choi et al. 2013).

15.11 Conclusion

Plant-derived metabolites with anti-obesity properties will go a long way to prevent fat metabolic disorders affecting people in different parts of the world, especially in developed countries like the United States where it is more common. Screening and evaluation of these metabolites will offer solution in tackling obesity and overweight in our society. Drugs commonly used such as orlistat and sibutramine are less effective with more adverse effects on the body and are therefore discouraged for consumption. Phytomolecules remain the best alternative for the treatment of obesity because it is of little or no side effects, easily accessible, cheaper, and safer for consumption. Hence joint efforts are required to explore and isolate natural products of plants for curing obesity and other ailments as well.

Suggestions and Recommendations
Exercises should be encouraged, and high-energy calorie food should be avoided to reduce the prevalence of obesity and overweight in our society. Natural plant products with anti-obesity properties should be used as best alternative in the treatment and management of obesity.

Conflict of Interest The author declares no interest of conflicts.

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Chapter 16
Chicory Inulin: A Versatile Biopolymer with Nutritional and Therapeutic Properties

Bisma Malik and Reiaz Ul Rehman

16.1 Introduction

Cichorium intybus L. commonly known as chicory is an erect perennial herb, 30–90 cm in height with a fleshy taproot up to 75 cm in length. *Cichorium intybus* belongs to the family Asteraceae, and it is commonly known as chicory in English and kasni in Urdu. It has been cultivated as vegetable as well as a medicinal herb by the ancient Greece and Romans (Plmuier 1972). It is native to temperate parts of old world, and the major producing countries of chicory are the United Kingdom, Belgium, France, the Netherlands, Germany, Switzerland, and South Africa. In India it is found wild in several states. It is cultivated in Bihar, Punjab, Jammu and Kashmir, Himachal Pradesh, Assam, Maharashtra, Gujrat, Tamil Nadu, Orissa, Andhra Pradesh, and Kerala. The members of chicory are cultivated across Europe for blanched buds (chicons), for salad leaves (Hocking and Withey 1987), for forage crops (Labreveux et al. 2006), and for spice and fructose production (Ricca et al. 2009) and for roots which after baking are used as coffee substitute (Bais and Ravishankar 2001) and feed additive (He et al. 2002).

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16.2 Chemical Constituents of Chicory

Chicory is rich in phytochemical constituents from its root to flowers, and the chicory contains a high amount of sesquiterpene lactones (Bais and Ravishankar 2001; Hance et al. 2007; Leclercq 1983; Rehman et al. 2003; Seto et al. 1988), flavonoids, and anthocyanin pigments (Bridle et al. 1984; Lante et al. 2011; Norbek et al. 2002; Vilkhu et al. 2008). The sesquiterpene lactones found mainly in chicory are lactucin, lactucopicrin, and 8-deoxylactucin, and these are responsible for the bitter taste of chicory, and it also contains caffeic, chlorogenic, neochlorogenic, 3-feruloylquinine, 3-p-coumaroylquinine, mono-caffeyl tartaric, di-caffeyl tartaric, and ferulic acids in good percentage (Lante et al. 2011). Chicory leaves also contain polyamines such as putrescine and spermidine (Krebsky et al. 1999), and the flavonoids present in chicory are coumarins such as esculetin (6,7-dihydrocumarin), umbelliferone, scopoletin, and cichorin (esculetin 7-O-β-D-glucoside) (Williams et al. 1996). The esculin is produced in the hairy root cultures and leaf cultures of *Cichorium intybus* L. cv. Lucknow Local (Bais et al. 1999; Rehman et al. 2003). Esculin is the glucosyl coumarin and is importantly known for its skin protective effects as well as vitamin P activity. Hydrolyzed esculin acts as an important microbiological marker for the detection of certain bacteria, such as *Yersinia*, *Clostridium thermocellum*, *Enterococci* sp., *Listeria* sp., and *Yersinia enterocolitica*, and fungi (differential isolation). Further, the flower heads cell free extract of chicory have been reported to transform cichoriin into esculin (George et al. 1999).

The roots are known to contain isochlorogenic acid, polyacetylenes (as ponticaepoxyde), fructans (mostly inulin), and free fructose (Ernst et al. 1995; Gupta et al. 1986, 1991; Gupta and Kaur 2000). Chicory also contains cytokinin, ribosyl zeatin, and a nucleotide sugar, uridine-5′-diphosphoglucose (de Kraker et al. 2003). The presence of the A guaianolide phytoalexin, cichoralexin, and condensed tannins has also been reported in chicory (Molan et al. 2003). These tannins increase the protein utilization efficiency among ruminants, and these have been reported to reduce intestinal parasites (Kidane et al. 2010). The carbohydrates present in the roots include a series of fructooligosaccharides and glucofructosans between sucrose and inulin besides glucose and fructose. Inulin is a linear polymer of β-2,1-linked fructosyl units with a single terminal glucose, and during storage the inulin present in the roots is converted into inulide and finally into fructose due to presence of an enzyme inulocoagulase, which coagulates inulin in the root juice (Dauchot et al. 2009; Roberfroid 2000). The earlier studies were more focused on cultivation, growth management, quality/yield, and breeding; however, due to its commercial cultivation (Cavin et al. 2005), the focus is now shifted toward the physiological and biochemical studies (Vilkhu et al. 2008), utilizing the extracts of chicory from root and leaf which have the potential for their uses in medicine such as anti-cancerous (Hazra et al. 2002), antifungal, fermentation, and functional food sector (Roberfroid et al. 1998).

16.3 Sources of Inulins

It has been reported that inulin occurs in around 36,000 plant species and historically inulin was isolated from *Inula helenium* (elecampane) (Meyer and Blaauwhoed 2009). Inulin, a carbohydrate, belongs to a class called fructans (containing fructose), and its biosynthesis takes place within a cell by a vacuolar enzyme, namely, sucrose fructosyl transferase (SST), in at least ten families of higher plants. They are present in many economically important and edible plants such as wheat, barley, artichoke, onions, and chicory (Abed et al. 2016; Meyer and Blaauwhoed 2009) (Table 16.1) which are consumed throughout the world either unprocessed or in processed form.

16.4 Properties of Inulin

Inulin is linear polymer composed of fructose units (2–60) connected with each other by β(2→1) glycosidic linkages with a single terminal α(1→2)-bonded d-glucose (Fig. 16.1) (Niness 1999), and mostly inulins are polydisperse mixture of fructan chains having a chain length (DP: degree of polymerization) which is dependent on its source. When the DP is lower than 20, it is considered as an oligosaccharide, and thus *β*-(2,1) fructans are called as oligofructose or fructooligosaccharides (FOS) because their DP is up to 10, but on an average DP is about 4. The solubility of inulins depends upon the chain length, and it has been observed that the longer the chain length, the more difficult it is to dissolve inulin. Inulins are prepared at high temperature (80 °C) and are stable up to 140 °C. They are susceptible to acid hydrolysis (pH less than 4) and have low viscosity. However, its combination with other ingredients helps in changing its rheological behavior and thus can be used for

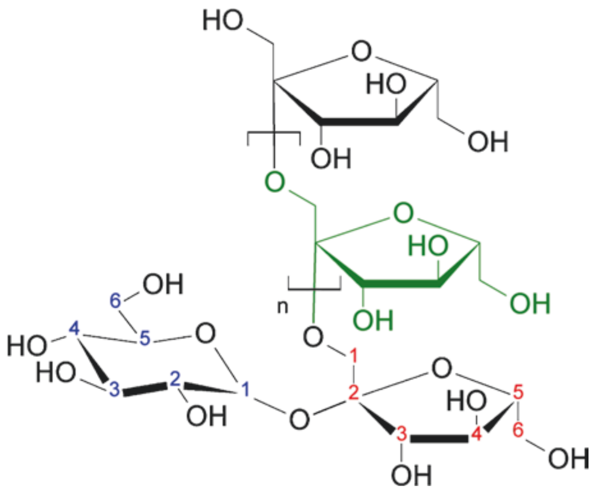

Fig. 16.1 Structural formula of inulin (fructan). Additional features (carbon numbers and repeated fructosyl unit) are highlighted with colors

Table 16.1 Plants as sources of inulin

Plant name(s)	Family	Common name(s)	Part used for inulin extraction	Reference(s)
Agave Americana L. *Agave fourcroydes* Lem.	Asparagaceae	Century plant, henequen	Lobes	Partida et al. (1998) and Villegas-Silva et al. (2014)
Allium ampeloprasum var. *porrum* (L.) J. Gay. *Allium cepa* L. *Allium sativum* L.	Amaryllidaceae	Leek Garden onion Garlic	Bulbs	van Loo et al. (1995), Ayyachamy et al. (2007) and Mahmoud et al. (2011)
Arctium sp.	Asteraceae	Wild rhubarb, *burdock*	Roots	van Loo et al. (1995)
Asparagus officinalis L. *Asparagus racemosus* Willd.	Liliaceae	Asparagus Shatavari, buttermilk root, climbing *asparagus*	Root tubers	Gupta and Kaur (1997)
Hordeum vulgare L.	Gramineae	Barley	Grains	van Loo et al. (1995)
Helianthus tuberosus L.	Asteraceae	Jerusalem artichoke	Root tubers	Sarchami and Rehmann (2015)
Camassia sp.	Liliaceae	Indian hyacinth, camash	Bulbs	van Loo et al. (1995)
Cichorium intybus L.	Asteraceae	Chicory	Roots	van Loo et al. (1995) and Gupta and Kaur (1997)
Cynara cardunculus L. *Cynara scolymus* L.	Asteraceae	Cardoon Globe artichoke	Leaves-Heads	De Leenheer (2007) and Leroy et al. (2010)
Dahlia sp.	Asteraceae	Dahlia	Root tubers	Zubaidah and Akhadiana (2013) and Rawat et al. (2015)
Dioscorea esculenta Lour.	Dioscoreaceae	Lesser yam	Root tubers	Zubaidah and Akhadiana (2013)
Echinops ritro L.	Asteraceae	Globe thistle	Roots	Vergauwen et al. (2003)
Fagopyrum esculentum Moench.	Polygonaceae	Buckwheat	Grains	Bonciu et al. (2012)
Helianthus annuus L.	Asteraceae	Sunflower	Root tubers	Chi et al. (2011), Ertan et al. (2003) and Sarchami and Rehmann (2015)
Lactuca sativa L.	Asteraceae	Lettuce	Roots	Hendry and Wallace (1993)

(continued)

Table 16.1 (continued)

Plant name(s)	Family	Common name(s)	Part used for inulin extraction	Reference(s)
Microseris lanceolata (Walp.) Sch.Bip.	Asteraceae	Yam daisy, murnong	Roots	van Loo et al. (1995)
Musa acuminate Colla *Musa cavendishii* Lamb. Ex Paxton	Musaceae	Banana	Fruits	van Loo et al. (1995)
Pachyrhizus erosus (L.) Urb.	Fabaceae	Yam bean, Mexican turnip, and Mexican potato	Root tubers	Zubaidah and Akhadiana (2013)
Polymnia sonchifolia Poeppig & Endl.	Asteraceae	Yacon	Root tubers	Braz de Oliveira et al. (2011)
Saussurea lappa (Falc.) Lipsch.	Asteraceae	Kusta, kuth	Roots	Kuniyal et al. (2005)
Scorzonera hispanica L.	Asteraceae	Oyster plant	Roots	Dolota and Dabrowska (2004)
Secale cereale L.	Gramineae	Cereal rye	Grains	van Loo et al. (1995)
Smallanthus sonchifolius (Poepp.) H.Rob.	Asteraceae	Apple of the Earth	Roots	De Leenheer (2007)
Taraxacum officinale L.	Asteraceae	Dandelion	Leaves	van Loo et al. (1995)
Tragopogon sp.	Asteraceae	Yellow salsify	Roots	Gupta and Kaur (1997)
Vernonia herbacea (Vell.) Rusby	Asteraceae	Brazilian Cerrado	Rhizome	De Carvalho and Dietrich (1993) and Pessoni et al. (1999)
Viguiera discolor Baker.	Asteraceae	Cerrado	Root tubers	Isejima et al. (1991)
Triticum aestivum L.	Gramineae	Common wheat	Grains	Moshfegh et al. (1999)

modifying textures of food products. Above 15% inulin concentration, it can form gel or cream, and the gel strength is dependent on chain length and temperature (Coussement and Franck 2001). Inulin when added to beverages along with thickeners (xanthan, pectin, or guar gum) makes the beverages homogenous. It also provides spreadability to gum-based fat-free dressings and sauces. Inulins when added to dairy products interact with its components (e.g., combined with κ-carrageenan) to provide a creamy mouth feel. Due to important properties (fat replacer, foam stabilizer, and bulking agent), inulin is used in dairy, bakery, and meat industries (Meyer and Blaauwhoed 2009). Sometimes inulin from a source (plants) is mixtures of certain polymers (2–50 fructose units) with different DP (Marx et al. 1997).

16.5 Chicory Inulin and Digestive Tolerance

The right balance of gut microbiota is essential for gut health and protects the body against disease, and this balance is maintained by inulin which stimulates the growth of beneficial bacteria (Marchesi et al. 2016). The increase in beneficial bacteria helps in improving digestion and immunity. Chicory is a rich source of inulin, and its roots are composed of 68% inulin on dry weight basis (Nwafor et al. 2017). The roots of chicory may be helpful in optimal gut health as the inulins and FOS present are digestive tolerant and act as prebiotic because they can stimulate lactobacillus and bifidobacteria. Studies have found that FOS is tolerant to 15 g–20 g/day with minor digestive complaints (Ellegard et al. 1997; van Dokum et al. 1999). Chicory inulin has been reported to help relieve constipation as one study found that people experienced more frequent bowel movements and improved stool consistency (Collado et al. 2014; Marteau et al. 2011). They act as prebiotic dietary fiber which passes undigested in the body, feeding the gut bacteria and completely fermented in the gut (colon). Studies show that chicory inulin 12 g per day relieved constipation by softening the stool and increasing the bowel frequency (Micka et al. 2017; Watson et al. 2019). Stimulation of beneficial bacteria plays an important role in reducing inflammation, improving absorption of minerals, and protecting the system from harmful bacteria (Carlson et al. 2018; Flamm et al. 2001; Kelly 2008; Macfarlane et al. 2008).

16.6 Chicory Inulin and Inflammatory Bowel Disease (IBD)

It is suggested that chicory inulin could also be used as a prebiotic as inulins have been shown to have good benefits for treating inflammatory bowel disease (IBD) by improving the gut microbiota and thus subsequently decreasing the gut inflammation (Akram et al. 2019). The inulin- and oligofructose-enriched supplementation in human studies have been found to reduce the symptoms of ulcerative colitis (Casellas et al. 2007) and inflammation markers in Crohn's disease (Lindsay et al. 2006).

16.7 Inulin Reduces Risk of Colon Cancer

It has been observed in some reports that inulin helps in the protection of cells in the colon because it is fermented into butyrate which has a protective role (Goncalves and Martel 2013). Several studies have shown that 88% of the groups (patients) which received inulin had a reduction in the precancerous colon growth (Pool-Zobel 2005). Another study reveals that inulin is beneficial in the reduction of colon cancers because it made the environment in the colon less favorable for the cancer development (Boutron-Ruault et al. 2005). In a study, rats were given inulin with particular dosage at particular time, and it was found that there occurred less inflammation and fewer precancerous cellular changes (Hijova et al. 2013).

16.8 Chicory Inulin: Regulating Blood Sugar for Diabetic Control

There are several studies suggesting the importance of inulin in controlling the blood sugar levels in pre-diabetes and diabetes (Guess et al. 2015; Pourghassem et al. 2013). The control of blood sugar levels depends upon the type of inulin, e.g., in one study, a high-performance (HP)-type inulin was found to decrease liver fat in prediabetic people (Guess et al. 2015; Niness 1999). This reduction activity in liver fat by inulin is of significance since fat reduces insulin resistance and thus reverses type 2 diabetes (Lim et al. 2011). In a study with type 2 diabetic females fed with HP inulin (10 g per day), the blood sugar decreased by 8.5%, and hemoglobin A1c fell by 10.4% (Dehghan et al. 2013). The chicory inulins from roots have been reported to be beneficial in controlling the blood sugar level especially in people suffering from diabetes. The role of inulin is that it promotes the beneficial bacteria in the gut which can metabolize carbohydrates in both insulin-sensitive and insulin-insensitive people and thus aid in the absorption of sugars from the blood (Guess et al. 2016; Pourghassem et al. 2013; Zhang et al. 2018). Furthermore, the chicory root fibers also contain compounds like chlorogenic and chicoric acids which increased the muscle sensitivity toward insulin in rodents (Azay-Milhau et al. 2013; Ferrare et al. 2018). In a study on 49 women with type 2 diabetes, 10 g of inulin per day was given for 2 months resulting in the decrease of blood sugar levels and hemoglobin A1c (Pourghassem et al. 2013).

16.9 Inulin and Heart Health

It has been revealed that inulin may improve the heart health by reducing the important markers as was observed in a study where females were fed for 8 weeks with 10 g of HP inulin that showed a significant decrease in both triglycerides and low-density lipoprotein (LDL) cholesterol (Dehghan et al. 2013).

16.10 Inulin Improved Mineral Absorption

Chicory root fiber improves absorption as the root fiber makes the colon more acidic, which increases the surface area that can absorb nutrients and makes more proteins that bind to calcium. It has been concluded from the animal studies that inulin improves the absorption of calcium and magnesium, thus improving the bone density. An FDA review suggests that the inulin-type fructans help in calcium absorption, thus benefiting the bone mineral density (Garcia-Vieyra et al. 2014; Legette et al. 2012).

16.11 Chicory Inulin in Weight Management

There are several reports suggesting that inulin is effective in weight loss (Chambers et al. 2015; Guess et al. 2015). In a weight loss study carried out for 18 weeks on prediabetic patients, it was found that inulin decreased more weight between 9 and 18 weeks (Guess et al. 2015). It has been suggested that the chicory root fiber may be helpful in managing weight possibly by regulating the appetite and decreasing the calorific intake. It was seen that there was a significant weight reduction at an average by 1 kg in 48 overweight adults which were fed with 21 g of chicory-oligofructose per day for 12 weeks. This reduction in weight was due to the decrease in level of hormone ghrelin (stimulates hunger) by oligofructose (Parnell and Reimer 2009). Similar findings have been reported with inulin or oligofructans from other sources (Arora et al. 2012; Guess et al. 2015).

16.12 Chicory Inulin Safety/Side Effects

The side effects of inulin in some people could be due to food allergy, and it may cause flatulence, bloating, abdominal pain, and diarrhea. It has been reported that the oligofructose derived from chicory can cause flatulence and bloating in some people taking 10 g per day (Bonnema et al. 2010). It was further reported that people can tolerate chicory inulin from roots at even higher doses than 10 g per day, but in some people, there was stomach discomfort even at low dosage of 7.8 g a day (Ripoll et al. 2010).

16.13 Chicory in Traditional System of Medicines

The cultivated chicory plants are used in Indian medicine as a tonic, curative in acne, ophthalmia, and inflamed throat. It is reported to be useful in fevers, vomiting, diarrhea, and enlargement of the spleen. The mild form is reported to be tonic, emmenagogue, and alexiteric. Chicory is also used for jaundice and is reported to be good for rheumatism. Added to coffee, it counteracts caffeine and helps in digestion. A tea made from chicory is beneficial in upset stomach. There are several brands available in the market containing chicory for ailments in humans as well as in animals; these include Bonnisan, Geriforte (GeriCare, StressCare), Herbolax

(LaxaCare), Liv.52 (LiverCare), Liv.52 Drops, Digyton, Geriforte Aqua, Geriforte Vet, Liv.52 Vet (Companion), Liv.52 DS, and PartySmart (http://www.himalaya-healthcare.com/herbfinder/h_cichor.htm, Himalayan Drug Company, India). The mixtures of leaf/root or the root alone are being used for lack of appetite and the mild dyspeptic disorders (Scholz 2006). Further, the use of the tea prepared from roots in digestive disorders and improving appetite was reported earlier (Madaus 1938). Some other sources have mentioned the medicinal uses of roots in the form of tonic as blood purifier, used in internal and external bleeding, jaundice, and catarrh of the stomach (Hoppe 1949, 1958). Further the root decoctions are being used as mild laxative, bitter tonic-stomachic for promoting liver function and diseases (Poletti 1990). The use of herb has also been reported as tea (Braun and Frohne 1994) and as tonicum (Hoppe 1958).

16.14 Elucidation of Drug Use

Various studies have been conducted to define and establish the medicinal properties of the chicory. The hepatoprotective role of chicory has been established with many studies, and it has been reported that chicory provides protection against the paracetamol- and CCl4-induced hepatotoxicity. The study was done in albino rats which were given the intraperitoneal aqueous seed extracts (Gadgoli and Mishra 1997). The hepatoprotective role against the paracetamol- and CCl4-induced toxicity was compared between the root extracts and the root callus extracts. The histopathology and analysis of hepatic enzymes revealed that the root callus extracts were better against the toxicity (Zafar and Mujahid Ali 1998). The in vitro study reported that the aqueous ethanolic extract of chicory protected the cells against the free radical-induced DNA damage (Sultana et al. 1995). Further, a phenolic compound AB-IV from the seeds showed a potent hepatoprotective activity when compared to the drug silymarin (Silybon-70) (Ahmed et al. 2003). The sesquiterpene glycoside compound (cichotyboside) from the seeds of chicory showed hepatoprotective effect against CCl4-induced toxicity. This compound showed the reduction of liver enzymes and proteins which were elevated after the toxicity (Ahmed et al. 2003, 2008). The choleretic effect was reported after intraduodenal administration of root extract prepared in water, and the reduction in cholesterol absorption in the jejunum and ileum of rats was also observed (Scholz 2006). The antioxidative effect of root extract on LDL (low-density lipoprotein) oxidation was reported by Kim and Yang (2001). Further, the sesquiterpene lactones such as lactucopicrin and lactucin from the roots showed anti-malarial activity in vitro against a HB3 clone of a Hondurus-1 strain of *Plasmodium falciparum* (Bischoff et al. 2004). These compounds showed analgesic effects similar to that of ibuprofen and lactucopicrin and were found to be more active (Wesolowska et al. 2006). The ethanolic extract of chicory was also studied for anti-diabetic activity in male Sprague Dawley rats, and it was found that there were no changes in insulin secretion; however, the activity of hepatic Glc-6-Pase was reduced (Pushparaj et al. 2007). The aqueous extract from chicory was found to inhibit the induced anaphylactic reactions in mice (Kim et al. 1999).

Further, the guaianolide 8-deoxylactucin present in the root extract of chicory was found to inhibit the COX-2 protein expression in human colon carcinoma cells (HT29 cells) (Cavin et al. 2005). The chicory root extract was also found to have tumor-inhibitory effect in mice (Hazra et al. 2002). The interaction of dried root of chicory fed to pigs for 15 days orally was studied in vivo on cytochrome P450, and it was found that it increases the mRNA expression of the protective liver enzymes (Rasmussen et al. 2011). In the chronic toxicity studies in male and female rats, it was found there was no treatment-related toxicity from the root extracts when fed for 28 days up to 1000 mg/kg/day (Schmidt et al. 2007). Further, the study on the aqueous extract of chicory leaves administered intraperitoneal resulted in the altered sex ratio in the offsprings (Behnam-Rassouli et al. 2010). It was observed that at the high dose of aqueous root suspension, 8.7 g/kg body weight in Swiss mice resulted in seminiferous tubule degeneration and Leydig cell atrophy (Roy-Choudhury and Venkatakrishna-Bhatt 1983); however, the mechanism of action of this high-dose toxicity is unknown. The chicory coffee when consumed for 1 week was shown to decrease the plasma and blood viscosity (Schumacher et al. 2011). The chicory root extract was studied for its effect on osteoarthritis, and it was found that there was some improvement in 20% patients in terms of pain and stiffness and thus it could be utilized for osteoarthritis management (Olsen et al. 2010).

16.15 Chicory in Functional and Health Foods

Inulin is a soluble fiber found in many plants and is composed of fructans (chains of fructose molecules). Prehistoric humans consumed much more inulin than we do today (Leach and Sobolik 2010). The molecules are linked in such a way that they cannot break down in the small intestine; instead, they feed the beneficial bacteria in the lower gut. The inulin is acted upon by the bacteria to convert them into short-chain fatty acids which nourish colon cells (Vandeputte et al. 2017). In the food industry, the manufactures add inulin to processed products to improve health benefits and to boost prebiotic content, alter food texture, and replace fat and sugar contents (Shoaib et al. 2016). The use of chicory root is not new since it has been in use since the time immemorial; it has been in use for cooking as well as the medicinal purposes and has been considered safe for most people. People might be using it unknowingly since it is used as an additive in the packaging of functional foods in the food industry as a sweetening supplement either in native form (unaltered) or in the altered form (treated chemically to make it sweeter) (Bonnema et al. 2010; Flamm et al. 2001). Fructose-rich syrup can be prepared by acid hydrolysis of aqueous root extract which can be used as sweetener, especially for diabetics. Mature roots are roasted and ground and blended with coffee to produce “French coffee” (65% coffee and 35% chicory).

The chicory powder increases the volume of coffee and improves the flavor and keeps the good quality of coffee powder. The addition of roasted chicory roots not only increases the coffee constituents but also enhances the flavor due to the presence of caffeol, one of the chief aromatic constituents in the brew. Chicory is known to produce a unique fiber inulin in shoots. This fiber has received a lot of attention for it being the source of fiber in functional and health foods. The inulin has building blocks of sugar (up to 90 monosaccharides) that is used as a reserve carbohydrate in several plants such as artichokes, Jerusalem artichokes, onions, and chicory. Further, the oligofructans are similar to inulin but have shorter chains. The industrial oligofructan is produced from the chicory inulin and is a very important dietary fiber used as a fat substitute which is low in calories. These dietary fibers are not broken down in the stomach or intestines, but these are broken down in the colon. These breakdown products are desirable for the lactobacilli growth and thus become unfavorable for other pathogenic microbes. So, inulin and oligofructans are the prebiotics which help in preventing digestive tract infections and also support the immune system (Roberfroid et al. 1998). These are used in dietary products and drinks which are sweet, are with consistency, and are low in calories. Chicory, being the primary source of inulin, starts to break down at the low temperatures; hence, it is required to genetically modify the chicory so that inulin is stabilized at the low temperatures. A gene from *Helianthus tuberosus* (Jerusalem artichoke) is having potential toward this goal. It has been shown that by genetically transforming the *chicory* by *Agrobacterium rhizogenes* resulted in a changed phenotype such as the annual flowering; however, inulin accumulation in tuberized endive roots was not changed by genetic transformation by *A. rhizogenes*, but at the end of the vegetative phase, free fructose was increased 8–10 times (Sun et al. 1991).

The transgenic studies on inulin (Kawakami et al. 2008; Li et al. 2007) are widely accepted. In chicory the inulin chain length shows variation in different seasons as well as in cultivars (Timmermans et al. 2001; Van den Ende et al. 1996). Chicory root is very commonly processed for extracting inulin and then used to increase the fiber content or as a fat or sugar substitute due to its sweet flavor and gelling properties. Chicory is available in the stores as whole root to be eaten as a vegetable or the roasted ground chicory which can be consumed as a rich beverage with caffeine (Madrigal and Sangronis 2007). However, it has been suggested that the native inulin is more tolerant as it causes less gas and bloating (Bonnema et al. 2010). The standard tolerable dosage of inulin is 10 gm per day in many studies, but a higher amount is also proposed for either altered or native inulins (Micka et al. 2017; Parnell and Reimer 2009). However, the chicory root dosage recommended in general for people is yet to be established. The pregnant and breastfeeding women need to take a professional advice before consuming it (Ernst 2002). Furthermore, people with issues of allergy toward birch or ragweed pollen should avoid chicory as it might trigger similar reactions (Cadot et al. 2003). Finally chicory root might be promising as an anti-hyperuricemia agent because it promotes renal excretion of urate by inhibiting its reabsorption. This property may be due to the downregulation of mRNA and protein expression of URAT1 and GLUT9 (Wang et al. 2019).

16.16 Conclusion

Chicory is one of the best sources of natural inulin which acts as a dietary fiber and has lots of health benefits. Besides it also has many reported nutritional as well as medicinal properties which make it an ideal candidate from which nutraceuticals and therapeutic products can be obtained at an industrial level. With increase in demand for the chicory inulin, its cultivation should be increased with the high-yielding varieties. Besides, there is a potential in genetic transformation technology for increasing the ratio of inulin by modifying the enzymes in inulin biosynthetic pathway. The higher accumulation of chicory inulin has been seen with overexpression of sucrose:sucrose 1-fructosyltransferase (1-SST). However, there is a need to improve some of the important characteristic of inulins like degree of polymerization (DP) which tends to decrease during extraction. Thus it is advisable to check the chicory cultivars and environmental conditions controlling the expression of enzymes: fructan:fructan 1-fructosyltransferase (1-FFT) and fructan 1-exohydrolase (1-FEH). So the cultivars with lower amounts of these enzymes and the environmental factors downregulating the genes encoding these enzymes are the best sources for improving the quality of inulin.

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Chapter 17
Medicinal Plant-Derived Antimicrobials' Fight Against Multidrug-Resistant Pathogens

Swapan Kumar Chowdhury, Debabrata Misra, and Vivekananda Mandal

17.1 Introduction

Nature is the source of various medicinal herbs, and human depends on it for the treatment of ailments and diseases over centuries as they are beneficial for their minimum side effect properties. The World Health Organization (WHO) has reported that 80% of the world population mainly the ethnic communities depend on plant-based traditional medicine to fulfill their primary healthcare needs (Mishra et al. 2013; Nitha et al. 2012). Phytochemicals, mainly the secondary metabolites of plants, are used in synthetic drugs of modern medicines worldwide. These are structurally diverse bioactive compounds derived from medicinal plants (Pandey and Kumar 2013). They have also been used as antimicrobial agents against pathogens to combat infectious diseases (Chandra et al. 2018; Mishra et al. 2013). Microorganisms such as bacteria, fungi, and viruses are the major causal agents of various infectious diseases in humans. More attention has been concentrated on the study of plant-derived compounds over the past decades which specially act against multidrug-resistant (MDR) Gram-negative and Gram-positive bacteria (Borges et al. 2015a, 2015b). Since the microbial sources are widely relied upon, the plant-originated pharmaceutical products distributed about 50% in which few are used as antimicrobials in the United States (Subramani et al. 2017). The golden era of the discovery of novel antibiotics was considered from the 1950s to 1970s (Aminov 2010). The term antimicrobial resistance (AMR) is often used to define the growth

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and spread of bacteria, fungi, viruses, protozoa, and nematodes in the presence of antibiotics or antimicrobial agents that was originally found to be effective for the treatment of infection caused by those microbes (Founou et al. 2017). The higher healthcare costs, increased duration of illness, and frequency of hospitalization enhanced due to uses of antibiotic resistance pathogens when compared with non-resistant common infections. It has been scientifically proven that the indiscriminate and inappropriate use of antibiotics has accelerated the emergence of multidrug-resistant strains (El Chakhtoura et al. 2018). Bacteria are the common microbes in which antibiotic resistance develops either through mutation of a target site protein, through the incorporation of an antibiotic resistance gene (ARG) that confers resistance through efflux or inactivation of the antibiotic, or through synthesis of a new target protein that is insensitive to the antibiotic (Davies and Davies 2010). The mortality rate for patients with infections caused by non-resistant bacteria is less than half of that of people with a resistant form of the same infection (Tiwari et al. 2013). However, the widespread use of antibiotics to control various human clinical pathogens has challenged the human immune system by the development of antibiotic resistance. The twentieth century is important for antibacterial therapy which is one of the most important medical developments and has become one of the pillars of modern medicine in preventing millions of premature deaths due to bacterial infection (Friedman et al. 2016).

Few bacterial pathogens have been recognized as the most terrible pathogens including β-lactamase producers *Escherichia coli* and *Klebsiella pneumoniae*, carbapenem-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa*, hospital-acquired methicillin-resistant *Staphylococcus aureus*, and vancomycin-resistant *Enterococcus* (Kumar and Varela 2013; Satlin and Walsh 2017; Talbot et al. 2006). The enteric pathogens *Pseudomonas aeruginosa, Streptococcus pneumoniae, S. aureus*, and *Mycobacterium tuberculosis* have modified themselves by developing resistance to multiple antibiotics (Ballal et al. 2014; Lowy 2003). On the other hand, On the other hand, *Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa*, and *Enterobacter* spp. are high-level drug-resistant bacteria referred together by the acronym ESKAPE which cause the majority of infections within the hospital environment (Pendleton et al. 2013). The broad range of AMR mechanisms used by the ESKAPE pathogens includes enzymatic inactivation, modification of drug targets, changing cell permeability through porin loss, or increasing the mechanical protection and expression of efflux pumps provided by biofilm formation. AMR in these pathogens is a major concern to public health systems worldwide and is likely to increase as resistance profiles change. Antibiotics have been saving an immense number of lives from many infectious diseases from more than a half-century. But due to increase in the AMR, the demand for alternative therapeutic compounds against those MDR pathogens has been emphasized for the last few decades. To overcome antibiotic resistance by developing more powerful antibiotics, which has been attempted by pharmaceutical companies, can lead to an only limited and temporary success and eventually contribute to developing greater resistance. There is an urgent need to develop some new medicinal plant-derived natural compounds which play an important role as antimicrobial agents with diverse chemical structures and novel mechanisms of action. Researchers and scientists from different research backgrounds have found thousands of phytochemicals with their antimicrobial usefulness on all types of microorganisms (Cowan 1999). Medicinal plants which are a rich source of valuable secondary metabolites, such as alkaloids, flavonoids, tannins, and terpenoids, quinones, and polyphenols with profound antimicrobial potentialities, have considerably drawn the interest of ethnopharmacologists, botanists, microbiologists, and chemists toward plant utilization as efficient, and safe natural products (Dhama et al. 2014; Edeoga et al. 2005; Tepe et al. 2004; Yadav et al. 2016). The number of bioactive compounds derived from plants has been estimated to be at least 2,00,000 produced by the species of medicinal plants growing on Earth (Efferth and Koch 2011). The biggest challenges include finding compounds with sufficiently lower minimum inhibitory concentrations (MICs), little toxicity, and ease of bioavailability for efficient and safe use in humans and animals. In recent years, the new approaches targeting specific regulatory pathways and bacterial virulence are becoming the paradigm of antibacterial-based therapeutics. The mechanisms of action of these compounds would certainly pave the path for the designing and development of novel drugs that could potentially control infectious diseases together with circumventing antimicrobial resistance. The results of these investigations show that phytochemicals modulate or modify resistance mechanisms in bacteria, suggesting that phytochemicals can be used in combination with antibiotics to increase the activity and decrease the doses of antibiotics (Santiago et al. 2015; Touani et al. 2014). The present study is a review focused on plant-derived antimicrobial compounds and their potential applications as novel antimicrobial agents against MDR pathogens and their mechanism of actions.

17.2 Plant Secondary Metabolites as Bioactive Molecules

Most of higher plants develop secondary metabolites, which are pharmacologically active. The development of secondary metabolites can either be a part of the plant's normal program of growth, or it can happen in response to pathogenic attack (such as phytoalexins). The secondary metabolites in plants can be divided into different categories according to their biosynthetic principles. A simple classification includes three main groups: terpenes such as mono-, di-, tri-, sesqui-, and tetraterpenes, saponins, steroids, cardiac glycosides, and sterols; phenolics such as phenolic acids, coumarins, lignans, stilbenes, flavonoids, tannins, and lignins; and nitrogen-containing compounds such as alkaloids and glucosinolates. The compounds within the groups do also have some similarities in their structures.

17.3 Historical Perspectives of the Use of Medicinal Plants as Antimicrobials

Medicinal plants have provided the source of various novel drug compounds since antiquity which have largely contributed to human health and well-being. Many plants derived phytochemicals used for the natural development of new drugs as well as phytomedicine to be employed for the treatment of infectious diseases. According to Hippocrates in the late century B.C., 300–400 plants having medicinal properties (Schultes 1978). The quinine alkaloid obtained from *Cinchona* sp. has a long history of therapeutic use in the treatment of malaria and to relieve nocturnal leg cramps and *Staphylococcus aureus*. The *Cephaelis ipecacuanha* The *Cephaelis ipecacuanha* (Brot.) A. Rich. (Family: Rubiaceae) controls the spread of *Escherichia histolytica* infections.

17.4 Emerging Infectious Diseases in Human: Causes and Consequences

Pathogenic microorganisms are known to cause various types of infection in human being leading to death worldwide. A brief overview of some highly disease-causing pathogenic microbes has been enumerated below.

17.4.1 Bacterial Diseases

Among the bacterial pathogens, *Enterobacteriaceae* members are responsible for causing several infections of humans (Fabio et al. 2007). Several hospital-acquired infections such as pneumonia, septicemia, and urinary tract and soft tissue infections are caused by the Gram-negative bacterium *Klebsiella pneumonia* (Wu and Li 2015). Another most pathogenic bacterium *Pseudomonas* sp. infects our body organs like the liver, brain, bones, and sinuses. *Escherichia coli* is a colon Gram-negative pathogenic bacterium that causes several diseases, viz., intestinal sickness, bloody diarrhea, bloody urine, pale skin, decreased urine output, bruising dehydration, biofilm formation, and pro-inflammatory interleukin-8 secretion, respectively (Ellis et al. 2020). *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are *Enterobacteriaceae* which are carbapenem-resistant reported worldwide (Bassetti et al. 2019). Neonatal sepsis and pneumonia are the dangerous diseases in human beings caused by *Streptococcus*. Among them, *Streptococcus mutans* causing dental caries disease have their surface protein antigens acting as the virulence factors (Nakano et al. 2008). The bacterium *Staphylococcus aureus* causes blood infections, nosocomial infections, and surgical wound infections (Haque et al. 2018).

17.4.2 Fungal Diseases

Fungi are responsible to causing several serious infectious diseases of human which differ fundamentally from other microbial infections in diverse ways. Based on the host's immunological status, multiple tissues of a patient may get infected. The invasive mycoses caused by fungi impact human health. The GAFFI (Global Action Fund for Fungal Infections) is the international agency focused on the devastating impact of focal fungal diseases of intact immune systems acquired by human being. Fungal keratitis affects human eyes causing more than one million blindness each year as reported by GAFFI (2018; Kneale et al. 2016). Fungi are responsible for causing serious infectious fungal disease such as *Histoplasma capsulatum* and *Mucormycetes* (endemic dimorphic fungi), *Aspergillus*, *Candida*, *Cryptococcus* species, *Pneumocystis jirovecii*, etc. The main agent of allergic fungal disease is *Aspergillus fumigatus*, mucosal disease caused by *Candida albicans*, and *Trichophyton rubrum* responsible for skin infections (Armstead et al. 2014; Rajasingham et al. 2017). GAFFI has prepared a fungal diseases list on the basis of their priority that are of public health importance along with diagnosis and better treatment procedure. These include pneumocystis pneumonia, cryptococcal meningitis, chronic pulmonary aspergillosis, disseminated histoplasmosis, and fungal keratitis (GAFFI 2017). Current global estimates have found that the cases of cryptococcal meningitis complicating HIV/AIDs are nearly 223,100, chronic pulmonary aspergillosis are 3,000,000, invasive candidiasis are 700,000, *Pneumocystis jirovecii* pneumonia are 500,000, disseminated histoplasmosis are 100,000, invasive aspergillosis are 250,000, fungal asthma are 10,000,000, and fungal keratitis are almost 1,000,000 annually (Brown et al. 2012).

17.4.3 Viral Diseases

Viruses cause many serious infections of human and other organisms due to their changeable genomic characters and diversity. The pandemic situations arise in the world due to deadly viruses which increased the risk of spreading viral diseases between continents (Drexler et al. 2010). New viral diseases have been reported continuously with severe health issues, and the lack of effective antiviral treatment makes them more severe (Kapoor et al. 2017). Several emerging diseases are caused by viruses, viz., respiratory disease, HIV, influenza, herpes simplex virus (HSV), dengue, chikungunya, Zika, hepatitis A (HSV), hepatitis B (HSB), hepatitis C (HCV), smallpox, etc. (Dyer 2015; Monath et al. 2016). At present, the world's population affected by the coronavirus disease (COVID-19) caused by a newly identified coronavirus has become pandemic (Ghildiyal et al. 2020). Human immunodeficiency virus (HIV) affects the immune system which is known as acquired immune deficiency syndrome (AIDS). Viruses found various strategies of invasion on host cells due to their unique configuration of surface molecules, efficient replication, and genetic variation linked to the host resources (Bekhit and Bekhit 2014). The cell-mediated and innate immunity system involves preventing the entry of viruses in the body. The diseases caused by viruses have a great risk to human health because of their uncontrolled infectious nature due to the viral genome's mutative properties (Yasuhara-Bell et al. 2010). So, the demands for medicinal plant-based novel antiviral agents with high protective capacity have been envisaged against the constant emergence of new resistant viral strains (Jassim and Naji 2003).

17.5 Drug Resistance in Bacteria: The Alarming Need for New Antibiotics

Antibiotics are the drugs used to significantly control the infectious diseases of human and various animals. Pests and pathogens in crop field are also controlled by the application of different types of antibiotics. The pathogenic microbes have become resistant uncertainly against some common antibiotics depending on the non-judicial use and the level of antibiotic consumption (Zaman et al. 2017). At present, the antimicrobial resistance (AMR) bacteria causing many serious infections have now captured global attention (Golkar et al. 2014). Many conventional antimicrobial drugs are now being tried to control these antibiotic-resistant pathogenic bacterial strains. MDR microbes have the capability to resist three or more antibiotics (Bender et al. 2018). Nowadays, emerging MDR "ESKAPE" pathogens are a serious threat to public health globally (Ma et al. 2019). So, plant-based novel antimicrobial sources are highly demanded to control the pathogenic microbes. The World Health Organization (WHO) published a list of 12 bacteria species with critical, high, and medium antibiotic resistance (Asokan et al. 2019). Therefore, novel antibiotics are desperately needed for battling these rapidly evolving pathogens. WHO priority drug-resistant pathogens and their current antibiotics of choice for their treatment are shown in Table 17.1.

Table 17.1 WHO global priority MDR pathogens and their currently used antibiotics

Priority category	Pathogens	Antibiotic resistance	Grams stain	Currently used antibiotics	References
Critical	*Acinetobacter baumannii*	Carbapenem-resistant	Gram-negative	Colistin, carbapenems, sulbactam, rifampin, and tigecycline	Viehman et al. (2014)
	Pseudomonas aeruginosa	Carbapenem-resistant	Gram-negative	Ticarcillin-clavulanate, ceftazidime, aztreonam, imipenem, ciprofloxacin, and colistin	Kanj and Kanafani (2011)
	Enterobacteriaceae, ESBL-producing *Enterobacteriaceae*	Carbapenem-resistant	Gram-negative	Polymyxins, fosfomycin, carbapenems, tigecycline, and aminoglycosides	Morrill et al. (2015)
	Klebsiella pneumonia	Third-generation cephalosporin-resistant	Both Gram-positive and Gram-negative	Cefepime, ceftriaxone, ceftazidime, rifampin clindamycin, ampicillin, vancomycin, teicoplanin	Moghnieh et al. (2015)
	Escherichia coli				
	Enterobacter spp.				
	Serratia spp.				
	Proteus spp.				
	Providencia spp.				
	Morganella spp.				

(continued)

Table 17.1 (continued)

Priority category	Pathogens	Antibiotic resistance	Grams stain	Currently used antibiotics	References
High	*Enterococcus faecium*	Vancomycin-resistant	Gram-positive	Streptogramin, linezolid, daptomycin, oritavancin, and tigecycline	Linden (2002)
	Staphylococcus aureus	Methicillin-resistant	Gram-positive	Vancomycin, trimethoprim-sulfamethoxazole, clindamycin, linezolid, tetracyclines, and daptomycin	LaPlante et al. (2008)
		Vancomycin intermediate and resistant		Tigecycline, chloramphenicol, erythromycin, rifampin, teicoplanin	Zhu et al. (2015)
	Helicobacter pylori	Clarithromycin-resistant	Gram-negative	Amoxicillin, esomeprazole, rabeprazole, omeprazole, metronidazole, levofloxacin, and clarithromycin	Kali (2015)
	Campylobacter	Fluoroquinolone-resistant	Gram-negative	Erythromycin, ciprofloxacin, and fluoroquinolones	Safavi et al. (2016)
	Salmonella spp.	Fluoroquinolone-resistant	Gram-negative	Ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin, ofloxacin	Cuypers et al. (2018)
	Neisseria gonorrhoeae	Third-generation cephalosporin-resistant	Gram-negative	Sulfonamides, penicillin, tetracyclines, and quinolones	Bala and Sood (2010)
		Fluoroquinolone-resistant		Ciprofloxacin	Espinosa et al. (2015)
Medium	*Streptococcus pneumoniae*	Penicillin-non-susceptible	Gram-positive	Penicillin	Pinto et al. (2019)
	Haemophilus influenzae	Ampicillin-resistant	Gram-negative	Ampicillin	Maddi et al. (2017)
	Shigella spp.	Fluoroquinolone-resistant	Gram-negative	Nalidixic acid; ciprofloxacin; norfloxacin; ofloxacin.	Pazhani et al. (2008)

17.6 Overview of the Mechanism of Development of Antibiotic Resistance

The microbes upon exposure to the antibiotics also tend to develop some mechanisms to survive against the lethal effect of the antimicrobials. The process of defense of these microbes against antibiotic drug is known as antibiotic resistance mechanisms (ARM) or multidrug resistance mechanisms (MRM). The bacterial pathogens with emerging antibiotic resistance are recognized as a major public health threat affecting human being worldwide. The key mechanisms responsible for resistance to antibiotics in bacteria are due to:

1. Characteristically different *surface membranes* on which antimicrobial peptides and polycationic antimicrobial agents provide a target (Epand et al. 2016).
2. *Peptidoglycan cell wall* on which many antimicrobial agents like β-lactam antibiotics, glycopeptides (like vancomycin), and phosphonic acids (like fosfomycin) are being targeted (Epand et al. 2009a, 2009b; Karageorgopoulos et al. 2012; Kong et al. 2010).
3. *Plasmids* carrying multiple antibiotic resistance genes ((Schultsz and Geerlings 2012; Beceiro et al. 2013).
4. *Inactivation of antibiotics* by the production of bacterial enzymes that chemically modify or degrade antibiotics (e.g., β-lactamase) and inactivate the drugs (Kong et al. 2010; Kumar and Varela 2013).
5. *Target site modification and drug degradation or inhibition* include mutations in RNA polymerase and DNA gyrase conferring resistance to the rifamycins and quinolones (Peterson and Kaur 2018).
6. *Preventing drug uptake* by changing the membrane permeability (Ang et al. 2004).
7. *Antibiotic efflux pump* (EP) mechanisms conferring resistance against several chemotherapeutic agents or antimicrobials, viz., tetracycline, the macrolides, and the fluoroquinolones (Du et al. 2018; Piddock 2006).
8. *Biofilm formation* {complex aggregation of microbial cells embedded in a common extra-cellular polymeric substance (EPS)}, conferring adherence to human tissues and medical devices, results in failure of antibiotic to penetrate or slow growth rate of organisms owing to slower metabolism (Hall and Mah 2017; Hoiby et al. 2010; Kostakioti et al. 2013).

Microbial efflux systems or multidrug transporters in bacteria are generally classified into five super-families based on their sequence similarity and cometabolite transport, namely, QacA major facilitators (MFS), NorM, multi-antimicrobial extrusion protein family (MATE), ATP-binding cassettes (ABC), LmrA, QacC small multidrug resistance family (SMR), MexAB, and resistance-nodulation-cell division (RND). The energy supply for each class is required for the active transport either from H+ protons (RND, SMR, and MFS), Na+ dependent (MATE), or by hydrolysis of ATP (ABC) (Dwivedi et al. 2017; Kourtesi et al. 2013; Sikri et al. 2018). These EPs are responsible for the export of antibiotics before they find their intracellular targets.

17.7 Plant Metabolites as Potential Antimicrobials Against MDR Strains

Continuous searching of novel antimicrobial compounds is an important line of research due to antibiotic resistance acquired by several microorganisms. Plants are the potent source of antimicrobial compounds. Various plant secondary metabolites like alkaloids including phenylalkylamines, pyrrolidines, pyrrolizidines, tropanes, and purine alkaloids; several flavonoids and tannins; alcoholic, aldehydal, phenolic, ketonic, and esterified derivatives of terpenoids; quinines; and resins have antimicrobial properties against many fungi and bacteria.

17.7.1 Antibacterial Agents

Many plant-derived compounds of different metabolite classes have antibacterial efficacy. Some of the potential metabolites and their specific antibacterial activity are shown in Table 17.2 and also shown in Fig. 17.1.

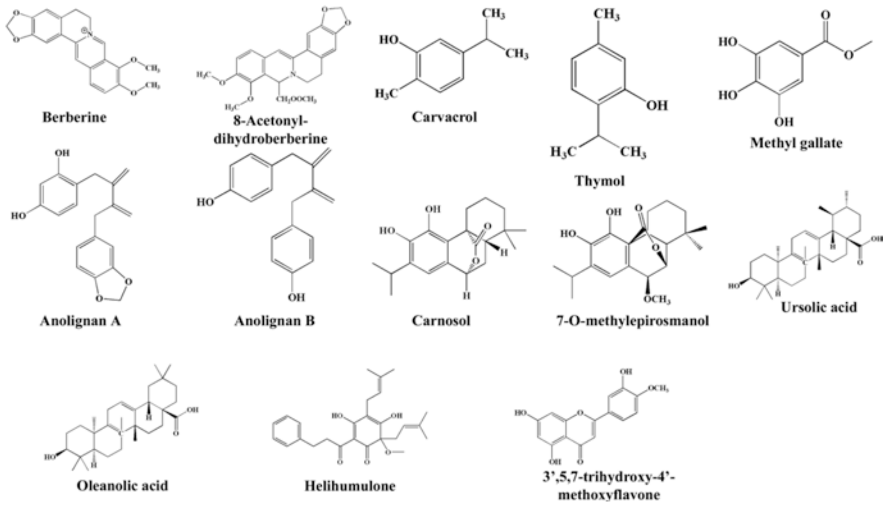

Fig. 17.1 Some potential phytochemicals used against the MDR bacterial strains

Table 17.2 Antibacterial activity of secondary metabolites produced from medicinal plants

S. No.	Lead molecules	Plant sources	Target microorganisms	Mode of action	References
1.	Anolignan B	*Terminalia sericea* Burch. ex DC. (Family: Combretaceae)	*B. subtilis*, *E. coli*, *K. pneumoniae*, *S. aureus*	Potential to selectively inhibit cyclooxygenase COX-1	Eldeen et al. (2006)
2.	(a) Benzoquinone (b) Benzopyran	*Gunnera perpensa* L. (Family: Gunneraceae)	*B. cereus*, *Cryptococcus neoformans*, *S. aureus*, *S. epidermis*	(a) Catalase enzyme inhibition (b) DNA gyrase B inhibition	Drewes et al. (2005)
3.	(a) Carnosol (b) 7-O-Methylepirosmanol	*Salvia chamelaeagnea* K. Bergius. (Family: Lamiaceae)	*B. cereus*, *S. aureus*	Leakage of cellular components	Kamatou et al. (2007)
4.	Diterpene and coumaric acids	*Baccharis grisebachii* Hieron. (Family: Asteraceae)	*S. aureus*	Disruption of bacterial cell membrane and binding to bacterial genomic DNA to inhibit cellular functions, ultimately leading to cell death	Feresin et al. (2003) and Lou et al. (2012)
5.	Ferruginol	*Juniperus excelsa* M. Bieb. (Family: Cupressaceae)	*M. smegmatis*,*M. intracellulare*,*M. chelonae*, *M. xenopi*	Potentiates oxacillin activity by acting as an efflux pump inhibitor	Topçu et al. (1999) and Smith et al. (2007)

(continued)

Table 17.2 (continued)

S. No.	Lead molecules	Plant sources	Target microorganisms	Mode of action	References
6.	Flavonoids	*Combretum erythrophyllum* (Burch.) Sond. (Family: Combretaceae) *Morus alba* L., *Morus mongolica* Schneider (Family: Moraceae) *Broussonetia papyrifera* (L.) Vent (Family: Moraceae) *Sophora flavescens* Ait. (Family: Leguminosae) *Echinosophora koreensis* Nakai (Family: Leguminosae)	*B. subtilis*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *S. aureus*, *S. aureus*, *Salmonella typhimurium*, *Staphylococcus aureus*, *S. epidermis*	Inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of the attachment and biofilm formation, inhibition of the porin on the cell membrane, alteration of the membrane permeability, and attenuation of the pathogenicity	Martini et al. (2004), Sohn et al. (2004) and Xie et al. (2015)
7.	Gingerols	*Zingiber officinale* Rosc. (Family: Zingiberaceae)	*M. avium*, *M. tuberculosis*	Disruption of the cell wall of bacteria causing cytoplasmic leakage	Hiserodt et al. (1998) and Park et al. 2008)

8.	Glucosinolates	*Aurinia sinuata* (L.) Griseb. (Family: Brassicaceae)	*Aeromonas hydrophila*, *B. cereus*, *Chryseobacteriumindologenes*, *Clostridium perfringens*, *E. faecalis*,*Enterobactersakazakii* ,*E. coli*, *Enterobacter cloacae*, *K. pneumoniae*, *Micrococcusluteus*, *P. aeruginosa*,*Pseudomonas luteola*, *S. aureus*, *Vibriovulnificus*	Disruption of the bacterial cell membranes	Blažević et al. (2010) and Borges et al. (2015a, 2015b)
9.	Helihumulone	*Helichrysum cymosum* (L.) D. Don (Family: Asteraceae)	*B. cereus*, *B. subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *S.aureus*	–	Mathekga et al. (2000)
10.	Methyl gallate and gallic acid	*Rhus chinensis* Mill. (Family: Anacardiaceae)	*Actinomycesviscosus*,*Lactobacilluscasei*, *L. acidophilus*,*L. salivarius*,*Porphyromonasgingivalis*, *Streptococcus mutans*, *S. sobrinus*, *Salmonella* spp.	DNA gyrase or ATPase inhibition	Kang et al. (2008) and Choi et al. (2014)
11.	Naphthoquinones	*Tabebuia avellanedae* Lorentz ex Griseb. (Family: Bignoniaceae)	*S. aureus*	Bacterial membrane binding and intracellular ROS generation leading to apoptosis	Machado et al. (2003) and Ravichandiran et al. (2019)
12.	Quassinoids	*Ailanthus altissima* (Mill.) Swingle (Family: Simaroubaceae)	*Mycobacterium tuberculosis*	–	Rahman et al. (1997)

(continued)

Table 17.2 (continued)

S. No.	Lead molecules	Plant sources	Target microorganisms	Mode of action	References
13.	Sesquiterpenoid	*Warburgia salutaris* (Bertol.f.) Chiov. (Family: Canellaceae)	*B. subtilis*, *E. coli*, *K. pneumoniae*, *S. aureus*	Inactivation of MurA enzyme of peptidoglycans biosynthesis	Rabe and van Staden (1997) and Bachelier et al. (2006)
14.	Terpenoids	*Spirostachys africana* Sond. (Family: Euphorbiaceae)	*E. coli*, *S. aureus*	Loss of cellular membrane integrity	Mathabe et al. (2006) and Guimarães et al. (2019)
15.	3,5,7-Trihydroxy-flavone (galangin)	*Helichrysum aureonitens* Sch. Bip. (Family: Asteraceae)	*Bacilluscereus*,*B. subtilis*,*Micrococcus kristinae*,*Staphylococcusaureus*	Disruption of the integrity of cytoplasmic membrane producing loss of potassium	Afoyalan and Meyer (1997) and Cushnie and Lamb (2005)
16.	Xanthones	*Canscora decussata* Schult. (Family: Gentianaceae)	*M. tuberculosis*	Molecular oligomerization of these amphipathic molecules is crucial in the disruption of the bacterial inner membrane and in causing bacterial death	Ghosal and Chaudhary (1975), Yasunaka et al. (2005) and Koh et al. (2018)

Table 17.3 Plant-derived antifungal compounds and target pathogens

Phytochemicals	Plant (part)	Target	Mode of action	References
Pinosylvin (phenolic compound)	*Pinus* spp. (Family: Pinaceae, Gymnosperm)	*C. albicans* and *Saccharomyces cerevisiae*	Adsorption and disruption of microbial membranes, interaction with enzymes, and metal ion deprivation	Lee et al. (2005)
4-Methoxy-5,7-dihydroxyflavone 6-C-glucoside (isocytisoside)	Leaves and stems of *Aquilegia vulgaris* L. (Family: Ranunculaceae)	*Aspergillus niger*	Decreases oxygen consumption in conidial germination and disrupts the integrity of the plasma membrane	Bylka et al. (2004) and Cotoras et al. (2011)
3,4',5,7-Tetraacetyl quercetin (flavonoid)	Wood of *Adina cordifolia* (Roxb.) Benth. & Hook.f. ex B.D.Jacks. (Family: Rubiaceae)	*Aspergillus fumigatus* and *Cryptococcus neoformans*	Enhances the disruption of the plasma membrane and mitochondrial dysfunction and inhibits cell wall formation, cell division, protein synthesis, and the efflux-mediated pumping system	Rao et al. (2002)
Scopoletin (hydroxycoumarin)	Seed kernels of *Melia azedarach* L. (Family: Meliaceae)	*Fusarium verticillioides*, *Candida tropicalis*	Interferes with the synthesis of essential fungal cell components and is able to disrupt both cell wall and plasma membrane and reduces preformed biofilms and its proliferation	Carpinella et al. (2005) and Lemos et al. (2020)
CAY-1 (triterpene saponin)	Capsicum annuum L. (Family: Solanaceae)	*Candida* spp., *Aspergillus fumigatus*	Disruption of the membrane integrity of fungal cells	Renault et al. (2003)
Smilagenin 3-O-β-D-glucopyranoside (spirostanol saponin)	Roots of *Smilax medica* Schltdl. & Cham. (Family: Smilacaceae)	*Candida albicans*, *C. glabrata*, and *C. tropicalis*	–	

(continued)

Table 17.3 (continued)

Phytochemicals	Plant (part)	Target	Mode of action	References
6-α-O-β-D-Xylopyranosyl-(1-->3)-β-D-quinovopyranosyl-(25R)-5α-spirostan-3β,23α-ol	*Solanum chrysotrichum* Schltdl. (Family: Solanaceae)	*Aspergillus niger* and *Candida albicans*	Possess a detergent effect that diminishes superficial tension, causing cell wall damage and fungal cell disintegration	Zamilpa et al. (2002) and Herrera-Arellano et al. (2013)
Jujubogenin 3-O-α-L-arabinofuranosyl(1-->2)-[β-D-glucopyranosyl(1-->3)]-α-L-arabinopyranoside	Stems of *Anomospermum grandifolium* Eichler (Family: Menispermaceae)	*C. albicans*	Weakens the virulence and kills fungi through destroying the cell membrane	Plaza et al. (2003) and Zhang et al. (2006)
Dioscin	Rhizomes of *Dioscorea cayenensis* Lam. (Family: Dioscoreaceae)	*C. albicans*, *C. glabrata*, and *C. tropicalis*	Membrane-disruptive mechanism	Sautour et al. (2004)
Tigogenin-3-O-β-D-glucopyranosyl-(1-->2)-[β-D-xylopyranosyl-(1-->3)]-βD-glucopyranosyl-(1-->4)-β-D-galactopyranoside	*Tribulus terrestris* L. (Family:Zygophyllaceae)	*C. neoformans* and *C. krusei*	Cell membrane disruption	Zhang et al. (2005)
1,7-Dihydroxy-4-methoxyxanthone	*Securidaca longipedunculata* Fresen. (Family: Polygalaceae)	*Aspergillus niger*, *A. fumigatus*, and *Penicillium* species	Effect on sterol biosynthesis by reducing the amount of ergosterol	Joseph et al. (2006) and Pinto et al. (2011)
3-Methoxysampangine (Alkaloid)	*Cleistopholis patens* (Benth.) Engl. & Diels (Family: Annonaceae)	*C. albicans*, *A. fumigatus*, and *C. neoformans*	–	Slobodnikova et al. (2004)

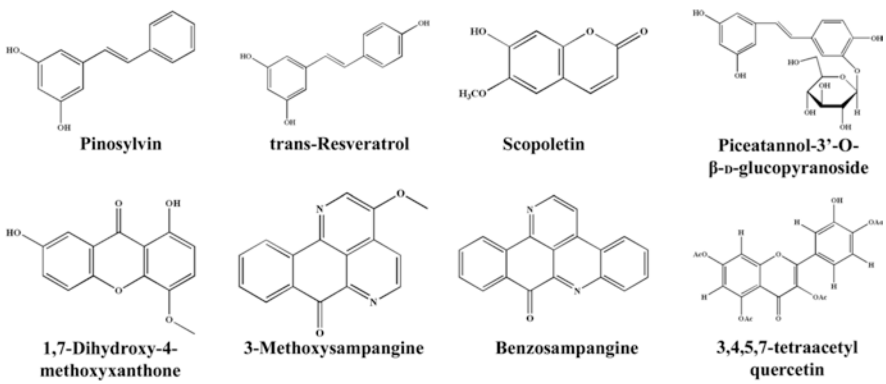

Fig. 17.2 Some potential phytochemicals used against the MDR fungal strains

17.7.2 Antifungal Agents

There are several antifungal compounds of various plant metabolite classes which have been isolated and identified from different plants. Such as dimethyl pyrrole, hydroxydihydrocornin aglycones, indole derivatives, etc. of various metabolite classes which have been identified and isolated from different plants (Schultes 1978). The clinically available antifungal agents may be classified into three natural product classes and four synthetic chemical classes. More than 600 plants having antifungal properties have been reported, most of which remains unexplored in terms of isolation and identification of the particular antifungal compounds (Arif et al. 2011). Some of the potent active antifungal agents derived from various plants are mentioned in Table 17.3 and also shown in Fig. 17.2.

17.7.3 Antiviral Agents

Medicinal plants are potent sources of antiviral agents with some advantages over conventional synthetic antiviral drugs to combat the global disease burden mainly due to viral infections by *Flaviviridae*, *Herpesviridae*, and *Picornaviridae* families in terms of their broad therapeutic potency and limited side effects. An insight into the Indian medicinal plant-derived antiviral compounds and their potential role in the treatment of viral infections is demonstrated in Table 17.4 and also shown in Fig. 17.3.

Table 17.4 Indian medicinal plants having antiviral potencies

Plant (part)	Phytochemicals	Target	Mode of action	References
Artocarpus lakoocha Roxb. (Family: Moraceae)	Oxyresveratrol	HSV-1, HSV-2	Inhibitory activity at the early and late phase of viral replication of HSV-1 and HSV-2. Inhibition of late protein synthesis	Chuanasa et al. (2008)
Citrus reticulata Blanco (Family: Rutaceae)	Tangeretin and nobiletin (polymethoxylated flavones)	RSV	Affected the intracellular replication of RSV. Tangeretin downregulated the expression of RSV phosphoprotein (P protein)	Scaglia et al. (2014)
Curcuma longa L. (Family: Zingiberaceae)	Curcumin	HIV	Inhibition of HIV-1 integrase	Barthelemy et al. (1998)
Humulus lupulus L. (Family: Cannabaceae)	Xanthohumol (chalcone)	BVDV	–	Zhang et al. (2010)
Papaver somniferum Linn. (Family: Papaveraceae)	Papaverine	HIV	Interfere with the envelope precursor protein gp 120 of HIV	Turano et al. (1989)
Phyllanthus emblica Linn. (Family: Euphorbiaceae)	–	HIV	Inhibition of HIV reverse transcriptase	Mekkawy et al. (1995)
Saraca indica Linn. (Family: Fabaceae)	–	HIV	HIV protease inhibition	Kusumoto et al. (1995)
Swietenia macrophylla King (Family: Meliaceae)	Limonoids (lignin)	HCV	–	Wu et al. (2012) and Cheng et al. (2014)
Terminalia arjuna (Roxb. ex DC.) Wight & Arn. (Family: Combretaceae)	–	HIV	HIV protease inhibition	Kusumoto et al. (1995)
Terminalia chebula Retz. (Family: Combretaceae)	–	HIV	Inhibition of HIV reverse transcriptase, inhibition of viral adsorption to cells	Nonaka et al. (1990), Weaver et al. (1992) and Mekkawy et al. (1995)
Ziziphus jujuba Mill. (Family: Rhamnaceae)	Jubanines (alkaloids)	PEDV	–	Kang et al. (2015)

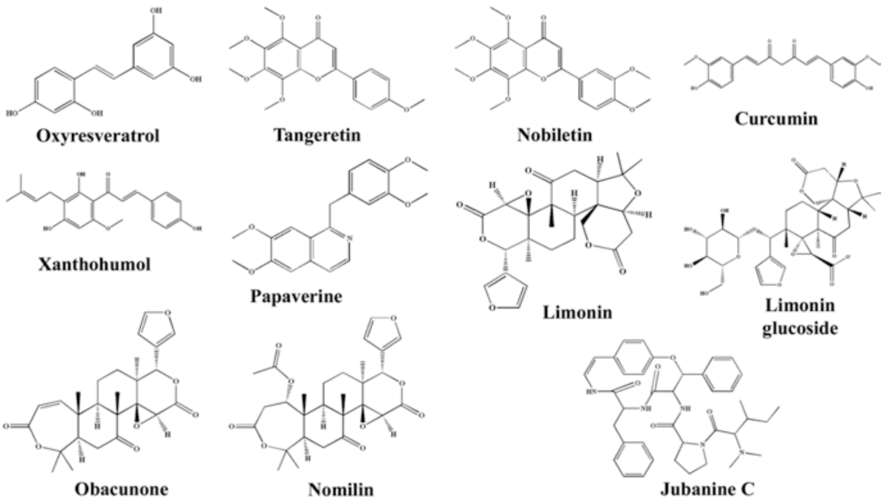

Fig. 17.3 Some potential phytochemicals used against the viral strains

17.8 Mechanism of Antimicrobial Activity of Plant Antimicrobials

Various mechanisms of antimicrobial activity of plant antimicrobials generally include damaging the bacterial cell membrane, inhibiting efflux pumps (EPs), inhibiting DNA and protein biosynthesis and also synergistic effects in combination with conventional antibiotic drugs leading to a direct impact on restoring microbial efficacy in resistant strains (Khameneh et al. 2019). The detailed mode of action of the plant antimicrobials is described under the following headings.

17.8.1 Mechanism of Antibacterial Activity

The mechanisms of antibacterial resistance mainly include the biofilm formation, activation of efflux pump, destroying the antibacterial agents through the destruction enzymes, modification of antibiotics by the means of modifying enzymes, and the alteration of target structures in the bacterium which have lower affinity for antibacterial recognition. An optimum concentration and the specific binding site are crucial for a particular antibacterial agent for its effect on reaching the site of action. Phytochemicals capable of alteration of each of these mechanisms have been separately discussed in the following paragraphs.

17.8.1.1 Inhibition of Biofilm Formation

Cell adherence is a prerequisite for colony formation; preventing bacterial adhesion would appear to be an ideal strategy for reducing biofilm formation (Kostakioti et al. 2013). Plant-derived compounds may influence biofilm formation by damaging microbial membrane structures, inhibiting peptidoglycan synthesis, and/or modulating quorum sensing. Quorum interference strategies include quorum sensing disruption through inhibition of signal molecule biosynthesis, signal molecule inactivation, and blockage of signal transduction. Flavonoid compounds exert antibiofilm effects via quorum sensing inhibition.

17.8.1.2 Efflux Pump (EP) Inhibitors

EPs act as an export system of antibacterial agent that is pumped out the bacterial cell in a faster rate than the time of its diffusion and can cause resistance against the antibacterial agent. Hence, the concentration of antibacterial drug within the bacterial cell becomes lesser for its effectiveness. While a few efflux pumps are capable of conferring clinical levels of resistance to antibiotics, many others marginally increase the minimum inhibitory concentrations (MIC) of antibiotics. Antimicrobial compounds of plant origin and their derivatives can act as more effective efflux pump inhibitors confronting bacterial resistance to antimicrobials either alone or as adjuvants with antibiotics, thereby restoring the antibacterial efficacy of antibiotics (Rao et al. 2018). Phytochemicals of different plants and effective concentration for inhibiting the efflux pumps of bacterial strains are summarized in Table 17.5.

17.8.1.3 Attenuating Bacterial Virulence

Antivirulence activity of plant antimicrobials against bacteria is mediated either by inhibition of cell attachment or by inhibition of preformed biofilm. *Calpurnia aurea* plant extracts are able to interfere with the quorum sensing (QS) system of *Pseudomonas aeruginosa*. The phytochemical compounds pentadecanol, dimethyl terephthalate, terephthalic acid, and methyl mannose identified from the plant suggested potent binding interactions and efficiency with the CviR protein active binding site of *Chromobacterium violaceum*. Further, the plant extracts decreased virulence factors by inhibiting the swimming and swarming motility of pathogenic bacteria like *P. aeruginosa*, *Vibrio cholerae*, *V. parahaemolyticus*, etc. Syntheses of virulence factors including pyocyanin, alkaline protease, elastases, exotoxin A,

Table 17.5 Effective concentrations of phytochemicals inhibiting efflux pumps of bacterial strains

Plants	Active compound	Effective concentration	Drug-resistant bacteria tested	Targeted efflux pumps	References
Acer saccharum Marshall (Family: Aceraceae)	Catechol	5 and 10 mg/ml	*P. aeruginosa* ATCC 15692 and UCBPPPA14, *E. coli* ATCC 700928, *P. mirabilis* HI4320	EtBr EP	
Alkanna orientalis (L.) Boiss. (Family: Boraginaceae)	Sarothrin	100μM	*Staphylococcus aureus* NCTC 8325-4	NorA	
Alpinia galanga (L.) Willd. (Family: Zingiberaceae)	1′-S-1′-Acetoxyeugenol acetate	3.12–25 mg/L	*Mycobacterium smegmatis* mc2 155	EtBr EP	
Ammannia baccifera L. (Family: Lythraceae)	4-Hydroxy-α-tetralone	125 μg/ml	MDR *E. coli*	YojI	
Berberis vulgaris L. (Family: Berberidaceae)	Berberine and palmatine	250–1000 μg/ml and 50–100 μg/ml	MDR *P. aeruginosa* (clinical isolates)	MexAB-OprM	
Capsicum annuum L. (Family: Solanaceae)	Capsaicin	25 μg/ml	*S. aureus* SA-1199B	NorA	
Catharanthus roseus (L.) G. Don (Family: Apocynaceae)	Catharanthine	25 mg/L	*P. aeruginosa*	EtBr EP	Dwivedi et al. (2017)
Chenopodium ambrosioides L. (Family: Chenopodiaceae)	Essential oil	170.6 μl/ml	*Staphylococcus aureus* IS-58	Tet(K) EP	
Cuminum cyminum L. (Family: Apiaceae)	Cumin-methanol extract	5 mg/ml	*S. aureus* MRSA OM505	LmrS	

(continued)

Table 17.5 (continued)

Plants	Active compound	Effective concentration	Drug-resistant bacteria tested	Targeted efflux pumps	References
Eucalyptus tereticornis Sm. (Family: Myrtaceae)	Ursolic acid and derivatives	25 and 50 µg/ml	MDR *E. coli* (KG4)	AcrA/B--TolC, MacB, and YojI	
Holarrhena antidysenterica (L.) Wall. ex A. DC. (Family: Apocynaceae)	Conessine	20 mg/L	*P. aeruginosa*	MexAB-OprM	
Hypericum olympicum L. (Family: Hypericaceae)	Olympicin A	50 µM	*S. aureus* 1199B	NorA	
Ipomoea muricata (L.) Jacq. (Family: Convolvulaceae)	Lysergol	10 µg/ml	*E. coli* MTCC1652 and KG4	YojI	
Persea lingue (HY) Nees ex Kopp (Family: Lauraceae)	Kaempferol rhamnoside	1.56 mg/L	*S. aureus* SA-1199B	NorA	
Salvia fruticosa Mill. (Family: Lamiaceae)	Essential oil	5 µl/ml	*S. epidermidis* (clinical isolates)	Tet(K) EP	
Scutellaria baicalensis Georgi (Family: Lamiaceae)	Baicalein	16 µg/ml	*S. aureus* SA-1199B	NorA	
*Terminalia chebula*Retz. (Family: Combretaceae)	Gallotannin	12.1–97.5 µg/ml	MDR uropathogenic *E. coli*	EtBr EP	
Wrightia tinctoria R. Br. (Family: Apocynaceae)	Indirubin	1.25 and 2.5 mg/L	*S. aureus* SA-1199B	NorA	

EtBr EP ethidium bromide efflux pump, *NorA* norfloxacin antibiotic efflux pump, *Tet(K) EP* tetracycline efflux pump, *YojI* microcin J25 efflux pump of *Escherichia coli*, *MexAB-OprM* resistance-nodulation-cell division (RND) proteins MexA, MexB, and OprM act as the MFP, RND transporter, and OMF, *AcrA/B-TolC*, RND-based tripartite efflux pump comprising of outer membrane protein TolC, periplasmic membrane fusion protein AcrA, and inner membrane transporter AcrB, *MacB* the enterotoxin and macrolide transporter, *LmrS* lincomycin multidrug resistance protein of *S. aureus* hemolysin, and lipase which are regulated by complex signaling system are inhibited by certain phytochemicals.

17.8.1.4 Synergism Between Phytochemicals

The synergistic capacity of many plant extracts with antibiotic drugs is promising. The study shows that tetracycline presents synergism with "guaco" (*Mikania glomerata* Sprengel), guava (*Psidium guajava* L.), clove (*Syzygium aromaticum* L.), garlic (*Allium sativum* L.), lemongrass (*Cymbopogon citratus* (DC.) Stapf), ginger (*Zingiber officinale* Roscoe), "carqueja" (*Baccharis trimera* (Less.) De Candolle), and mint (*Mentha piperita* L.) extracts against *Staphylococcus aureus* strains. The possible activities of phytochemicals on ribosome structure and bacterial enzymes inhibition appear to be related with synergism profile between plant extracts and antibiotics. Plant-derived antimicrobials used in combination with some conventional antibiotics showing synergistic activity against clinically important bacteria in vitro are presented in Table 17.6.

17.8.2 Mechanism of Antifungal Activity of Plant Antimicrobials

The antifungal compounds exert their impacts on the pathogenic fungal cells by any of the six different mechanisms, i.e., inhibition of cell wall formation, cell membrane disruption, dysfunction of the fungal mitochondria, inhibition of cell division, inhibition of RNA/DNA synthesis or protein synthesis, and inhibition of efflux pumps which are described in the following paragraphs. The mechanism of action is being elaborated below.

17.8.2.1 Inhibition of Cell Wall Formation

Phenolic compounds exert their antifungal action through inhibition of cell wall formation. The cell wall integrity is disrupted due to the inhibition of the synthesis of the cell wall components β-glucans and chitin (Walker and White 2011). Lectins (naturally occurring proteins found in most plants serve a protective function for plants) interfere with the biosynthesis of chitin and inhibit cell wall formation (Smith et al. 2007). Naphthoquinone also acts through interference with the fungal cell wall; however, the exact mechanism is yet not known. In general, the sulfur compounds show hindrance to the polysaccharide as well as cell wall formation in fungal cells.

Table 17.6 Synergistic activity of different plant-derived antimicrobials and antibiotics

Plant studied	Plant-based antimicrobials + antibiotics	Bacteria tested and FIC	Observations and inferences	References
Berberis vulgaris L. (Family: Berberidaceae)	Berberine + azithromycin	MRSA (0.375)	MIC was reduced by 50–96.9% of antibiotic dose	Zuo et al. (2012)
B. vulgaris L. (Family: Berberidaceae)	B-Acetonyl-dihydroberberine + levofloxacin + tetrandrine + cefazolin	MRSA (0.188)	Greater penetration of B-acetonyl-dihydroberberine tothe MRSA membrane	Zuo et al. (2012)
Hydrocotyle javanica Thunb. (Family: Araliaceae)	Dillapiole + kanamycin	*E. coli* (0.50)	Efficiency improved by 16 fold	Mandal and Mandal (2018)
Monochoria hastata (L.) Solms (Family: Pontederiaceae)	Tridecanoic acid methyl ester + ampicillin	*E. coli* (0.50)	Efficiency improved by tenfold	Misra and Mandal (2018)
Stephania tetrandra S. Moore (Family: Menispermaceae)	Tetrandrine + cefazolin	MRSA (0.250)	MIC was reduced by 75%–94% of antibiotic dose	Zuo et al. (2011)
S. tetrandra S. Moore (Family: Menispermaceae)	Demethyltetrandrine + cefazolin	MRSA (0.188)	MIC was reduced by 50%–94% of antibiotic dose	Zuo et al. (2011)
Thymus broussonetii Boiss. (Family: Lamiaceae)	Carvacrol/borneol + pristinamycin	*K. pneumoniae* (0.500)	Carvacrol destabilizes the cytoplasmic membrane of bacteria by reducing pH	Fadli et al. (2012)
T. maroccanus Ball (Family: Lamiaceae)	Carvacrol/thymol + ciprofloxacin	*V. cholerae* (0.140), *K. pneumoniae* (0.37), *S. aureus* (0.26), *P. aeruginosa* (0.15)	Demonstrated broad-spectrum synergistic activity against both Gram-positive and Gram-negative bacteria	Fadli et al. (2012)

Plant studied	Plant-based antimicrobials + antibiotics	Bacteria tested and FIC	Observations and inferences	References
T. maroccanus Ball (Family: Lamiaceae)	Carvacrol/thymol + gentamycin	*P. aeruginosa* (0.180)	Carvacrol content is higher in *T. maroccanus* (89.15%) than *T. broussonetii* (21.31%)	Fadli et al. (2012)
Zataria multiflora Boiss. (Family: Lamiaceae)	Thymol/carvacrol + vancomycin	*S. aureus* (0.185)	MIC was lowered from 1 μg/mL to 0.125 μg/mL when used in combination	Mahboubi and Bidgoli (2010)
Z. multiflora Boiss. (Family: Lamiaceae)	Thymol/carvacrol + vancomycin	MRSA (0.320)	First report on the synergistic effects of *Z. multiflora* against MRSA	Mahboubi and Bidgoli (2010)

S. aureus Staphylococcus aureus, *MRSA* methicillin-resistant *S. aureus*, *K. pneumoniae Klebsiella pneumoniae*, *V. cholerae Vibrio cholerae*, *P. aeruginosa Pseudomonas aeruginosa*, *MIC* minimum inhibitory concentration, *FIC* fractional inhibitory concentration (μg/ml)

17.8.2.2 Cell Membrane Disruption

Ergosterols are the essential components of the fungal cell membrane. The antifungal drugs target either to bind with these sterols or to inhibit its synthesis (Walker and White 2011). Thus, the cell membrane's integrity is disrupted, and the membrane becomes leaky. The fungicidal effect of phenylpropanoid eugenol of clove essential oil results due to impaired biosynthesis of ergosterol that causes direct damage to the cell membrane leaving a lesion (Pinto et al. 2019). The monoterpenes and the sesquiterpenes act as antifungal compounds through cell membrane disruption. The saponins (saikosaponins) and steryl glycosides have detergent-like property which acts on cell membrane disruption. They anchor with their lipophilic moiety in the lipophilic membrane bilayer after complexing with cholesterol and the hydrophilic moiety outside the cell. Essential oils are typically lipophilic and disrupt the membrane structure while passing through it. Lipophilic terpenoids of tea tree essential oil exert damage of membrane and make it permeable. Phenolic compounds, terpenoid alcohols, and polyacetylenes also have a direct action on the biomembrane. Studies assumed that the main mechanism of the flavonoid papyriflavonol A and polygodial is the cell membrane disruption, but it did not confirm how it disrupted the membrane. Nitrogenous compounds may cause cell membrane disruption by inhibition of ergosterol biosynthesis or its complexing.

17.8.2.3 Dysfunction of the Fungal Mitochondria

Inhibition of the mitochondrial electron transport will result in reduction in mitochondrial membrane potential leading to apoptosis or subsequent programmed cell death of the target cells. The phenolic compounds, phenylpropanoids, monoterpenes, and the sesquiterpenes can destroy fungal mitochondria. Essential oil from dill seeds (*Anethum graveolens* L., family Apiaceae), containing mainly carvone, limonene monoterpenes, and a phenylpropanoid apiol, impacts on mitochondrial dysfunction by reactive oxygen species (ROS) accumulation, inhibition of electron transport or proton pump, and inhibition of ATPase activity in the mitochondria. While the sesquiterpene polygodial isolated from *Polygonum punctatum* Elliott var. *punctatum* of family Polygonaceae targets the mitochondrial ATP synthase, polyphenol plagiochin E shows antifungal activity through ROS accumulation. The flavonoid baicalein shows antifungal activity by affecting themitochondrial homeostasis, without elevating the intracellular ROS level (Kang et al. 2010). The sulfur-containing compounds of leaves of ramson plant (*Allium ursinum* L., family Amaryllidaceae) act as uncouplers of proton translocation in the mitochondrial membrane and hamper ADP phosphorylation.

17.8.2.4 Inhibition of Cell Division

Most of the antifungal compounds derived from plants inhibit cell division by inhibiting the formation of the mitotic spindle through arresting the microtubule polymerization process (Walker and White 2011). A polyphenol resveratrol produced in various nut plants arrests the cell cycle at the S-phase and thereby affects fungal cell growth inhibiting cell division processes in the tested fungus (Davidson and Taylor 2007).

17.8.2.5 Inhibition of RNA/DNA Synthesis or Protein Synthesis

The antifungal phytochemical agents enter the fungal cells via active transport by ATPases and interfere with the DNA transcription resulting in faulty RNA synthesis. The naphthoquinones act through two different mechanisms, i.e., interference with mRNA transcription and interference with the protein synthesis. The sulfur compounds with some specific functional groups interfere with the fungal membrane integrity; inhibit synthesis of DNA, RNA, and proteins; and stop the production or activity of associated enzymes.

17.8.2.6 Inhibition of Efflux Pumps

Efflux pumps transport toxic substances and accumulated drugs out of the fungal cells. Drug resistance developed by overexpression of efflux pumps can be reduced by inhibiting them using plant-based phytochemicals. Anthraquinone inhibits the energy-dependent efflux pumps (Kang et al. 2010), while carvacrol, a terpenoid alcohol, activates specific signaling pathways that results in calcium stress and inhibits the target of rapamycin pathway of nutrient sensing and exerted dose-dependent inhibition on yeast growth.

17.8.3 Mechanism of Antiviral Activity of Phytochemicals

Plant-derived antiviral compounds mainly function as inhibitors of replication and terminators of RNA and protein synthesis. Following are some mechanisms of action of some phytochemicals derived from medicinal plants used as antiviral compounds (Kapoor et al. 2017).

Alkaloids Alkaloids block virus binding and inhibit the virus growth. It reduces the viral titers in lungs in case of HIV and HSV.

Flavonoids Flavonoids have inhibitory effect on reverse transcriptase and block RNA synthesis in HIV, HSV, and influenza.

Lectins Lectins act by arresting reverse transcriptase and N-glycohydrolase activity and thus inhibit HIV virus penetration in human.

Polyphenols Polyphenols inhibit the viral cell entry by modulating the viral surface structure and effect the expression of virus proteins on cell surface.

Polysaccharides These compounds inhibit viral replication and viral binding to host cell.

Proteins GAP31 (*Gelonium* anti-HIV protein of 31 kDa) anti-HIV plant proteins that are isolated from the medicinal plants *Momordica charantia* (Family: Cucurbitaceae) and *Gelonium multiflorum* A.Juss. (Family:) inhibit viral DNA integration and viral replication. Panaxagin inhibits reverse transcriptase and inhibits viral protein synthesis in HIV.

Terpenes Saponins inhibit the replication in HSV and influenza viruses.

17.9 Conclusion and Future Prospects of Drug Discovery in Pharmaceuticals

The antibacterial activity of phytochemicals is a well-known phenomenon, and in recent years, knowledge regarding these bioactive compounds has widely increased. Many mechanisms of antimicrobial action have been suggested. The purpose of this study is to investigate the prospect of antimicrobials of plant origin and to study the correlation between the type of natural compounds and its antifungal mechanism of action. Generally phytochemicals cause damage to the bacterial membrane; suppression of some virulence factors, including enzymes and toxins; and inhibition of bacterial biofilm formation. Many antiviral crude and purified compounds successfully obtained from various plants have efficacy toward different viruses. Fungal infections of human are cured by photoactive secondary metabolites derived from medicinal plants. Hence, antimicrobial phytochemicals have widely been used as preventative and curative therapeutic solutions against multidrug-resistant pathogens. Some plant-based antimicrobials have antibiotic resistance-modifying activities besides their direct antimicrobial activity. These compounds have the synergistic effect in combination with conventional antibiotics at lower MIC dose against drug-resistant bacteria. From this review, it has been evidenced that some secondary metabolites were proven to be potent antimicrobial agents against drug-resistant pathogens which mainly include bacteria, fungi, and viruses. The findings show that it is difficult to simplify the mechanism of antimicrobial action of plant secondary metabolites as there is an array of bioactivities of a particular phytochemical compound. There are some correlations between few secondary metabolite classes and its antimicrobial mechanism of action, but many of the compounds act through more than one mechanism of action. Hence, it is necessary to study the mechanism of action of each biomolecule instead of a group of compounds.

The pharmaceutical companies are engaged in developing more powerful antimicrobials from medicinal plant-derived natural products which have diverse chemical structures and novel mechanisms of action to control antibiotic resistance by MDR pathogens. With the emergence of antibiotics, resistance developed by microbial strains creates a serious problem to combat against different infectious diseases. In this situation, there is an urgent need to engage scientists of different research backgrounds to handle with thousands of phytochemicals for their antimicrobial activity in vitro as well as in vivo against all types of pathogenic organisms. Medicinal plant-derived secondary metabolites including alkaloids, flavonoids, tannins, and terpenoids have shown antimicrobial potentialities against several MDR microbial pathogens (Fig. 17.4). Botanists, microbiologists, ethnopharmacologists, and natural product chemists should work in combination on utilization of these natural products and structural modification either synthetically or via biotransformation. The finding of antimicrobial compounds with sufficiently lower minimum inhibitory concentrations (MICs), little toxicity, and optimum bioavailability for efficient and safe use in humans and animals is the biggest challenge. The isolation of such antimicrobials from thousands of biomolecules in plant bodies is tough and laborious. The application of sophisticated tools, viz., column purification, high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS), liquid chromatography-mass spectrometry (LC-MS), liquid chromatography-nuclear magnetic resonance-mass spectrometry (LC-NMR-MS), capillary NMR (cap-NMR) spectroscopy, LC-solid phase extraction (SPE)-NMR along with bioassay-guided fractionation, and high-throughput bioassays, will accelerate the access of plant-derived natural products. The green synthesis of various metallic nanoparticles from medicinal plants which act against MDR pathogens is a recent trend in searching novel antimicrobial agents. Hence, more budgetary allocation by the government and multinational pharmaceutical companies in research and development of such plant-based antimicrobials is necessary.

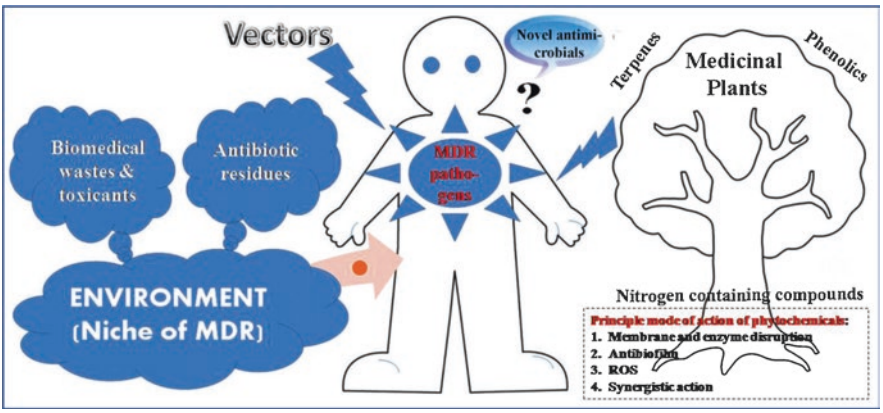

Fig. 17.4 Schematic overview of the causes of MRD strain infections in human individuals and the role of medicinal plant-derived antimicrobials fighting against MDR pathogens

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Chapter 18
Biosynthesis of Lemongrass Essential Oil and the Underlying Mechanism for Its Insecticidal Activity

Mohammad Mukarram, M. Masroor A. Khan, Andleeb Zehra, Sadaf Choudhary, M. Naeem, and Tariq Aftab

18.1 Introduction

Lemongrass (*Cymbopogon flexuosus*) is a perennial crop from grass family Poaceae with numerous industrial applications. The term lemongrass is coined after its 'lemony' essence. Lemongrass is endogenous to India, Sri Lanka, Brazil and Myanmar. India is a major exporter of lemongrass among these with an exportation capacity of about 1000 tons worldwide every year, predominantly to America, England and Australia (Ganjewala and Gupta 2013).

Lemongrass is chiefly grown for its essential oil (EO) that has multiple medicinal (anticancer, analgesic and antimicrobial) and cosmetic usage (Chandrashekar and Prasanna 2010; Ganjewala 2009). In addition to this, lemongrass is a rich source of vitamins A, B complex and C, folate and mineral nutrients including magnesium, phosphorous, manganese, copper, potassium, calcium, zinc and iron. It is also used in Thai cuisine as well as in the form of herbal tea (Fig. 18.1). Recent developments have also explored lemongrass as biofuels enhancing its worth even more (Dhinesh et al. 2016; Alagumalai 2015). In addition to these, lemongrass essential oil has extensive utilisation in the synthesis of eco-friendly pesticides because of its insect-repellent nature (Zheljazkov et al. 2011; Ganjewala 2009). Given the high amount of plant volatiles, lemongrass oil has also been used in the making of high-grade deodorant or air fresheners (Srivastava et al. 2013).

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Fig. 18.1 A 150-day-old lemongrass (*Cymbopogon flexuosus*) plant in the pot

18.2 Lemongrass Essential Oil

In principle, plants synthesise essential oils (EOs) for defence and communication (Hassoun and Çoban 2017; Pavela and Benelli 2016). They provide defence against multiple phenomena including but not limited to bacteria, viruses, fungi and insects (Swamy et al. 2016; Vergis et al. 2015). Due to these, EOs find high use in pharmaceutical, cosmetic and food industries. As of now, almost 10–15% of the total, i.e. about 3000, different EOs are being used on a commercial scale for various purposes (Hassoun and Çoban 2017). The EOs have a strong flavour or essence and thus are called 'essential' oils. This property of EOs is highly exploited by perfumery and flavour industries (Pavela and Benelli 2016).

Lemongrass EO is one from about 400–500 commercially produced EOs (Tisserand and Young 2013). Lemongrass EO has rich spectra of compounds enriched with rigorous medicinal properties, e.g. antibacterial, antiviral, antifungal, antifungal, anticancer and antitumor (Sharma et al. 2009). It also has limonene and borneol that are attributed for analgesic and anaesthetic properties (Sharma et al. 2009). However, other closely related species to lemongrass do not have the identical chemical composition of their EOs. *Cymbopogon flexuous* is an aldehyde type of grass and mainly contains citral (60–80%) which is an isomeric mixture of neral and geranial (higher in quantity than neral) (Abdulazeez et al. 2016; Kakarla and Ganjewala 2009). This citral content can be used as quality marker for lemongrass EO (Abdulazeez et al. 2016). The remaining constituents are elemol, geraniol, geranyl acetate, nerol, isoneral, citronellal, isogeranial and citronellyl acetate which together make up about 10–20% of lemongrass EO. Other related species are *Cymbopogon winterianus* (Java citronella) and *Cymbopogon martinii* (palmarosa). These species are distinguished for their comparatively higher alcohol (e.g. nerol and geraniol) content than lemongrass (Meena et al. 2016). Java citronella is an intermediate type, i.e. both aldehyde and alcohol are found in moderation, while palmarosa is an alcohol type, i.e. it has more alcohol than aldehyde (Meena et al. 2016).

18.3 Essential Oil Biosynthesis in Lemongrass

The biosynthesis of monoterpenes has been a point of interest since long (Banthorpe and Charlwood 1980; Banthorpe et al. 1972; Croteau 1988; Croteau 1987). Various approaches were adopted with the advent of technology including the use of radioactive compounds (Ganjewala and Luthra 2007). Though these attempts provided significant insights, essential oil biosynthesis in lemongrass could not be entirely comprehended and still has research gaps.

Essential oil in lemongrass is primarily a mixture of cyclic and acyclic monoterpenes which are chiefly derived from geranyl diphosphate (GPP) which acts as the precursor for monoterpenes biosynthesis (Ganjewala and Gupta 2013). The GPP is formed by the condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) units. This IPP can either be synthesised in plastid (MEP pathway) or in cytoplasm (MVA pathway). The IPP units generated by MVA pathway could be transferred to plastids for synthesising monoterpenes along with other IPP units generated through MEP pathway. Therefore, essential oil biosynthesis in lemongrass leaves occur either through cytoplasmic MVA pathway or through plastidic MEP pathway (Ganjewala and Gupta 2013).

18.3.1 Mevalonate Pathway

In the first step of acetate-MVA pathway, two acetyl-CoA molecules condense to generate acetoacetyl-CoA. This condensation is catalysed by acetoacetyl-CoA thiolase enzyme (E.C. 2.3.1.9). In the second reaction step, acetoacetyl-CoA is further condensed into 3-hydroxy-methylglutaryl-CoA (HMG-CoA) through HMG-CoA synthase (E.C. 2.3.3.10) with the addition of another acetyl-CoA molecule. HMG-CoA is then reduced into mevalonate (MVA) by HMG-CoA reductase (E.C. 1.1.1.34). Mevalonate plays a crucial role in MVA pathway by acting as a precursor for the biosynthesis of IPP units.

Final steps can be marked as the generation of IPP units from MVA. However, this conversion is a multistep process which includes phosphorylation and decarboxylation of mevalonate. Mevalonate kinase (MVK) (E.C. 2.7.1.36) catalyses the phosphorylation of mevalonate into mevalonate-5-phosphate which is further phosphorylated into mevalonate-5-diphosphate (MVAPP) by phosphomevalonate kinase (PMK) (E.C. 2.7.4.2). Subsequent decarboxylation of mevalonate-5-diphosphate through mevalonate-5-diphosphate decarboxylase (MVD) (E.C. 4.1.1.33) generates IPP units (Fig. 18.2).

18.3.2 Mevalonate-Independent Pathway (MEP Pathway)

The MVA pathway was considered to be the only pathway for IPP biosynthesis in plants until an alternate non-mevalonate pathway was discovered (Rohmer 1999). This mevalonate-independent pathway also known as MEP pathway starts with the conversion of glyceraldehyde 3-phosphate and pyruvate to 1-deoxy-D-xylulose-5-phosphate (DXP) (Richard et al. 2001). This conversion is catalysed by DXP synthase (E.C. 4.1.3.37) (Gräwert et al. 2011). DXP reductoisomerase (E.C. 1.1.1.267) then rearranges DXP into a branched intermediate aldose with the subsequent formation of 2-C-methyl-D-erythritol-4-phosphate (MEP) (Gräwert et al. 2011). In the third reaction step, MEP cytidylyltransferase (E.C. 2.7.7.60) converts MEP into 4-diphosphocytidyl-2-C-methyl-D-erythritol (CDP-ME) (Kuzuyama 2002). CDP-ME kinase (CMK) (E.C. 2.7.1.148) catalyses phosphorylation of CDP-ME through the transfer of a phosphate residue provided by ATP. The subsequent product (CDP-ME 2-phosphate (CDP-MEP)) in the fifth reaction step is further converted into 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (MEcPP) through intramolecular transphosphorylation. This step is catalysed by MEcPP synthase (MCS) (E.C. 4.6.1.12). In the sixth reaction step, MEcPP is converted into 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate (HMBPP) through HMBPP synthase (HDS) (E.C. 1.17.4.3). In the final step of IPP formation, HMBPP is further to a mixture of IPP and its isomer DMAPP by the enzyme HMBPP reductase (HDR enzyme) (E.C. 1.17.1.2) (Fig. 18.3).

The IPP generated through MVA pathway as well as through MEP pathway is converted to produce GPP by GPP synthase (GPS) (E.C. 2.5.1.1). All the cyclic and acyclic monoterpenes present in the lemongrass essential oil, e.g. geranial, neral and citronellal, are produced by GPP through different reactions (Fig. 18.4).

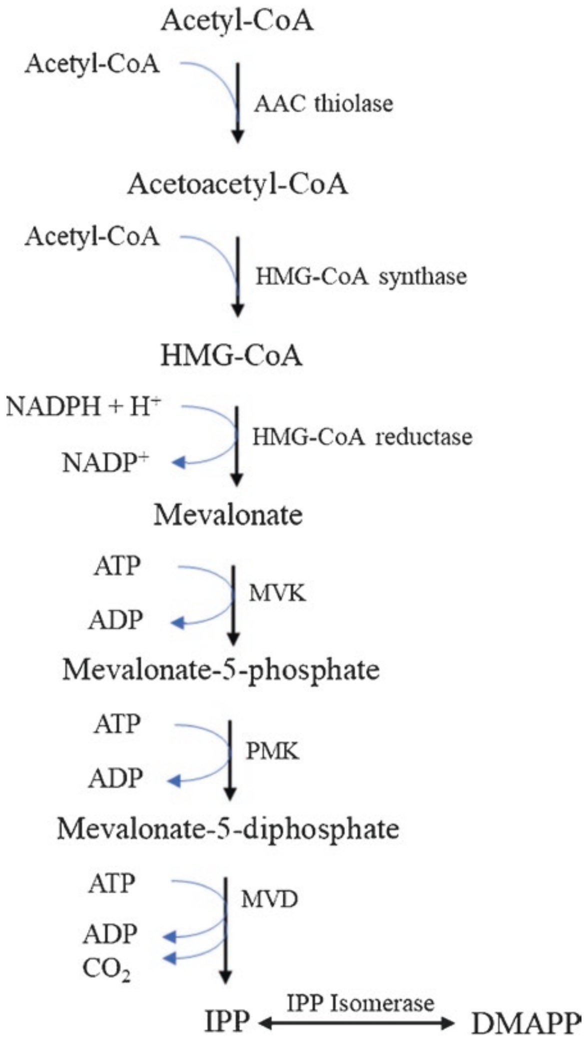

Fig. 18.2 Mevalonate pathway for the biosynthesis of IPP and DMAPP. AAC thiolase, acetoacetyl coenzyme A thiolase; HMG-CoA, 3-hydroxy-methylglutaryl coenzyme A; MVK, mevalonate kinase; PMK, phosphomevalonate kinase; MVD, mevalonate-5-diphosphate decarboxylase; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate

18.4 Essential Oil Storage

Lemongrass plants store EO in its leaf parenchymal cell, also known as 'oil cells' (Ganjewala and Gupta 2013). As lemongrass leaves expands, they synthesise EO more rapidly during early days of their development. Acetylation potential is also on peak at this stage. This renders higher content of geranyl acetate and citronellyl acetate than geraniol and citronellol, respectively. Cytosolic MVA pathway is more active in younger leaves that explains higher EO content in young lemongrass leaves. In older leaves, geranyl acetate and citronellyl acetate content start to diminish as a result of negligible acetylation capacity (Ganjewala and Luthra 2007).

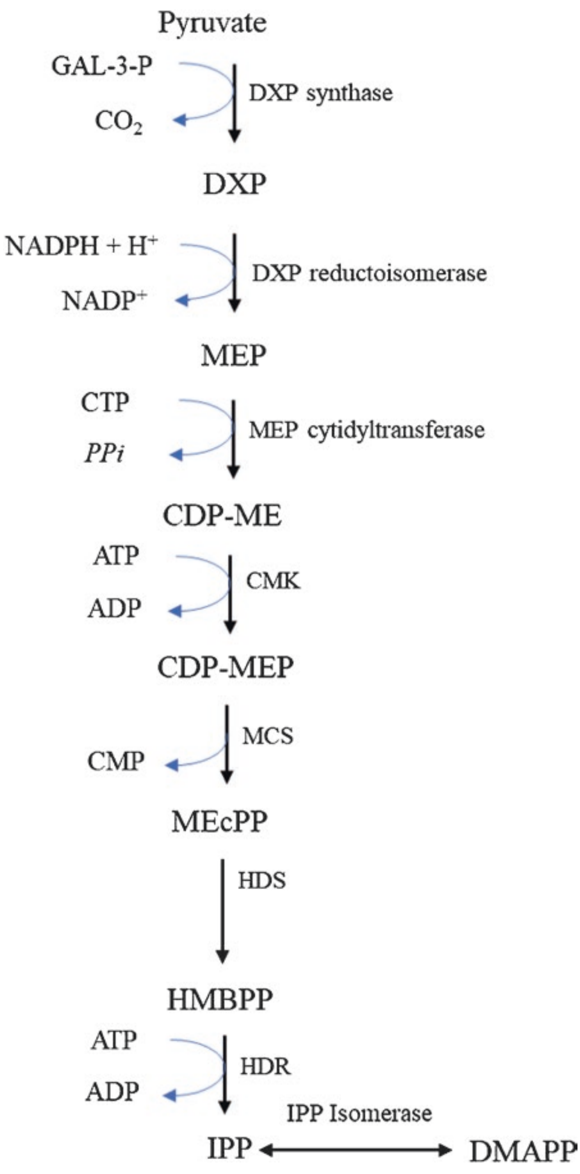

Fig. 18.3 Mevalonate-independent pathway (MEP pathway) for the biosynthesis of IPP and DMAPP. GAL-3-P, glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose-5-phosphate; MEP, 2-C-methyl-D-erythritol-4-phosphate; CDP-ME, 4-diphosphocytidyl-2-C-methyl-D-erythritol; CMK, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase; CDP-MEP, 4-diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate; MCS, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; MEcPP, 2-C-methyl-D-erythritol 2,4-cyclodiphosphate; HDS, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase; HMBPP, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate; HDR, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate

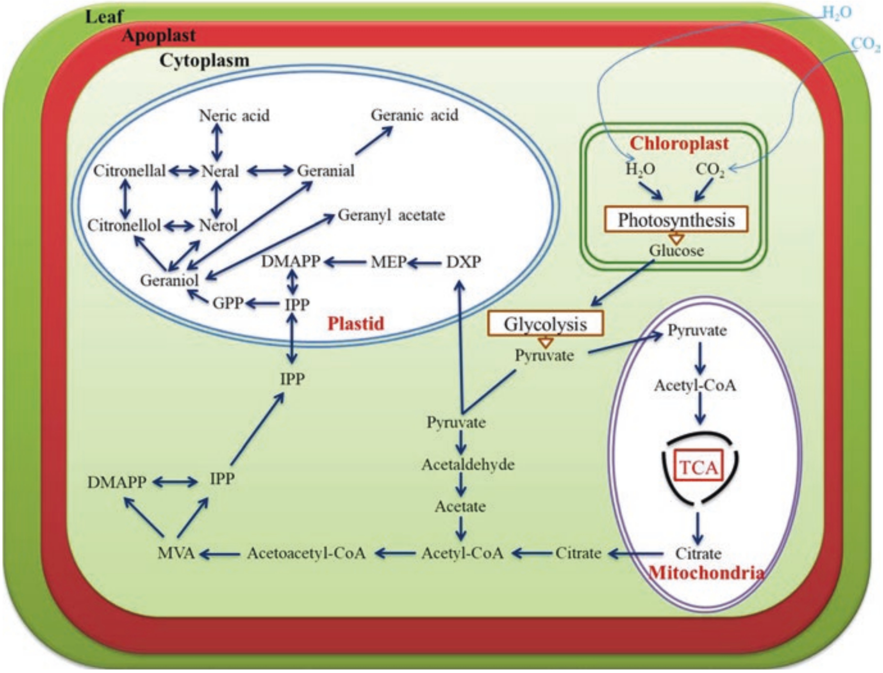

Fig. 18.4 Demonstrative representation for essential oil biosynthesis through MVA and MEP pathways and their interaction with other metabolic processes in the lemongrass. *DMAPP* dimethylallyl diphosphate, *MEP* 2-C-methyl-D-erythritol-4-phosphate, *DXP* 1-deoxy-D-xylulose-5-phosphate, *GPP* geranyl diphosphate, *IPP* isopentenyl diphosphate, *MVA* mevalonate

Moreover, photosynthetic rate lowered down in older leaves that might further account for decreased yield (i.e. EO content) in the older lemongrass leaves (Maffei et al. 1988).

18.5 Insecticidal Property of Lemongrass Essential Oil

Our heavy dependence on chemical pesticides till date has posed a serious health concerns over the decade. These pesticides are serious threat to nontarget hosts including humans that might cause major setbacks in ecological food web. Recent developments are more focused on searching sustainable insecticidal options and causing minimal side-effects and bioaccumulation. Essential oils have played a key role in the management of various insects of economic importance including pests, mites, mosquitoes and aphids (Feroz 2020; Manh and Tuyet 2020; Huong et al. 2019). Essential oils (EOs) are the volatile mixture extracted from various plant species which have cytotoxic potential and can be exploited as insecticides (Al-Sarar et al. 2020; Manh and Tuyet 2020). Along with cytotoxicity, EOs are attributed to cause neurological damages and organ failure in target organisms (Afify et al. 2019). Other sublethal effects can include irritation, repellence and complications in reproduction and proliferation (Muturi et al. 2020). Essential oils can induce detrimental responses in stored product insects by entering into their digestive system (Devi et al. 2019). Moreover, usage of EOs as insecticides is garnering attention because of their non-toxicity to nontarget hosts, inexpensiveness, biodigestibility and negligible bioaccumulation (Eden et al. 2020).

Table 18.1 Chemical composition of lemongrass (*Cymbopogon flexuosus* (Steud.) Wats) essential oil collected in Aligarh, India. The compositional analysis of essential oil was carried out by gas chromatography-mass spectrometry (GC-MS, Shimadzu QP-2010 Plus) with thermal desorption system TD 20

No.	Retention time (min)	Molecular formula	Molecular mass	Compound	Abundance in EO (%)
1.	7.433	$C_{10}H_{16}$	136	α-Pinene	0.08
2.	8.020	$C_{10}H_{16}$	136	Camphene	0.48
3.	9.488	$C_8H_{14}O$	126	6-methyl-5-hepten-2-one	0.19
4.	11.250	$C_{10}H_{16}$	136	Limonene	0.83
5.	13.185	$C_9H_{18}O$	142	Pentyl propyl ketone	0.92
6.	14.529	$C_{10}H_{18}O$	154	Linalool	0.96
7.	16.444	$C_{10}H_{16}O$	152	6-octenal, 7-methyl-3-methylene	0.20
8.	16.622	$C_{10}H_{18}O$	154	(trans-)Chrysanthemol	0.54
9.	16.854	$C_{10}H_{18}O$	154	Citronellal	1.22
10.	17.301	$C_{10}H_{16}O$	152	Isoneral	1.11
11.	18.172	$C_{10}H_{16}O$	152	Isogeranial	1.49
12.	20.615	$C_{10}H_{20}O$	156	Citronellol	1.52
13.	21.051	$C_{10}H_{16}O$	152	Neral	28.20
14.	21.710	$C_{10}H_{18}O$	154	Geraniol	6.33
15.	22.504	$C_{10}H_{16}O$	152	Geranial	39.05
16.	25.803	$C_{12}H_{22}O_2$	198	Citronellyl acetate	0.87
17.	27.050	$C_{12}H_{20}O_2$	196	Geranyl acetate	2.46
18.	27.397	$C_{15}H_{24}$	204	β-Elemene	0.60
19.	28.586	$C_{15}H_{24}$	204	(E)-Caryophyllene	0.57
20.	31.151	$C_{15}H_{24}$	204	Germacrene D	0.53
21.	32.205	$C_{15}H_{24}$	204	β-Elemene	0.37
22.	32.485	$C_{15}H_{24}$	204	γ-Cadinene	0.27
23.	34.026	$C_{15}H_{26}O$	222	α-Elemol	5.85
24.	35.033	$C_{15}H_{26}O$	222	Germacrene D	2.24
25.	35.153	$C_{15}H_{24}O$	220	Caryophyllene oxide	0.58
26.	38.066	$C_{15}H_{26}O$	222	Cadinenol	1.26
27.	38.696	$C_{15}H_{26}O$	222	α-Elemol	1.25

Essential oil constituents were identified on the basis of their retention time (RT) during GC-MS analysis and are listed according to their order of elution on solid phase. Abundance (%) = Percent peak area of essential oil component

Essential oils from lemongrass family (*Cymbopogon* spp.) have been reported highly effective against various diseases of animals, plants as well as processed and stored food products caused by insects and other related organisms, e.g. mites (Li et al. 2020; Devi et al. 2019). The insecticidal property of lemongrass EO is accredited to the various bioactive cyclic and acyclic terpene constituents present in its oil (Eden et al. 2020) (Table 18.1). Citral (i.e. a mixture of geranial and neral) is considered to be the key contributor for the insecticidal property of lemongrass EO (Eden et al. 2020; Solomon et al. 2012). It was also found to impart sublethal responses on larval growth and production of the cabbage looper (Tak and Isman 2016).

In addition to its major constituent, minor EO components could also exhibit synergistic or antagonising effects in insects (Devi et al. 2019). Lemongrass essential oil comprises germacrene D (2.24%), caryophyllene (0.57%) and caryophyllene oxide (0.58%) in limited quantity. These bioactive compounds, however, carry substantial insecticidal and repellent activities against various insects including common housefly and malarial vector mosquitoes (El Mokhtari et al. 2020). However, percentage abundance of these constituents can vary depending on the geography, temperature, plant developmental stage, method used for essential oil extraction as well as solvent used for the extraction (Muturi et al. 2020). Given such dynamic composition, different responses or varying severities of such responses against the same insect can be exhibited by the essential oils (Al-Sarar et al. 2020) (Fig. 18.5).

Fig. 18.5 Lemongrass and its interaction with insects. The volatiles from lemongrass plant (orange plumes) consist a mixture of cyclic and acyclic monoterpenes that possess a pungent lemony smell with insect-repellent potential

18.6 Mechanism for Insecticidal Activity

Essential oils can act on different aspects of insect physiology. It could exhibit detrimental effects on the growth, development and reproduction as well as interfere with respiratory enzymes of mitochondrial membrane and insect nervous system itself (Feroz 2020). Essential oils can easily enter into insect's respiratory system given their highly volatile nature. Once in the respiratory system, bioactive components of EOs target olfactory receptors hijacking insect respiration (Devi et al. 2019). The activation of a subset of the olfactory receptor neurons by lemongrass EO has also been reported in the insect (Afify et al. 2019). Along with olfactory neurons, essential oils can incite other neurotoxic responses as well (Gaire et al. 2019; Politi et al. 2017). It can inhibit the activity of different neurotransmitters including acetylcholine esterase and octopamine (Singh et al. 2017; Rajashekar et al. 2014; Price and Berry 2006). Fumigant toxicity of EOs can interfere with insect metabolism and can exhibit a wide response range with lethal to sublethal impact including the reduction in the DNA synthesis rate (Feroz 2020; Devi et al. 2019). These responses and neurotoxic efficiency of EOs is further assisted by the fact that EOs might ultimately have the potential to hijack various receptors associated with GABA pathway and neurotransmission such as octopamine and acetylcholine esterase receptors in insects (Manh et al. 2020).

Furthermore, EOs can also induce cytotoxicity in mitochondria and damage its membrane by reducing membrane potential through depolarisation (Feroz 2020). This also alters the permeability of mitochondrial membrane (Döll-Boscardin et al. 2012). Mitochondrial dehydrogenase activity and in turn overall mitochondrial metabolic rate could thus be reduced under EO exposure (Feroz 2020).

A major constituent in lemongrass EO, citral, can regulate cell proliferation through interacting with intracellular oxygen radicals and oxidative stress (Sanches et al. 2017; Kapur et al. 2016). Additionally, citral along with other EO components might act on neuroreceptors, hinder signal transduction and cause hormonal imbalance, membrane damage as well as cytotoxicity in the host (Feroz 2020; Manh and Tuyet 2020; Manh et al. 2020; Rattan 2010). In addition to citral, different other components might target different sites in insect, enabling essential oils to attack on multiple target sites simultaneously (Suwannayod et al. 2019). Components of EOs could exhibit synergistic effect in insects which further intensifies their insecticidal properties. Interestingly, insects can minimise the toxic effects of various major components through their defence and detoxification system (Scalerandi et al. 2018). However, the underrated minor components are the ones that could impart graver consequences in insects by evading their detoxification (Scalerandi et al. 2018). Such constituents can induce cytotoxicity in various insect cell lines including ovarian cell line (Tak et al. 2016; Brari and Thakur 2015).

Essential oils might also target specific vital metabolic enzymes. This can be correlated to various acidic phenolic components of EOs including geranial and neral given their elicited reactivity with most enzyme active sites which can even lead to inactivation of the active sites (Ngongang et al. 2019). Phenoloxidase is one such enzyme that is considered as an important marker for insect defence system (Feroz 2020; Huang et al. 2016). It provides resistance against various insecticides, and any change in its activity can be used to determine the toxicity level of any chemical (Bali and Kaur 2013). Essential oils might interfere with the activation cascade of phenoloxidase, thus hampering its activity (Kalita and Devi 2016). Such downregulation in phenoloxidase activity by EOs weakens insect immune system and exhibits serious damage to the insects in the long run. Similarly, lactate dehydrogenase activity was also found to be enhanced with EO application (Qari et al. 2017). Lactate dehydrogenase activity is a potential marker for the evaluation of cellular damage, and the enhancement in its concentration is directly proportional to the damage caused in the organism (Brown et al. 2012). It is thought that EOs might modulate the expression of genes associated with the biosynthesis of these enzymes (Qari et al. 2017).

Essential oils from different *Cymbopogon* spp. were also traced to hamper haemocyte viability in two different insect larvae populations (Feroz 2020). Haemocytes are considered to have significant part in maintaining cellular and humoral immune system in insects (Kalita and Devi 2016). Others (Kalita et al. 2017; Kalita and Devi 2016) suggested that haemocyte viability can be modulated either by targeting constituents of insect immune systems or by regulating immune responses. Such alterations negatively influence the hematopoietic system along with the aberration in endocrine gland activity (Feroz 2020). This retards overall insect growth and development.

Components of EOs can be effective against various insects to a different degree. Moreover, they can target different insect systems with varying intensities. These variations could be categorised into two possibilities: plant-dependent factors and insect-dependent factors. While the plant-dependent factors can be correlated with the varying concentrations of EO constituents and developmental stage of the plant along with various phenological and environmental conditions, the insect-dependent factors could comprise level of EO absorption by the insect as well as developmental stage, cuticle thickness and weight of the insect (Devi et al. 2019). Essential oils, which are a mixture of such components, thus can target the nervous system, respiratory system, reproduction system as well as defence system in insects (Devi et al. 2019). Various recent reports have suggested that EOs can utilise neurological mechanism to impart substantial damage in insects (Moore 2014; Kain et al. 2013; Hamid et al. 2011; Isman 2006).

18.7 Conclusion and Future Perspective

Lemongrass plant is mainly cultivated for its essential oil that has numerous pharmacological and industrial applications. Apart from its heavy use in cosmetics and food industries, lemongrass oil has substantial potential in the synthesis of environmentally friendly insecticides. Essential oil constituents of lemongrass have a wide spectrum of biological activities. These components could also exhibit cytotoxic as well as neurotoxic response in different insects of economic importance. However, the full potential of lemongrass EO has not still reached. Much research could be performed to:

1. Synthesise more potent insecticides from lemongrass EO.
2. Enhance longevity and durability of such herbal insecticides.
3. Exploit the cytotoxic nature of lemongrass EO in the treatment of human diseases such as tumour.

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Chapter 19
Multiple-Usage Shrubs: Medicinal and Pharmaceutical Usage and Their Environmental Beneficiations

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19.1 Jojoba (*Simmondsia chinensis*)

Jojoba (*Simmondsia chinensis*) is dioecious monogenetic grey-green, drought renitent, wind-pollinated shrub (Al-Obaidi et al. 2013), belonging to Simmondsiaceae family, with other common names including lemon leaf, coffee nut, wild hazel and buck nut. Jojoba is almost woody, eternal green, everlasting shrub, producing tiny seeds which contain fluid wax extremely analogous to sperm of whale in prominence (Al-Obaidi 2019).

The origin of *S. chinensis* shrub is the Sonoran Desert of Northern Mexico and the United States in Baja California and South-West Arizona (Azzam et al. 2012), where it is utilized as treatment shrub for different diseases including the healing of wounds, curing some types of ulcers and treatment of skin scratches (Ibrahim et al. 2017). Worldwide, economical value of the plant leading several states such as Argentina, Chile, the Palestinian territories, Egypt, Saudi Arabia, Mexico, India and Australia to cultivate jojoba for more than three decades attributable to economic significance (Abdel-Mageed et al. 2014). *S. chinensis* has waxy esters containing a chain of alcohols and fatty acids (Miklaszewska and Banaś 2016).

Jojoba roots can permeate into 4–5 m deepness, and this elongating shape of root enables them to survive in arid circumstances, and leaves are oval 20–30 mm long

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and 10–15 mm wide, blue-green or grey-green, leather in shape and elongated (Ashour et al. 2013). In a normal environment, *S. chinensis* can tolerate high temperatures and dehydration and, therefore, may be classified as a xerophytic plant (Arya and Khan 2016). Commonly male shoots begin to flower more early than female; the flower originates through the second year of plant development (Ince and Karaca 2011). Flowers are small, green to yellow, 5–6 protecting sepals without petals, female flower initiates independently at another branching node, soft green with elongated sessile with no insects attracting odour in female, meantime the male flowers arise as clusters (Ibrahim et al. 2017). Jojoba grows in tough, sandy soil that allows water to drain at a moderate rate and without water in 5–8 pH. Furthermore, the shrub prefers highly sanded soil with minimum organic materials; also it can resist salinity conditions, with minimum or no fertilization (Aldababseh et al. 2018). Jojoba grows in rainfall standards of 220–400 mm/year (Al-Hamamre 2013). Soil temperature is around 20 °C, and the plant can grow in high temperature, the optimum being 27–33 °C (El Mogy 2012). Extremely low temperature below −3 °C particularly at flowering may kill new seedling plantations, although mature saplings may combat frozen temperatures and the frost may not affect their viability but may decrease yield (Sandouqa and Al-Hamamre 2019). Reproduction of jojoba may be via seed, which is the simplest and more inexpensive process resulting in more male than females; the ratio may reach 5:1 (Inoti 2017), another propagation methods by tissue cultures (Eed and Burgoyne 2015). Vegetative reproduction is preferable more than others due to its ability to resolve the problem of male bias, and only the eligible plant can be selected (Al-Obaidi et al. 2017b). To speed up the plant growth, plantations should be in warming periods of the year especially when the temperature of soil around or higher than 21 °C, with depths of 20–33 mm (Kumar et al. 2013b). Jojoba blooming takes place at the beginning of winter through March to May (Genaidy et al. 2016). At the end of the fourth year of a plantation, jojoba will produce higher economic yield (Khan et al. 2017).

Distinguishably, jojoba differs from other related oil seed shrubs such as corn, peanut and soybean in which the production of oils as the essential storage lipid, jojoba wax comprise 50% of seed dry mass (Al-Obaidi et al. 2013). Previously, jojoba oil has been recorded to have a strong potency in skin treatment and cosmetics industry; these valuable oils are vital in the manufacture of skin care moisturizers (Zięba et al. 2015). In addition to that, jojoba is utilized to fight and reduce desertification in wide areas (Sánchez et al. 2016) and also in the production of hair conditioner and curing agent for psoriasis and acne (Odoms 2012). Easily-removed lubricants anti-inflammatory efficacy (Guirguis et al. 2013). In recent years, it is used as biofuel and easily-removed lubricants (Shah et al. 2010). The shrub has powerful activity in food improvement (Kassem et al. 2011).

19.1.1 Medicinal and Pharmaceutical Significances of Jojoba

Local American citizens use *S. chinensis* as medical agent for cold recovery, wounds, sore throats and verruca (McKeon 2016). Insecticidal, antifungal and antifeedant properties of jojoba have been recorded previously (Clericuzio et al. 2014). Oily extracts of jojoba are also observed to have antimicrobial action against many pathogenic microorganisms (Al-Ghamdi et al. 2019). In Sudan, a prior study indicated the antiproliferative and antimicrobial activities of jojoba oil (Elnimiri and Nimir 2011).

Jojoba extracts and milky fluids displayed functional antimicrobial activities against some pathogens such as *Escherichia coli*, *Salmonella typhimurium*, *Candida albicans* and *Clostridium perfringens* (Abu-Salem and Ibrahim 2014). Oily extracts of jojoba showed anti-inflammatory properties in rat, causing a decrease in prostaglandin rate and fluid accumulation; also alcoholic seed extract revealed antioxidant activity which in turn can keep rat liver in safe versus fumonisin B1-stimulated hepatotoxicity (Abdel-Wahhab et al. 2016). Additionally, crudely seed extracts of the shrub recorded important antimicrobial action against yeast and bacteria (Dey et al. 2014). Coats of seed combined with jojoba male and female leaves possess antibacterial and cytotoxic ability against cell lines of cancer (Hani et al. 2014). Cyclooxygenase-2, an enzyme responsible for pain and inflammation, and its value in carcinogenesis appear in high productivity of prostaglandins, preventing apoptosis; modifying pro-carcinogens into carcinogens, angiogenesis and increased pervasiveness; and modifying immune system (Wang et al. 2018). Jojoba releases cyclooxygenase inhibitor which was shown to have antitumour activity (Abdel-Mageed et al. 2016). Flavonoids also considered valuable antioxidant compounds having a beneficial role in the treatment of disturbances such as inflammation, cancer and asthma (Abdel-Mageed et al. 2014). Lipoxygenase inhibitor and antioxidant isolated from jojoba is an enzyme which has importance in leukotriene synthesis, which was indexed to play a serious role in the elimination of free radical disorders.

Simmondsin and its derivatives, 4-demethylsimmondsin-2′-ferulate, simmondsin-3′-ferulate and 4,5-didemethylsimmondsin, are correlated with the antioxidant activity of jojoba (Hani et al. 2014). Stability of jojoba liquid wax and its tolerance to rancidity and oxidation (Mishra et al. 2011) reported to have stability in penicillin products; jojoba oil is the optimum liquid wax in this matter (Bilin et al. 2018). In pharmaceutical industries, Egyptian jojoba oils are preferred due to the high content of triglycerides and carboxylic acids (Al-Ghamdi et al. 2017). Mixing 30 ml of 0.5,1 N (HCL, NaOH) with 70 ml ethyl alcohol, is the most promising extraction method for the assessment of antibacterial and antioxidant activities of all handling processes especially in phytomedicines, the output is natural compounds and the remaining particles utilized to carbon activation (Akl et al. 2019).

Jojoba therapy by natural extracts may create new prospects for medicinal inventions of coriaceous wounds (Pazyar et al. 2014). Jojoba oils are suitable for all types of skin; it encourages wound healing by enhancing collagen production in tissues (Ranzato et al. 2011). Human sebaceous gland secretions contain wax esters obtained from the redistribution of purified plant oil including omega-7 fatty acid and purified oil of jojoba due to its unparalleled chemical structure with high similarity to human sebum (Brown et al. 2016). Chemical analysis study revealed that jojoba oil own antioxidant characteristics use for repression and tackling several illnesses such as diabetes and stroke (Manoharan et al. 2016).

Alcohol derivatives of jojoba oil such as 15-tetracosenol, 13-docosenol and 11-eicosenol, possess many pharmacological properties particularly as antiviral activity; these traits make jojoba oil worthy (Sánchez et al. 2015). Moreover, they work as carrier oils valuable in hair healthiness (He et al. 2017). Micro-emulsion of jojoba oil is reported to have ability in decreasing the troublesome of Tazorac which is the ethyl ester of tazarotene acid, increasing its skin sedimentation and enhancing treatment of psoriatic illness (Nasr et al. 2017). Jojoba wax owning anti-inflammatory properties, according to that, they can eliminate neutrophil seepage as noticed by lowering myeloperoxidase efficacy and decreasing carcinoma necrosis and nitrous oxide production, it was shown that jojoba waxy compounds may be valuable armoury against psoriasis (Pazyar and Yaghoobi 2016).

Bright significance of jojoba oil is to restrain shaving wound, segmented lips, windburn and sunburn (Arafat and Basuny 2018). Antimicrobial activity of jojoba oil through mixing with hydrated lime combined with saline plays an active role in exceeding the rehabilitation of invading microorganisms *Enterococcus faecalis* and *Candida albicans* on teeth (Fayed et al. 2018). A study on callus culture of jojoba showed that the synergistic effect between 2,4-D and kinetin resulting in the production of phytochemical active compounds; in addition to that this mechanism may be used medically as the origin of antimicrobial items (Solliman et al. 2017).

When hyperglycaemia happens by insulin disturbance through diabetes, and raising of reactive oxygen species standards will lead to cellular defect, polyphenolic acid will be the strongest weapon to decrease the oxidative stress (Plummer et al. 2016). The affluence and variation in jojoba seed particles make the shrub a vigorous agent to inhibit the devastation of RINm5f beta cells encouraged by hyperglycaemia, lessening 59% of simmondsin and 69% of reactive O_2 species for watery extract (Belhadj et al. 2018). Jojoba volatile oils, one of the unique properties that can be used with other essential oils, can work as carriers for massage oils in aromatherapy process (Michalak 2018).

Rapid penetration of jojoba oils showed that serum non-esterified fatty acid (NEFA) levels are increased half an hour after transcutaneous donating of jojoba oil, mode of action for the raising NEFA levels attributable for increased lipolysis via patatin-like phospholipase upregulation in the liver and minimizing fatty acids runoff by fatty acid transport proteins in skin (Matsumoto et al. 2019). Jojoba oil physical and chemical characteristics close to meibomian gland excretions, used in either treatment of dry eye symptoms or for medications acceptance by meibomian tissues. Moreover, jojoba wax tears utilized to extenuate eye harassment and irritability as well as blur, jojoba wax is efficacious upon local application to permeate the lid edge until reaching gland tissues where may impact as therapeutic agent alone, or with an assistant agent (Maskin 2013).

19.1.2 Industrial and Commercial Uses of Jojoba

S. chinensis is a remarkable industrial plant; with attentions raised about the obtainability of perfect raw substances for several industries to depress the cost of the product, jojoba industry relies on its oil; also the remaining seed materials are important in biofuel, animal meals and cosmetics (Agarwal and Khan 2018).

Bio-lubricants are preferable more than traditional lubricants, due to valid viscosity, little emissions, less volatility and high flash properties (Pathak et al. 2019) and higher flashing point activity of shrub-related bio-lubricants obtained from the high molecular weight of neutral fats (Matiliunaite and Paulauskiene 2019). Naturally, industrialization processes require more lubricants; regrettably, the wide usage of petroleum-dependent oils contributes to ecological pollution; thus natural vegetable oils are an excellent solution for providing bio-lubricants which are more friendly to the environment (Al-Obaidi et al. 2017a), in addition to its high-performance hydropower oil applications (Bhaumik et al. 2019). Jojoba lubricants preferable more over the world in term of high tolerance to pressure and elevated temperature, consequently to high boiling point 389 °C of jojoba oils, their consistency in the cutting process is impressive (Bilin et al. 2018). Formulation mode of jojoba bio-lubricant performing by mixing it with some engine materials, increasing its specialization in term of industrial standards for 10 W oil products synergistically, resulting 238 °C flashpoint, 242.2 viscosity point and low stream point at 5 °C (Hassan et al. 2019). Mixing a small amount of jojoba oil and mineral oil showed greater friction than mineral alone, adding nano-graphene particles to 20% of jojoba oil which had increased lubrication potential than mineral oil; therefore this ratio of combining may be superior to replace mineral lubrication process (Alotaibi and Yousif 2016; Kannan and Rameshbabu 2018). The environmental analysis showed that using jojoba bio-diesel leading to restrict emissions and better engine streamline, a combination of 10% of jojoba bio-lubricant resulted in perfect records in comparison with SAE20W40 mineral oil (Gupta et al. 2020). During the last 50 years, the energy demand has been steadily growing, and the scientists show that this may increase with time, and then the number of harmful gas emissions will increase and motivate global warming (Du et al. 2018). Blending properties of jojoba wax may be developed; enhancing physio-chemical properties of wax will lead to using it as an alternative fuel source, initially by blending wax with diesel resulting in an effective tool for decreasing engine troubles related with high viscosity of jojoba methyl ester (Agarwal et al. 2020). Jojoba oil is known to be incentively converted into bio-diesel, the energy can be increased and CO_2 emissions will be lowered by 52% by utilizing composts as fertilizers, 50% of fossil fuel used in esterification step and all types of diesel-fuel for road cars and agriculture machines, can be replaced by jojoba bio-diesel (Sandouqa and Al-Hamamre 2019). Another study by using raw oil of jojoba when blended with petroleum-diesel leading to a noticeable reduction in hydrocarbon and nitrogen oxide emissions, associated with reverse effect with electromagnetic waves to heater walls (Al Omari et al. 2019). Jojoba meal is a valuable term in the food industry; after oilseed purification, the remainder consists of 26–33% protein as crude, fibres, vitamins and carbohydrates (Chaudhary and Tripathi 2017). Jojoba seed can be used in frying due to high-temperature tolerance, also used to liquefy salad despite being indigestible (Krist 2020). Researchers recommend that it is useful if the farmers adding jojoba meal as a constituent of fish feeding, its ability in enhancing animal general health, improving blood system, increase resistant to invading pathogens and disease recovery (Sarhan et al. 2019).

Jojoba oil also has anticorrosion activity showed by preparation of three green rust inhibitors, through the reaction of brominated-jojoba oil with several amines, it was recorded that concentration of rusted iron decreasing, relating with rising of jojoba oil inhibitor, under chemical analysis procedure (Nasser 2017). Another significance of using jojoba oil as an antifoaming agent in the hydrocarbon industry, low amount of shrub natural oil having a good value of foam inhibition under low temperature 20 °C, heat stability of its oil also beneficial in calorimetric methods (Cevada et al. 2017; Khairi 2019). In the cosmetic industry, jojoba oil has a precious impact in main markets, and many main companies use it as raw materials (Bilin et al. 2018). In Northwestern Mexico, three ecospecies of jojoba analysed by Inter Simple Sequence Repeat (ISSR) markers revealed about 83% polymorphism over many other genotypes for usage in the cosmetic industry (Araiza-Lizarde et al. 2017).

19.1.3 Environmental Influence

The ability of *S. chinensis* to grow in arid and hot environment 35–48 °C will lead to acclimation with variable undesirable habitats of the world; nowadays agriculture of jojoba is wide in the United States, Australia, Peru, India and some regions of Asia, on marginal areas (Sturtevant et al. 2020). Despite the solar radiation intensity and dry conditions, jojoba has typical adaptation system; a study proved that regional environment may affect plant expression of dimorphic properties, during midday jojoba direct its leaves to the sun due to the lack of *Paraheliotropic* leaf movement (Eskander et al. 2018).

In terms of bioremediation, *S. chinensis* seeds were examined and extracted with many solvents, at all concentrations, the result showing a significant larvicidal effect on *Culex pipiens* fourth instar larvae, which considered a vector of many diseases of birds, humans and other animals. Also, LC50 of 1.905% for jojoba oils against *Spodoptera littoralis* second instar larvae invading cotton in Egypt, results indicated a decrease in pupal, larval intervals for the second instar larvae survival (EL-Sabagh et al. 2019). Cooperation between jojoba roots and endophytes, promoting rhizogenesis of the shrub, stress restriction caused by biotic, abiotic agents, activating defence mechanisms in shrub through enhancing enzymes activities of jojoba (Perez-Rosales et al. 2018). The usage of jojoba seed residues in the removal of dyes and heavy metals such as ferric ions from wastewater, these dyes are mutagenic, dangerous and poses a threat not only to human but also environment, so the treatment of these toxicants is by adsorption potential of jojoba seeds (Al-Zoubi et al. 2020).

Globally, thousands of hectares of crops suffering from salinity, thus adding of proline to jojoba soil causing superior influence in reducing salinity and rising salt tolerance of other shrubs for the same soil in related areas (Alotaibi et al. 2019). Previous work concluded that plant-bacteria interaction is a useful pattern in reducing salinity, inoculation of *Azospirillum brasilense* Az39 and Cd strains on jojoba soil leading to reduce the deleterious effects of NaCl on jojoba root formation, increasing plant salt tolerance (Gonzalez et al. 2015).

Low conservation, drought-tolerant, prolonged-live and low-burning hazards of *S. chinensis*, so it can be utilized on highways and roads as wayside implantations and fences, as well as soil stabilizer in green straps surrounding desert towns anguished from air-particles pollution (Khan et al. 2017). In Sinai desert, Egypt, plantation of jojoba as the antidesertification plant is remarkable; S-700, S-B and S-610 are superior jojoba clones providing altitude production and may be recommended for trade plantations in arid and semi-arid areas such as Saudi Arabia and Iraq (Bakeer et al. 2017). In the same desert, jojoba cultivated as antidrought shrub due to high tolerance to water stress according to several morphological alterations such as leaf thickness increasing and leaf dimensions detraction, originally jojoba influenced by high irrigation intervals (Khattab and Amin 2018). Supporting research in transcriptional profiling after RNA sequencing had detected the genes responsible for jojoba antidrought advantage; samples were collected under normal and high water stress conditions in Saudi Arabia (Al-Dossary et al. 2020).

S. chinensis AA-5 was tested previously to strengthen the phytoremediation capacity of Jojoba in the aquatic system; results showed the ability to remove nickel and cadmium heavy metals (Kara et al. 2013). In India, plantation of jojoba is considered safe and environmentally friendly, leading agricultural companies which suffering from gerbils and rodents, to use protein meal fat-free of jojoba as antifeedant, according to dose-dependent, this meal has an extraordinary mortal effect on farm-destructive animals (Chaudhary and Tripathi 2017).

19.2 *Aloe vera*

Mentioned throughout recorded history and given a high ranking as an all-purpose herbal plant, *Aloe vera* is considered to be one of the most potent *Aloe* species, with commercial importance and popularity in research (Kumar et al. 2017a). *A. vera* is well-known for its considerable medicinal properties; it is one of the richest natural sources of health for human beings. Various parts of the plant contain approximately 75 nutrients (Radha and Laxmipriya 2015), as well as tens of active compounds including amino acids, vitamins, carbohydrate, minerals, saponins, anthraquinones, lignin and salicylic acid (Misir et al. 2014). Historical findings regarding *A. vera* date back to hundreds of years B.C. in old Egypt and Mesopotamia (Manvitha and Bidya 2014). Old Egyptians, Arabs, Romans and Indians also recognized the healing properties of this plant, especially that related to skin and hair treatment (Joseph and Raj 2010). *A. barbadensis* Miller, commonly referred to as *A. vera*, is one of few hundred species of *Aloe* belonging to family Liliaceae that is native to Arabian peninsula but have been found also in dry tropical and subtropical areas (Kumar et al. 2020).

Complex carbohydrates obtained from natural sources such as plants have shown diverse biological activities such as wound healing, enhancement of the reticuloendothelial system, stimulation of the immune system, treatment of tumours and effects on the haematopoietic system (Talmadge et al. 2004). Generally, *A. vera* has many potential uses both for humans and animals such as gel, latex and the whole leaf extract, whose biological ingredients may act alone or in synergy (Christaki and Florou-Paneri 2010). *A. vera* also well-known for its anti-inflammatory, antioxidant, antidiabetic, relief of sunburn, immune booster, antiageing and anticancer properties (Langmead et al. 2004). *A. vera* is a perennial shrub with the ability to store water in the leaves to survive during the dry season/condition. The thick fleshy leaves of *A. vera* plants contain not only cell wall carbohydrates such as cellulose but also carbohydrates such as acetylated mannans (Ni and Tizard 2004). *A. vera* leaves have a long history as a therapeutic plant with diverse healing applications. Many biological activities were linked to the presence of polysaccharides in the leaves gel (Hamman 2008). But those *A. vera* properties could also be linked to volatile compounds, cellulose, lignin, pectin, hemicellulose phenolic compounds, minerals and vitamins (Hęś et al. 2019).

19.2.1 Medicinal and Pharmaceutical Applications

The plant *A. vera* is used in ayurvedic, homoeopathic and allopathic streams of medicine**,** and not an only tribal community but also most of the people for food and medicine (Sahu et al. 2013). The chemistry of the plant has revealed tens of different compounds which include many medicinal and pharmaceutical with potential clinical applications (Nazeam et al. 2020). *A. vera* is anthelmintic (Mwale and Masika 2015), laxative (Guo and Mei 2016), aperients, carminative (Thompson 2008), deobstruent, depurative, diuretic, stomachic and emmenagogue (Chinnadurai et al. 2018). Pharmacologically, it is considered an immunity booster with detoxifying properties; it is also recommended for supporting treatment with antibiotics to eliminate drug-induced gastritis and other adverse effects. It has been reported that the polysaccharides in *A. vera* gel have potential therapeutic properties, antioxidant, wound healing, antibacterial, antiviral, antifungal, antidiabetic and antineoplastic activities (Kaushik et al. 2014; Kumar et al. 2019).

A. vera has been reported to have antifungal activity, hypoglycaemic activity, anti-inflammatory, anticancer, immunomodulatory, gastroprotective and antihepatotoxic properties (Hamman 2008). It is good for conjunctivitis, dysentery, kidney pains sprains, stomatitis, sore throat, skin burns, scalds, scrapes, sunburns, wounds pimples, acne, cuts dandruff, hair fall and baldness (Alapati and Sulthana 2015).

Dried juice of leaves has the potential to be used in dysmenorrhoea and diseases of the liver. It is used in jaundice due to viral hepatitis and in spleen disorders. Gel topically is emollient, anti-inflammatory and antimicrobial used for wound healing and sunburn. *A. vera* detoxifies the body and is considered as the best colon cleanser. It prevents constipation, controls diabetes and clears acne and skin allergies and dark spots (Verma et al. 2013).

A. vera also exhibits skin protective properties, and it has been used in this role throughout history since ancient times (Qadir 2009). It has been demonstrated that *A. vera* and its active compounds have effective therapeutic properties on several chronic skin diseases such as psoriasis and eczema (Khan et al. 2016). *A. vera* gel can be used for the treatment of pimples and protection of the skin from ageing, and it is thus used in the cosmetics industries providing moisturizing effects (Sahu et al. 2013). The leaf gel materials of *A. vera* and few other *Aloe* species was shown an in vivo study shown the ability to rehydrate skin (Fox et al. 2014). *A. vera* juice showed promising repair properties for UV-induced human hair damage (Daud and Kulkarni 2011). Also study on rats indicated that extract of *A. vera* gel can promote hair growth (Pamudji et al. 2015).

The results of the recent study depicted that *A. vera* gel extract has significant anti-inflammatory property particularly in an increased dose of gel extract (Afzal et al. 2020). The ability of healing wounds has been reported in many earlier studies (Rahmani et al. 2014; Keorochana et al. 2015; Oryan et al. 2016; Hormozi et al. 2017; Ranjbar and Yousefi 2018; Oryan et al. 2019; Abdel-Mohsen et al. 2020b; Zhang et al. 2020). In a comparison study of *A. vera* gel dressing with a simple dressing on the wound immediately after caesarean section. In the *A. vera*-treated group, wound healing and pain reduced faster than the control group in the first 24 hours (Molazem et al. 2015). In another study, wounds of patients were treated with *A. vera* gel two times a day in combination with current treatments, and the control group only used conventional treatments, while the *A. vera*-treated patients wound healing occurred in about a month, while in the control group it took 2 months (Avijgan et al. 2016). *A. vera*, together with other two *Aloe* species, have shown the ability to speed up the healing of wounds in keratinocytes with negligible toxicity on normal human keratinocyte cells (Fox et al. 2017). *A. vera* gel used to treat nulliparous episiotomy patients, the pain reduced after 10 days of treatment (Sabzaligol et al. 2014). The dental and oral disease also reported being treated by *A. vera* gel (Nair et al. 2016).

Acetone extract from *A. vera* shown growth inhibition effect on pathogenic bacterial species *Staphylococcus aureus* and *Salmonella enterica* (Adzitey et al. 2019). The results showed *A. vera* inner gel expresses antibacterial properties against *Helicobacter pylori* and therefore, in combination with antibiotics, could represent a novel strategy for the treatment of the infection of *H. pylori*, especially in cases of multiresistant (Cellini et al. 2014). The orally administered *A. vera* supplemented probiotic lassi was found to prevent *Shigella dysenteriae*-induced infection (Hussain et al. 2017).

Aqueous extract of *A. vera* shows hepatoprotective activity against carbon tetrachloride CCl4-induced hepatotoxicity in rats (Salama et al. 2016). While ethanolic extract of *A. vera* leaves shows renoprotective action against gentamicin-induced nephrotoxicity in rats (Virani et al. 2016). On the other hand, *A. vera* gel could suppress the mRNA expression of lipogenic genes in mice by modulating various pharmacological pathways (Kumar et al. 2013a). *A. vera*-positive effect on the treatment of diabetes was observed with the intervention of its supplementation significant reduction which was observed in blood glucose, lipid profile and blood pressure of the diabetic patients (Choudhary et al. 2014). A clinical study has shown the hyperglycaemic activity of *A. vera* gel patients with type 2 diabetes (Pothuraju et al. 2016).

In vitro and in vivo antioxidant activities of *A. vera* gel constituents have been reported (Kang et al. 2014). Numerous studies have shown that different compounds in *A. vera* leaves have anticancer properties by modulating the growth of various cancers (Ahmet et al. 2016). Another study also demonstrated that aloe-emodin caused cell apoptosis in colon cancer cells (Suboj et al. 2012). An immunomodulatory study on rats showed that *A. vera* gel extract is taken orally at a dose of 200 and 400 mg/kg exhibiting significant phagocytic activity as compared with aspirin (Bhalsinge et al. 2018). *A. vera* extract revealed nerve protective activity by reducing the mitochondrial damage in the rat brain (Wang et al. 2010) and antiparkinson and antioxidant activities in mice (Bagewadi and Rathor 2014).

A herbal product used with an aqueous extract of *A. vera* showed antiviral activity against influenza A and influenza B viruses (Glatthaar-Saalmüller et al. 2015). *A. vera* gruel plays a significant role in the adjuvant therapy of HIV infection (Olatunya et al. 2012). In summary, compounds found in *A. vera* exhibited antioxidation activities in vitro and in vivo, suggesting that *A. vera* could be beneficial in combating diseases related to oxidation stress in the body such as cancer, diabetes as well as cardiovascular disease (Yang et al. 2017).

19.2.2 The Industrial Importance of A. vera

A. vera gel enables the plant to hold water for a long time and has soothing effects as well. So, *A. vera* has found an extensive application in cosmetic and skincare industries, such as cleansers, moisturizers, deodorants, sun lotions, toothpaste and shaving creams (Benítez et al. 2015). The use of *A. vera* gel extract in the preparation of functional foods started 40 years ago in North America and Europe (Maan et al. 2018). Since then its applications have been expanded to the functional and nutraceutical foods especially for the preparation of health drinks, also used as a flavouring component and preservative (Park and Jo 2006). The addition of basil seed mucilage to *A. vera* gel or their application as an edible coating could be a promising approach to maintain postharvest quality and control physiological process of apricot fruits during cold storage (Nourozi and Sayyari 2020).

Also chitosan-*A. vera* combination can be used to extend the storage life of mango fruit (Shah and Hashmi 2020). Edible films based on potato starch, chitosan and *A. vera* gel (AV) as modifiers were evaluated towards their potential application in food or cosmetics industry. All the samples with *A. vera* gain the increase resistance to microbial action (Bajer et al. 2020). Development of water-resistant carboxymethyl cellulose-coly vinyl alcohol - *A. vera* packaging film; it delayed lipid peroxidation and inhibited the growth of bacteria in the minced chicken meat (Kanatt and Makwana 2020).

Green synthesis of cubic In_2O_3 nanoparticles (NPs) (5–50 nm) was reported using indium acetylacetonate and *A. vera* plant extract solution (Dinesh et al. 2015). Highly stable and spherical ZnONPs using *A. vera* extract have also been synthesized (Abdullaeva 2017).

The most promising materials for wounds and burns dressing are still based on natural polymers such as polysaccharides (alginates, chitin, chitosan, heparin, chondroitin), proteoglycans and proteins (collagen, gelatin, fibrin, keratin, silk fibroin, eggshell membrane) (Mogoşanu and Grumezescu 2014). Recently reports illustrated the importance of *A. vera* in wound dressing manufacturing (Abdel-Mohsen et al. 2020a; Dadashzadeh et al. 2020). Preparation of solid lipid nanoparticles modified with *A. vera* for antiviral drug delivery applications showed promising result (Joshy et al. 2016). The mucilaginous nature of *A. vera* gel also helps it to serve as a protective barrier between skin and beard in shaving creams (Lad and Murthy 2013). The *A. vera* fibre-reinforced biocomposites have a huge potential to be used in applications such as automobile interior trims, consumer electronics like mobile phone body parts as well as non-toxic toys for children (Chaitanya and Singh 2018).

19.2.3 Environmental Impact

Recently, *A. vera* and its by-products have attracted attention for employment in environmental applications; this plant grows readily in hot and dry climates. It has been reported that different agro-climatic conditions have effects on phytochemical diversity and antioxidant potential of *A. vera* plant (Rahman et al. 2017). Antioxidant activity was higher in *A. vera* plants grown in India concluding that more metabolites are produced in plants under low-temperature stress conditions (Kumar et al. 2017b). Investigation shows that the *A. vera* is effective and inexpensive adsorbent for the removal of Pb, Cd, Ni, Cu, Cr (III) and (VI) from contaminated soil by heavy metals (Shokri et al. 2016). The surface properties of activated carbon from *A. vera* leaf shells were modified by sulfuric acid *A. vera*-acid activated carbon (AV-AAC) and then used as a novel adsorbent to remove para-chlorophenol (p-CP) and methylene blue (MB) from aqueous solutions (Zeydouni et al. 2020). An effective, low-cost, rapid adsorption rate and high adsorption capacity adsorbent synthesized from waste *A. vera* leaves, which has been used to remove the maximum concentration of Cr (VI) from the synthetic solution (Prajapati et al. 2020). *A. vera* waste biomass-based sorbents, as well as modified counterparts, are used for the removal of heavy metals, dyes and other pollutants from aqueous media (Giannakoudakis et al. 2018). The emergence of environmental issues such as air pollution has greatly required the need for cheap, adaptable and eco-friendly remediation systems for purifying the environment. *A. vera* emits oxygen at night; it is almost a “no-maintenance” plant and caters to a lot of beauty benefits too (Kulkarni and Zambare 2018).

The pyrolysis treatment of *A. vera* leaf is cost-effective, friendly to the environment and profitable, with which the highest performance in biochar and condensable fuel is obtained with lower gas production (Strubinger et al. 2017). Green composites (biocomposites) comprising lignocellulosic fibres are low-cost sustainable materials having huge potential to replace petroleum-derived commodity and engineering plastics (lignocellulosic). *A. vera* fibres exhibited eco-friendly fibre to limit the environmental hazards of conventionally used chemical fibre (Chaitanya and Singh 2016, 2018; Ramesh et al. 2020).

19.3 *Moringa oleifera*

Highly content of minerals and vitamins as well as food diversity in *Moringa oleifera* Lam has been led scientists to name it the miracle tree it is a type of native medical grass which is common in the equatorial and subequatorial areas (Abdull Razis et al. 2014). Moringa has seven-time vitamin C more than oranges, ten-time vitamin A more than carrots (Gopalakrishnan et al. 2016), 17-time calcium more than milk, nine-time protein more than yoghurt, 15-time potassium more than bananas and 25-time iron more than spinach (Rockwood et al. 2013). As the most economic prominent species among others belong to the family Moringaceae, there are other names for this shrub as drumstick or horseradish, Mulangay, Benzolive and Mlonge; the name drumstick came from the shape of the tote bag or pendulous, like the tender and turned stick used for flogging the drum. Perhaps the name radish came from the pendant, the slim and tender shape of the unripe fruits of the tree so much as the siliqua of the radish (Abdull Razis et al. 2014; Sandeep et al. 2019). India is one of the bigger producers of moringa, a yearly harvest of about 2.2 million tonnes (Raja et al. 2016).

The vegetative parts of *M. oleifera* consist of stems, twigs, compound leaves, flowers, fruity pods as well as the seeds; all parts are traditionally utilized for various purposes; leaves are usually the most utilized in the world (Mallenakuppe et al. 2019). Moringa is rapid growing and retains green leaves (Chukwuebuka 2015); the leaves have two or three leaflets with the latter being the most common where it takes spiral shape on the branch (Agyepong 2009). The flower is aromatic and androgynous, with five yellowed-white petals and contain different veins. In more fixed seasonal heat and with stable rainfall, bloom can occur two times or even along the year (Alhakmani et al. 2013).

At the maturity, the tree become grown and divide vertically from the three corners along with the pods; then the blackish-brown seeds are released; adult *Moringa* tree of 15–20 feet high can produce thousands of seed pods and therefore produce uncounted seeds every year (Zaku et al. 2015).

M. oleifera proliferates by seed plantation, seedling growing and parts of the stem. Seed plantation is favoured, seeds often sow at the deepness of 2 cm, deeper than that may cause a decrease in the growth rate (Aslam et al. 2019). Moringa can live a broad extent of soil situation, but predominating choose neutral acidic soil where it takes to grow 5–12 days after planting (Mallenakuppe et al. 2015; Raja et al. 2016). *M. oleifera* is considered as a worthy food resource; it is utilized in the food industry as well as in the development of stable feed in animal feeding which has been palliated (Sahay et al. 2017). Some researchers have highlighted the use of *M. oleifera* as supplement therapy or for use in the prevention of disease (Mohammed et al. 2012; Gadzirayi et al. 2012; Alegbeleye 2018). *M. oleifera* extracts are often added with canned foods to preserve their chemical and physical properties, as well as increase the shelf life of the product (Gull et al. 2016). Different parts of the *M. oleifera* shrub have shown antimicrobial activities (Zaffer et al. 2014) and antioxidant and antidiabetic properties (Vargas-Sánchez et al. 2019). *M. oleifera* has been used to treat over 300 diseases in India and Africa continent (Gopalakrishnan et al. 2016).

19.3.1 Medicinal Uses and Pharmaceutical Applications

Considerably all parts of *M. oleifera* have potential use in treating different infections and inflammatory diseases relating to cardiac, gastrointestinal, haematologic and tumour maladies (Abdull Razis et al. 2014). *M. oleifera* leaves provide important sources for antioxidant, group of different vitamins, metals and amino acids and a wide range of protein sources (Saini et al. 2016). Additionally, anticancer activity of the plant is relating to its antiproliferative action; in addition to that cancer cells can be targeted by reactive O_2 species from *M. oleifera* (Tiloke et al. 2013; Reetu et al. 2020). Phenolic compounds such as Robigenin and Flavin meletin indicated by HPLC having a potential antiproliferative action against apoptosis, thus indicating that extracts of *M. oleifera* leaves have potential of cancer prevention and sometimes may be used as cure site for cancer (Sreelatha et al. 2011). Nectar production by shrub flower may have anti-inflammatory action (Gopalakrishnan et al. 2016). Some cases of skin infections, some conditions of anaemia and gastrointestinal ulcers may be treated with selectively wood extracts medications termed alkaloid spirochim (Vergara-Jimenez et al. 2017).

Depending on the massive medicinal qualifications of *M. oleifera*, leaf extracts from this plant possess antioxidant and phytochemical properties (Yaméogo et al. 2011). Moreover, medical co-agents contributing to decreasing cholesterol level enhance the immune system, antiulcer, toxin illuminating agent, diuresis and treating of spasticity also observed (Okiki et al. 2015). *M. oleifera* has inhibition effect against microbial pathogenicity combined with recently organized studies indicating that aqueous extract of the plant showed a noticeable effect against *Bacillus*, *S. aureus* and *Escherichia coli* with scheduled doses (Saadabi and Zaid 2011). This shrub has great antifungal properties versus *Aspergillus niger* and slightly lower with *Aspergillus nidulans* and *Aspergillus terreus* (Kekuda et al. 2010). Leaf extracts in ethyl alcohol of this plant also had an inhibition effect indicated against the earth-worm *Pheretima posthuma* (Rastogi et al. 2009). In a comparison study between moringa seed extract and nonsteroidal anti-inflammatory aspirin, the study showed the high anti-inflammatory effect of seed extracts considerably reduced inflammation signals and increased detoxification in lipopolysaccharide-induced bone marrow macrophages (Jaja-Chimedza et al. 2017). The shining side of pharmaceutical applications for *M. oleifera* is wound healing (Tang et al. 2017; Li et al. 2020). Mixing an equal percentage of calcium-alginic acid-polyethene glycol ethyl ether methacrylate (PEGMA) with *M. oleifera* and *Aloe vera* increase cell viability after few days, in comparison with scaffolds free of shrub extracts showing that two plant extracts combination with calcium-alginic acid-PEGMA possessing considerable chance in wound healing medications (Rubio-Elizalde et al. 2019). These equal amount of both two plant extracts combined with calcium-alginic acid-PEGMA highly affects human skin cells to proliferate rapidly after 7–10 days due to existence of bioactive compounds in the gel of both *M. oleifera* and *A. vera* (Skotti et al. 2014). Recent studies signalize utilizing of naturalistic nontoxic compounds which rely on substitution of chemical compounds with plant natural products, for instance, in slabs, moisturizer, suspension and salve (van Wyk and Wink 2015; Hwang and Lyga 2017; Young 2019). *M. oleifera* gum production is a real pharmaceutical example of synthetic cream polymers used in wound healing or injuries events prescribed by pharmacologists (Uphadek et al. 2018). Diabetes considered to be one of the most traditional illnesses is included in the applications of *M. oleifera* pharmacology leaves of moringa used to treat type 1 and type 2 of diabetes (Taweerutchana et al. 2017). Once a prior investigation for utilizing the hydrous extract of leaves to recover streptozotocin of type 1 and insulin hormone of type 2 with rat laboratory animals (Cerf 2013).

As shown previously, all parts of the shrub extracts are of medical value and also in terms of the bark of *M. oleifera* stem can be used in different properties in preventing pain in addition to elimination irritant reactions, thus providing results using traditionally in inflammatory cases (Kumbhare and Sivakumar 2011). Petroleum naphtha, ethyl ethanoate and methanolic leaf extracts of *M. oleifera* were discussed in many published papers to have an analgesic effect (Manaheji et al. 2011; Dubey et al. 2013; Al-Abri et al. 2018). Antipyretic characteristic has also been examined practically with baker's yeast; therefore according to previous results, methanolic extracts showed an elevated level of effectiveness (Reddy and Kuber 2017). Natural compounds of *M. oleifera* may be developed using amelioration of anti-inflammatory medications relating to chronic diseases (Muangnoi et al. 2012). Substantially phytochemical ingredients of *M. oleifera* such as amphipathic glycosides, tannic acid, phenolics, flavonoids, limonene and alkaloid organic compounds; the presence of these substances with diverse shape among the same genus will lead to broad pharmacological uses with wide circumstances (Rani et al. 2018). Polyphenolic antioxidant natural components can lower the oxidation level in cells by free radicals (Vergara-Jimenez et al. 2017). In return to the high ratio of *M. oleifera* polyphenols combined with studies of methanol extraction of leaf materials such as sophoretin, meletin, 3-caffeoylquinate 3-caffeoylquinic acid, sophorin and condensed tannin, all contributing to decreasing tissue damage (Gopalakrishnan et al. 2016). In addition to a group of leaf extracted polyphenols such as $C_{16}H_{12}O_5$ flavon, Apigenin-8-C-glucoside flavone, ortho-coumaric acid 2-hydroxycinnamic polyphenolic acid, β-(4-hydroxyphenyl) acrylic polyphenolic acid and vanillate indicated to have anticarcinogen activity (Joshi and Mehta 2010). Furthermore, with the pharmaceutical benefits of leaves active compounds, beta-carotenoids, alpha-Tocopherol and amphipathic glycosides having pain-reducing conducts and some alleviating properties (Tejas et al. 2012). Group of polyphenolic extracts and pharmaceutical applications also is revealed in stem bark and root (Maurya and Singh 2014). *M. oleifera* leaf extracts show in vivo ability to modulate metabolic disorder features (Irfan et al. 2020) and adjust adiponectin besides inhibiting adipogenesis in 3 T3-L1 adipocytes cells (Muni Swamy et al. 2020). Additionally, histopathological studies proved that *M. oleifera* minimizes cirrhosis of the liver (Hamza 2010). Extraction experiment of seed protein interacts with sodium lauryl sulphate was reported that protein had important antiseptic attitude, related protein is with integrated sites with sodium lauryl sulphate and markup protein-lauryl sulphate component (Bhaskar et al. 2013). Oily extracts of *M. oleifera* can be used as an aromatherapy machine and skin softener with good stability on the skin and moisturizing agent (George et al. 2018). Ethanolic extracts of shuck have a reliable hypotensive action at 0.03 gm/kg. isolation of $ROC(=S)NR_2$ and isothiocyanates from plant leaves, assisted by production of O-ethyl-four-[(α -l-rhamnosy loxy)-benzyl] carbamic acid, β-sitosterol and others showing elevated hypotensive activity (Biswas et al. 2012).

19.3.2 The Industrial Importance of **Moringa oleifera**

There are more than 90 chemical compounds in *M. oleifera* of high nutritional value, inclusive of carbohydrates, proteins, fats and feeding fibres; protein is the most considerable in the part of plant among sundry nourishment, forming about 25% of arid weight (Anwar et al. 2007). *M. oleifera* is utilized in the tropics as a feeding resource to process the lack of nutrition, especially in newborns (Brilhante et al. 2017). *M. oleifera* seeds can be applied as physical treatment of water in civil and rustic regions, lowering of microorganism loading and hold off parasitic worms (Mangale Sapana et al. 2012). *M. oleifera* utilized to decrease 90% of turbidness and colour of polluted water; *M. oleifera* seeds were frugal and more active than $Al_2(SO_4)_3$ in decreasing the turbidness of polluted water with 95% efficiency compared to 80% of $Al_2(SO_4)$ (Gámez et al. 2015).

Also, this plant has been used to limit the spread of some harmful species of insects (De Oliveira et al. 2011). *M. oleifera* has insecticide, larvicide and ovicide action versus the transports of *Anopheles stephensi* and *Aedes aegypti* (Prabhu et al. 2011). The larvicide action of proteins, such the water-dissoluble *M. oleifera* lectin acquired from seeds, has been versus organophosphate-reluctant phase four *A. aegypti* maggot (Agra-Neto et al. 2014).

The cultivation of aquatic plants for food in developed countries considered a public effort, to decrease ecological harm caused by contaminants. To reduce the use of chemicals and antibiotics for water treating, *M. oleifera* could acts as an alternate (Adesina and Omitoyin 2011). Suspensions acquired from *M. oleifera* shrivelled seeds decrease organic compounds and turbidness, on account of the efficiency of the seed extract, which remove acids of humus from water; together, these seeds encourage deposition and minimize bacterial loading in polluted water (Ferreira et al. 2011). The germicide possibility of *M. oleifera* against fungi and bacteria, return to a normal state from water animal farming merit interest, since these microbial considered a major cause of economic losses in the animal and agriculture field, besides, health troubles (Brilhante et al. 2017).

Furthermore, besides the repressing effects of *M. oleifera* on various microorganisms, it has been notified that raw extracts of leaves and seeds also prevent an enzyme that breaks down proteins and peptides of microorganism. It is acting as a muscular degenerating of fish and prawn during storage (Bijina et al. 2011). Moringa oil can be a perfect thermic, oxidative and cooking stabilization (Ogunsina et al. 2014).

A group of scholars highlighted the importance of mixing of *M. oleifera* seeds powder with wheat powder for cake products, raising the protein ratio approximately from 13% to 23%, as well as the ratio of fibres, and Fe, Zn, Ca, fats and dust also increased (Dachana et al. 2010). *M. oleifera* oil contribution as biofuel also been reported (Chinma et al. 2014).

19.3.3 Environmental Impact

M. oleifera is a type of equatorial multi-function shrub which is adapted to a semi-dry environment (Noulèkoun et al. 2018). *M. oleifera* utilized in water treating especially seeds and leaves. They have no noticeable secondary impact and are safe and decomposed by bacteria or other living organisms (Eman et al. 2014). *M. oleifera* considered a positive polymer that has several ionizable groups, which have shown to be efficient in water treating as a replacement for aluminium sulphate (Araújo et al. 2013).

M. oleifera is considered God's gift to some countries, especially in Asia and Africa (Mahmood et al. 2010). One of the several functions of *M. oleifera* shrub being able to contribute in the elimination of heavy minerals from water. *M. oleifera* removed most of the iron, 98% copper and cadmium (Shan et al. 2017). Fungus contamination is causing several diseases that infect plants which cause remarkable losses due to the reduction of yield (Labandeira and Prevec 2014). The aggressiveness of fungal diseases is related to toxic and dangerous impacts on the environment treated usually using chemicals (Yadav et al. 2013). *M. oleifera* proteins revealed an antifungal impact in vitro (Lacerda et al. 2014). *M. oleifera* Chitin Binding Protein

(MOCBP) is a stable glycoprotein efficiency in extremely high heat and pH (Batista et al. 2014). MOCBP3 is having wide activity versus some fungi diseases as *Fusarium oxysporum* and *Colletotrichum* spp. (Gifoni et al. 2012). *M. oleifera* seeds play a significant role in reducing pollution of palm oil waste that contains many organic and inorganic substances (del Real-Olvera et al. 2016). The oils extracted from moringa seeds have shown their effectiveness as a healthy natural coagulant for treatment of liquid waste (Othman et al. 2011). Moringa seeds worked as a good fertilizer for maize farms by improving and diversifying the solid nutrients (Emmanuel et al. 2011).

Agronomical investigations are based on the use of genetically engineered *M. oleifera* seeds to fertilize maize, resulting in refinement of soil nutritional contents. *M. oleifera* leaf extracts were exercised in curing the land polluted by crude oil, repairing processes for soil to prepare it for agriculture (Abd El-Hack et al. 2018). Hydrocarbon declination rate can be monitored and stimulated using moringa oil (Ukpaka 2016). Moringa is an exciting tree in extenuating climate counter changes, which are a real threat for a long time in countries in the Arabic region like Saudi Arabia (Robiansyah et al. 2014). *M. oleifera* cultivated widely as a forest shrub which plays an important role in reducing the temperature, aerial CO_2 concentration and sedimentation (Mridha 2015). The plant has high CO_2 absorption rate which is about 20 times more than other shrubs; the ability to store carbon makes the plant as a good potential shrub to reduce carbon footprint (Daba 2016). *M. oleifera* leaf extract is considered a natural shrub growth improver, not only enhancing plant regeneration and mass production but also encouraging bearing under stress events (Yasmeen et al. 2012). Leaf extracts are considered as a tool to overcome injuries affecting maize and increase productivity rates of genetically engineered maize seeds at minimum temperatures (Afzal et al. 2012). Leaf extracts of *M. oleifera* play a vital role as antidrought in wheat farms (Yasmeen et al. 2013).

In Asia, particularly in Saudi Arabia harsh and dry circumstances leading cultivars to farm large areas of *M. oleifera*, as a desertification-reducing agent (Mridha 2015). A similar scenario applied in African regions such as southern Ethiopia (Lemma and Mohammed 2016). Also, a study in Sudan showed that *M. oleifera* can grow under the little amount of water with no rains up to 1 month, due to the high nutritional value especially with proteins, K, Fe and Ca, versus *Moringa peregrina* which has few proteins and metals (Ali et al. 2015).

Industry in some countries like Australia relies on *M. oleifera* as a source of biodiesel production (Omonhinmin et al. 2020) to meet emission requirements. Despite low level of performance and high NO_x releasing versus fossil fuels, biofuel has decreased emissions of hydrocarbon, carbon oxide and carbon dioxide in contrast to fossil fuel (Azad et al. 2015). Biodegradable and coagulation properties, numerous sources, leading to use *M. oleifera* taking off chemical O_2 demand and overall dissolved solids in local landfill wastes, attributing that valuable and frugal alternative over another classical method (Sivakumar 2013).

19.4 *Acacia*

Genus *Acacia* has a hard history in the world; it is classified as large trees and bushes. Some *Acacia* sorts are important, while others saw them as harmful plants (Rahman et al. 2019). Nourishment instability, lack and poor diet considered an issue worrying many countries around the world, particularly in drier regions. One of the most important reasons behind food deficiency is the increment in desertification and changing climatic conditions which lead to the lack of interest in some important wild species (Tasisa and Nemomissa 2019). *Acacia* is one of the shrubs that lives in the drier regions on earth, as it represents the largest part of the arid regions of the Middle East and Africa (Winters et al. 2018). *Acacia* has over 1000 different species distributed worldwide around the arid and semi-arid areas (Lake 2015). Acacias also considered native to equatorial and subequatorial zones, in particular Australia where they are called wattles (Khan and Sahito 2013). They are also known as mimosa and Arabic gum (*Acacia senegal*) (Choudhary et al. 2020).

Acacias' special leaf takes the shape of small split leaflets that give the leafstalk a plumy or fernlike look. In most Australian and Pacific Ocean species, the leaflets are repressed or absent completely; a petiole may be perpendicularly coordinated and carry forks or severely turned needles at their bas;, these are familiar as phyllodes, which are a winged leaf stalk that functions as a leaf (Maiti et al. 2016).

The flowers possess five tiny petals, roughly unobserved by the tall of the male fertilizing organ of a bloom, and are coordinated in dense groups or cylinder mass; they are xanthous or pale yellow coloured in various species, milky in some or lilacs such as *Acacia purpureopetala* or red such as *Acacia leprosa* (Ghouila et al. 2012; Gleed and Franklin 2018). Acacia flower can be featured from the flower of genus *Albizia*, by their male fertilizing organs (stamens), which are not connected at the basis. Also, different from mimosa flowers, *Acacia* possesses more than ten stamens (Prenner 2011).

Acacia seeds are distributed generally by being thrown away from the legume when it crackles, commonly under the impact of the sun. In some time the grains may stay suspended in the air by a filamentous stalk from the unfold legume, the chromatic funicle and aril effective as a substance that attracts bird (Abdelbasit et al. 2014). Most of *Acacia* species are important economically (Nigussie et al. 2020). The adhesive substance of *Acacia* especially *A. senegal* produces Arabic gum, a substance used in glue, as a medicinal drug, pontiff, desserts and other products (Nasir et al. 2012). A few types of *Acacia* include alkaloids which affect the mind, and some include potassium fluoroacetate, a rodent venom (Gonzales and Tolentino 2014).

19.4.1 Medicinal Uses and Pharmaceutical Applications

Medicinal plants made about 70–80% of world people plantation fundamentally in the developing countries, it is usually utilized for major health care, healthier lifestyle combining the ability to cure some disease with the minim side effect. Various part of the plant has different various biologically active compounds and diverse pharmacologic behaviour (Carvalho et al. 2014). *Acacia* species is one of the wealthiest biological resources, which include flavonoids, morphine, quinine, phenolic resins, saponins, carbohydrate and other organic compounds (Jelassi et al. 2016). Several parts of *Acacia* have various phytochemical compositions and various pharmacological activities (Amadou et al. 2020). Bark extracts of *Acacia catechu* had a role in treating skin pain, scarring, mouth ulcers and bronchitis (Sunil et al. 2019a). Also showed good efficacy in the possibility of using it as a catalyst for the immune system (Sunil et al. 2019b).

It has been observed that the ethanol *Acacia* extracts play a major role in wound healing, especially plant's bark because of the high content of flavonoids and alkalis (Suriyamoorthy et al. 2014). Boiling pods of *Acacia* is useful in the diseases of both urinary and genital organ and stops early ejaculation (Castillo-Mitre et al. 2017). Also, pods are utilized in aired cough (Farzana and Tharique 2014). The pods and leaf extracts are utilized for body revitalize (Dasgupta and Roy 2010). *Acacia nilotica* is used as contractile, bleeding stops, and emollient (Akbar 2020).

The fruits of *Acacia* tree are beneficial in treating infection of the intestines, diarrhoea and diabetes (Semenya and Maroyi 2012). A study estimated extracts of *Acacia farnesiana* for diabetes treatment in mice, showing that aqueous extract safely lowered glucose grade in the blood (Akbar 2020). Root's ash reported as an agent that can help in stopping diarrhoea and decrease uric acid in the urine (Das et al. 2015). Beneficial in wounding healing and helpful in burning (Iftikhar et al. 2010). Different plants parts are utilized to strengthen hair relieve ear pain and treat cholera, purge and contagious diseases (Wuthi-udomlert and Vallisuta 2011). Other studies evaluated the slough healing activity of *Acacia farnesiana* methanol leaf extracts against ulcer-motivated sample in rats. Outcomes offered the methanol extracts decreased the ulcer indicator contrast to monitoring ranitidine (Dwarakanath et al. 2013). Also demonstrated that *Acacia auriculiformis* extracts act to inhibit acetylcholine esterase and in turn enhance memory (Sharma et al. 2014a). It has been reported that the *Acacia* plant extracts had a more positive effect than a placebo gel on mouth infections, especially gingival and teeth broach (Rajvaidhya et al. 2012). In in vitro study, on Swiss albino mice, *Acacia* gum gave faster-wound renewal; also it can maintain proper moisture level at wounding surface (Parwani et al. 2019). A mixture of *Acacia* gum-sodium alginate gel with low concentrations of ZnONPs used to treat wounds and limited the growth of some pathogens (Raguvaran et al. 2017). In another study, Arabic gum was used to decrease C-reactive protein levels, which can be used as natural fibres for treatment Sickle cell disease (SCD) patients (Kaddam and Kaddam 2020). In vivo research shows that in 74 patients having haemoglobin SS, Acacia gum reduced transaminase and bilirubin level in the blood, without affecting aspartame about renal function (Kaddam et al. 2019). *A. nilotica* is an important attractive appearance medical plant; it is a source of many active compounds which can be used for medicine expansion with a greater possibility of prosperity, where flavonoids and alkalis form a large percentage of the plant extract with anti-inflammatory and antioxidative properties (Rather and Mohammad 2015).

A. nilotica showed high content of alkali lignin, which acts as an antioxidant (Barapatre et al. 2015). Unpurified root extracts of *A .nilotica* reveal notable effect against a *Plasmodium berghei* in mice, due to its high content of terpenes, diterpenes, sesquiterpenes and derivatives of gallic acid such as tannins (Jigam et al. 2010).

Acacia polyacantha methanolic extracts revealed the highest antioxidant properties compared to extraction using acetone (Mohammedelnour et al. 2017). It has been observed that there is a correlation between the contents of phenolic compound and antioxidant activity in *Acacia mearnsii* (Missio et al. 2017). Extract of *A. nilotica* seeds shows the ability to lower arterial blood pressure and supplies guide of antihypertensive effectiveness (Malviya et al. 2011).

Safari and his group explained the water leaf extracts s of *A. nilotica* have antinociceptive and anti-inflammatory effects, also showing the ability to reduce fever in albino mice (Safari et al. 2016). These properties attributed to the existence of compounds as alkaloid, phenolic acid and plant pigments (Jigam et al. 2010). *Acacia modesta* has tranquillizer and anti-inflammatory effects, as well as analgesic effects related to the opioid regulation (Bukhari et al. 2010). The flower extracts of *Acacia dealbata* contain approximately 25% phenolic compounds, leading to possess it different biological properties such as tumour cells killing, antitoxins and anti-inflammatory (Casas et al. 2020).

The stem bark extracts of *A. nilotica* have a strong antibacterial effect against *S. aureus*, *Proteus mirabilis* and *Klebsiella pneumoniae*; phytochemical analyses of extracts showed existences anthraquinones, saponins, tannins and flavonoids (Deshpande 2013). In another study, the outcomes showed that the methanolic extracts of *A. farnesiana* exhibited good antimicrobial effect, getting suppression zones approximately 2.8 cm against *Vibrio cholerae*, as well as, has been isolated and diagnosed methyl gallate compound which has efficient against the last microbe (Sánchez et al. 2013). *A. polyacantha* leaf extracts also showed significant anti-staphylococcal action, this activity is attributed to the existence of epicatechin compound in the extracts (Ashu et al. 2020). The extracts of *Acacia* pods and leaves showed antimalarial and antibacterial properties (Sadiq et al. 2017).

A. nilotica used in the folk medication to prevent the growth of some pathogen fungi (Safavi et al. 2015). *A. nilotica* showed potent activity versus *Aspergillus fumigatus*, due to its nilobamate as a modern compound for better treatment against some pathogenic fungi (Mbatchou and Oumar 2012). *A. nilotica* has an antiparasite activity, specifically to reduce the growth of trypanosomes (Sulaiman et al. 2020).

Also, an aqueous extract of *A. nilotica* leaves showed inhibition impacts against some type of fungi including *Candida albicans*, *Aspergillus niger* and *A. fumigatus* (Sharma et al. 2014b). All the extracts of the *Acacia sinuata* contain a considerable amount of phenol and various types of plant pigments (flavonoids), and they also exhibited antifungal activity (Vijayasekhar et al. 2016). Leaves and fruits of *A. nilotica* aqueous extracts showed inhibitory effects against Bovine Herpes Virus type-1(BHV1); the highest ratio of inhibition was 61.1% at concentration 0.156 mg/ml for leaf extract (Goel et al. 2011). Leaf extract of *Acacia mellifera* showed anti-HBV efficiency (Malviya et al. 2011). Caffeoyl and coumaroyl extracted from leaves of *Acacia cochliacantha* had anthelmintic action against *Haemonchus contortus* (Castillo-Mitre et al. 2017).

19.4.2 The Industrial Importance of Acacia

Acacia is an important species and plays a varied role in the livelihoods of rustic populations in all sides of the world (Miller et al. 2011). While *Acacia* lumber was previously utilized for building boats or ships as well as bridges, currently, it is utilized to produce furniture. Researchers worked to introduce even better wood quality cultivation of *Acacia* such as *Acacia* hybrid and second-generation *Acacia mangium* (Jusoh et al. 2014).

The existence of chemicals used for antiinsect applications is considered harmful to cultivated plants and animals in nature, and it is considered a big environmental concern and their unfavourable effect on human health (Gill and Garg 2014). Coal of *Acacia etbaica* was found to be an efficient and cheap alternative for the elimination of a large group of pesticides and other synthetic organic compounds with chlorinated aromatic molecules from water solutions (Gebrekidan et al. 2015). *A. nilotica* extract is used to colour woollen yarns, which showed high stability after washing; also, it possesses antioxidant and antipoisoning activity (Rather et al. 2017). In a previous study, the various solvents for obtaining colour from a tropical *Acacia* (*A. nilotica*) bark and their effectiveness, water, acidic, alcoholic and alkali solvents were studied. A dye obtaining in alkali exhibited high colour potential with more stability (Kumar et al. 2015). The product of biofuel intended as a substitute for diesel from *A. nilotica* seeds oil, which contains a high level of unsaturated fatty acid, is also highlighted (Adhikesavan et al. 2015). *Acacia* biomass (*A. mangium* and *A. auriculiformis*) may be used as a raw material in heating decomposition (Ahmed et al. 2018). *A. mangium* was also used to produce fuel pellets (Amirta et al. 2018). *Acacia concinna* were used to synthesize eco-friendly silver nanoparticles which could have many industrial and medical applications (Kumar Sur et al. 2018). In the food biotechnology industry, the leaf extracts of *Acacia leptoloba*, *Acacia auriculiformis* and *Acacia disparrima* have the potential to prevent bacteria which caused food toxicity (Cock 2012). *Acacia* gum can be used to limit some biological activities, by adding it in the culture media as an antibacterial agent (Dwivedi et al. 2020). Gum powder of *A. modesta* may be utilized for decreasing shrink, internal treating inside the Source Code Control System (SCCS), for promoting the viscidity of the cementitious systems (Rizwan et al. 2018). Moreover, the wide range of antimicrobial ability indicates the possibility to use *Acacia* trees extracts as natural feed keeper (Cock 2017). Also, Arabic gum utilized with canella and garlic as an alternative, natural meat and fishes preservatives (Rakshit and Ramalingam 2013).

High level of phenolic compounds in the flowers *Acacia* extracts showed activity against *Salmonella enterica* and *Escherichia coli*; this led to using them as preservative substances in the feed, medication and the manufacture of cosmetic (Stankov et al. 2018). Another study in vivo proved that the cream which contains *Acacia* bark extracts decreases sebaceous glands excretions (Atif Ali et al. 2012). As well, *Acacia concinna* pods support hair growth and prevent appearance small pieces of dead skin (Gediya et al. 2011). Recently, *Acacia* trees have contributed to the manufacturing of furniture, fibre concrete, gates and car parts characterized by rapidly degradable, friendly of environment, lightweight and strong (Muhammad et al. 2019).

19.4.3 Environmental Impact

In case of environmental alteration and its negative impact to forests globally, *Acacia* trees are considered one of the most successful forest trees to resist drought and climate change in the world due to its root system, where *Acacia tortilis* has a major ability to resist the cruelty of drought (Aref et al. 2013; Winters et al. 2018). *A. mangium* is a species with significant potential for phytoremediation and its capacity to transport heavy minerals such as aluminium and iron (Zuhaidi and Vijayanathan 2018). Other minerals such as zinc, copper and cadmium can be treated by using *A. mangium* (Majid et al. 2011). It has been shown that extracts of *A. concinna* pods had high performance and qualification as anticorrosion substances (Haldhar et al. 2020). *Acacia saligna* and *A. polyacantha* exhibited guide of cumulation of Ni, Cu and Fe and thus may be utilized for phytoremediation (Masvodza et al. 2013). Although *A. mangium* have a positive impact on the ecosystems but may be negatively interfering with other types of trees (Koutika and Richardson 2019). *Acacia seyal* negatively affected sorghum yield, due to competition for shading and nutrients (Deng et al. 2017). Mixing between *A. mangium* and *Eucalyptus grandis* led to the exploration of deep soil with a positive impact of forest (Germon et al. 2018). *A. nilotica* can be used as an indicator of the presence of air pollutants such as copper and cobalt (Attahiru et al. 2015). *Acacia raddiana* showed the ability to boost the potential of microbial groups and enhances carbon and nitrogen content in soil (Fterich et al. 2012; Pereira et al. 2018). Polypropylene compounds extraction from dark *Acacia* bark have good properties, such as higher strength and extreme crystallization heat and thermic stabilization (Taflick et al. 2015). *A. concinna* pod extracts exhibited a repressive effect against weeds in agriculture crops (Boonmee and Kato-Noguchi 2017). The water extracts of the phyllodes, roots, stalks, seeds and blooms of *Acacia cyanophylla* exhibited inhibition on the seed germination of two weeds *Silybum marianum* L. and *Peganum harmala* L. (El Ayeb et al. 2013). *Acacia seyal* seeds have impacted on growing and production of *Sorghum bicolor* positively in *Striga hermonthica*-invaded region (Eltayb et al. 2013). *Acacia* trees help to reduce the temperature surrounding urban environment, by increasing the shade (Aguilera et al. 2015). It also improves water and air quality through the absorbance of pollutants and interferes with dust and small particles (Attahiru et al. 2015), as well as oxygen production and reduction of ozone erosion (Abbasi and Abbasi 2017). It reduces wind risk and speed (Wittmann et al. 2018).

19.5 Conclusion

Multi-usage plants have been utilized and cultivated by humans for thousands of years for different purposes. It is all started by the utilization for food consumption of the wild plants and then growing those plants for remedies, and then it progressed to be utilized in different aspects of life. The examples of this are the four plants given within this chapter representing a group of plants which could be a good candidate to be cultivated for medicinal and pharmaceutical purposes and at the same time directly or indirectly will be of great benefit to the environment in terms of increasing oxygen rate and reducing the carbon footprint. More efforts from government and non-governmental organization need to be directed to the cultivation of these plants especially at the local communities in many developing countries for economical, environmental and social benefits.

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Chapter 20
Aromatic and Medicinal Plants for Phytoremediation: A Sustainable Approach

Jitender Kumar, Nazir Ahmad Malik, and Narender Singh Atri

20.1 Introduction

There are two burning issues in current scenario – one is depletion of agricultural fertile lands due to the long-term contamination of heavy metal (HMs) and organic pollutants in soil through natural and anthropogenic activities and another is the overexploitation of indigenous aromatic and medicinal plants in wild. Heavy metals/metalloids are chemical elements having high density (>5) and high atomic weight and which are toxic even at a very low concentration (<1 ppm) (Ali and Khan 2018a; Olaniran et al. 2013). Most of the HMs are persistent and non-biodegradable and represents a serious threat to the fauna and flora of the earth. Chronic exposure of these metals in humans results in various complications as they are well-known toxic and carcinogenic agents (Sarwar et al. 2017). HMs and organic pollutants in soil result in soil quality deterioration, crop yield reduction, and poor quality of agricultural products (Saxena and Bharagava 2017).

Therefore, the growing number of HMs that contaminated areas increases the importance of the removal of these HMs from the contaminated matrix to safeguard the environment and human health. The cleanup of soils contaminated with HMs is one of the most difficult tasks. Remediation using physical, chemical, and biological methods has been adopted to solve the problem. The chemical and physical

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techniques generally generate toxic by-products and are not cost-effective (Lajayar et al. 2017). Thus, an eco-friendly and sustainable solution is required for the treatment and management of contaminated sites, which is biological methods. The biological methods, using plants, are efficient and on an average are about tenfold cheaper than other remediation methods. The remediation of contaminated sites using non-food plants as compared to food plants is a newly emerging concept. Non-food crops such as aromatic and medicinal plants are a better choice for remediation of contaminated sites as compared to other non-food crops and are currently under research due to its various merits. In this chapter, the concept of phytoremediation and preferred selection of aromatic and medicinal plants for this purpose; different types of soil contaminants, their source, and their lethal effects; current research on the phytoremediation of contaminated soils using aromatic and medicinal plants; different types of remediation techniques used by these plants; terrestrial and aquatic aromatic and medicinal plant diversity for phytoremediation in HMs and organic pollutant-contaminated soils; plant microbe interactions and the mechanisms used by microbes and plants for environmental remediation; transgenic approaches to modify the hyperaccumulating plants and associated microbes were explained.

20.2 Phytoremediation

Plants are not only source of fuel, food, fiber, and medicine but also environmental counterbalances to anthropogenic and natural pollution. Plants which grow in HM-polluted soil and withstand this abiotic stress are known as metallophytes (Wenzel and Jockwer 1998). Use of these metallophytes to clean up HMs and synthetic organic compounds from the contaminated water and soils is known as phytoremediation. The term “phytoremediation” is made up of two words, i.e., Greek head “phyto” (means plant) and Latin root “remedium” (means to correct or remove an evil). It is the process of transformation, stabilization, degradation, and detoxification of a toxic compound with the aid of plants and/or its associated microbial communities by making use of the inherent biological mechanisms of microorganisms and plants (Chirakkara et al. 2016; Dixit et al. 2015). Phytoremediation has been tested using various plant species, and most promising plants for phytoremediation of contaminated sites have been identified from families – Araceae, Asteraceae, Brassicaceae, Fabaceae, Geraniaceae, Lamiaceae, Poaceae, and Pontedericeae. Remediation of HMs is more difficult than that of organic contaminants, as the latter can be mineralized into carbon dioxide and water, but HMs cannot be mineralized and can only be converted to less toxic form or immobilized to reduce their bioavailability (Sharm et al. 2018).

20.3 Why Aromatic and Medicinal Plants for Phytoremediation

A sustainable approach to mitigate contaminants of environment is the need of the hour. However, the use of food and fodder plants for phytoremediation is not safe as HMs can enter the food chain upon consumption and can cause some deleterious effects on humans or animals (Ali and Khan 2018b). Medicinal and aromatic plants are a better choice for phytoremediation, as these plants are primarily grown for secondary metabolites and oil production (Angelova 2012). The essential oils and secondary metabolites from these plants are not contaminated by HMs significantly, as these are extracted through the distillation process (Abu-Darwish 2009). The content of the essential oil obtained from aromatic and medicinal plants is either not affected or increased in HMs contaminated areas (Pandeya et al. 2018). Zheljazkov and Nielsen (1996) found that aromatic and medicinal plant peppermint can extract significant quantities of HMs from the polluted soil without its essential oil contamination. Three aromatic and medicinal plants, chamomile (*Matricaria recutita*), sage (*Salvia officinalis*), and thyme (*Thymus vulgaris*), are extensively used since ancient times both for their medicinal and aromatic use and are recently preferred for efficient phytoremediation (Rašković et al. 2015; Shebbo et al. 2020; Wang et al. 1998). Regardless of the metal accumulation, the quality and quantity of essential oil from these plants were not affected, and extracted essential oils were free of HMs, indicating that HM-contaminated areas can be used for the production of essential oils and secondary metabolites using medicinal and aromatic plants (Lydakis-Simantiris et al. 2016).

Phytoremediation of a contaminated soil becomes more economical and sustainable if it is done with such plant species which also have industrial application and generate large amount of biomass for bioenergy and biofuel production (Sheoran et al. 2009). Various aromatic and medicinal plants which are used for phytoremediation are currently being exploited to generate economy and bioenergy (Tabinda et al. 2020). These plants can be commercialized, and employment can be generated, because these plants are also important source of timber, food, fiber, medicine, and metals and bioenergy (Abu-Darwish 2009; Mahar et al. 2016; Sheoran et al. 2009). Thus, cultivation of aromatic and medicinal plants in contaminated areas fulfils the purpose of remediation alongside monetary benefits. The essential oils or secondary metabolites from these plants have potential market in pharma, perfumery, cosmetic industry, and aromatherapy. Aromatic and medicinal plants such as *Cymbopogon winterianus* (citronella), *C. flexuosus* (lemon grass), *C. martini* (rosha grass), *Artemisia annua* (sweet sagewort), *Vetiveria zizanioides* (vetiver), *Matricaria chamomilla* (chamomile), *Melissa officinalis* (common balm), *Ocimum basilicum* (basil), *Salvia sclarea* (Europe sage), *Salvia officinalis* (common sage), *Helianthus annuus* (sunflower), *Lavandula angustifolia* (lavender), *Portulaca oleracea* (purslane), *Mentha arvensis* (wild mint), *Mentha piperita* (peppermint), and *Mentha citrata* (lemon mint) are mainly used for this purpose (Ali et al. 2020; Angelova et al. 2016a, 2016b; Gupta et al. 2013; Kovacik et al. 2006; Lajayar et al. 2017;

Lee et al. 2014; Prasad et al. 2010; Putwattana et al. 2010; Shams et al. 2010; Suelee et al. 2017). They can be successfully used in phytoremediation of HMs as well as organic pollutants and can replace food and fodder crops grown under the same set of contaminated conditions. Therefore, one of the most sustainable and innovative approaches for addressing reclamation of contaminated sites in recent years is the use of aromatic and medicinal plants as it is environmentally safe with high monetary value.

20.4 Soil Contaminants Remediated Using Aromatic and Medicinal Plants, Their Source, and Lethal Effects

Soil contaminants such as heavy metals/metalloids, organophosphate insecticides, radionuclides (U, Cs, Sr), explosives, pesticides surfactants, and other organic pollutants are major health concern for plant and animal. HMs such as cadmium (Cd), mercury (Hg), aluminum (Al), arsenic (As), silver (Ag), lead (Pb), cobalt (Co), and chromium (Cr) possess biological toxicity (Ali and Khan 2018a). If the concentration of these hazardous HMs exceeds the maximum permissible concentration in soil and water, they adversely affect the environment. The maximum permissible limit given by the Comprehensive Environmental Response Compensation and Liability Act, USA, for Cd, Ag, Cr, Ar, Pb, and Hg is 0.05 mg L^{-1}, 0.01 mg L^{-1}, 0.01 mg L^{-1}, 0.015 mg L^{-1}, 0.05 mg L^{-1}, 0.002 mg L^{-1}, respectively (United States Environmental Protection Agency 2000). Cd and Pb are the most toxic HMs and are measure concern for environmental biologist. There are a number of other HMs like zinc (Zn), iron (Fe), copper (Cu), nickel (Ni), selenium (Se), and manganese (Mn) which are otherwise essential micronutrients for plant growth and a wide variety of cellular processes but are reported to become toxic only above threshold limit.

HM contamination in soil and water occur naturally, i.e., through processes such as the weathering of rocks, volcanic eruption, and/or anthropogenic activities such as the mining, processing and smelting of ores, industrial wastes, emission of automobiles, fuel production disposal of municipal sewage, nuclear and automotive industries wastewater, waste incineration, and the agricultural application of some pesticides and fertilizers (Ali et al. 2020). The total HM content in a soil is the sum of the concentrations of the elements originating from the parent material (lithogenic source) and addition from the various anthropogenic sources (Lydakis-Simantiris et al. 2016). Inputs of HMs to agricultural land through these anthropogenic activities are increasing in the environment. The precarious and excessive utilization of fertilizers, herbicides, pesticides, and wastewater irrigation on agricultural land has raised the concentration of Cd, Pb, Cu, As, Ni, and Zn in soil (Pirzadah et al. 2018). HM pollution through anthropogenic activities has made agriculture soil unsuitable as it directly affects the plant production yield. These pollutants are also major source of oxidative stress in plants (Chandra et al. 2015b; Mani 2015). Generally, HMs inactivate the enzymes by binding to specific group, for instance, binding of HMs like cadmium (Cd) to sulfhydryl group of enzymes and proteins either results in misfolding or inhibition of enzyme action. Besides these, HM stress in plants causes stunted growth, reduced biomass, smaller root size, chlorosis, necrosis, turgor loss, decreased respiration and photosynthetic activity, apoptosis, and finally death of the plant (Szopinski et al. 2019). HM poisoning in human affects the immune system; damages the nervous system; impairs speech and hearing; causes loss of coordination, paralysis, weakness of the muscles, gangrene, skin disorders, cancer, respiratory and pulmonary diseases; and is harmful to developing foetuses, while severe exposure in human causes life-threatening degenerative diseases such as Alzheimer's disease, muscular dystrophy, atherosclerosis, and Parkinson's disease (Giacoppo et al. 2014; Lee et al. 2018).

Organic pollutants in the environment are mostly man-made and are toxic and/or carcinogenic. Sources of organic pollutant in the environment include industrial activities (e.g., chemical, petrochemical), accidental release (using fuels, solvents), military activities (e.g., explosives, chemical weapons), and agriculture (e.g., pesticides, herbicides). There are several classes of organic pollutants: explosives petroleum products including benzene, toluene, ethylbenzene, and xylene (BTEX); polycyclic aromatic hydrocarbons (PAHs); polychlorinated biphenyls (PCBs); and herbicides/ pesticides (i.e., atrazine, chlorpyrifos, 2,4-D). Explosive compounds are used for numerous applications such as mining, construction, and military munitions. Military training ranges and production units serve as most common sites for pollutants, where explosives and their transformed products permeate into the soil and leach into the groundwater (Quinn et al. 2009). Major soil contaminants found in ammunition evaluation facilities, artillery ranges, army depots, and disposal sites include the toxic explosive compounds 2,4,6-trinitrotoluene (TNT), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and composition B (Comp B), which is a commonly used military formulation. TNT causes liver damage and anemia, while RDX causes renal, gastrointestinal, and central nervous system toxicity (Garcia-Reyero et al. 2011). Organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) are primarily formed by incomplete combustion or pyrolysis of organic matter into the soil. PAHs are of great concern owing to their toxic and/or carcinogenic and mutagenic properties (Wei and Pan 2010). The releases of anthropogenic chemicals such as BTEX, PAHs, and PCBs into the environment due to increasingly industrialized global economy have resulted in contamination of many areas on Earth. Many of these ring-structured compounds are strong carcinogens and cause growth inhibition, reduced transpiration, wilting, and chlorosis in plants (Xiao et al. 2015). Pesticides can cause chronic abnormalities in humans and plants. The remediation of soil polluted by these organic and inorganic contaminants is urgent and imperative, due to their persistence and non-biodegradable nature.

20.5 Phytoremediation Approaches Used by Aromatic and Medicinal Plants for Environmental Cleanup

In general, phytoremediation can be applied for different types of contaminants and based on the mode of the action of plants for removing or reducing the toxic contaminants from the soils (Fig. 20.1). It can be subdivided into six basic types, phytoextraction, phytodegradation, rhizofiltration, phytostabilization, phytostimulation, and phytovolatilization (Ali et al. 2013; Sarwar et al. 2017), of which phytoextraction, phytodegradation, and phytostabilization are commonly used by aromatic and medicinal plants for the phytoremediation of HMs and organics in contaminated soils.

20.5.1 Phytoextraction

Among the phytoremediation techniques, phytoextraction refers to the extraction and accumulation of HMs or organic contaminants from the matrix through absorption by plant roots and translocation/sequestration to aboveground portions of the plants without any apparent phytotoxic effect (Mahar et al. 2016). It is also referred to as phytoaccumulation or phytoabsorption or phytosequestration. It is the most preferred method used by plants for the remediation of polluted environments as it

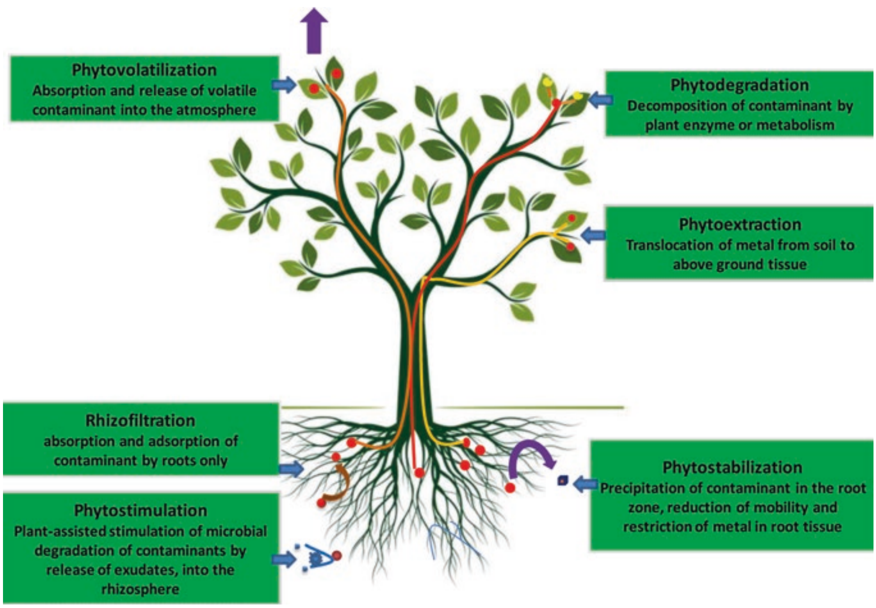

Fig. 20.1 Different phytoremediation approaches used by plants for environmental cleanup

is sometimes enhanced by plant growth-promoting rhizobacteria (PGPR) associated with the plant roots (Konkolewska et al. 2020). Phytoextraction involves the uptake and movement of metal pollutants into aboveground parts of the plants, using the mechanism of hyperaccumulation (Lajayar et al. 2017). Plants that can accumulate very high amount of HMs from soils were termed as "hyperaccumulator plants." Such hyperaccumulator plants overcome phytotoxicity due to the presence of a versatile property known as hypertolerance, making them as sensitive. Metal hyperaccumulating aromatic and medicinal plants are mainly preferred for phytoremediation of contaminated sites.

20.5.1.1 Aromatic and Medicinal Hyperaccumulating Plants for Phytoremediation

Plants that accumulate very high metal concentration (metallophytes), i.e., 100–1000-fold higher than those found in non-hyperaccumulating species, without any visible symptom of toxicity are regarded as hyperaccumulators, and the overall process is termed as hyperaccumulation (Rascio and Navari-Izzo 2011; Reeves 2003). The content limits of some metal elements in dry biomass of plants to be termed hyperaccumulators should be 100 mg/kg of Cd and As; 1000 mg/kg of Pb, Cu, Co, Se, Cr, and Ni; and 10,000 mg/kg of Mn and Zn (Reeves and Baker 2000). To date, several aromatic and medicinal hyperaccumulator metallophytes have been used in the phytoremediation of HM polluted sites. For examples, medicinal plant *Pteris vittata* (Chinese brake fern), which have antioxidant, antiproliferative, or anticancer potential (Kaur et al. 2014), can accumulate up to 35,303 and 20,707 mg/kg dry weight (DW) of Cr and As, respectively (Kalve et al. 2011). The aromatic plant *Tagetes minuta* (marigold), which is commercially grown for its essential oils (Tagetes oil), can accumulate Pb up to 380.5 μg g^{-1} DW, and medicinal herb *Bidens pilosa* (hairy beggarticks) can accumulate 100.6 μg g^{-1} DW, of Pb concentration in leaves (Salazar and Pignata 2013). Therefore, *Pteris vittata* has a considerable hyperaccumulation and phytoremediation potential for Cr and As, whereas *Tagetes minuta* and *Bidens pilosa* for lead polluted soils. Some medicinal and aromatic plant genera have better hyperaccumulator species, e.g., genus *Alyssum* which is used for its essential oil and medicinal property (Golkar and Fotoohi 2019; Mahmoudi et al. 2019) hyperaccumulate Ni in 48 out of 170 species (Brooks et al. 1979; Brooks and Radford 1978). Among the best known hyperaccumulators species of family Brassicaceae is *Alyssum murale* commonly known as yellow tuft. It has been reported to accumulate up to 9129 mg/kg Ni in fertilized soil and 8483 mg/kg Ni in unfertilized soil (Bani et al. 2007). *Brassica juncea* (Indian mustard) and *Helianthus annuus* (sunflower) which is used for its essential oil and therapeutic potentials (Yu et al. 2003) have been found to HMs and organic pollutants hyperaccumulators in contaminated soil. The site of accumulation of HMs differs from one species to another or also within the same species based on their ecotype and populations (Rascio and Navari-Izzo 2011). Angelova et al. (2016b) reported that accumulation of Pb in organs of sunflower was selective since 59% Pb is accumulated in the leaves and only 1% is accumulated in the seeds. Similarly, *Sesbania drummondii*, which is used in veterinary medicine (Bunma and Balslev 2019), reported to be a Pb hyperaccumulator in shoot while maintaining a high biomass yield (Sahi et al. 2002). Aromatic and medicinal hyperaccumulating plants are specific to and hyper-accumulate one or more HMs (Table 20.1) and are preferred choice for the phytoremediation.

20.5.1.2 Selection Criteria for Aromatic and Medicinal Hyperaccumulating Plants for Phytoremediation

The screening of hyperaccumulating plants is mainly done on serpentine/ultramafic soils which is the most abundant metalliferous soil on earth. Plants inhabiting such soils are known as "serpentinophytes" which are of great significance in phytoremediation. Several medicinal plant species like *Alyssum bertolonii*, *Crotalaria micans, Leucaena leucocephala, Bidens pilosa, Pueraria lobata,* and *Conyza canadensis* have been reported to remove Ni and Zn from a serpentine site for successful phytoremediation applications (Ho et al. 2013). Bini et al. (2017) have suggested the use of *Alyssum bertolonii* in the remediation of Ni-contaminated soils with both the phytostabilization and phytoextraction because it accumulates high concentration of Ni (i.e., 2118 mg $kg-^{1}$ in aerial parts) from serpentine soils. Similarly, medicinal plant Greek oregano (*Origanum vulgare*) exhibited a rather exceptional capacity to bioaccumulate Cr in the aerial part (up to 1200 mg of total Cr kg^{-1} DM) and the roots (4300 mg kg^{-1} DM), much above the hyperaccumulation range (Levizou et al. 2019). Thus, serpentinophytes are the key plants amicable for the phytoremediation and phytomining. The ideal hyperaccumulating aromatic and medicinal plants for phytoremediation should possess: (Chandra et al. 2015a, 2015b; Levizou et al. 2019) (a) tolerance to high salt, pH, and temperature variation, (b) resistance to high concentration of toxic metal, (c) ability to preferably hyperaccumulate HMs in the aboveground parts, (d) fast growth, (e) high biomass, (f) resistant to diseases and pests, (g) easy to cultivate and harvest, (h) widespread highly branched roots, (i) safe disposal, and (j) economic to process for metal recovery. The phytoextraction and hyperaccumulation efficiency of plants primarily depend on three factors: (1) bioconcentration factor (BCF), (2) translocation factor (TF), and (3) biological accumulation factor (BAF). BCF is the ratio of metal concentration in root and metal concentration in soil and represents metal accumulation; whereas TF is the ratio of metal concentration in shoot and metal concentration in root and represents metal translocation, BAF is ratio of HMs concentration in shoot and soil (Ali et al. 2013). Plant with a value higher than 1 in these parameters are regarded as efficient in phytoextraction and/or phytoaccumution. For example, phytoremediation potential of medicinal plant *Urtica dioica* (Stinging nettle) was evaluated according to TF, BCF, and finally, BAF. It is reported that nettles are hyperaccumulating zinc ($TF = 1.1 \pm 0.1$) in shoot, while the TF of lead was 0.4 ± 0.1 indicating its accumulation in root tissues and was hardly translocated to the green plant parts at all.

Table 20.1 Aromatic (A) and medicinal (M) hyperaccumulating plant species used in phytoextraction of HMs

Species	Family	HMs	A/M	References
Alyssum murale L.	Brassicaceae	Ni	A/M	Bani et al. (2007)
Ocimum basilicum L.	Lamiaceae	Cd	A/M	Putwattana et al. (2010)
Helianthus annuus L.	Asteraceae	Pb, Cd, Zn	A/M	Angelova et al. (2016b)
Pelargonium species	Geraniaceae	Pb	A/M	Arshad et al. (2008)
Salvia sclarea L.	Lamiaceae	Pb	A	Angelova et al. (2016a)
Arabidopsis halleri L.	Brassicaceae	Cd	M	Meyer and Verbruggen (2012)
Arabidopsis halleri L. *ssp. Germmifera*	Brassicaceae	Zn, Cd	M	Zhang et al. (2017)
Astragalus bisulcatus (Hook.) A. Gray	Fabaceae	As	M	Freeman et al. (2006)
Stanleya pinnata (Pursh) Britton	Brassicaceae	As	M	Freeman et al. (2006)
Euphorbia cheiradenia Boiss. & Hohen	Euphorbiaceae	Cu, Fe, Pb, Zn	M	Nematian and Kazemeini (2013)
Urtica dioica L.	Urticaceae	Zn	M	Viktorova et al. (2016)
Pteris vittata L.	Pteridaceae	As	M	Xie et al. (2009) and Chen et al. (2016)
		Hg	M	Su et al. (2008)
Viola baoshanensis Shu	Violaceae	Pb, Zn, Cd	M	Zhuang et al. (2005)
Solanum nigrum L.	Solanaceae	Cd	M	Peng et al. (2009)
Kalimeris indica (L.) Sch.Bip	Asteraceae	Pb	M	Sun et al. (2016)
Cirsium setosum (Willd.) Bess.	Asteraceae			
Cynanchum chinense R. Br	Apocynaceae			
Eleusine indica (L.) Gaertn.	Poaceae			
Humulus japonicus Siebold & Zucc.	Cannabaceae			
Metaplexis japonica (Thunb.) Makino	Apocynaceae			
Imperata cylindrica (L.) P. Beauv	Poaceae	Fe	M	Fuente et al. (2016)
Miscanthus floridulus (Labill.) Warb. ex K. Schum. & Lauterb.	Poaceae	Cd	M	Cheng et al. (2016)
Rosmarinus officinalis L.	Lamiaceae	Ni	A	Divrikli et al. (2006)
Matricaria chamomilla L.	Asteraceae	Cd	A	Kovacik et al. (2006)
Leersia hexandra Sw.	Poaceae	Cr	M	Zhang et al. (2007)
Spirodela polyrhiza (L.) Schleid.	Lemnaceae	As	M	Rahman et al. (2007)
Bidens pilosa L.	Asteraceae	Cd	M	Sun et al. (2009)
Lobelia chinensis Lour	Campanulaceae	Cd	M	Peng et al. (2006)

20.5.2 Phytostabilization

Phytoremediation of soil using plants by reducing the mobility and migration potential of contaminants in soil is known as phytostabilization (Berti and Cunningham 2000). Plants inactivate and immobilize contaminants at their place involving absorption/adsorption on roots, and precipitation, complexation, and reduction of metal valences in rhizosphere, e.g., medicinal plant mesquite (*Prosopis* spp.) uptake Cr^{6+} and causes its reduction to much less toxic and insoluble Cr^{3+} using the mechanism of phytostabilization (Aldrich et al. 2003). Thus, this technique reduces the mobility of contaminants and prevents their migration to groundwater (leaching) or air and entry into the food chain. Plants having broad root system and low mobility of metals from roots to shoots determine the success of phytostabilization (Berti and Cunningham 2000). This process mostly addresses the pollutants such as Cd, Pb, Zn, Cu, As, Se, Cr, U, PCBs, PAHs, dioxins, furans, DDT, pentachlorophenol, and dieldrin (Suelee et al. 2017). Significant phytostabilization of HM metals especially Cr and Ni were observed in the roots of aromatic and medicinal plant *Nerium oleander* (Elloumi et al. 2017).

Aromatic plant vetiver (*Chrysopogon zizanioides, Vetiveria zizanioides*) can be used as an excellent phytostabilization agent for remediation and reclamation of iron-ore mine spoil-dumps (Banerjee et al. 2016; Suelee et al. 2017). The phytostabilization ability of a plant could be increased by changing the organic matter content by addition of biochar or compost and by pH which will increase plant yield and immobilize the more metals in the roots from soil (Palansooriyaa et al. 2020; Visconti et al. 2020). Various soil additives such as mineral sorbents are nowadays, used for enhanced phytostabilization (Radziemska 2018). Aided phytostabilization reduces metal toxicity, enhances microbial activity, and improves soil fertility (Touceda-Gonzalez et al. 2017; Visconti et al. 2020). Many species of aromatic and medicinal plants have potential of the aided phytostabilization of HMs. For example, significant phytostabilization of HMs such as Pb, Cd, and Zn was achieved by roots of *Festuca rubra* in the presence of minerals dolomite, chalcedonite, and halloysite (Radziemska 2018). Two medicinal plant species *Brassica juncea* and *Dactylis glomerata* were used for the efficient phytostabilization of mine soils amended with compost or biochar (Visconti et al. 2020). Hence, phytostabilization with soil additives has proved as a better choice in remediation of multi-metal-contaminated soils. Medicinal plants are much better alternative for phytostabilization in situ because this method increases the economic value of plants along with phytoremediation and decreases the chances of food chain contamination.

20.5.3 Phytodegradation

It is the breakdown of complex organic contaminants into less toxic or non-toxic forms by plant metabolism or by the activity of plant enzymes within plant tissues (Castro et al. 2001; Mani 2015). It is also known as phytotransformation and planted-assisted degradation. This technique involves the uptake of partially or completely degraded organic molecules into plant tissues, which further undergo enzymatic breakdown into simpler forms and can be utilized by plants for metabolic growth processes. Phytodegradation mainly depends on the action of plant root exudates, plant enzymes, and activity of rhizosphere microbes. This process converts highly toxic organic pollutants such as DDT, atrazine, PAHs, bisphenol A, organophosphorus compounds, ethylene dibromide (EDB), trichloroethylene (TCE), petroleum hydrocarbon, methyl benzotriazole (MBT), explosives (RDX, TNT), PAHs, PCBs, into simpler or non-toxic form (Castro et al. 2001). Canna plant which is known for antidiarrhea, hepatoprotective, and antimicrobial activity was reported to detoxify various xenobiotics by undergoing phytotransformation (Boonsaner et al. 2011). The phytodegradation of MBT with *Helianthus annuus* was achieved in aqueous solution with no signs of toxicity to plant (Castro et al. 2001). The tropical tree *Leucaena leucocephala* (coffee bush) which is used for its essential oil and medicinal property (Zayed and Samling 2016) was also reported effective for the removal of EDB and TCE through phytodegradation (Doty et al. 2003). Remediation of surface water and groundwater (if the water table is within the zone tapped by deep-rooted plants) via phytodegradation can be achieved in situ. The US Army Corps of Engineers have achieved the phytodegradation of explosive-contaminated groundwater (RDX and TNT) at Milan Army Ammunition Plant (Schnoor et al. 1995). The two most effective species for TNT uptake and phytotransformation reported to date are aromatic and medicinal plants vetiver grass (*Chrysopogon zizanioides*) and Eurasian water milfoil (*Myriophyllum spicatum*). For RDX phytodegradation from explosive-contaminated soils reed canary grass (*Phalaris arundinacea*) and fox sedge grass (*Carex vulpinoidea*) also has shown promising results (Kiiskila et al. 2015).

20.5.4 Phytovolatilization

It is the conversion of contaminants into a volatile form via metabolic conversion within plants and their release into the troposphere through stomata (Kang 2014; Tangahu et al. 2011). It is also known as phytoevaporation. It is both a transformation and transport process. This technique addresses soil, sediments, contaminated waste waters, and sludges and is used against pollutants such as chlorinated solvents like carbon tetrachloride, methylene chloride, trichloroethylene, tetrachloroethylene, 1,1,1 trichloroethane, carbontetrachloride, radioactive substances, As, Hg, and Se (Dushenkov 2003; Limmer and Burken 2016). In this technique, the conversion of inorganic and organic contaminants into volatile forms is due to the metabolic potentials of the plants. This process is extensively studied for the remediation of Se and Hg from organic and inorganic Se and Hg containing compounds (Dhillon and Banuelos 2017). For example, medicinal plant *Nicotiana tabacum* has the ability to absorb highly toxic methyl mercury from contaminated sites and convert it to the less toxic (elemental Hg) volatile form that escapes through the leaves to the atmosphere (Heaton et al. 2005). This potential of tobacco plant is due to Hg reductase genes. To exploit this potential, the attempts have been made in inserting Hg reductase genes in the genome of other plants to enable phytovolatilization (Dhillon and Banuelos 2017). Phytovolatilization of As has been carried out on medicinal pteridophyte *Pteris vittata*, where up to 90% of As was observed to volatize at laboratory scale (Sakakibara et al. 2010).

20.5.5 Rhizofiltration

It is also known as phytofiltration. This process involves the elimination of toxic substances or pollutants from the aquatic environment or groundwater in low concentration through absorption, concentration, and precipitation by the root of plants (Dushenkov et al. 1995). Once the plant has adsorbed or absorbed all the contaminants, they can be harvested and used in similar manner to phytoextraction (Dushenkov et al. 1995). Rhizofiltration is used for the treatment of the agricultural runoff, industrial discharge, metals, and radioactive contaminant (Verma et al. 2006). It work through the mechanism of rhizosphere accumulation mainly on Pb, Zn, Cd, Cu, Ni, radionuclides (Cs, Sr, U), and hydrophobic organics (US Environmental Protection Agency 2000). Rhizofiltration using aromatic and medicinal plant *Helianthus annuus* (sunflower) has been achieved in the remediation of radionuclides (Cs, U, and Sr) from surface water and ground water (Alsabbagh and Abuqudaira 2017). Phytoremediation efficiency of *Helianthus annuus* was tested in reducing uranium content from uranium-rich carbonate soil. It was observed that most of the uranium was accumulated in the roots through rhizofiltration, and only 3% of the uranium passed to the aboveground parts (Alsabbagh and Abuqudaira 2017). Moreover, the plants were much resistant to toxicity of uranium and biomass was not affected by increasing concentration of U in the soil. Both terrestrial and aquatic plants resistant to high concentrations of toxic metals and widespread fibrous root system which accumulate high concentrations of HMs are better for rhizofiltration (Raskin and Ensley 2000).

20.5.6 Phytostimulation

This technique relies on the symbiotic effect of plant and plant growth-promoting microbes in rhizosphere. Phytostimulation is plant-assisted stimulation of microbial and fungal degradation by release of exudates, organic compounds (inducers), co-metabolites, or enzymes into the rhizosphere (Zahoor et al. 2017). The stimulation of the microbial biodegradation and mineralization of contaminants in rhizosphere due to secretion of plant by-products, exudates, and enzymes is known as phytostimulation. It is used for remediation of both soil and water. It is reported that when species of *Brassica* are grown for phytoremediation purpose along with endophytic fungi, and arbuscular mycorrhizal fungi (*Mucor* sp.), isolated from HMs-contaminated site, plant showed reduction in HMs content by 94% in edible parts along with increased phytostimulation (Zahoor et al. 2017). Some other examples of aromatic (A) and medicinal plants (M) of different phytoremediation technologies are mentioned in Table 20.2.

20.6 Terrestrial Aromatic and Medicinal Plant Diversity for Phytoremediation

Numerous aromatic and medicinal trees, herbs, and grasses have the ability to remediate organic and inorganic pollutant when grown under natural conditions. Despite the grasses are more heavily represented in the literature (Table 20.3), the effectiveness of trees and grasses for rhizoremediation of contaminants have their own advantage. However, the greater depth and biomass of tree roots can show phytoremediation, up to great depth (Cook and Hesterberg 2013). Most of the hyperaccumulator plants act as phytomonitor and have ability to grow on metalliferous soils and to accumulate HMs without displaying phytotoxic effects (Rascio and Navari-Izzo 2011). Sage (*Salvia* sp. family – Lamiaceae) was found to be the most efficient hyperaccumulator crop among those tested in HM-polluted sites (Chandra et al. 2015b). Medicinal plant *Datura innoxia* (Solanaceae) is one of the Ni and Co hyperaccumulating plants in Africa which is also recommended as a phytomonitor (Bhattacharjee et al. 2004). Similarly, medicinally used *Senecio coronatus* (Asteraceae) is a HM-tolerant species and a hyperaccumulator of Ni (Przybylowics et al. 1995). Likewise, medicinal plants *Blepharis diversispina* (Acanthaceae), *Sagittaria montevidensis* (Alismataceae), and *Helichrysum candolleanum* (Asteraceae) are also able to accumulate high concentrations of HMs when grown in contaminated sites (Demarco et al. 2019; Nkoane et al. 2005). A medicinal plant *Canna indica* (Cannaceae) was tested on different amendments of industrial sludge, and it was reported that the accumulation of Fe, Cr, Cd, Pb, Cu, Ni, Zn, and Mn in different parts of *C. indica* increased with time and increasing doses of sludge amendments. Overall, *C. indica* was found suitable for phytoremediation of most of the HMs in industrial sludge (Bose et al. 2008). In Ghana, the levels of HMs were

Table 20.2 Examples of aromatic (A) and medicinal plants (M) involved in different phytoremediation technologies

Phytoremediation method	Plants used	A/M	Contaminants	References
Phytostabilizer	*Dactylis glomerata* L.	M	As, Cd, Pb	Visconti et al. (2020)
	Chrysopogon zizanioides (L.) Roberty and *Vetiveria zizanioides* (L.) Nash	A/M	Cd, Pb, Cu, As, Hg, Fe, Cr, Ni, Sn, Zn	Banerjee et al. (2016) and Suelee et al. (2017)
	Cymbopogon citratus (DC.) Stapf	A/M	Cd, Hg, Pb, Cu, Ni, Cr	Gautam et al. (2017)
	Cymbopogon martinii (Roxb.) Wats.	A	Cr, Cd, Pb, Ni, Fe, Zn, Cu	Pandey et al. (2015)
	Cymbopogon winterianus Jowitt ex Bor.	A/M	Cd, Cr	Sinha et al. (2013)
	Ocimum basilicum L.	A/M	Cr, Cd, Ni, Pb, As, Zn	Prasad et al. (2010)
	Salvia officinalis L.	A/M	Cd, Pb, Cu	Stancheva et al. (2014)
	Mentha × piperita L.	A/M	Pb, Ni, Cu, Cd	Zheljazkov et al. (2006)
	Rosmarinus officinalis L.	A	Pb, As, Sb, Zn, Cu	Madejon et al. (2009)
	Zygophyllum fabago L.	M	Cd, Pb, Zn Cu	Kabas et al. (2014)
Rhizofiltration	*Helianthus annuus* L.	A	Radionuclides (Cs, U and Sr) from surface water and ground water	Dushenkov (2003) and Alsabbagh and Abuqudaira (2017)
	Sagittaria montevidensis Cham. & Schltdl.	M	Al, V, Fe, As, Cu, Zn, Pb, Cd, Ni, Cr	Demarco et al. (2019)
	Eichhornia crassipes (Mart.) Solms	M	As, Cd Cr, Cu, Ni, Se	Zhu et al. (1999)

(continued)

Table 20.2 (continued)

Phytoremediation method	Plants used	A/M	Contaminants	References
Bio-monitor, as well as Phytoaccumulator	*Rosmarinus officinalis* L.	A	Ni. Hg, Pb, Cu, Zn, Cd, Ni, Fe, As, Sb	El-Rjoob et al. (2008)
	Pelargonium roseum (Andrews) DC.	A	Cd, Ni, Pb, Zn	Shahid et al. (2012)
	Matricaria recutita (L.) Rauschert	A	Cd, Pb, Zn	Stancheva et al. (2014)
	Portulaca oleracea L.	M	Cr	Kale et al. (2015)
	Chrysopogon zizanioides (L.) Roberty	A	Mn and Cu	Gautam et al. (2017)
	Helianthus annuus L.	A	Pb, Cd, Zn, Cu, Fe, and As	Chauhan and Mathur (2020)
	Cymbopogon martini (Roxb.) Wats.	A/M	Mn and Cu	Gautam et al. (2017)
Phytodegradation	*Helianthus annuus* L.	A	Methyl benzotriazole	Castro et al. (2003)
	Scirpus microcarpus J.Presl & C.Presl, *Phalaris arundinacea* L.	M	TNT, RDX	Schnoor et al. (1995)
	Leucaena leucocephala (Lam.) de Wit	M	Tetrachloroethane (TCE), EDB	Doty et al. (2003)
	Nicotiana tabacum L.	M	Perchlorate	Sundberg et al. (2003)
	Canna indica L.	A/M	Simazine	Knuteson et al. (2002)
	Arabidopsis thaliana (L.) Heynh.	M	TNT (2,4,6-trinitrotoluene)	Subramanian et al. (2006)
	Kandelia candel (L.) Druce	M	Pyrene	Ke et al. (2003a)
	Bruguiera gymnorrhiza (L.) Lam.	M	Pyrene	Ke et al. (2003b)
	Chrysopogon zizanioides (L.) Roberty, *Phalaris arundinacea* L.	A/M	RDX, TNT	Kiiskila et al. (2015)

(continued)

Table 20.2 (continued)

Phytoremediation method	Plants used	A/M	Contaminants	References
Phytostimulation	*Ceratophyllum deinersuin* L.	M	Atrazine	Rupassara et al. (2002)
	Vetiveria zizanioides (L.) Nash, *Bidens pilosa* L., *Eleusine indica* (L.) Gaertn.	A/M	Petroleum hydrocarbons	Kuo et al. (2014)
	Ludwigia octovalvis (Jacq.) Raven	M	As	Titaha et al. (2013)
Phytovolatilization	*Nicotiana tabacum* L.	M	Methyl mercury	Rahman et al. (2008)
	Pteris vittata L.	M	As	Sakakibara et al. (2010)

studied in 27 medicinal plant species collected from their natural habitat. Some species, especially *Ocimum canum* (Lamiaceae), *Clausena anisata* (Rutaceae), and *Rauwolfia vomitoria* (Apocynaceae) were reported to be cadmium (Cd) and iron (Fe) hyperaccumulator (Annan et al. 2010). Some medicinal plants such as *Cannabis sativa* (Cannabaceae) has the potential to yield high biomass which could be used for production of bioenergy and can be used as phytoremedial agent for removal of toxic metals from contaminated sites (Kumar et al. 2017a).

Regeneration of HM-polluted and HM-degraded sites are largely dependent on judicious selection of the plant adapted to harsh environmental conditions. The suitability of *Leucaena leucocephala* (Fabaceae) for phytoremediation of HMs-polluted and degraded sites was assessed. This species has fast growth and can produce large quantities of phytomass that can accumulate HMs through phytoextraction (Ssenku et al. 2017). *Leucaena leucocephala* produce a large amount of seeds that can germinate into seedlings to carry on further remediation of the polluted site. Vetiver (*Chrysopogon zizanioides* or *Vetiveria zizanioides*; family – Poaceae) is tolerant to wide ranges of pollutants and can be used for restoration of HMs contaminated sites as it stabilizes Cd, Pb, As, Cu, V, and Zn (Singh et al. 2017; Ng et al. 2017). *Vetiveria zizanioides* is an excellent candidate for remediating fly ash genotoxicity and reclamation of fly ash dump sites (Ghosh et al. 2015). It is commonly used in old landfills lead mine zone for HMs removal and land reclamation. It is also used for the phytoextraction of Cd in aboveground parts and phytostabilization of Pb in roots (Xia 2004). Pb accumulation in roots also enhances the oil content of vetiver (Rotkittikhun et al. 2010). Vetiver grass, *Vetiveria zizanioides*, removes about 77–78% of Cr and around 80–94% Pb from the synthetic wastewater samples. Different species of genus *Cymbopogon* such as *Cymbopogon citratus*, *Cymbopogon flexuosus, Cymbopogon winterianus* (citronella), and *Cymbopogon martini* have a great potential for phytoremediation (Handique and Handique 2009). *Cymbopogon citratus* is an effective adsorbent plant for the treatment of wastewater and Cu mine and acts as a potential accumulator of Cd, Al, Zn, Pb, As, Cr, and Ni and efficient phytostabi-

Table 20.3 Terrestrial aromatic and medicinal plant species used for phytoremediation

Plant species	Family	Contaminant/substrate	Accumulated contaminant	References
Catharanthus roseus (L.) G.Don	Apocynaceae	Chromium, sludges derived from tanneries	Chromium	Ahmad and Misra (2014)
Trifolium repens L.	Fabaceae	Pentachlorophenol (PCP)-contaminated soil	PCP	Hechmi et al. (2014)
Cymbopogon citratus (DC.) Stapf	Poaceae	Soil amended with biowastes	Fe, Mn, Cu, Al, Zn, Cd, Pb, Cr, As, and Ni	Gautam et al. (2017)
		Polluted sites	Cd, As, Ni, Cu, Fe	Jisha et al. (2017)
Vetiveria zizanioides (L.) Nash.	Poaceae	Amended soil	Cd and Pb	Ng et al. (2017)
		Petroleum hydrocarbon-contaminated soils	Petroleum hydrocarbon	Brandt et al. (2006)
		Pb/Zn and Cu mine tailings amended with manure compost and sewage sludge	Pb and Zn	Chiu et al. (2006)
		TNT in hydroponic media	TNT	Das et al. (2010)
		Tannery-wastewater-contaminated soil	Cr	Sinha et al. (2013)
		Soils contaminated with fly ash from thermal power plants	Toxic metals from the fly ash	Ghosh et al. (2015)
		Metal-contaminated water	Cu, Fe, Mn, Pb, Zn	Suelee et al. (2017)
		Sb- and As-amended soil	Sb, As	Mirza et al. (2017)
		EDTA-amended soil	Cd, Pb, Cu, and Zn	Ng et al. (2019)
		CaO-activated Si-based slag amended soil	Cd, Cr, Cu, Pb, and Zn	Mu et al. (2019)
		Metal smelter factory	Multi-metals	Siyar et al. (2020)
Leucaena leucocephala (Lam.) de Wit	Fabaceae	Soil and groundwater contaminants	Ethylene dibromide and trichloroethylene	Doty et al. (2003)
Ocimum basilicum L.	Lamiaceae	Cd and nanoparticles amended soil	Cd, Cd-ionic, and nanoparticles	Alamo-Nole and Su (2017)
		Cd-contaminated calcareous soil	Cd	Zahedifara et al. (2016)

(continued)

Table 20.3 (continued)

Plant species	Family	Contaminant/substrate	Accumulated contaminant	References
Ocimum gratissimum L.	Lamiaceae	Polluted agricultural soil	Cd	Prapagdee and Khonsue (2015)
Ricinus communis L.	Euphorbiaceae	DDTs and cadmium co-contaminated soil	DDT, Cd	Huang et al. (2011)
Euphorbia hirta L.	Euphorbiaceae	Radioactive waste	Radioactive substances	Hu et al. (2014)
Aloe barbadensis Mill.	Asphodelaceae	Cr contaminated agricultural land	Cr	Sharma and Adholeya (2011)
Hypericum perforatum L.	Hypericaceae	Cadmium-contaminated agricultural land	Cd	Chizzola and Lukas (2006)
Senecio coronatus (Thunb.) Harv.	Asteraceae	Contaminated soil	Ni	Przybylowics et al. (1995)
Viola boashanensis Shu	Violaceae	Leaching lead/zinc mine	Pb, Zn, Cd	Zhuang et al. (2005)
Brassica juncea (L.) Czern.	Brassicaceae	HMs	Se, Zn, Cu, Pb	Prasad (2003)
Bacopa monnieri (L.) Pennell	Plantaginaceae	Sewerage wastes, chlorosodical industrial wastes	Hg, Cd	Hussain et al. (2011)
Artemisia vulgaris L.	Asteraceae	Sludges, compost, waste paper and from retteries	Zn, Cu, Pb, Cd, Ni	Porębska and Ostrowska (1999)
Cannabis sativa L.	Cannabaceae	Heavy metals	Pb, Cu, Zn, Cd, Ni	Girdhar et al. (2014)
Hibiscus cannabinus L.	Malvaceae	Dredging sludge	Cadmium and zinc	Arbaoui et al. (2014)
Mentha arvensis L.	Lamiaceae	Land irrigated with sewage water	Pb Cu	Anwar et al. (2016)
		Agricultural land	Cu, Zn	Malinowska and Jankowski (2017)
Urtica dioica L.	Urticaceae	Chromium	Cr	Shams et al. (2010)
		PCB-contaminated soil and mining ore	Pb, Zn, As and chlorinated biphenyls	Viktorova et al. 2016
Astragalus racemosus Pursh	Fabaceae	Naturally seleniferous soil	Se	Lindblom et al. (2013)

(continued)

Table 20.3 (continued)

Plant species	Family	Contaminant/substrate	Accumulated contaminant	References
Lobelia chinensis Lour.	Campanulaceae	Contaminated sites, mine-tailing	Cd	Peng et al. (2006, 2009)
Solanum nigrum L.	Solanaceae		Cd, Pb, Zn, Cu	Peng et al. (2006, 2009)
Imperata cylindrica (L.) P.Beauv, *Cynodon dactylon (L.) Pers.*	Poaceae	HMS contaminated sites, mine-tailing	Cd, Pb, Zn, Cu	Peng et al. (2006)
Phytolacca americana L.	Phytolaccaceae			
Xanthium strumarium L.	Asteraceae			
Polygonum hydropiper L.	Polygonaceae			
Pelargonium hortorum L.H.Bailey	Geraniaceae	Heavy metal contaminated soils	Multi-metals	Orroño and Lavado (2009)
		Soil spiked with heavy metal	Cd, Cr, Cu, Pb, Ni, Zn	Orroño et al. (2012)
Pelargonium roseum (Andrews) DC.	Geraniaceae	Contaminated field	Multi-metals	Shahid et al. (2012)
Pelargonium graveolens L'Herit.	Geraniaceae	In heavy metal polluted soil	Multi-metals	Chand et al. (2016)
Lavandula officinalis L.	Lamiaceae	Soils contaminated with heavy metals	Pb, Zn, Cd	Angelova et al. (2015)
Acorus calamus L.	Acoraceae	Cd- and PAH-treated soil	Cd-PAHs	Jeelani et al. (2017)
Rosmarinus officinalis L.	Lamiaceae	Abandoned gold ore processing site	Multi metals	Boechat et al. (2016)

lizer of Mn, Fe, and Cu (Das and Maiti 2009; Gautam et al. 2017). Wastewater irrigations produced more lemongrass herb on contamination in the field, and essential oil content also increases with increase in contamination in plant. *Cymbopogon flexuosus*, *Cymbopogon winterianus*, and *Cymbopogon martini* can act as a potential phytostabilizer of Cd, Cr, and As and can be adopted as a potential candidate for phytostabilization of HMs (Pandey et al. 2015; Sinha et al. 2013). *Cymbopogon martini* is also responsible for phytoextraction of Mn and Cu and phytostabilizes Fe, Zn, Ni, Pb, Cd, and Cr in roots (Gautam et al. 2017).

Geranium (*Pelargonium* sp., family – Geraniaceae) is also a multi-metal hyperaccumulator which makes it suitable for phytoremediation of contaminated sites (Orroño et al. 2012; Shahid et al. 2012). Different species of genus *Pelargonium* such as *Pelargonium hortorum, Pelargonium roseum*, and *Pelargonium graveolens* are most resistant to HMs toxicity and are used for phytoremediation in contaminated soil (Arshad et al. 2008; Chand et al. 2016; Patel and Patra 2015; Shahid et al. 2012). Similarly, *Miscanthus* sp. a hybrid, perennial, medicinal, and bio-energy crop developed in South Korea has a potential to absorb As, Cu, Pb, Ni, Cd, and Zn from contaminated soil. The results of the BCF, TF, and enrichment coefficient tests indicate that *Miscanthus* sp. is an As hyperaccumulator and can be used for phytoremediation of As contaminated ecosystem more efficiently (Bang et al. 2015). Adewole et al. (2010) investigated the phytoremediation ability of two aromatic plant species *Helianthus annuus* and *Tithonia diversifolia* on soil contaminated with effluents from the paint industry. It was observed that both aromatic plants were efficient plant for the removal of Cu, Pb, and Cd from soil contaminated with effluents from the paint industry without affecting the essential oil content.

Some aromatic and medicinal plants grown for phytoremediation cannot be used for consumption when intention is remediation of contaminants in order to avoid food chain contamination. Mint (*Mentha* sp.; family – Lamiaceae) is mostly phytostabilizer in HM-contaminated sites and can be used for consumption, because most of the metals are accumulated in the roots with less translocation to shoot (Anwar et al. 2016; Malinowska and Jankowski 2017). However, use of silicate fertilizer also eliminates the Cd transport from roots to shoots which reduce the HMs concentration in the edible parts of mint plants (Putwattana et al. 2010). The HM treatment brings about the increased level of linalool and essential oil eugenol and increases the commercial potential of this plant (Kunwar et al. 2015). Peppermints (*M. piperita*) can phytostabilize Cd, Cu, Ni, and Pb and the essential oil amount was not influenced by these metals (Zheljazkov et al. 2006). In the genus *Mentha*, *M. piperita* is most suitable species for phytoremediation, followed by *M. arvensis* and *M. citrate. M. spicata* can be grown in tannery waste sludge at 75:25 ratios of sludge and soil for better oil yield (Patel et al. 2016). Similar to mint, basil (*Ocimum* sp., family – Lamiaceae) also acts as a potential phytostabilizer and phytoextractor for many HMs. *Ocimum basilicum* is used for the phytoextraction of Pb, Cd, and Zn (Putwattana et al. 2010; Stancheva et al. 2014) and phytostabilization of Cr-, Cd-, Pb-, and Ni-contaminated soils (Alamo-Nole and Su 2017; Prasad et al. 2010).

Remediation with aromatic and medicinal plant is a promising solution for even co-contaminated sites with organic and inorganic pollutant. For example, the medicinal plant *Acorus calamus* was grown in different concentration of PAHs (phenanthrene and pyrene) and Cd. After 60 days of growth, no visible symptoms of contaminant toxicity were observed. It was observed that plant biomass and concentration of both contaminants was significantly higher in combined treatments as compared to all other treatments (Jeelani et al. 2017), suggesting that a certain concentration of PAHs can increase root and shoot biomass and phytoremediation ability. Similarly, for medicinal plant *Juncus subsecundus* (Juncaceae), the interactive effect of both Cd and PAHs on plant biomass was higher for the combined treatment (Cd-PAH) as compared to Cd treatment alone (Zhang et al. 2012). Similar results were observed for *Tagetes patula* (Asteraceae) and *Solanum nigrum* (Solanaceae) when grown in combined treatment of B[a]P-Cd-contaminated soil (Sun et al. 2011;

yang et al. 2011). Use of these plants will be a good choice in the long run for remediation of co-contaminated soil.

Chamomile (*Matricaria recutita*, *Matricaria chamomilla asteraceae*) mainly acts as a metal excluder or phytostabilizer not hyperaccumulator. *Matricaria recutita* phytostabilizes Cd, Ni, and Zn to a great extent (Stancheva et al. 2014), while *Matricaria chamomilla* acts as potential phytostabilizer for Cd, Cr and Cu (Kovacik et al. 2006, 2009; Kovacik and Backor 2007) and as a Pb excluder (Farzadfar et al. 2013). Aromatic and medicinal plant rosemary (*Rosmarinus officinalis*, Lamiaceae) can also act as a potential hyperaccumulator, phytostabilizer, as well as bio-monitor and show good tolerance when grown in HM-contaminated soil (Parra et al. 2014). It is documented that in rosemary, different parts of plant are specific for accumulation of different metals (Abdul-Wahab et al. 2008). Lavender (*Lavandula* sp. family – Lamiaceae) is a potential hyperaccumulator of Cd, Pb, and Zn (Angelova et al. 2015). Zheljazkov and Astatkie (2011) reported that lavender is a hyperaccumulator of Cd and Pb with maximum accumulation in leaves and stem, respectively, and essential oil was not contaminated by HMs.

Sulfonated anthraquinones are recalcitrant pollutant from textile and industrial effluents. It is precursors of many synthetic pigments and dyes. Two medicinal plants *Rheum rabarbarum* (rhubarb) and *Rumex acetosa* (common sorrel) are the most efficient plants for the phytoremediation of sulfonated aromatic compounds (Page and Schwitzguébel 2009). Similarly, medicinal plant canna (*Canna*×*generalis*) could accumulate BTEX (benzene, toluene, ethylbenzene, and xylenes) from rhizome zone and root zone and show significant translocation of these compounds to aboveground parts (Boonsaner et al. 2011). The canna could remove about 80% of BTEX in the root zone and rhizome zone soil in 21 days, and the sequences of accumulation in the shoot, root, and rhizome strongly depend upon physicochemical properties of contaminant and soil. Phytoremediation of petroleum contaminants depends on interactions among microbes, plants, and soils. Trees and grasses are commonly used for rhizoremediation, with grasses typically being used for remediation of PAHs and total petroleum hydrocarbons, while trees are more commonly chosen for remediation of BTEX (Cook and Hesterberg 2013). Three promising phytoextracting perennial medicinal plant species *Chrysanthemum leucanthemum* L. (ox-eye daisy), *Solidago canadensis* L. (Canada goldenrod), and *Rumex crispus* L. (curly dock) were analyzed at two polychlorinated biphenyl (PCB)-contaminated sites in southern Ontario (Ficko et al. 2011). It was reported that, these medicinal plant species, are efficient PCB accumulator and total plant biomass and shoot PCB concentrations are dependent on life cycle (vegetative and reproductive stages) and plant age. *Betula pendula* (Silver birch) trees have the significant potential to phytoscreen and possibly phytoremediate trichloroethylene (TCE) comparable to other effective tree species and related compounds, but not efficient to phytoextract HMs when compared with other hyperaccumulator plants (Lewis et al. 2015). In a more recent study, a number of agricultural, ornamental, aromatic, and medicinal plants were investigated for their ability to remediate explosive-contaminated soils, and Vetiver grass was reported to be better candidate for the phytoremediation of explosives (Kiiskila et al. 2015). In order to select appropriate plant species for phytoremediation of explosive compounds, uptake proficiency, phytotoxicity, ability of the plant to degrade and/or transform the compounds, and environmental factors are important. The environmental factors comprise soil type, climatic attributes, the water environment, bioavailability, and root penetration depth. While researchers are working on the identification of potential plants for phytoremediation of explosives for years, very few field studies have been carried out so far.

20.7 Aquatic Aromatic and Medicinal Plant Diversity for Phytoremediation

Phytoremediation is also a resourceful cleanup technique of constructed wetlands, municipal wastewater, spillage areas, sewage water, and polluted aquatic ecosystem. This technique has been used successfully to detoxify large volumes of wastewater with dilute concentrations of contaminants, including petroleum, hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals, and radionucleids (Sasmaz et al. 2008, 2016; Tangahu et al. 2011). Some aquatic plants are well adapted to contaminated site and always develop an extensive hairy root system which makes them the best option for the uptake of contaminants in their roots and shoots. A wide array of free-floating, submerged, and emergent aquatic plants has displayed tremendous ability for the phytoremediation of numerous kinds of wastewater. Medicinal free-floating aquatic plants (the plants with floating leaves and submerged roots) such as water hyacinth (*Eichhornia crassipes*), water ferns (*Salvinia minima*), duck weeds (*Lemna minor*), water lettuce (*Pistia stratiotes*), and water cress (*Nasturtium officinale*) have potential for the elimination of HMs from contaminated water (Abbas et al. 2019; Chen et al. 2018; Maine et al. 2001; Tabinda et al. 2020), because they (1) can tolerate heavily polluted environment, (2) have high absorption rate of different contaminants, and (3) have a high rate of biomass production. Phytoremediation employing aquatic macrophytes showed promising results in the successful remediation of HMs originated from industrial and domestic wastewater streams, sewage and mining effluents and landfill leachate. Aquatic plants have the ability to withstand the landfill leachate pollution without showing any significant cutback in growth rate and biomass. Medicinal aquatic plants such as *Eichhornia crassipes*, *Gynerium sagittatum*, and *Heliconia psittacorum* have shown great phytoremediation potential for the remediation of landfill leachate (Madera-Parra et al. 2015). *Eichhornia crassipes* (Pontedericeae) possesses greater ability to remediate contaminants like Cd, Zn, As, Fe, Cu, Hg, Cr, Pb, and Ni from domestic wastewater streams, sewage, and mining effluents (El-Gendy et al. 2006; Goswami and Das 2018; Tabinda et al. 2020). *Pistia stratiotes* and *Lemna minor* also reported to show high removal efficiencies for various HMs form the landfill leachate generated from the landfill site (Maine et al. 2016). Non-living plant biomass of aquatic plants which act as a simple biosorbent substance to remove the metals from water has gained popularity in recent years, because it is a cost-effective and natural approach (Han et al. 2018; Yadav et al. 2018). For example, dead biomass of different aquatic aromatic and medicinal plant species such as *Salvinia herzogii* and *Eichhornia crassipes* was successfully used as biosorbent material for the removal of Pb, Cr, Zn, Cd, Cu, and Ni, effectively (Goswami and Das 2018; Priya and Selvan 2017).

Printing and dyeing process in textile industry produces both organic and inorganic contaminants, which are more toxic and dangerous for public health. Among different aquatic plants, *Eichhornia crassipes* is a bioindicator of water polluted by organic pollutants, di-*n*-hexylphthalate, pentabromodiphenyl ether, nitenpyram, acetamiprid, and bis(3-*tert*-butyl-4-hydroxy-6-methylphenyl) sulfide (Laet et al. 2019) and reported to be better contender for the eradication of Cd, Cu, Cr, and Zn from textile wastewater (Priya and Selvan 2017). Submerged aquatic medicinal macrophytes like *Ceratophyllum demersum*, *Hydrilla verticillata*, and *Myriophyllum spicatum* are used for the phytoremediation of mine effluents as they have great ability to accumulate HMs in their whole-body biomass (Pat-Espadas et al. 2018). Water fern (*Salvinia auriculata*), a small free-floating macrophyte, is used as a bioindicator of pollution in water body and for phytoremediation of Cd, Fe, Ni, Mn, Pb, and Zn as well from wastewater and constructed wetland (Gardner and Al-Hamdani 1997).

Pistia stratiotes has the capacity of reducing chemical oxygen demand (COD), biological oxygen demand (BOD), dissolved oxygen (DO), pH, ammonia (NH3), nitrite (NO2−), nitrate (NO3−), As, Cu, Zn, Fe, Cr, Cd, and Hg from surface water, drinking water, sewage water, and industrial wastewater (Ansari et al. 2020; Valadi et al. 2019). Uptake of these contaminants have no any harmful effect on the plant growth, development, and biomass which makes *Pistia stratiotes* eligible to be used as a hyperaccumulators plant for the remediation of toxic metals and organic contaminants. Free-floating medicinal herbs species *Lemna minor*, *L. gibba*, and *Spirodela polyrhiza* are also the most suitable plants for phytoremediation of different HMs (Cr, As, Cu, Hg, Zn, Al, Ag, Cd and Pb), organic contaminants, and pesticides from industrial and agricultural wastewater and has potential to remediate the water-contaminated with textile effluents and toluidine blue dyes (Chen et al. 2018; Ekperusi et al. 2019; Kara 2004; Neag et al. 2018; Rahman et al. 2007). Some of the most common submerged aquatic plants such as *Ceratophyllum demersum*, *Potamogeton pectinatus*, *Mentha aquatica*, and *Vallisneria spiralis* are well known for their ability to accumulate Zn, Cr, Fe, Cu, Cd, Ni, Hg, and Pb from contaminated water (Ansari et al. 2020). The use of different aquatic plants to mitigate different contaminants is mentioned in Table 20.4.

Emergent aquatic plants concentrate most of the HMs in roots from water and sediments. For example, some common medicinal plants such as common reed (*Phragmites australis*), cat tail (*Typha latifolia*), and smartweed (*Polygonum amphibium*) are the best emergent aquatic medicinal plant species having a high tolerance and removal efficiency for Cd, Zn, Pb Mn, Cr, Ni, and Fe released from industrial and municipal wastewater (Maine et al. 2017; Sasmaz et al. 2008). Similarly, another common medicinal aquatic emergent macrophytes such as *Hydrilla verticillata*, *Ipomea aquatica Phragmites australis*, and *Marsilea quadri-*

Table 20.4 Aquatic aromatic and medicinal plants used for phytoremediation

Aquatic plant	Family	Contaminant/ substrate	Contaminant removed	References
Eichhornia crassipes (Mart.) Solms	Pontedericeae	Contaminated artificial reservoirs	Hg	Molisani et al. (2006)
		Water polluted by organic pollutants	di-*n*-hexylphthalate, pentabromodiphenyl ether, nitenpyram, acetamiprid and bis (3-*tert*-butyl-4-hydroxy-6-methylphenyl) sulfide	Laet et al. (2019)
		Cu-amended solution	Cu	Tabinda et al. (2020)
		Wastewater from industries	Zn, Cr, Cu, Cd, Pb, Ag, and Ni	Odjegba and Fasidi (2007)
		Aquarium contaminated with different concentrations of toxic wastewaters	ammonium, $PO4^{(3-)}$, COD, and BOD	Victor et al. (2016)
Pistia stratiotes L.	Araceae	Heavy metal amended solution	Cr, Zn, Fe, Mn, Cu	Maine et al. (2004)
		Cu and Cr amended solution	Cu and Cr	Tabinda et al. (2020)
Salvinia minima Baker	Salviniaceae	Heavy metal amended solution	Pb, Cr, Cd	Olguín et al. (2002)
		Heavy metal amended solution	Cd Pb, Zn, and Cu	Basile et al. (2012)
Lemna gibba L.	Araceae	Secondary effluents	As, U, B	Sasmaz and Obek (2009)
		Water of chromium mining areas	Cr, Ni, Co	Sasmaz et al. (2016)
Spirodela intermedia W.Koch	Araceae	Heavy metal-amended solution	Fe, Zn, Mn, Cu, Cr, Pb	Miretzky et al. (2004)

(continued)

Table 20.4 (continued)

Aquatic plant	Family	Contaminant/ substrate	Contaminant removed	References
Nasturtium officinale Aiton	Brassicaceae	Contaminated wastewater	Cr	Zurayk et al. (2001)
		Polluted wetlands	Ni	Duman and Ozturk (2010)
Potamogeton nodosus Poir.	Potamogetonaceae	Explosive-contaminated groundwater	TNT	Best et al. (1997)
Ceratophyllum demersum L.	Ceratophyllaceae	Explosive-contaminated groundwater	TNT	Best et al. (1997)
Typha angustifolia L.	Typhaceae	Water and sediment of the lake	Fe, Mn, Cu, and Pb	Branković et al. (2012)
Thelypteris palustris Schott	Thelypteridaceae	Wastewater	Zn, Mn, Ni, Fe, Pb, Cu	Hejna et al. (2020)
Mentha aquatica L.	Lamiaceae	Water and sediment of the polluted lake	Fe, Mn, Cu, and Pb	Branković et al. (2012)
Lycopus europaeus L.				
Bidens tripartita L.	Asteraceae	Polluted lake	Fe, Mn, Cu, and Pb	Branković et al. (2012)
Polygonum amphibium L.	Polygonaceae	Water and sediment of the lake	Fe, Mn, Cu, and Pb	Branković et al. (2012)

folia showed much better accumulation potential and translocation factor (TF) value for Fe, Cr, Zn, Pb, As, Hg, Cd, and Cu from the industrial effluents and municipal wastewater as compared to the hyperaccumulator terrestrial plants (Ansari et al. 2020).

20.8 Physiological Mechanism of Metal Resistance, Uptake, and Transport in Aromatic and Medicinal Plant

Physiological mechanism of metal resistance, uptake, and transport in aromatic and medicinal plants is similar to other plants. It utilizes the biological mechanisms within plants to eradicate hazardous pollutants such as HMs and organic pollutants (Ayangbenro and Babalola 2017). HMs at toxic levels acts as an obstacle to metabolic processes by producing ROS responsible for the physiological damage. Plants have developed various protective mechanisms to reduce or eliminate ROS. The oxidative stress produced by ROS is removed by active antioxidant systems which occur naturally in plants. These ROS are detoxified by a number of enzymes including glutathione-S-transferase (GST), glutathione reductase (GR), peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT). Phytoremediation potential of medicinal plant *Catharanthus roseus* was tested in the sludge collected from Banthar Industrial Pollution Control Company (BIPCC), UP, India. *C. roseus* was reported to absorb up to about 38% of the amount of Cr present in sludge-amended soil through roots and accumulate about 22% in leaves, and antioxidant enzyme peroxidase (POD) and detoxification enzyme glutathione-S-transferase (GST) from leaves were also observed at higher concentration as compared to control (Ahmad and Misra 2014).

Tolerance to metals depends on multiple mechanisms which include cell wall binding, active transport of ions into the vacuole, and formation of complexes with peptides or organic acids. The most important mechanism for metal detoxification is chelation of metals by low-molecular-weight metallothioneins proteins and the phytochelatins peptide ligands (Memon and Schröder 2009). Metal stress in plants also induces production of metallothioneins and enzyme phytochelatin synthase (Shukla et al. 2012; Thakur et al. 2016). These metallo-proteins have higher affinity for a wide range of metals such as Pb, Zn, Cu, Hg, As, Cd, etc. by binding via thiol group (–SH) of cysteine residues and thus are involved in the cell homeostasis and detoxification of HMs (Pirzadah et al. 2018). Similarly, phytochelatins synthase produces low-molecular-weight short-chain thiol-rich peptides phytochelatin from sulfur-rich glutathione which act as defensive mechanism of plants against various abiotic stresses including HMs. PCs are used as biomarkers in plants for the early detection of HMs stress (Wu et al. 2010). These metallo-proteins also act against oxidative stress by removing free radicals. However, the interaction between metal binding and ROS scavenging is not clear yet.

Vacuoles in plant cells are considered as the ideal and preferred sites of metal sequestration (Wu et al. 2010). Plants take toxic metals from water or soil via roots and bind them either in cell wall or translocate to the shoots. In the shoot toxic metals are actively transported to vacuoles to prevent their interactions with other metabolic processes in the cytosol (Ali et al. 2013; Chandra et al. 2015a). In plants, there are several gene families that encode many types of transporters engaged in transport of metal ions through plasma membrane and tonoplast of vacuoles (Schmidt et al. 2007; Koźmińska et al. 2018). Membrane transporter proteins (MTPs) such as ATPases, zinc-iron permease (ZIP), pleiotropic drug resistance (PDR) transporter, cation diffusion facilitators (CDF), natural resistance-associated macrophage protein (NRAMP) transporter, copper transporters (COPTs), multidrug resistance-associated proteins (MRP) transporters, ATP-binding cassettes (ABC), and cation exchangers (CAXs) and heavy metal ATPase (HMA) such as P1B-ATPase, HMA4, and HMA5 help in the uptake and transport of metals across the cell membrane and vacuole and facilitate their detoxification (Sarwar et al. 2017; Koźmińska et al. 2018). The metal transporter NRAMP is responsible to transport a wide variety of metal ions viz., Fe^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+}, and Co^{2+} across membranes (Nevo and Nelson 2006). In plants, vacuoles are considered as main site for accumulation of phytochelatins-HMs complexes. PDR, MRP and HMT1transporters are involved in detoxification of chelated HMs complexes inside the vacuole in an Mg-ATP-dependent manner (Lee et al. 2005, 2014; Yazaki 2006). ABC transporter family, a large group of genes encoding proteins localized in the tonoplast, are also responsible for detoxification and sequestration of metals in vacuolar lumen (Yazaki 2006; Koźmińska et al. 2018). These examples suggest that physiological mechanism of phytoremediation of HMs in plants is very complex, and further comprehensive study is required to understand the cross-talk between HMs and plant response.

20.9 Phytoremediation Mechanisms of Aromatic and Medicinal Plants for Organic Pollutant-Contaminated Soil

It has been speculated that aromatic and medicinal plants also use remediation pathway for the transformation of organic contaminants similar to other plants. To avoid the toxic effects of the organic contaminants, the simplest way to avoid these contaminants is in the form of excretion. Similar to HMs, organic contaminants are taken up by aromatic and medicinal plants and are than translocated from the roots to other plant tissues. In the shoot they are either volatilized or stored unchanged or degraded partially or completely (Gao et al. 2014; Xiao et al. 2015). However, there are little evidences that organic contaminants are accumulated considerably in plants tissues. The primary mechanism for the remediation of organic pollutant is rhizospheric microbial degradation, where soil microbes use organic compounds as carbon substrates for growth. Tejeda-Agredano et al. (2013) investigated the influence of the rhizosphere on the ability of soil microbiota to reduce PAH levels, and it was observed that the removal rate of total PAHs increased by 16% within 90 days with the help of cultivating *Helianthus annuus* as compared to contaminated soil without plants. Root exudates can be used as carbon sources or electron donors or to support metabolism of microbes in the rhizosphere. The soluble fraction of root exudates is composed of low-molecular-weight compounds such as amino acids, carbohydrates, organic acid anions, and various secondary metabolites which play important role in phytoremediation (Walker et al. 2003). Amino acids, such as glutamine and isoleucine, are powerful chemoattractants for bacterial species. Protein amino acid ornithine plays an important protective role against stress for cell membranes. Enzyme secreted by exudates such as dehydrogenase, oxidase, POD, CAT, and urease in rhizosphere soil was strongly correlated with a higher degradation performance of plant growing in organic pollutant contaminated soil (Liu et al. 2015a). Practically, single approach is never sufficient for effective cleanup contaminants from soil. The integrated approaches, such as microbe-assisted phytoremediation and use of genetic engineering, is essential for effective phytoremediation using aromatic and medicinal plants and their rhizospheric microorganisms in the future.

Table 20.5 Example of aromatic and medicinal plant and associated microorganisms used for phytoremediation

Plant used for phytoremediation in association with microbes	Family	Microorganism used	Pollutant remediated	References
Brassica juncea (L.) Czern,	Brassicaceae	*Burkholderia phytofirmans* PsJN[T]	Mine and smelter area	Konkolewska et al. (2020)
Medicago sativa L.	Fabaceae			
Cymbopogon citratus (DC.) Stapf	Poaceae	*Bacillus Cereus*	Cr (VI), Fe, Mn, Cu, Cd, Ni and Zn	Nayak et al. (2019)
Solanum torvum Sw.	Solanaceae			
Solanum nigrum L.	Solanaceae	*Enterobacter* sp., *Kocuria* sp., and *Kosakonia* sp.	As	Mukherjee et al. (2018)
Lolium multiflorum Lam.	Poaceae	Bacteria (*Acinetobacter* sp.), AM fungi (*Glomus mosseae*)	PAHs	Wu et al. (2014)
Lolium perenne L.	Poaceae	*Glomus caledonium* and *Glomus mosseae*	Cd	Hu et al. (2013)
		Endophyte *Cadophora* sp. and AM (*Funneliformis mosseae*)	Cd/Zn/ Pb-polluted soil.	Berthelot et al. (2018)
Polygonum pubescens Blume	Polygonaceae	*Enterobacter* sp. and *Klebsiella* sp.	Cd	Jing et al. (2014)
Sesbania cannabina (Retz.) Poir.	Fabaceae	Rhizobia (*Ensifer* sp.) AMF (*Glomus mosseae*)	PAHs	Ren et al. (2017)
Costus scaber Ruiz & Pav.	Costaceae	*Rhizophagus*, *Glomus*, *Acaulospora*, and *Archaeospora*	Crude oil	Garcés-Ruiz et al. (2017)
Sedum Alfredii Hance	Crassulaceae	*Glomus caledonium, Glomus mosseae*	Cd	Hu et al. (2013)
Sedum plumbizincicola X.H.Guo and S.B.Zhou ex L.H.Wu	Crassulaceae	*Phyllobacterium myrsinacearum*	HMs	Ma et al. (2013)
		Endophyte *Bacillus pumilus*	Cd, Zn	Ma et al. (2015a)
Robinia pseudoacacia L.	Fabaceae	*Funneliformis mosseae*	Pb	Huang et al. (2019)
Helianthus annuus L.	Asteraceae	*Ralstonia eutropha* and *Chryseobacterium humi*	Zn Cd	Marques et al. (2013)
Alnus firma Siebold & Zucc	Betulaceae	*Bacillus thuringiensis*	As	Babu et al. (2013)

20.10 Plant-Microbe Interactions for Enhanced Phytoremediation

Plants and microbes coexist in adapting to metalliferous environments and can thus be used to improve microbe-assisted phytoremediation (Table 20.5). Plant root exudates are useful energy and nutrient sources for rhizosphere microorganisms, with which they establish communication systems with plants (Ma et al. 2011a, 2011b). The main factors which limit the phytoextraction and phytostabilization rate are biomass production and metal accumulation in plant tissue. Applications of both rhizospheric and endophytic bacteria and fungi in soils/plants improve plant growth and significantly increase the phytoremediation potential of plants by enhancing metal bioavailability in soil through various mechanisms such as acidification, complexation, precipitation, redox reactions, and chelation (Konkolewska et al. 2020; Ma et al. 2016). Inoculating phytoremediation plant with growth-promoting rhizobacteria is more environmentally friendly than adding chelators and fertilizers. Cultured microbes are also added to the soil to enhance the phytoremediation, and this process is known as bioaugmentation. The combination phytoremediation and bioaugmentation results in better remediation of pollutant and is among the most common and efficient biological methods of treating HMs pollution in soil (Konkolewska et al. 2020).

The root-colonizing, plant growth-promoting rhizobacteria (PGPR) have been reported to enhance host plant growth and assist phytoremediation of contaminated sites (Ma et al. 2009, 2011b, 2015b; Yuan et al. 2013). Major drawback of phytoremediation, i.e., biomass production and metal accumulation, is enhanced using PGPR in contaminated sites. *Brassica juncea* and *Ricinus communis* inoculation with *Psychrobacter* sp. SRS8 and *Pseudomonas* sp. A3R3 from serpentine soil revealed a significant increase on plant biomass as well as translocation and accumulation of Ni, Zn, and Fe grown on metal-contaminated serpentine soil (Ma et al. 2015b). The effects of PGPR strains on the growth and phytoremediation potential of *Helianthus annuus* grown in Zn-Cd-contaminated soil were assessed, and it was found that inoculation with PGPR strains is an effective way to enhance the biomass and phytoremediation potential of this plant (Marques et al. 2013). This significant increase in biomass and hyperaccumulation is attributed to production of metabolites by bacteria that stimulate plant growth and mobilize metals. PGPR influence plants growth by producing plant hormones (auxins, gibberellins, cytokinins) and siderophore secretion (Jing et al. 2014; Konkolewska et al. 2020). PGPR siderophores are able to bind HMs other than iron and thus enhance their bioavailability to plants. Other strains of PGPR such as *Pseudomonas aeruginosa, P. putida, Enterobacter cloacae, Serratia liquefaciens, Bacillus thuringiensis, Burkholderia phytofirmans*, and *Azospirillum brasilense* can be used to increase the biomass and phytoremediation efficiency of aromatic and medicinal plants (Konkolewska et al. 2020). PGPR also play important role in the remediation of polycyclic aromatic hydrocarbons, pesticide, and disinfectant. *Sphingobium chlorophenolicum* is reported as a pentachlorophenol (organochlorine compound used as a pesticide and a disinfectant) degrader and bioaugmentation with combined use of *S. chlorophenolicum* and medicinal, and aromatic plants can be used for degradation of pesticides in soil (Dams et al. 2007).

Endophytes reside in the inner tissues of plants which help in plant growth promotion and development under biotic or abiotic stress similar to PGPR (Gaiero et al. 2013). They are also able to tolerate high HMs and hence lower toxicity to remediating plants. Like PGPR, they also produce phytohormones, organic acids, enzymes, siderophores, growth regulators, and biosurfactants that help in various physiological processes of plant (Ma et al. 2016). Endophytic bacteria mainly improve metal accumulation capacity in roots by excreting metal immobilizing extracellular polymeric substances, which increases the phytostabilization potential of remediating plant and reduce HMs translocation to plant shoots (Berthelot et al. 2018; Gaiero et al. 2013; Ma et al. 2015a, 2016). For example, co-inoculation of *Lolium perenne* with AM fungi (*Funneliformis mosseae*) and the dark septate endophyte (*Cadophora* Sp.) decreases the oxidative stress and Cd accumulation in plant shoot (Berthelot et al. 2018).

Arbuscular mycorrhizal fungi (AMF) have been reported to tolerate high HM concentration and considered to be a tool to enhance phytoremediation. Underground mycelium network acts as a bridge between plant roots and rhizosphere microorganisms, which significantly increases the area of a plant root to access nutrients and contaminants from soil. These fungi in association with plant roots lower toxicity to remediating plants through their mobilization from soil and thus assist in phytoremediation (Khan et al. 2000; Kuo et al. 2011). The possible mechanisms by which AMF protect their host plants from HMs include (Hu et al. 2013; Meier et al. 2012) (a) chelation of HMs; (b) binding of metals to the cell wall; (c) immobilization in the plasma membrane once metals cross the cell wall; (d) membrane transportation of metals from the soil to the cytosol; (e) intracellular chelation with organic acids and amino acids; and (f) sequestration of metals into vacuoles. AM fungi in association with hyperaccumulating medicinal plants have potential application in both extraction and stabilization of contaminant in soil. Gildon and Tinker (1981) first reported that HM-tolerant AM fungi, *Glomus mosseae*, could increase HMs uptake by *Trifolium repens* (clover) in soil co-contaminated with Zn and Cd. The phytoextraction efficiencies of Cd were increased when hyperaccumulating medicinal plants Alfred stonecrop (*Sedum alfredii* Hance) were inoculated with two AMF *Glomus mosseae* and *Glomus caledonium* in a Cd-contaminated acidic soil (Hu et al. 2013). Thus Alfred stonecrop associated with AM fungi can be used for both extraction and stabilization of Cd in Cd-contaminated acidic soil. Mycorrhizal plants increase the bioavailability and uptake of HMs and transfer them to plant shoots (phytoextraction) or immobilize them in soil (phytostabilization) depending on factors such as the soil properties, pH, climate, and the HM-plant-fungus combination (Mishra et al. 2017). AM fungus *Funneliformis mosseae* in association with medicinal plant *Robinia pseudoacacia* enhances root length, surface area, volume, short root number, and Pb phytostabilization in Pb-contaminated soil (Huang et al. 2019).

The combined treatments of mycorrhiza and PAH-degrading bacteria in phytoremediation of contaminated soil have proved more helpful than their individual treatment (Ren et al. 2017; Wu et al. 2014). For example, medicinal plant *Lolium multiflorum* (ryegrass), in symbiotic association with AMF (*Glomus mosseae*) and PAHs-degrading bacteria (*Acinetobacter* sp.), was used for cleaning up PAH-contaminated soil (Wu et al. 2014; Yu et al. 2011). Higher dissipation rates were observed in bacteria-mycorrhizae-ryegrass treatments as compared to mycorrhizae-ryegrass and bacteria-ryegrass treatment. The growth of ryegrass in association with these microbes significantly increased soil peroxidase activities, leading to enhanced dissipation of pyrene (PYR) and phenanthrene (PHE) (Yu et al. 2011). Since biomass production and metal accumulation limit the phytoextraction, to achieve the desirable remediation goal, plants should be used in association with microorganisms for phytoremediation.

20.11 Criteria/Characteristics of Aromatic and Medicinal Plants for Phytoremediation

In order to be suitable for an eco-sustainable phytoremediation technology, plant species should meet the following criteria (Dixit et al. 2015; Mani and Kumar 2013; Prasad 2003; Sheoran et al. 2009): (i) native hardiness and metal resistant, (ii) fast growth and high biomass yield, (iii) a profuse root system for absorption of large amount of contaminants, (iv) uptake of a large amount of HMs and enhanced root-to-shoot translocation, (v) able to accumulate and degrade considerable amounts of the organic pollutants depending on the level of contamination, as well as several chemical and physicochemical properties of the contaminated soil, (vi) easy to dispose of as a hazardous waste by composting, compacting, drying, thermal decomposition, and (vii) economically important. Since most metal hyperaccumulators are slow growing and have low biomass, bioengineering of non-accumulators having high biomass is essential for effective phytoremediation. So, extensive research is required to testify the phytoremediation potential of hyperaccumulating medicinal and aromatic plants for the treatment and management of HMs contaminated sites.

Table 20.6 Medicinal transgenic plant, source of gene for transgenosis, and their enhanced phytoremediation effect

Target gene	Source	Recipient	Contaminant	Transgenosis effect	References
1. Stress response gene					
CYP450-like gene (Os08g01480)	Rice	*Arabidopsis*	Multi-metals	Heavy metal and other abiotic Stress tolerance	Rai et al. (2015)
MuSI (multiple stress response gene I)	*Ipomoea batatas*	*N. tabacum*	Cd	Enhanced Cd tolerance, and less root-to-shoot translocation	Kim et al. (2011)
gsh1 (γ-glutamylcysteine synthetase) and gsh2 (glutathione synthetase)	*E. coli*	*A. thaliana,*	Hg, As	Increased As and Hg tolerance and accumulation	Li et al. (2006a)
	Cynodon dactylon	*N. tabacum*	Cd	Increase Cd tolerance and accumulation	Li et al. (2006b)
	Ceratophyllum demersum	*N. tabacum*	Cd, As	Enhanced Cd and As root accumulation	Shukla et al. (2012)
	Nelumbo nucifera	*A. thaliana*	Cd	Enhanced Cd accumulation	Liu et al. (2012)
2. Stress response and metal binding protein phytochelatin synthesizing gene					
PCS1 (phytochelatin synthase) and GSH1	*Allium sativum* (PCS1), *S. cerevisiae* (GSH1)	*A. thaliana* co-expression Cd, As	Cd, As	Enhanced Cd and As tolerance and shoot accumulation	Guo et al. (2008)
c-GCS cysteine synthase	*Spinacia oleracea*	*N. tabacum*	Cd, Se, Ni	Increased Cd, Se, and Ni tolerance, enhanced Cd shoot accumulation	Kawashima et al. (2004)
3. Metal binding protein metallothionein synthesizing gene					
CUP1 (metallothionein)	*Saccharomyces cerevisiae*	*N. tabacum*	Cd	Increased Cd tolerance, increased levels in roots	Krystofova et al. (2012)
			Cu	Enhanced Cu accumulation	Thomas et al. (2003)
SpMTl	*Sedum plumbizincicola*	*Sedum plumbizincicola*	Cd	Overexpression of gene results in Cd hyperaccumulation and hypertolerance	Peng et al. (2017)

OsMT2c	*Oryza sativa L.*	*A. thaliana*	Cu	Enhanced tolerance to Cu stress and increased ROS scavenging	Liu et al. (2015b)
MT2b (metallothionein)	*A. sativum*	*A. thaliana*	Cd	Enhanced Cd tolerance and accumulation	Zhang et al. (2006)
MT2b (metallothionein) and HMA4 (P1B-ATPase)	*A. thaliana*	*N. tabacum* co-expression Cd, Zn	Cd, Zn	Enhanced Cd tolerance, enhanced Cd and Zn root-to-shoot transport	Grispen et al. (2011)
merP (Hg2+-binding protein)	*Bacillus megaterium*	*A. thaliana*	Hg, Cd, Pb	Enhanced Hg, Cd and Pb tolerance and accumulation	Hsieh et al. 2009
4. Genes for increasing the bioavailability of metal ions					
Nicotianamine synthase (*NAS*) and nicotianamine amino transferase (NAAT), involved in phytosiderophore biosynthesis	wheat	Can be used in aromatic and medicinal plants for enhanced phytoremediation	Cd, Pb, and Ni	Phytosiderophore increases bioavailability of the contaminants in the soil	Gupta and Singh (2017)
nicotianamine synthase (NAS gene)	Root secreted nicotianamine from *A. halleri*	Can be used for other medicinal plants	Zn	Phytosiderophore increases Zn bioavailability	Tsednee et al. (2014)
5. Degradation gene					
merA	mercury(II) reductase Tn21 transposon	*Nicotiana tabacum*	Hg	Enhanced mercury volatilization	He et al. (2001)
merB (organomercurial lyase)	*Escherichia coli*	*A. thaliana*	Organomercurials	Increased tolerance to organomercurials	Bizily et al. (1999)
6. Heavy metal Transporter gene					
OsMTP1metal tolerance protein gene	Rice	*Nicotiana tabacum*	Cd	Enhanced cadmium accumulation and tolerance	Das et al. (2016)

(continued)

Table 20.6 (continued)

Target gene	Source	Recipient	Contaminant	Transgenosis effect	References
merC (HM transporter)	*Acidithiobacillus ferrooxidans*	*A. thaliana*, *N. tabacum*	Hg	Hg hypersensitivity, enhanced Hg uptake	Sasaki et al. (2006)
	Specific membrane targeting Tn21 transposon	*A. thaliana*	Cd	Increased Cd tolerance and accumulation	Kiyono et al. (2012)
HMT1 gene phytochelatin (PC)-cadmium transporter	*Schizosaccharomyces pombe* Fission yeast	*A. thaliana*	Cd	Lowers seed cadmium through phytochelatin-dependent vacuolar sequestration	Huang et al. (2012)
SnYSL3 cadmium-regulated yellow stripe-like transporter	*Solanum nigrum*	*A. thaliana*	Cd	Gene is upregulated in the excess of Cd and enhances translocation of Cd to the shoots	Feng et al. (2017)
tzn1 (high specificity Zn transporter)	*Neurospora crassa*	*N. tabacum*	Zn	Enhanced Zn accumulation	Dixit et al. (2010)
MRP7 (ATP-binding cassette transporter)	*A. thaliana*	*N. tabacum*	Cd	Increased Cd tolerance, enhanced Cd accumulation in leaf vacuoles	Wojas et al. (2009)
CET3, CET4 (cation-efflux transporters)	*B. juncea*	*N. tabacum*	Cd	Enhanced Cd tolerance, altered Cd accumulation	Lang et al. (2011)
7. Gene for accelerated growth rate, enhanced plant biomass and phytoremediation					
Hairy roots gene *copC*	*Pseudomonas fluorescens*	*N. tabacum*	Cu	Transgenic plant with high root biomass, hyperaccumulation of Cu and low values of the oxidative stress index	Pérez-Palacios et al. (2017)
Agropine-type plasmid pRiA4 (genes nptII and rol B) and the binary vector pESC4	*Agrobacterium rhizogenes*	*Scirpus americanus*	Pb, Cr	Hairy roots, more root biomass and removed 3.8 and 1.2 times more Pb and Cr, respectively, than control	Alfaro-Saldaña et al. (2016)

20.12 Biotechnology and Use of Genetic Engineering in Phytoremediation Using Aromatic and Medicinal Plants

Although plants have the inherent ability to detoxify pollutant, this process is either slow or they generally lack the catabolic pathway for the complete degradation of toxic compounds as compared to microorganisms. The cleanup of polluted environments using indigenous microorganisms or plant alone has not given much positive results. Therefore, phytoremediation efficiency of plants can be enhanced using genetic engineering. Two medicinal transgenic plants *Arabidopsis thaliana* and *Nicotiana tabacum* are first time practiced for significant removal of Cd and Hg as compared to control (Misra and Gedamu 1989; Rugh et al. 1996). Since transgenic plants have proved very effective in phytoremediation, very few medicinal and aromatic plants are engineered (Table 20.6) for efficient phytoremediation of contaminated site in field. Transgenic approaches could be used to enhance the phytoremediation potential of some medicinal and aromatic plant. This will increase the number of plant species that can be used for efficient phytoremediation. In transgenic plants, genes involved in oxidative stress and metal uptake, transport, sequestration, and degradation of hazardous pollutants have been introduced from microbes, plants, and animals successfully (Cherian and Oliveira 2011; Yan et al. 2020). Transgenic plants exhibiting biodegradation capabilities of microorganisms bring the promise of an efficient and environmental-friendly technology for cleaning up polluted soils. For example, an extracellular fungal enzyme, laccase gene of *Coriolus versicolor*, was introduced in tobacco plants and the transgenic tobacco plant, was able to remove bisphenol or pentachlorophenol from rhizosphere apparently greater than that of control lines (Sonoki et al. 2005). It is also possible with the help of biotechnology to introduce and overexpress the gene for maximum resistance and phytoremediation. HM tolerance is usually manifested by the strength of oxidative stress defense system by overexpression of genes involved in antioxidant machinery (Das et al. 2016; Kozminska et al. 2018). For example, overexpressing LAC1 gene of transgenic *Arabidopsis thaliana* exhibited enhanced phytoremediation and resistance to several phenolic allelochemicals and 2,4,6-trichlorophenol (Wang et al. 2004).

Since most have low biomass and are slow growing, bioengineering of aromatic and medicinal plants for multiple traits such as high biomass, hyperaccumulation, and tolerance to of multiple HMs provides better phytoremediation options. Transgenic tobacco having more dense hairy roots were developed to hyperaccumulate high Cu concentrations from waste waters by expressing, copC gene from *Pseudomonas fluorescens,* and transgenic hairy root biomass was significantly higher and accumulated twice the amount of copper as compared to control roots. The transgenic tobacco plant expressing modified merA genes are more resistant and at least ten times more Hg accumulator than control lines (Mani and Kumar 2013). It is reported that transfer of the complete operons from bacteria for pollutant metabolism will be necessary for high expression of enzymes and to achieve more effective phytoremediation (Sylvestre et al. 2009; van Aken 2009). Complete operon for PCB metabolism was introduced into transgenic tobacco plants to achieve more effective PCB phytoremediation (Sylvestre et al. 2009). Therefore, plants engineering with genes for the bacterial pathway offer a more effective technology for remediation of recalcitrant and toxic pollutant. Moreover, another approach for increasing the efficiency of phytoremediation is to engineer plant-associated bacteria (Kotrba 2013). Plasmid with the *bph* operon was transferred into *Sinorhizobium meliloti*, and resulting bacterial strain was than inoculated in the rhizosphere of alfalfa. It was observed that modified strain resulted in significantly better degradation of 2,3,4-trichlorobiphenyl as compared to plants inoculated with the wild-type strain (Chen et al. 2005).

The plant genes encoding HMs transporters proteins, usually represented by large gene families, are potential candidate for transformation toward improved HMs phytoextraction in shoots and/or enhanced phytostabilization in roots. The candidate genes for transgenosis are isolated from microbes in order to increase the phytoremediation efficiency of plants. HMs transport in plants includes transport through both the cytoplasmic membrane and tonoplast. Alteration of these transport processes via genetic modification therefore appears to be an option to develop a transgenic aromatic and medicinal plant suitable for HMs phytoextraction or phytostabilization. For example, a vacuolar glutathione S-conjugate pump associated with multi-drug resistance, yeast cadmium factor protein gene (YCF1; HM protein transporter), was introduced into *Brassica juncea*, and the resultant transformants have enhanced tolerance to Cd and Pb and also an increased accumulation of these HMs, about 1.5- to 2-fold compared to the wild type in shoots (Bhuiyan et al. 2010). Upon Cd exposure, the resultant transgenic plants exhibited reduced Cd toxicity symptoms and increased Cd content in the aerial tissue compared to non-transgenic plants. Similarly, *B. juncea* and *Arabidopsis thaliana* plants expressing AtATM3, (mitochondrial membrane ABC transporter), exhibited increased Cd and Pb tolerance and shoot accumulation compared to the wild-type plant. These changes in transgenic metallophenotype were due to increased expression levels of intrinsic phytochelatin synthase 1 and glutathione synthetase II induced by AtATM3 overexpression (Bhuiyan et al. 2011). More examples of HM transporters employed in plant transgenosis are listed the Table 20.6.

To date, chelation and subcellular sequestration seems to be best detoxification mechanisms in plants (Verbruggen et al. 2009). HMs that enters the cell is sequestered by organic acids, glutathione (GSH), or metal-binding ligands such as MTs (diverse proteins encoded by a superfamily) and PCs (synthesized from GSH by PC synthase) (Verbruggen et al. 2009). *Nelumbo nucifera* homologue of PCs phytochelatin synthase1 and MT2b from *Arabidopsis thaliana* was isolated and functionally expressed in response to Cd, Pb As, Cu, and Zn stress in *A. thaliana* and *Nicotiana tabacum*, respectively, and represents a useful target gene for the phytoremediation of these HMs (Grispen et al. 2011; Liu et al. 2012; Shukla et al. 2012). Therefore, modifying the levels of MTs and PCs in aromatic and medicinal plants is increasing nowadays.

Moreover co-expression of multiple genes will be an extra boost to the efficiency of phytoremediation and plant biomass production. Transgenic plants with more tolerance and enhanced As accumulation in the shoots were developed by co-expressing two bacterial genes. The *E. coli*, arsC gene (arsenate reductase) was expressed in leaves and γ-ECS gene (γ-glutamylcysteine synthetase – a rate-limiting enzyme in glutathione biosynthesis, which plays a central role in detoxification of ROS and in HMs chelation) was expressed in both roots and shoots. These double transgenic plants show almost three times higher hyperaccumulated As in the aboveground part and 17 times higher biomass than wild-type plants. This is a significant proof-of-concept of transgenic plants for biomass production and phytoremediation.

Tobacco plants engineered with the bacterial gene for a NADPH-dependent nitroreductase and *Arabidopsis* plants carrying the xplA gene from *Rhodococcus rhodochrous* bacteria tolerate and degrade high levels of TNT and RDX, a level nearly ten times higher than non-transgenic plants, respectively (Cherian and Oliveira 2011). Both xplA and xplB co-expression in transgenic plants resulted in a 30-fold higher RDX removal rates, than with xplA alone (Jackson et al. 2007). Transgenic plant technology is also used to improve phytoremediation of pesticides. The atzA gene (encoding atrazine chlorohydrolase) was expressed in transgenic plants and causes dechlorination of atrazine to hydroxyatrazine in all of the plant organs (Wang et al. 2005). The transgenic *Arabidopsis*, tobacco and alfalfa actively expressed atzA, resulting in enhanced tolerance and degradation to a wide range of atrazine concentrations. Since many mammalian CYP enzymes have the capability to metabolize these harmful contaminants into relatively safe products, their gene can be used to create transgenic plants for efficient phytoremediation. The mammalian cytochrome P450 genes, CYP1A1, and CYP1A2 (involved in Phase I metabolism and remediation of many xenobiotics) were allowed to express in a transgenic tobacco cell culture, resulting in increased metabolism of atrazine (Bode et al. 2004). One of the most widespread of the organic pollutants is the solvent trichloroethylene (TCE). Plants utilize a TCE degradation pathway similar to mammals, since trichloroethanol metabolite is produced in both the cases. However, phytoremediation of TCE is not so effective due to low expression of the cytochrome P450 gene. Transgenic tobacco plants expressing mammalian cytochrome P450 2E1 also have increased removal of volatile organic compounds such as TCE, ethylene dibromide, vinyl chloride, and carbon tetrachloride (James et al. 2008).

Over the last two decades, progress has been made in the applicability of phytoremediation techniques, including the use of both natural and GM medicinal and aromatic plants. Although the results to date are promising, much of the work has been limited to laboratory settings. So field applications are needed for better efficacy of technique. There are many sites that are contaminated with several chemicals, but phytoremediation technologies are still available for a few pollutants. Therefore, aromatic and medicinal plants need to be engineered with multiple stacked genes in order to meet the requirements of specific sites and different toxic elements. Hyperaccumulator aromatic and medicinal plants with fast growth and high biomass production should be identified and modified through genetic engineering to efficiently extract HMs from the environment.

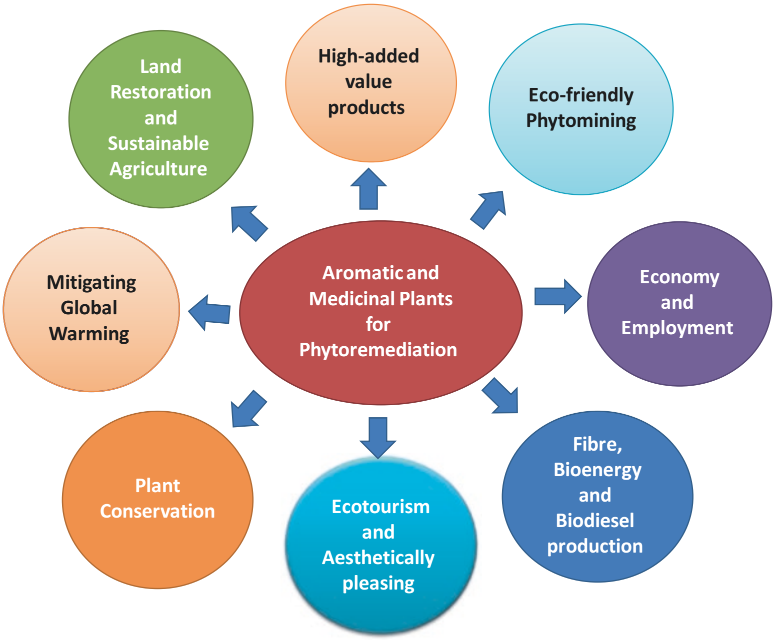

Fig. 20.2 Various uses of aromatic and medicinal plants when used for phytoremediation

20.13 Benefits or Advantages of Aromatic and Medicinal Plants When Used for Phytoremediation

Till date, few data are available where aromatic and medicinal plants were utilizing as prominent phytoremediation crops. Therefore, the main thrust was currently focused on aromatic plants which are used for various purposes along with phytoremediation (Fig. 20.2). Several aromatic and medicinal plants have been reported that have the potential for ecofriendly remediation and production of useful products as discussed below.

20.13.1 Environmental Aspect

The major environmental aspect regarding use of aromatic plants and medicinal plants for phytoremediation is that being a non-food crop contaminant's entry into the food chain can be reduced (Gupta et al. 2013; Zheljazkov and Nielsen 1996). Other positive aspect of using aromatic and medicinal plant for phytoremediation is that they can tolerate toxic environments as well as reduce soil erosion and simultaneously enhance the quality of soil. With the help of proper management techniques, wastelands, contaminated, and degraded sites have much potential for the growth of hyperaccumulator aromatic and medicinal plants, to prevent further soil degradation and desertification of land. The fast-growing aromatic and medicinal grasses like vetiver, *miscanthus*, switchgrass, lemongrass, palmarosa, citronella, etc. hold the soil surface and check the soil degradation along with phytoremediation (Patel and Patra 2017). This technique can also be used to re-establish vegetation at sites where natural vegetation is lacking due to physical disturbances or high metal concentrations in surface soils. Metal-tolerant and hyperaccumulating species can be used to restore vegetation to the sites, thereby decreasing the migration of contamination through wind and leaching to groundwater. Being a hyperaccumulator plant for several metals, some medicinal and aromatic plants such as *Ricinus communis* can be sustainably used for the reclamation of saline or sodic soil (Kumar et al. 2017b). *Cannabis sativa* and *Hibiscus cannabinus* has the potential to accumulate contaminants and yields high biomass which could be used for production of eco-friendly and reliable bioenergy to meet energy demand of future generations.

20.13.2 Sustainable Agricultural Development

Land restoration could be achieved for sustainable agricultural development using aromatic and medicinal plants. The applicability of phyto-remediation for the rehabilitation of contaminated sites for agriculture has been proven by numerous scientific projects (Stephenson and Black 2014; Verma et al. 2014). Medicinal plant species *Leucaena leucocephala* and *Astragalus racemosus* which is used for phytoremediation have inherent ability of nitrogen fixation through nodule formation and can substantially revitalize microbial bioactivities for re-establishment of self-sustaining plant communities and crop production over the polluted sites (Doty et al. 2003; Lindblom et al. 2013; Ssenku et al. 2017). These plants can optimize nitrogen content of the polluted soils because of its flexibility to nodulate with rhizobia of other legumes and vice versa. Utilizing aromatic and medicinal plants is a novel and economic approach to rejuvenate contaminated soils for agriculture. Currently a number of plants, viz., vetiver, palmarosa, lemon grass, citronella, geranium, river tamarind, mint, tulsi, salvia, and *Arabidopsis*, are ecologically feasible and viable for agricultural land restoration (Kumar et al. 2017a; Mahar et al. 2016).

20.13.3 Economic Aspect

Essential oils obtained from aromatic plant and secondary metabolites obtained from medicinal plants are of high demand at the international market because of their significant role in different industrial sectors, viz., pharma, ayurveda, perfumery, aromatherapy, and cosmetic industry. It is predicted that the potential market for essential oils would reach to US$5 trillion by 2050 (Verma et al. 2014). Therefore, to meet the ever-increasing demand of these products, aromatic and medicinal plants can be grown on contaminated sites which can help in their restoration. Moreover, wild and domestic animals do not damage to aromatic plants due to its fragrance and hence can be cultivated on large scale. Among several medicinal plant species used in phytoremediation, *Cannabis sativa*, a multipurpose herbaceous plant species, has a wide range of application as medicine, industrial fiber, livestock feed, seed oil, as well as significant place in religious and spiritual practices (Muhammad et al. 2013; Vishnoi and Singh 2017). After artificial culturing, aromatic and medicinal plants could also be sold as garden plant which will result in economic benefits and save a large amount of disposal expenses. Heavy metal-laden phytomass of *Leucaena leucocephala* (River tamarind) molds into furniture and is used or sold for construction purposes to gain economic benefit after phytoremediation thus eliminate the costs of disposal during the phytoremediation program.

20.13.4 High-Added Value Products

There are several aromatic and medicinal plants which have not only the tolerance ability against the environmental contaminants, but also high-added value products extracted from these plants. Plants like *Ocimum basilicum*, *Mentha* sp., *Cannabis sativa*, *Hibiscus cannabinus, Ricinus communis, Cymbopogon citratus*, etc. have been found to have substantial efficiency to accumulate and are generally used to extract the essential oil through steam distillation (Jisha et al. 2017; Saba et al. 2015). Aromatic plants products are extensively used in food, pastry, condiment, soaps, detergents, perfumery, insect repellents, cosmetics and food processing (Gupta et al. 2013; Lajayar et al. 2017). *Hibiscus cannabinus* and *Ricinus communis* seed oil is used for treatment of various health disorders like blood pressure, cholesterol level, etc. These are also sold as traditional medicine since the human civilization first steps (Kumar et al. 2017a).

20.13.5 Mental and Psychological Effect

Phytoremediation being interdisciplinary in nature is also aesthetically acceptable technique for soil restoration in the form of garden (Marques et al. 2013). Aromatic and medicinal plants are of vital significance to the forestation and beautification of degraded and contaminated sites in cities, which could directly influence people's mental and psychological state. Successful cultivation of market-oriented aromatic and medicinal plants having considerable market value may be a promising means for the economic upliftment of the rural people.

20.13.6 Bioenergy Production

Biomass-based energy (bioenergy) is gaining popularity as it is relatively renewable, clean, ecofriendly, and carbon-neutral alternative to fossil fuels. Coupling phytoremediation with bioenergy production using fast-growing hyperaccumulating aromatic and medicinal plants appears to be a rather attractive and economic opportunity. Energy can be generated from aromatic and medicinal plant biomass through the direct burning or production of biogas through the gasification of biomass obtained after phytoremediation (Mahar et al. 2016; Saba et al. 2015). Essential oil-bearing crops, like *Ocimum basilicum*, *Brassica juncea, Cannabis sativa*, *Mentha piperita*, *Hibiscus cannabinus***,** *Chrysopogon zizanioides, Vetiveria zizanioides*, *Cymbopogon citratus*, *Helianthus annuus*, etc., accumulate toxic metals. After harvesting the oil, residual biomass of these plants is utilized for the production of eco-friendly and reliable bioenergy to meet energy demand of future generations (Jisha et al. 2017). This integrated approach will help to reduce the cost of petroleum oil and will be a sustainable model in mitigation of many environmental issues. This clean and green energy production will help to reduce greenhouse gases, pollution alleviation, etc. The energy yield of medicinal plants, *Cannabis sativa*, *Millettia pinnata*, and *Hibiscus cannabinus* for biofuel and biogas production, has been reported comparable to most of the energy crops (Kumar et al. 2017b; Muhammad et al. 2013). Combining both bioenergy production and phytoremediation of contaminated sites apparently improves the overall ecosystem sustainability and economic viability of the individual techniques.

20.13.7 Mitigating Global Warming

Many medicinal plants which are used as a phytoremediation agent also sequester many greenhouse gases such as carbon dioxide and thus contribute in reducing the impact of global warming. Various grasses like vetiver, *miscanthus*, switchgrass, lemongrass, etc. have been used as an ideal phytoextractant help in carbon sequestration (Patel and Patra 2017).

20.13.8 Fiber Production

Medicinal plant *Hibiscus cannabinus* (Malvaceae) and *Cannabis sativa* are high biomass-yielding, important fiber plant which has numerous industrial applications and are potential candidate for phytoremediation of HMs and oils (Muhammad et al. 2013). It is mainly cultivated for fibers and bast which are used in making of absorbents, ropes, twine, composites, bedding materials, fabrics, highly efficient paper, and building materials**.**

20.13.9 Ecotourism

A contaminated area can be developed for ecotourism by growing aromatic and medicinal plants to preserve the aesthetic beauty of the land. Plant tourism is a worldwide industry worth billions of US dollars annually, and as a tourism resource, aromatic and medicinal plants have a huge potential along with employment.

20.13.10 Phytomining

To eliminate the use of conventional mining, plants with fast growth rate, more biomass, and high metal tolerance and accumulation are mainly preferred for phytoextraction. It could be an economically viable technology because hyperaccumulator plants can be utilized as "bio-ore" to extract the functional and valuable metals, and this process is technically known as phytomining (Chandra et al. 2015b). In addition, phytomining can generate employment opportunities and revenue.

20.13.11 Biodiesel Production

Remediation using plants from polluted water or soil are also able to produce biodiesel. *Jatropha curcas* is a widely used plant species, with many advantages for remediation and biodiesel production (Abioye et al. 2017; Agamuthu et al. 2010). *Acacia nilotica* and *Azadirachta indica* also have phytoremediation capability in soil and water medium along with tremendous medicinal and biodiesel production. The biodiesel derived from both tree species is efficient in terms of energy and meets the international standards requirement (Tiwari et al. 2017).

20.13.12 Conservation

Aromatic and medicinal plants have a high market potential as the world demand for their products is growing. Herbal market has grown substantially in the past decades because of having no side effects compared to the synthetic chemicals. 75–80% of the people in developing countries rely on traditional natural medicines (Ekor 2014). Cultivation of aromatic and medicinal plants in polluted land will also reduce the burden on these plants in the wild because around 90% of the medicinal plants used in pharmaceutical industries today are collected from the wild. Thus, phytoremediation will be helpful for the species conservation which is going to extinct in wild.

20.14 Limitations

Possible disadvantages associated with aromatic and medicinal plants for phytoremediation/plant-assisted remediation techniques include:

1. Phytoremediation using aromatic and medicinal plant is a slower process due to slow growth and low biomass of plants, and more time is required for remediation, usually more than one or several growing season.
2. Treatment is generally applicable to limited depth of soils.
3. Use of phytoremediation using aromatic and medicinal plants is currently limited to research activities and limited field testing.
4. Only few aromatic and medicinal plants having metal hyperaccumulator's capability are reported so far.
5. In order to be effective for phytoremediation, plants need to produce large biomass and only very few plants produce high biomass when grown in contaminated soil.

20.15 Future Prospects and Conclusion

The use of aromatic and medicinal plant in the remediation of contaminated soil is an excellent alternative as compared to other plants and has attracted more attention in recent years. Phytoremediation using aromatic and medicinal plant is a safe, economically feasible, and eco-friendly sustainable approach. This will also be very helpful in the economy of many nations by exploiting the essential oil and medicinal products from aromatic and medicinal plants, because complete utilization of by-products would always be the key criteria for universal acceptance of phytoremediation technologies in waste management industries. Since time is the main drawback in phytoremediation, to achieve the desirable remediation goal, high biomass plants should be used in association with microorganisms (rhizobacteria, endophytes, and mycorrhiza), to shorten the time period and cost-effectiveness. In addition, research integrating biotechnological approaches is needed to improve plant biomass, tolerance and hyperaccumulation of toxic metals in plants. In order to successfully use transgenic plants for phytoremediation in harsh environmental conditions, it is essential to preserve the recombinant bacterial population in the soil. In a nutshell, extensive research is needed for utilizing full phytoremediation potential of aromatic plants and medicinal plants, which may lead to "Green scented technology."

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Chapter 21
Environmental Pollutants and Their Remediation Using Medicinal and Aromatic Plants

Mohammad Saleem Wani, Nazir Ahmad Malik, Naseema Aqbar Wani, and Younas Rasheed Tantray

21.1 Introduction

The global population will exceed seven billion and will rapidly reach eight billion. This growing population is disrupting existing natural resources and causing measurable waste worldwide. When pollution is manageable, land, water, and atmospheric ecosystems can naturally dilute, destroy, or absorb pollution. The increasing burden of pollution requires further measures to prevent its harmful effects (Kjellstrom et al. 2006). Pollution is a threat to the environment because of its toxicity and unrecoverable behavior. Extensive industrialization and urbanization are the major culprits for the steady deterioration in environmental quality. The release of natural and pollution has been a for the past few decades many types of contamination are constantly causing problems, some of them easily cured, but not many. Plants serve as a Green Livers for the ecosystem to explain the harmful effects of climate pollution and toxicity (Sandermann Jr 1994).

An environment may be polluted or contaminated. Contamination is different from pollution; but contaminants may be pollutants and pose a detrimental effect on the environment. In the literature, pollution is the direct or indirect cause of harmful effects of injecting materials or energy into the environment, damaging living resources, causing hazards to human health, disrupting environmental activities,

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impairing quality for use of the environment, and reducing amenities. Contamination, on the other hand, is the concentration of substances in the atmosphere and the organism, above the natural background level. Environmental pollution can refer to an unwanted and undesired change in the physical, chemical, and biological properties of air, water, and soil which can be hazardous for animals and plants. Pollution usually takes the form of chemical substances or energy, such as noise, heat, or light (Ko and Hui 2012).

Pollutants, the elements of pollution, can either be foreign substances/energies or naturally occurring contaminants.

21.2 Types of Pollutants

21.2.1 Heavy Metals

Heavy metals are a common term applied to a group of metals and metalloids, and it has an atomic density higher than 4000 kg/m^3 (Edelstein and Ben-Hur 2018; Vardhan et al. 2019). Virtually all heavy metals are hazardous to people even at low metal ions (Ali et al. 2019; Engwa et al. 2019). Examples of heavy metals are copper, cadmium, zinc, chromium, arsenic, boron, cobalt, titanium, strontium, tin, vanadium, nickel, molybdenum, mercury, lead, etc. While heavy metals such as copper, zinc, boron, iron, molybdenum, and nickel are essential needs for which contribute to plant growth, these heavy metals are damaging the organisms and plants when their levels exceed the allowable limits. Some heavy metals, for instance, lead, mercury, cadmium, and arsenic, are not needed for the development of plants and animals. Soil contamination is caused by heavy metals due to the use of commercial wastewater, sewage sludge, fertilizers, and treated wastewater in the land application and weathering of earth minerals (Aladesanmi et al. 2019).

Heavy metals not only contaminate the soil but also affect food production, quality, and well-being (Steffan et al. 2018). Some heavy metals are toxic to plants although, at a very low concentration, other heavy metals accumulated in plant tissues cause mild abnormalities without apparent negative effects or reduction in yield (Saranya et al. 2018; Tangahu et al. 2011). The growth of plants in these heavy metal-contaminated areas alters their metabolic, physiological, and biochemical pathways, resulting in reduced metal accumulation, reduced biomass production, and decreased biomass growth. Heavy metal toxicity depends on the species. For instance, some plants known as metal-tolerant plants and hyperaccumulators (the concentration in dry weight foliar tissue is not less than >100 μg/g for cadmium (Cd); >300 μg/g for cobalt (Co) and copper (Cu); >1000 μg/g for nickel (Ni), arsenic (As), and lead (Pb); >3000 μg/g for zinc (Zn); and >10,000 μg/g for manganese (Mn) when the plants are grown in their natural habitat) (Leitenmaier and Küpper 2013; Peng et al. 2020) have barrier systems to prevent the damage caused by heavy metal-initiated pressure; however, the magnitude of exposure and other natural conditions add to the effect of heavy metal (Di Toppi and Gabbrielli 1999). Folks contaminated with heavy metals in large amounts may experience the ill effects of various ailments.

21.2.2 Effect of Heavy Metal-Polluted Soil on Plant Growth and Humans

Heavy metals which are available for plant usage are easily soluble in soil solution or through root exudates (Blaylock 2000). Although some heavy metals are required for plant growth and maintenance, large amounts of these metals can become hazardous to plants. The capacity of plants to extract the necessary metals allows them to obtain other unwanted metals (DalCorso et al. 2019). As metals cannot be broken down, when the plant's concentrations surpass the proper levels, they can, directly and indirectly, affect the plant.

Some of the direct harmful effects caused by high metal concentration are the inhibition of cytoplasmic enzymes and the loss of cell structures because of oxidative stress (Bansal 2018; Kurutas 2015; Tan et al. 2018). The conversion of essential nutrients at cation exchange sites of plants is an example of an indirect toxic effect (Sisay 2019). Besides, heavy metals can adversely affect the growth and activity of soil microorganisms and indirectly affect plant growth. For example, a decrease in the number of valuable soil microorganisms as a result of high metal concentration leads to a decrease in organic matter and a decrease in soil nutrients. Due to heavy metal intrusion in soil microbial activity, enzyme activity that is beneficial for plant metabolism is also hindered. These toxic effects lead to a decrease in plant growth and subsequently to plant death (Shah et al. 2010).

The effect of heavy metal toxicity on plant development differs depending on the specific heavy metal involved in the process. Table 21.1 shows an overview of the hazardous effects of specific metals on growth, biochemistry, and physiology of various plants. For metals such as Pb, Cd, Hg, and As which have no suitable role in plant growth, negative effects were reported at very low levels of these metals in the growth medium. *Azadirachta indica* grown in mercury-contaminated soils significantly reduced its vegetation growth and photosynthetic pigmentation as per Ahammad et al. (2018). Most of the decrease in growth parameters of plants growing in contaminated soils can be related to decreased photosynthetic activities, plant mineral nutrition, and reduced activity of some enzymes (Shah et al. 2010).

For other metals which are advantageous to plants, "small" levels of these metals in the soil increase plant growth and development. Nevertheless, at larger levels of these metals, a decrease in plant growth has been recorded. For example, Jayakumar et al. (2013) described that at 50 mgCo/kg, there was a rise in the nutrient content of tomato plants weighed against the control. In contrast, a reduction in plant nutrients from 100 mgCo/kg to 250 mgCo/kg was recorded. Similarly, Khan et al. (2006) described that Ni at more than 50 μg Ni g^{-1} dry weight adversely influences plant

Table 21.1 The toxic effect of some heavy metals on plants and humans

Heavy metal			Toxic effect on plant and human	References
As	Plant	Rice (*Oryza sativa*)	Reduction in seed germination, decrease in seedling height reduced leaf area, and dry matter production	Abedin et al. (2002), Marin et al. (1993)
		Tomato (*Lycopersicon esculentum*)	Reduced fruit yield, decrease in leaf fresh weight	Barrachina et al. (1995)
		Canola (*Brassica napus*)	Stunted growth, chlorosis, wilting	Cox et al. (1996)
	Human		Affects essential cellular processes such as oxidative phosphorylation and ATP synthesis, is highly carcinogenic, and can cause cancer of the lungs, liver, bladder, and skin, cardiovascular and peripheral vascular disease, developmental anomalies, neurologic and neurobehavioral disorders, diabetes, hearing loss, portal fibrosis, and hematologic disorders (anemia, leukopenia, and eosinophilia)	Centeno et al. (2006), Jaishankar et al. (2014), Tchounwou et al. (2004), Tripathi et al. (2007), Wilbur et al. (2012)
Cd	Plant	Wheat (*Triticum* sp.)	Reduction in seed germination, decrease in plant nutrient content, reduced shoot and root length	Ahmad et al. (2012), Yourtchi and Bayat (2013)
		Garlic (*Allium sativum*)	Reduced shoot growth, Cd accumulation	Jiang et al. (2001)
		Maize (*Zea mays*)	Reduced shoot growth, inhibition of root growth	Wang et al. (2007)
	Human		Carcinogenic, mutagenic, endocrine disruptor, lung damage and fragile bones, affects calcium regulation in biological systems, osteoporosis, hypercalciuria, and kidney disease	Bernard (2008), Degraeve (1981), Jaishankar et al. (2014), Salem et al. (2000)

(continued)

Table 21.1 (continued)

Heavy metal			Toxic effect on plant and human	References
Co	Plant	Tomato (*Lycopersicon esculentum*)	Reduction in plant nutrient content	Jayakumar et al. (2013)
		Mung bean (*Vigna radiata*)	Reduction in antioxidant enzyme activities, decrease in plant sugar, starch, amino acids, and protein content	Jayakumar et al. (2008)
		Radish (*Raphanus sativus*)	Reduction in shoot length, root length, and total leaf area, decrease in chlorophyll content, reduction in plant nutrient content and antioxidant enzyme activity, decrease in plant sugar, amino acid, and protein content	Jayakumar et al. (2007)
		Cowpea (*Vigna unguiculata*)	Inhibited seedling growth. Caused chlorosis in young leaves Reduced the Mn concentration in roots and Fe concentration in leaves thus inhibited plant growth	Liu et al. (2000)
	Human		Neurological (e.g., hearing and visual impairment), cardiovascular, and endocrine deficits	Leyssens et al. (2017)
Cr	Plant	Wheat (*Triticum* sp.)	Reduced shoot and root growth	Panda and Patra (2000), Sharma and Sharma (1993)
		Tomato (*Lycopersicon esculentum*)	Decrease in plant nutrient acquisition	Moral et al. (1996, 1995)
		Onion (*Allium cepa*)	Inhibition of germination process, reduction of plant biomass	Nematshahi et al. (2012)
	Human		Hair loss	Salem et al. (2000)

(continued)

Table 21.1 (continued)

Heavy metal			Toxic effect on plant and human	References
Cu	Plant	Bean (*Phaseolus vulgaris*)	Accumulation of Cu in plant roots, root malformation and reduction	Cook et al. (1998)
		Black bindweed (*Polygonum convolvulus*)	Plant mortality, reduced biomass and seed production	Kjær and Elmegaard (1996)
		Rhodes grass (*Chloris gayana*)	Root growth reduction	Sheldon and Menzies (2005)
	Humans		Brain and kidney damage, elevated levels resulting in liver cirrhosis and chronic anemia, stomach and intestine irritation	Salem et al. (2000), Wuana and Okieimen (2011)
Hg	Plant	Rice (*Oryza sativa*)	Decrease in plant height, reduced tiller and panicle formation, yield reduction, bioaccumulation in shoot and root of seedlings	Du et al. (2005), Kibra (2008)
		Tomato (*Lycopersicon esculentum*)	Reduction in germination percentage, reduced plant height, reduction in flowering and fruit weight, chlorosis	Shekar et al. (2011)
	Humans		Autoimmune diseases, depression, drowsiness, fatigue, hair loss, insomnia, loss of memory, restlessness, disturbance of vision, tremors, temper outbursts, brain damage, lung and kidney failure	Neustadt and Pieczenik (2007), Gulati et al. (2010)

(continued)

Table 21.1 (continued)

Heavy metal			Toxic effect on plant and human	References
Mn	Plant	Broad bean (*Vicia faba*)	Mn accumulation shoot and root, reduction in shoot and root length, chlorosis	Arya and Roy (2011)
		Spearmint (*Mentha spicata*)	Decrease in chlorophyll a and carotenoid content, accumulation of Mn in plant roots	Asrar et al. (2005)
		Pea (*Pisum sativum*)	Reduction in chlorophylls a and b content, reduction in relative growth rate, reduced photosynthetic O2 evolution activity and photosystem II activity	Doncheva et al. (2005)
		Tomato (*Lycopersicon esculentum*)	Slower plant growth, decrease in chlorophyll concentration	Shenker et al. (2004)
	Humans			
Ni	Plant	Pigeon pea (*Cajanus cajan*)	Decrease in chlorophyll content and stomatalconductance, decreased enzyme activity which affected Calvin cycle and CO2 fixation	Sheoran et al. (1990)
		Rye grass (*Lolium perenne*)	Reduction in plant nutrient acquisition, decrease in shoot yield, chlorosis	Khalid and Tinsley (1980)
		Wheat (*Triticum* sp.)	Reduction in plant nutrient acquisition	Pandolfini et al. (1992), Barsukova and Gamzikova (1999)
		Rice (*Oryza sativa*)	Inhibition of root growth	Lin and Kao (2005)
	Humans		Allergic skin diseases such as itching, cancer of the lungs, nose, sinuses, throat through continuous inhalation, immunotoxicity, neurotoxicity, genotoxicity, affects fertility, hair loss	Salem et al. (2000), Khan et al. (2007), Duda-Chodak and Blaszczyk (2008)

(continued)

Table 21.1 (continued)

Heavy metal			Toxic effect on plant and human	References
Pb	Plant	Maize (*Zea mays*)	Reduction in germination percentage, suppressed growth, reduced plant biomass, decrease in plant protein content	Hussain et al. (2013)
		Portia tree (*Thespesia populnea*)	Reduction in number of leaves and leaf area, reduced plant height, decrease in plant biomass	Kabir et al. (2009)
		Oat (*Avena sativa*)	Inhibition of enzyme activity which affected CO2 fixation	Moustakas et al. (1994)
	Humans		Excess exposure in children causes impaired development, reduced intelligence, short-term memory loss, disabilities in learning and coordination problems, risk of cardiovascular disease	Salem et al. (2000), Wuana and Okieimen (2011), Padmavathiamma and Li (2007)
Zn	Plant	Cluster bean (*Cyamopsis tetragonoloba*)	Reduction in germination percentage, reduced plant height and biomass, decrease in chlorophyll, carotenoid, sugar, starch, and amino acid content	Manivasagaperumal et al. (2011)
		Pea (*Pisum sativum*)	Reduction in chlorophyll content, alteration in the structure of chloroplast, reduction in photosystem II activity, reduced plant growth	Manivasagaperumal et al. (2011)
		Rye grass (*Lolium perenne*)	Accumulation of Zn in plant leaves, growth reduction, decrease in plant nutrient content, reduced efficiency of photosynthetic energy conversion	Bonnet et al. (2000)
	Humans		Dizziness, fatigue, etc.	Hess and Schmid (2002)

growth at multiple levels such as morphological, physiological, and biochemical. It is interesting to note that in most real-life scenarios where the soil is contaminated with multiple heavy metals, both the conflicting and coherent associations between heavy metals may affect plant metallic poisoning. Nicholls and Mal (2003) noted that the mixture of Pb and Cu at both high concentration (1000 mg/kg each) and low concentration (500 mg/kg) led to the speedy and total death of the leaves and stem of *Lythrum salicaria*. The experts reported that there was no synergistic interaction between these heavy metals possibly because the concentrations utilized in the experiment were too high for the interactive relationship to be observed between the metals. Another study by Ghani (2010) analyzed the effect of six heavy metals (Cd, Cr, Co, Mn, Hg, and Pb) on the growth of maize. The result indicated that the presence of these metals in soil decreased the growth and protein content of maize. The toxicity of these metals happened in the following order: Cd > Co > Hg > Mn > Pb > Cr. It is also seen that the combined effect of several heavy metals in this study is as dangerous as the effect of the very toxic heavy metal. The researcher traced this outcome to the paradoxical relationship between heavy metals.

Heavy metals are systemic toxins with unique neurotoxic, nephrotoxic, fetotoxic, and teratogenic effects (Nordberg 1999). Heavy metals have a direct impact on individual behavior by disrupting their psychological and neural activity, and they can overcome the placental barrier, and they are found in breast milk, brain, and the developing nervous system in children (Bağdat and Eid 2007) Table 21.1.

21.2.3 Organic Pollutants

Organic pollutants are manufactured and recurring in nature. These organic xenobiotics are stable in the environment and are very toxic. They are called persistent organic pollutants (POPs) because they are not quickly destroyed. Pesticides, petroleum products, pharmaceuticals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) are a few of the active organic pollutants (Abhilash and Singh 2009). The 12 important POPs are referred to as the "filthy dozen" and are needed for the removal and phasing out by the United Nations Environmental Plan ((US), 2000). Aldrin, dieldrin, chlordane, DDT, endrin, heptachlor, mirex, toxaphene, PCBs, HCBs, dibenzodioxins, and dibenzofurans are the 12 most hazardous associated with organic pollution. Organic pollutants are a real threat to the environment for their persistence in the environmental surroundings, lipophilic character, and high bioconcentration potential. These pollutants tend to get transferred in the adipose tissues of organisms (POPs, WHO Report 2008). For some time, pollutants achieve a high level of toxicity due to their high bioconcentration potential. Soil contaminated with organic pollutants leads to soil microflora death and reduces plant growth and yield.

POPs can cause critical unwanted effects, such as congenital disorders, certain cancers and tumors at numerous sites, immune system problems, reproductive issues, and impairments of the ability to fight diseases and of growth, and permanently disrupt brain function (Kim et al. 2019). POPs are suspected carcinogens and have been implicated in endometriosis, increased likelihood of diabetes, and neurobehavioral impairment, including learning problems, reduced efficiency on standard tests, and changes in behavior (Ashraf 2017; Bolt and Degen 2002).

21.2.4 Radioactive Contaminants

Radiation is all around us, and radioactive products have great benefits for humans and society and enjoy an important position in everyday life. Applications for radiation include scientific, medical, agricultural, commercial, and power production (Waltar 2003). It is therefore imperative that such widespread activities result in the generation of radioactive waste. The International Atomic Energy Agency (IAEA 2010) defines radioactive waste as any substance that contains a greater amount of radioactive emitting particles than is considered safe on national and international standards. For instance, harmful radioactive waste is produced at every stage of the uranium nuclear fuel cycle. It is regrettable that currently no country has recognized lasting repositories and storage services for the most dangerous high-grade nuclear wastes from nuclear power plants. Radioactive waste may be solid, liquid or gas from a varied group of procedures and activities and accidents, but regardless of its source, radioactivity poses a risk to human health and the environment and damage to ecosystems. The nuclear crash in Chernobyl, Ukraine, increases the risk of cancer in humans by 0.1% (Dushenkov 2003; IAEA 2005). Besides, a recent record from Fukushima, Japan, found high levels of radioactive cesium in woody plants after the accident (Yoshihara et al. 2013). There is a constant radioactive decay experienced by radionuclides, resulting in alpha, beta, and gamma cells (Fulekar et al. 2010; Ghosh and Singh 2005). One of the main causes of exposure to humans is the consumption of food crops and contaminated water by radionuclides. The stability of radiation in the atmosphere can last for billions of years; as such, it can cause permanent damage to organisms along with the ecosystem Table 21.2 (Malhotra et al. 2014).

21.3 The Role of Medicinal Plants in Phytoremediation

In general, the aromatic plants have many characteristics such as social and environmental advantages, industrial/commercial crops, multiple crops in 1 year, tolerance against biotic and abiotic stress situations, invaluable nature, etc. (Verma et al. 2014). Several medicinal plants have been described to be capable of collecting environmental pollutants (Gupta et al. 2013). These plants can be applied simultaneously for the remediation of environmental pollutants and serve many purposes, such as the production of the pharmaceutical element, energy production, carbon sequestration, and pollution remediation.

Aromatic and medicinal plants can be utilized to remediate the contaminated sites since they are less likely to transfer metal from soil to essential oil or to change its composition (Zheljazkov et al. 2006) due to the process used to extract oil (Bernstein et al. 2009; Pandey and Singh 2015; Scora and Chang 1997). Additionally, it is proved that metals remain in residues of plants during oil removal by steam distillation (Zheljazkov and Nielsen 1996).

Table 21.2 Adverse impacts of radionuclides on humans

Radionuclide	Radiation emitted	Effects and danger
Barium-140	β	Second event emitter binds to DNA. Of concern in assessing effects of nuclear atmospheric tests and accidents
Tritium H3	β	Evidence of serious genetic effects in invertebrate development at very low doses, short range of β decay causes high ionization density
Sulphur-35	β	Sulfur also a part of internal macromolecules in living systems. Transmutes to a reactive gas, chlorine
Strontium-90	β	Second event nuclide with daughter Y-90 of concern since it binds to DNA
Krypton-85	β	Very soluble in fats and therefore can build up in body fat (beast tissue, lymphatic tissue) following inhalation
Caesium-134, 137	βγ	Binds to muscle, ulcers, erythema, tissue necrosis
Radium-226	α	Bone cancer, lymphoma, aplastic anemia, and leukemia
Uranium-238	α	Carcinogenic, mutagenic, birth defects
Thorium-232	α	Carcinogenic

21.4 Phytoremediation Potential of Medicinal and Aromatic Plants

Phytoremediation is the use of plants or plant products to eliminate environmental toxins. Although this technology is almost 2–3-year-olds, recent developments have made it more appealing and efficient. Initially, when technology appeared, searching of plants capable of accumulating contaminants, especially toxic metals, was given more emphasis. Many plants have been researched as phytoremediator, and most of them are stated as hyperaccumulators. Plant selection is an important step in phytoremediation to eliminate contaminants. Researchers are currently seeking to get plants that are capable to accumulate heavy metals and pollutants and may offer some value-added products simultaneously. It has been found that many medicinal and aromatic plants are resistant to biotic and abiotic stresses and capable of collecting pollutants that can be used as a phytoremediator plant (Table 21.3).

There is no doubt that edible plants are also employed for phytoremediation applications, but crops have some disadvantages as hazardous metals can enter into the food chain and triggers some harmful effects. Not only are aromatic plants an important source of essential oils (Lal et al. 2013), but animals are also not harmful to aromatic plants, so it can be grown in large quantities. Numerous aromatic and medicinal plants are capable of accumulating hazardous metals, which are now used in phytoremediation technology. Therefore, aromatic and herbaceous plants provide a novel approach to address contaminated soils.

Table 21.3 Medicinal plants studied for their phytoremediation potential of different heavy metals, radioactive wastes, and organic pollutants

Plant	Family	Metal	Reference
Bacopa monnieri	Plantaginaceae	Hg, Cd, Cu, Cr, Mn, Pb	Sinha and Chandra (1990), Sinha et al. (1996); Sinha (1999), Hussain et al. (2011)
Ocimum basilicum	Lamiaceae	Cd, Pb, Cr, Zn, Ni	Putwattana et al. (2010), Dineshkumar et al. (2019), Zahedifar et al. (2016), Prasad et al. (2010), Alamo-Nole and Su (2017) Stancheva et al. (2014), Prasad et al. (2010), Przybyłowicz et al. (1995), Rai et al. (2004), Ramazanpour (2015), Rascio and Navari-Izzo (2011), Sá et al. (2015), Schmidt et al. (2007)
Catharanthus roseus	Apocynaceae	Cd, Pb, Ni, Cr	Pandey et al. (2007), Subhashini and Swamy (2013), Ahmad and Misra (2014)
Mentha sp.	Lamiaceae	Cr, Ni, Cd, Pb, Ni, Cr, Al	Zurayk et al. (2001), Zurayk et al. (2002), Sá et al. (2015), Manikandan et al. (2015)
Aloe vera	Xanthorrhoeaceae	Cr	Seema and Alok (2011)
Cannabis sativa	Cannabaceae	Ni, Pb, Cr, Cd	Linger et al. (2002), Citterio et al. (2003), Shi and Cai (2009)
Cymbopogon citratus	Poaceae	Cr, Ni, Pb, Cd, As, Cu, Fe, Zn	Pandey and Singh (2015), Jisha et al. (2017), Babarinde et al. (2016), Lee et al. (2014)
Ricinus communis	Euphorbiaceae	Cd, Ni	Shi and Cai (2009), Bauddh and Singh (2012), Bauddh and Singh (2015), Sobh et al. (2014)
Azadirachta indica	Meliaceae	Cd, Cu, Zn, Cr, Pb	Thangaswamy et al. (2015)
Withania somnifera	Solanaceae	Cu	Varun et al. (2012)
Euphorbia hirta	Euphorbiaceae	Cd, radioactive waste	Hamzah et al. (2016), Hu et al. (2014)
Chrysopogon zizanioides	Poaceae	As, Cu, Zn, Pb, Hg	Datta et al. (2011), Chen et al. (2004), Mangkoedihardjo and Triastuti (2011)
Vetiveria zizanioides	Gramineae	Cd, Pb, Cu, Sn, Zn, Ni	Ng et al. (2017), Vo et al. (2011), Gunwal et al. (2014)
Mentha arvensis	Lamiaceae	Cu, Zn	Malinowska and Jankowski (2017)
Cymbopogon citratus	Poaceae	Pb, Cd, Zn	Babarinde et al. (2016)
Mentha crispa	Lamiaceae	Pb	Sá et al. (2015)
Lavandula vera	Lamiaceae	Pb, Zn, Cd	Angelova et al. (2015)

(continued)

Table 21.3 (continued)

Plant	Family	Metal	Reference
Rosmarinus officinalis	Lamiaceae	Cd, Pb	Ramazanpour (2015)
Cymbopogon flexuosus	Poaceae	Cr	Patra et al. (2015)
Matricaria chamomilla	Asteraceae	Cd, Pb, Zn	Stancheva et al. (2014), Voyslavov et al. (2013)
Ocimum tenuiflorum, *O. gratissimum*	Lamiaceae	As, Cr	Siddiqui et al. (2013), Rai et al. (2004)
Cymbopogon winterianus	Poaceae	Cr	Sinha et al. (2013)
Pelargonium sp.	Geraniaceae	Pb	Abdullah and Sarem (2010)
Hypericum sp.	Hypericaceae	Cd	Tirillini et al. (2006)
H. perforatum	Hypericaceae	Cd	Schneider and Marquard (1995)
Senecio coronatus	Asteraceae	Ni	Przybyłowicz et al. (1995)
Centella asiatica	Apiaceae	Cd, Cu, Fe, Ni, Pb, Zn	Yap et al. (2010)
Orthosiphon stamineus	Lamiaceae	Cd, Cr, Zn, Cu, Pb	Arifin et al. (2011)
Acacia mangium	Fabaceae	Zn, Cd, Pb, Cr, Cu	Majid et al. (2012b)
Pluchea indica	Asteraceae	Cd	Majid et al. (2012a)
Jatropha curcas	Euphorbiaceae	Cu, Fe, Pb, Al, Zn	Majid et al. (2012c)
Hibiscus cannabinus	Malvaceae	Cu, Pb	Hasfalina et al. (2012)
Salvinia molesta	Salviniaceae	Diesel	Abdulwahab (2011)
Paspalum vaginatum	Poaceae	Diesel	Salmi et al. (2012)
Nymphaea spontanea	Nymphaeaceae	Cr	Choo et al. (2006)
Solanum melongena, *Ipomoea batatas*, *Allium cepa*	Solanaceae, Convolvulaceae, Amaryllidaceae	Fe, Zn, Cd, Mn, Pb, Cu, Cr	Ismail et al. (2005)
Eichhornia crassipes	Pontederiaceae	Petroleum refinery effluents	Ismail and Beddri (2009)
Nicotiana tabacum	Solanaceae	Cd	Chitra et al. (2011)
Salix viminalis	Salicaceae	Cd, Zn	Hammer et al. (2003)
Salix spp.	Salicaceae	Cd	Vandecasteele et al. (2005)
A. indica	Meliaceae	Cd, Hg, Cr	Tiwari et al. (2017)
Acer rubrum, *Liquidambar styraciflua*, *Liriodendron tulipifera*	Sapindaceae, Altingiaceae, Magnoliaceae	^{244}Cm, ^{137}Cs, ^{238}Pu, ^{226}Ra, ^{90}Sr	McLeod et al. (1984)
Cocos nucifera	Arecaceae	^{137}Cs	Robison and Stone (1992)

(continued)

Table 21.3 (continued)

Plant	Family	Metal	Reference
Pinus radiata	Pinaceae	^{137}Cs, ^{90}Sr	Entry et al. (1993)
Cannabis sativa	Cannabaceae	^{137}Cs, ^{90}Sr	Hoseini et al. (2012)
Portulaca oleracea	Portulacaceae	Cr	Kale et al. (2015)
Portulaca oleracea	Portulacaceae	Pb, Ni, Zn	Amer et al. (2013)
Ocimum gratissimum	Lamiaceae	Cd, Zn	Chaiyarat et al. (2011)
Artemisia annua	Asteraceae	As	Rai et al. (2011)
Nasturtium officinale	Brassicaceae	Ni	Duman and Ozturk (2010)

Chrysopogon zizanioides typically called as vetiver grass has been discovered to be ideal to remove organic (e.g., 2,4,6-trinitrotoluene, phenol, and petroleum hydrocarbon) along with inorganic (hazardous metals such as lead, cadmium, copper, zinc, and arsenic) pollutants (Balasankar et al. 2013; Brandt et al. 2006; Chen et al. 2004; Datta et al. 2011; Ho et al. 2013; Makris et al. 2007; Singh et al. 2008; Singhakant et al. 2009). Because of its large root biomass and capability to enter deep levels of soil, plant vetiver can be utilized to reduce deep contaminated soil (Pichai et al. 2001; Truong 2000). Truong (2000) noted that this plant has the potential to recover the soil filled with mining waste. In a trial done by Datta et al. (2011), vetiver grass was discovered to be a possible phytoremediator of As when grown in various kinds of soils. The plant was discovered to possess substantial As elimination efficiency, i.e., around 10.6% when grown in 45 mg As/kg of soil (Millhopper soil).

Hamzah et al. (2016) had researched to measure the cadmium buildup performance of some native plants. They discovered that *Euphorbia hirta*, an industrially crucial plant, has considerable Cd bioaccumulation efficacy. *E. hirta* has been also discovered to reduce radioactive contamination (Hu et al. 2014). *Hypericum perforatum* L. is an imperative medicinal plant typically used as an antidepressive agent and has an outstanding power to store a significant quantity of Cd (Chizzola and Lukas 2006; Đurović et al. 2013; Verotta 2003). The workers proposed that *Hypericum* sp. can increase the great content of Cd inside their aerial components without major adverse outcomes on the growth and dry biomass. Hypericin, an essential part of essential oil, was not negatively affected by Cr when grown in Cr-contaminated media (Tirillini et al. 2006).

Ricinus communis also referred to as castor has been used since ancient times as an anti-implantation, anti-inflammatory, antitumor, anti-asthmatic, etc. and has just become a powerful phytoremediator for the cleaning of several pollutants, both organic and inorganic types (Bauddh et al. 2015; Kiran and Prasad 2017; Rissato et al. 2015). The plant has the potential to develop easily up to 150 mg/kg Cd and acquire a considerable quantity of metal in their roots and shoots (Bauddh et al. 2016). The plant has excellent resistance to biotic and abiotic stresses. The plant has been discovered to build up heavy metals like Cd, Ni, Pb, Cu, As, Cr, Zn, Ba, etc.

(Abreu et al. 2012; Adhikari and Kumar 2012; Khan et al. 2019). The plantation of *R. communis* has also been noted to enhance the soil physicochemical properties when grown in wasteland soils (Wu et al. 2012).

Hypericum perforatum, a plant that is important in depression (Mir et al. 2019), has been discovered to work in solving Cd from soil (Pavlova et al. 2015). The plant has no undesirable impacts on growth and dry biomass production. Dinu et al. (2020) discovered that the metal gathering capacity of *Ocimum basilicum* varies in different plant organs: Cd, Co, Cr, and Pb were accumulated in roots while Cu, Ni, and Zn in flowers. Also, the heavy metal intake depends on the plant development stages; thus, Cd, Cr, and Pb were accumulated more in mature plant leaves. The plant has produced a protection system by limiting the metal in their sources. A current study showed that *O. basilicum* is favorable for phytoremediation of Cd-contaminated soil, which was more enhanced when the plants were provided with a variety of fertilizers (Zahedifar et al. 2016). Rai et al. (2004) described that *O. tenuiflorum* L. can cope with the phytotoxicity of Cr by altering various metabolic processes.

The phytoremediation efficiencies of six wild plants, that is, *Malva parviflora*, *Datura stramonium, Citrullus colocynthis*, *Rhazya stricta*, *Phragmites australis*, and *Lycium shawii*, were tested for Cd, Zn, Cu, Ni, and Pb content by MM et al. (2013). They discovered a medicinal plant called *D. stramonium*, ideal for phytostabilization of soil contaminated with Ni and Cu.

The phytoremediation efficiency of *Cymbopogon martinii*, *C. flexuosus*, and *Vetiveria zizanioides* was estimated for Cd by Lal et al. (2008) and Singh et al. (2020). *V. zizanioides* has been discovered to possess high Cd tolerance. Many authors have reported the tolerance and bioaccumulation potential of numerous *Mentha* species to metals (i.e., Ni, Cr, Cd, Al, etc.) (Saxena et al. 2019; Zurayk et al. 2002, 2001). Zurayk et al. (2001) grown 12 different hydrophyte species along with 4 *Mentha* species, viz., *M. longifolia*, *M. aquatica*, *M. pulegium*, and *M. sylvestris*, in 1.0 ppm Cr, Ni, and Cd contamination. They discovered that all *Mentha* species collect a significant quantity of all metals studied. *M. longifolia* was found to build up Cr (1076.8 µg Cr plant^{-1}), while *M. sylvestris* gathered Ni (1822 µg Cr plant^{-1}) which was the maximum level of the metals gathered among all (12) considered plants.

Two species of *Mentha*, namely, *M. aquatica* and *M. sylvestris*, were grown in the solutions containing 1.0, 2.0, 4.0, and 8.0 mg Ni L^{-1} for 14 days (Zurayk et al. 2002). Both the species gathered a large quantity of Ni within their roots (8327 mg Ni kg^{-1} dry weight in *M. aquatica* and 6762 mg/kg dry weight in *M. sylvestris*) indicating that this plant is a powerful phytoremediator. Two medicinally vital plants (*Centella asiatica* and *Orthosiphon stamineus*) have been reviewed due to their phytoremediation potential of heavy metals by many analysts (Abd Manan et al. 2015; Arifin et al. 2011; Mohd Salim et al. 2013). In a study done by Abd Manan et al. (2015), the evaluation was completed in two medicinal plants (*C. asiatica* and *O. stamineus*) because of their Zn, Cu, and Pb buildup and translocation, and it was discovered that *C. asiatica* has translocation component of more than one for all the considered metals (Zn = 1.34, Cu = 2.77, and Pb = 1.42). Translocation factor more than one for all the metals suggests that *C. asiatica* may be utilized for phytoextraction. But another plant *O. stamineus* has a translocation factor of significantly less than one but was discovered to amass a significant quantity of metals and might be ideal for phytostabilization.

Allium sativum has been discovered to possess excellent ability to accumulate cadmium in its roots which was 1826 times greater than control (Jiang et al. 2001). The plant revealed sufficient tolerance toward Cd and showed no harmful results at low concentrations. *Cannabis sativa* L., a commercial and medicinal therapeutic plant grown in Ni-, Pb-, and Cd-contaminated soil to evaluate their phytoremediation potential and outcomes of these metals on the quality of fiber, was investigated by Linger et al. (2002). They discovered that the greatest amount of all studied metals was accumulated in plant leaves. Furthermore, they discovered that none of the studied metal affected fiber quality. Citterio et al. (2003) grew *C. sativa* in Cd-, Ni-, and Cr-contaminated soil, and following 2 months of seed germination, sowing was discovered to have no significant changes in plant growth. Metals resulted in the improved making of phytochelatins which revealed a stronger defense against metal poisoning.

21.5 Conclusion

Nowadays, phytoremediation offers an answer to the most destructive issue of pollution faced by mankind. Phytoremediation not merely solves the issue of pollution but additionally furnishes many ecosystem benefits along with making it a suitable and plausible approach. In particular, the use of medicinal and aromatic plants and tree species can lead to an overall development of the ecosystem and its population. Because it is economically practicable, it can be encouraged to be adapted by the masses for decontamination of the sites. A wide range of contaminants can be remediated by plants at a lower cost which is an admirable feat. Technological and biological amendments can be made to increase plant capacity for pollution solutions. It is of huge significance for the benefit of our environment to promote phytoremediation.

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Chapter 22
Toxicity: Its Assessment and Remediation in Important Medicinal Plants

Sahil, Manvi Sharma, Adhip Das, Shubham Saxena, and Shabir A. Rather

22.1 Introduction

From the time immemorial, plant-derived products have been used as phytomedicines. Plants are a rich source of potential phyto-drugs that are of immense importance to humankind, and their key role in human health and benefits has grown to a greater extent. In recent years of revolutionizations, the utility of these phytomedicines has seen a significant advancement due to the fact they are as effective as synthetic drugs and show minimum to no side effects. The fact mentioned above holds authenticity, only if these phyto-drugs are consumed as per their dietary intake values.

Phytochemicals (derived from a Greek word 'phyto' meaning 'plants') are predominantly found in medicinal plants as organic and inorganic bioactive substances that protect plants from stress and damage. These substances are mainly found accumulated in various parts of a plant such as in roots, stem, leaves, or even seeds also. They are synthesized as primary or secondary metabolites. Phytochemicals are mainly classified under some major categories of compounds like carbohydrates, alkaloids, lipids, terpenoids, phenolic acids, and nitrogen compounds, comprising various essential biological properties for human welfare. These phyto-active compounds are also distributed into some major classes, about the type of function they

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perform in the prevention of various human diseases – antioxidants, anticancer, antibiotic, immunity boosters, neuropharmacological agents, etc. All of these classes consist of an enormous range of phytochemicals obtained with different levels of potency, and also, it can be possible for one particular phytochemical to perform roles higher than one (Saxena et al. 2013).

As stated earlier, phytomedicines are beneficial only if consumed appropriately; otherwise, it may lead to phytotoxicity. Phytomedicines are most prevalent in developing countries because of their nominal cost and are readily available. Since these are considered natural, it is believed that they are always ‘safe’, but some of the evidence suggests the other corner of the story. However, there are very few adverse effects of phytomedicines, and also very small numbers of people are likely to get affected because of these toxic effects of phytoconstituents of medicines. Researchers face difficulties in detecting toxic effects of phytomedicines because it is comparatively more convenient to estimate the toxic effect of a single compound which is precisely the case with drugs that consist of a single chemical compound. Since medicinal plants comprise of 400 or more phytochemicals, it becomes next to impossible to figure out all the interactions and synergies taking place between these phytoconstituents. Also, many beneficial constituents might counteract the toxic effect of another constituent. Moreover, this becomes a major reason why the toxicity of phytochemicals remains undetected in many medicinal plants (George 2011).

By virtue of different chemical constituents of medicinal plants, toxicity both in terms of mutagenic and carcinogenic effects is also evident. Some of them are found to be potentially genotoxic as well (Fennell et al. 2004). The relative potential of a chemical to show unfavourable or adverse effects in an organism is known as toxicity. Also, toxicity can be reviewed as the level or magnitude up to which tissue is exposed to a harmful substance, is affected and further, beneficial effect on the organism as well as its substructural components. Toxicity is also referred to as the study of harmful effects of a substance on an organism as well as its complete mechanism of action, symptoms, and treatments involved in therapy. Toxicity can further be demarcated into two categories mainly acute toxicity and chronic toxicity. Acute toxicity studies involve observing the toxic effects of a constituent after administering a single large dose of a compound for not more than 24 hours. Chronic toxicity occurs when the chemical or toxin is administered for a more extended time period, i.e. the exposure time is for months or even years. Chronic toxicity may also cause irreversible toxicity in an organism (Mensah et al. 2019).

Poisoning or toxicity from traditional medicinal plants occurs mainly because of the misidentification of plants required for medicines, wrong preparations of extracts or its inappropriate administration, and incorrect dose concentration. The dose/quantity of a chemical constituent to be administered is very important; keeping in mind the toxic effects of chemicals. As it is said, a non-toxic chemical can become harmful or toxic in large doses, and a toxic chemical can be safe at low doses (Mensah et al. 2019).

Many plants show toxic effects among the living organisms, for example, *Lantana camara*, which is used as an antimalarial drug, has also shown major hepatotoxic effects in animals, and this can be a major issue of concern for its utility as medicine for humans. Another example is of *Momordica charantia*, which is a prevalent source for treating diabetes and antimalarial drugs. However, it is also sometimes used as an abortifacient in some countries and has also shown hypoglycaemic effects among children (Mensah et al. 2019). For the safe use of medicinal plants, three groups of herbs have been designated (Nasri 2013). The first group of plants involves harmful constituents near to pharmaceutical concentrations which should not be consumed by locals or unqualified individuals except in the case of the homeopathic arena. Examples for this group are *Digitalis* spp., *Atropa belladonna*, and other plants. The second group involves plants that show powerful effects often vomiting or nausea, for example, *Lobelia* and *Euonymus* spp. The third group consists of some typical plants that show particular toxic effects. Plants containing pyrrolizidine alkaloid are the best examples of this category such as Comfrey *(Symphytum)* (Fig. 22.1).

22.2 The Global Pharmaceutical Market

The pharmaceutical sector has remained a vital role player on the grounds of health care. It is counted as one of the prime industries that can significantly contribute to economic development. The industry has also been one of the frontliners when it comes to value-added products, the constant development of drugs, and also employing flourishing minds. Over the years, it has evolved both in terms of technological and research aspects. For a long time, the pharmaceutical industry has shown tremendous growth globally, and there has been an exponential rise in the multi-folds of the ever-potentiating sector. Various global pharmaceutical markets are showing immense involvement in the emerging markets across the countries in order to stay in business and become sustainable. The sudden paradigm shift towards local emerging markets across the globe has also pushed India's pharmaceutical sector to become one of the largest generic pharmaceutical producers of the world. When it comes to the global pharmaceutical market, the links between commercial and retail units are of utmost importance. In order to gain maximum profit and be mutually beneficial, there has been an extensive search for newer forms of cooperation in the pharmaceutical commodity network. In the present-day conditions, the interaction between production units has intensified enormously, and this substantial increase has resulted in meeting ever elevating needs of the population. The statistical data analysis reported that in 2016 107.0% increase in the volume of the pharmaceutical market was achieved. However, contrastingly it has slowed down in recent years bringing around 5–6% change in the average market volume.

Fig. 22.1 Some important medicinal plants: (**a**) Aegle marmelos, (**b**) Tinospora cordifolia, (**c**) Zingiber officinale, (**d**) Bacopa monnieri, (**e**) Terminalia chebula, (**f**) Aloe barbadensis, (**g**) Curcuma longa, (**h**) Murraya koenigii, (**i**) Mentha, (**j**) Saussurea simpsoniana, (**k**) Saussurea costus, (**l**) Azadirachta indica, (**m**) Ocimum sanctum

In this world of innovation and development, the pharmaceutical industry has also spread its wings venturing more into the herbal medicine field. Herbal medicine is termed as the precursor of modern medicine and drug development. Over the last few decades, significant growth has been observed in the herbal drugs market around the world. The usage of synthetic drugs has received a strong competition in the global scenario of consumption due to growing awareness among people about the rising cost as well as relative side effects related to synthetic drugs. Traditional medicinal systems encompass herbal medicine as its primary component and have been widely used around the world. India has been home to various medicine systems, namely, Ayurveda, Unani, and Siddha, and makes use of more than 90% plant-based formulations. The enormous growth of the pharmaceutical industry has led to efficient use of knowledge, research, and development by allowing the economic market to foster the production of useful drugs against new diseases (Singh et al. 2019). Overall, one can say that the pharmaceutical market has received great value.

22.3 Medicinal Plants and Their Importance as Herbal Remedies

Nature offers different plants that are a valuable source in the therapeutic domain. Such plants often referred to as medicinal plants offer a wide range of anti-inflammatory, antidiabetic, cancer-preventive, or per se pharmacologically and phytochemically important compounds (Da-Yong and Ting-Ren 2019). Herbal remedies of therapeutically significant medicinal plants are widely accessible and used in traditional medicinal systems. The ancient wisdom and the knowledge base of important compounds of medicinal plants could be utilized for focused research in the field of newer, safer, and naturally effective drug inventions also further leading to the major drug discovery processes in the future.

22.3.1 Anticancer Herbs (Oncologic)

1. *Moringa peregrina*: Native to Indian subcontinent, this plant belonging to the Moringaceae family has been found as a potential remedial herb comprising of various bioactive compounds including flavonoids as the most prevalent effect-causing agent of pharmacological significance (Mansour et al. 2019). The action knows its role in cancer prevention by inducing cell apoptosis and enhanced antioxidant effect leading to cancerous cell arrest providing long-term benefits to the human body. No wonder it has extensive use as a functional food in the pharmacology.
2. *Crocus sativus:* Commonly known as 'saffron', it has also shown an extensive use in the chemopreventive processes. Its cancer-preventive role is attributed to its bioactive compounds, that is, safranal, crocetin, and crocin (Naeimi et al. 2019). These three bioactive compounds by making use of different mechanisms which include suppressing cancer cell proliferation, regulating host immune response, and apoptosis induction among a few. These potential underlying mechanisms have been found to be effective in reducing malignancies associated with gastrointestinal (GI) cancers worldwide (Naeimi et al. 2019).

22.3.2 Antidiabetic Herbs

1. *Syzygium cumini:* This has come into picture in the wide spectrum of diabetes treatment and has been found to be useful in the successful reduction of diabetes mellitus complications and effective in reduction of symptoms associated with the same. The aqueous and ethanolic seed extracts of the *S. cumini* are found to be playing an essential role in reducing the oxidative damage, providing antihyperglycemic effect, and increasing bioavailability of the compounds that are

found to help control diabetes effectively, thereby making *S.cumini* a medicinally potent plant.

22.3.3 Respiratory Tract Ailment Preventing Herbs

1. *Acacia arabica*: The pharmacological properties of *Acacia arabica* finds utilization of the parts of the plant brings put to medicinal use. In the Indian traditional medicine system, the medicinal uses of *Acacia arabica* include the treatment of respiratory ailments, diabetes, skin disease, and cancer. The effects like antiviral, astringent, anthelmintic, antimicrobial, antidiarrheal, antioxidant, and enhanced nutritional value are to be counted as some of the important ones that are contributed by fresh plant part that is termed as a significant pharmacological agent (Rajvaidhya et al. 2012).

22.3.4 Herbs Used in Autoimmune Disorders

1. *Ocimum sanctum*: Commonly known as Tulsi or holy basil, this found a primordial place in the Indian households as a sacred plant and now in the traditional medicine system due to its unparallel effects like anticancer, antiasthmatic, antidiabetic, antifertility, hepatoprotective, hypotensive, antistress, and many more in the human body. It has become a joint herbal agent as a remedy for various ailments including autoimmune disorders. The autoimmune effect can be attributed to the mechanism of effective and increased production of WBC and RBCs; the immunomodulatory effect contributed by the production of antibodies is noted as one of the actions (Jeba et al. 2011). Different plant parts of *Ocimum sanctum*, e.g. leaves, flowers, seeds, etc., are widely used for their curative effects in providing substantial relief in gastric issues, immunity building, infection prevention, etc.

22.3.5 Anti-inflammatory Herbs

1. *Curcuma longa*: Commonly referred to as turmeric, this is widely used in the Indian kitchen and is considered as a wonder spice. Its active compound curcuminoid found in the form of curcumin extract has found its place in the medical system for its anti-inflammatory effect. The mechanism which the compound supports is by suppressing mutagens and reducing oxidative stress over the cells by free radicle scavenging (Bitencourt 2020). Curcumin has another significant role to play in lipid peroxidation and increasing the antioxidant activity of other

enzymes like catalase and superoxide dismutase making it a profound antioxidant and free radical scavenger (Ramadan et al. 2011).

2. *Zingiber officinale*: Commonly known as ginger, this is also counted as an important dietary spice. Ginger consists of some pharmacologically significant pungent components such as gingerol, shogaol, and zingerone which are effective in providing a treatment alternative as a medicinal drug by contributing majorly as an anti-inflammatory agent in therapeutic handling of disorders like arthritis and also in cancer (Ahmad et al. 2008). Estimated sales of different pharmaceutical drugs in the year 2018 has been shown in Fig. 22.2.

22.4 Contamination of Medicinal Plants and Plant Products

Medicinal plants are being used for many years now. Their usage has been increased because of the general assumption of natural as harmless. However, along with their safe and effective herbal use, they also have many ill effects on the human body contributed by the toxic elements present in these medicinal plants. Recent studies have divulged that some of the medicinal plants can be toxic and cancer-causing and can also cause mutations (Higashimoto et al. 1993; Schimmer et al. 1988, 1994; De Sã Ferrira and Ferrão Vargas 1999; Kassie et al. 1996). Some of the toxic elements get accumulated in plants, growing in polluted soil (due to the use of harmful chemical fertilizers, various anthropogenic activities) and irrigated with polluted water. The availability of chemical elements and soil parent minerals proves to be an important source of toxic metals in plants (Golubović and Blagojević 2013). So, the concentration of toxic elements in plants depends on the surrounding environment: polluted soil, polluted water, and the ability of plants to accumulate particular elements. The different elements, both essential and non-essential elements, can prove

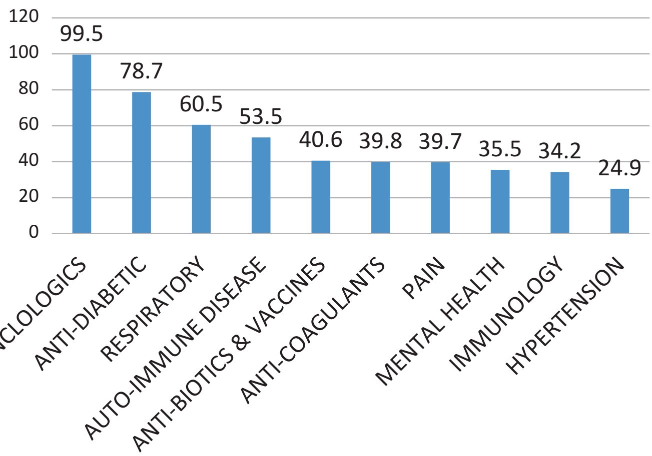

Fig. 22.2 Estimated sales of top ten pharmaceutical classes of the year 2018 (in billion USD)

toxic to the plants depending on the concentration of these elements. For example, some elements like zinc, manganese, iron, nickel, etc. are essential for plant growth but are toxic to the plant at higher concentrations.

Not only the toxic elements present in the soil contribute to contamination of medicinal plants and herbal products, but there are different other contaminants involved in contaminating medicinal plants as well as their herbal products. These contaminants are broadly classified into biological and chemical contaminants, as shown in Fig. 22.3:

22.4.1 Biological Contaminants

Living organisms, like bacteria, viruses, fungus, protozoa, insects, and other organisms that are present as adulterants in medicinal plants, their preparations, and the different plant products, are considered as biological contaminants. The most common contaminants of medicinal plants (mostly the aerial parts) are moulds as these are ubiquitous in the atmosphere. The microbial contamination mostly occurs due to mishandling during the manufacturing and packaging of these herbal products. Microbes present in the air and with humans contribute to contamination and can also spread this through cross-contamination. Fertilizers like animal manure are also responsible for microbial contamination. Useful medicinal herbs are identified

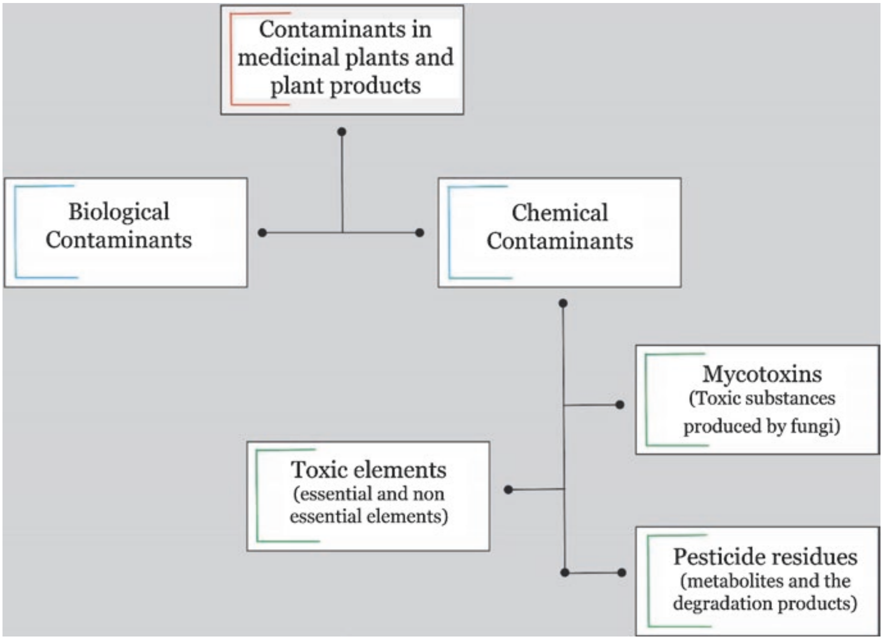

Fig. 22.3 Classification of different contaminants in medicinal plants and plant products

by the number of microbes present, and satisfactory herbal products are the ones that are without certain bacteria like *Salmonella* spp. and *Shigella* spp. According to the Directorate for the Quality of Medicines and Health Care (EDQM) of the Council of Europe (2007), it is mandatory to assess the type of microbes that contaminate herbal plants and plant products and presence of a particular substrate that could lead to the growth of specific microbes. This recipient is going to intake the herbal products. There can be contamination by certain bacteria that are resistant to antibiotics in plant products as studied by Brown and Jiang (2008). Brown and Jiang (2008) found different bacterial species, like *Ewingella americana*, *Bacillus* spp., *Erwinia* spp., etc. in 20 herbal supplements bought from the USA-based store. These bacterial species were resistant to readily available antibiotics like ampicillin, streptomycin, nalidixic acid, etc.

These antibiotic-resistant bacteria are also found in medicinal teas, but the number of microbes can be lowered by using proper preparation or brewing methods. Hauer et al. (1999) identified a strain of bacteria – *Acinetobacter baumannii* – present in tea infusions that were responsible for spreading infection in the neurology care unit. Wilson et al. (2004) studied fennel, peppermint, chamomile, and fruit teas for the presence of particular microbes. To check the number of microbes that arose as a result of an error in preparation methods, some tea bags were made to brew up at 90 °C for 5 minutes, and others were dipped in hot water (67 °C). Lesser moulds and bacteria were observed in tea bags that are brewed at 90 °C, whereas tea bags dipped in water kept at 67 °C had more number of microbes (Wilson et al. 2004). This proved that the preparation methods contribute to the number of contaminants in plant products.

Herbal plant products could also be contaminated with spores of particular microbes that could prove harmful for the humans; for example, honey has been found to contain spores of *Clostridium* spp. Moreover, these spores are very harmful as they are known to cause infant botulism (Schocken-Iturrino et al. 1999; Nakano et al. 1990).

22.4.2 Chemical Contaminants

22.4.2.1 Mycotoxins

Mycotoxins are specific secondary metabolites that are toxic and are synthesized by fungus/moulds. It has been studied that more than 50% of the herbs collected from the Brazilian market exceed the limits set by US Pharmacopeia regarding the microbial count (Bugno et al. 2006). The moulds that contributed the most to the mycotoxin contamination, in the above study, belonged to the *Aspergillus* genus and the *Penicillium* genus.

The following factors govern the ability of the mould to produce particular mycotoxin:

1. Genetic inheritance of the ability to synthesize mycotoxins
2. Humidity
3. Substrate for growth
4. CO_2/O_2 level ratio
5. Application of fungicides
6. Presence of other microbial species

Ochratoxin A (OTA) is a secondary metabolite of low molecular weight, synthesized by numerous fungi, for example, *Penicillium verrucosum* and *Aspergillus niger*. Ochratoxin A (OTA) is a kind of mycotoxin which is cancer-causing as declared by the International Agency for Research on Cancer (IARC) (1993). This mycotoxin is also immunotoxic, teratogenic, and nephrotoxic. OTA can contaminate different foodstuffs like cereals, spices, coffee, etc. (Pitt 2000; Leslie et al. 2008).

Fumonisins are another type of mycotoxins produced by *Fusarium* spp. According to IARC, fumonisins can be carcinogenic. These fumonisins mainly affect maize and maize products.

22.4.2.2 Toxic Elements

Plants growing in a particular area, already contaminated with different toxic elements, have been found to have increased accumulation of these toxic elements within themselves. The concentration of these toxic elements varies in different plant parts in different plant species. Toxic elements in the environment are contributed by different anthropogenic activities that ultimately contaminate soil and water. Certain essential elements have an important biological role but become toxic when given in excessive amounts (Donkin et al. 2000; Obi et al. 2006).

Bioaccumulation of toxic elements in plants depends on the following factors (Slaveska et al. 1998):

1. Genetic inheritance of the ability to bioaccumulate different elements
2. Accumulation in different plant parts
3. Other geo-climatic factors

Consumption of toxic elements like arsenic, lead, cadmium, etc. is quite hazardous for human health. Arsenic is very harmful to human health as it causes vitamin A deficiency resulting in heart problems and night blindness (Saha et al. 1999). Extended exposure to arsenic can lead to skin cancer, skin pigmentation, and liver damage (Shraim et al. 2003; Tseng 2004). Cadmium is carcinogenic and toxic at low concentrations because it is antagonistic to zinc, copper, calcium, and iron (Tchounwou et al. 2012). Lead toxicity causes kidney damage, neurotoxicity, and gastrointestinal and neurodevelopmental defects (Tchounwou et al. 2012).

22.4.2.3 Pesticide Residues

Pesticides are the chemicals used to eliminate pests, mostly from the agricultural fields. There are different types of pesticides like insecticides, fungicides, herbicides, etc. (Britt 2000).

Pesticides have been grouped into four categories based on their chemical structure:

1. Organochlorine pesticides (OCPs) that include benzene hexachloride (BHC), dichlorodiphenyltrichloroethane (DDT), lindane, and hexachlorocyclohexane (HCH)
2. Organophosphorus pesticides (OPs) that include malathion, ethion, parathion, and many more
3. Nitrogen-containing pesticides that include propazin, atrazin, and simazin
4. Pesticides of plant origin that include rotenoids and pyrethroids

OCPs were used for several years in agriculture and the control of malaria but were discontinued because of their harmful effects on human health. They can accumulate in the environment because of their slow rate of degradation, making them more hazardous for the environment. Among OCPs, DDT has been known to be very harmful as it can accumulate in the adipose tissue of humans (Ling et al. 1999).

OPs have also been proved to be harmful because being lipophilic they can get readily absorbed through ingestion, inhalation, or the skin. They are involved in the inhibition of enzyme acetylcholinesterase, thereby resulting in the agglomeration of acetylcholine. This leads to repeated stimulation of cholinergic synapses. One of the prime symptoms of OP exposure is delayed neuropathy (Britt 2000).

The residues of pesticides, either the metabolites or the degradation products, lead to environmental pollution and prove harmful to plant health.

22.5 Toxicity Assessment of Important Medicinal Plants

Indigenous medicinal plants are considered as one of the important sources of modern-day medicines among various other sources. Herbal remedies still contribute significantly to healthcare systems especially in developing countries since developing countries face limitations in basic healthcare infrastructure and medicines. Although several methods have been developed for the synthesis of pharmaceuticals, herbal medicines are still being used as a prime source of new substances and compounds, particularly because plants can synthesize products that are often strenuous to be procured via chemical synthesis. There is a significant difference between a medicinal plant and any synthetic drug. Synthetic drugs generally contain one single chemical compound, whereas a plant contains a huge mixture of chemical compounds. Medicinal value of plants is particularly because of the presence of phytochemicals. Substantially, remedies associated with plant products are considered unthreatened unless a potential risk is actually ascertained to it in individuals internalizing the medicine. But there are several case reports that appraise chronic toxicities resulting from their use. Most of the information available in the public domain remains invalidated with factual scientific information. Moreover, because of this reason, validation of toxicity assessment is immensely important so that the adverse effect of those toxic elements could be determined and minimized on their consumption.

22.5.1 Toxic Ingredients Present in Different Medicinal Plants

Toxicity has been a significant concern in different important plant species. Presence of various alkaloids in solanaceous vegetables; alpha-gliadin in the rye, oat, and wheat; and thiocyanates of different *Brassica* vegetables is often considered as potentially poisonous. Carol. A. Newall, one of the co-authors of the book *Herbal Medicines: A Guide for Health-care Professionals*, stated that a natural product does not necessarily signify that it has to be always safe. Recently, there are pieces of evidence that depict some herbs that are associated with potential health hazards which were considered safe for several decades. With the advancement of technology, the detection of toxic and carcinogenic chemicals in various herbs even at minute quantities and evaluation of hazardous effects has become very easy. Some of the botanicals with toxic constituents are given in Table 22.1.

22.5.2 Toxicity Assessment of Different Medicinal Plants

22.5.2.1 Toxicity Assessment of Different Medicinal Mushrooms on Neurite Outgrowth

Neurite outgrowth has been considered as a prime process at neuronal migration and differentiation. It is considered to be indispensable for conditions associated with neurodegeneration such as Alzheimer's and Parkinson's diseases. Therefore, treatments that promote neurite outgrowth are utterly necessary. Medicinal mushrooms like *Pleurotus giganteus* (popularly known as cow's stomach mushroom), *Pleurotus pulmonarius* (grey oyster mushroom), and *Ganoderma neo-japonicum* (purple Reishi) are an important source of antioxidants and also considered as neurite outgrowth simulator. *Lignosus rhinocerotis* (tiger milk mushroom) and *Ganoderma lucidum* (Reishi) have anticancer properties, and *Cordyceps militaris* (winter worm, summer grass) and *Grifola frondosa* (Hen of the Woods) have anti-inflammatory properties, and *Hericium erinaceus* (lion's mane mushroom or Yamabushitake) has anti-ulcerous property and also serves as a neurite outgrowth simulator. Out of these eight species, *Pleurotus giganteus*, *Pleurotus pulmonarius*,

Table 22.1 Different medicinal plant species and their toxic ingredients as well as toxic effects

Species	Common name	Toxic ingredient present	Toxic effect	References
Symphytum officinale L	Comfrey	Pyrrolizidine alkaloids	Impedes flow of blood to the liver, thereby accelerating hepatotoxic reaction and often considered to be carcinogenic	George (2011)
Medicago sativa	Alfalfa	Medicarpin	Causes systemic lupus erythematosus (SLE) symptoms in individuals already exposed to this condition	George (2011), Cosgrove and Undersander (2003)
Ginkgo biloba	Ginkgo	Flavonol glycosides; isorhamnetin, quercetin, and kaempferol	The extract of the plant has the potential to cause inhibition to platelet-activating factor. Therefore, its continuous usage may lead to a delay in blood clotting, and also it can cause haemorrhage	Rowin and Lewis (1996), Zhang et al. (2015), Lin et al. (2014)
Ephedra sinica	Ephedra	Ephedrine	It is associated with high blood pressure, liver damage	George (2011)
Glycyrrhiza glabra	Liquorice	Glycyrrhizinic acid	Has the potential to foster potassium depletion if consumed in large quantities. Therefore, it is hazardous for people who have problems with blood pressure, kidney, and heart disease. It is also associated with hypertension and oedema	George (2011)
Panax ginseng	Ginseng	Ginsenosides	Causes hypertension and mastalgia. Also, continuous usage may cause blood thinning, thereby leading to a delay in blood clotting	Becker et al. (1996), Rokot et al. (2016)
Sassafras albidum	Sassafras	Safrole	The root bark has a very minute quantity of carcinogenic compounds toxic to the liver if taken for a prolonged period or if consumed in large quantities	George (2011)

(continued)

Table 22.1 (continued)

Species	Common name	Toxic ingredient present	Toxic effect	References
Silybum marianum	Milk thistle, Saint Mary's thistle, Mary thistle, Marian thistle	Mycotoxin, potassium nitrate	Toxic for ruminants. The bacteria present in their stomach break down the compound, thereby giving rise to nitrite ions. These ions then react with haemoglobin, producing methemoglobin which blocks oxygen transport that results in oxygen scarcity in the body	George (2011)
Senna alexandrina	Senna	Glycosides	Continuous use of this herb leads to decrease in potassium levels in the body, causing fatalities. It may also lead to seizures, hypertension, and anaphylactic reactions	George (2011)
Hypericum perforatum	St. John's wort	Hypericin and hyperforin	Restlessness and skin irritation	George (2011)
Curcuma longa	Turmeric	Curcumin	In the case of excessive consumption, curcumin causes gastrointestinal troubles, thereby causing diarrhoea and also nausea	George (2011)

Hericium erinaceus, and *Grifola frondosa* come under culinary-medicinal mushroom group, and *Ganoderma neo-japonicum*, *Lignosus rhinocerotis*, *Ganoderma lucidum*, and *Cordyceps militaris* come under nonculinary-medicinal mushroom group. The effect of eight mushroom species on neurite outgrowth was assessed by using mouse neuroblastoma (N2a) cells (Phan et al. 2013). The Image-Pro Insight processor system was used to measure neurite length. Fluorescence immunocytochemical staining of neurofilaments was performed for further validation of neuritogenesis activity. Mouse embryonic fibroblast (BALB/3T3) and N2a cells were used for investigating any embryo- and neurotoxic effects. The aqueous extracts of *Ganoderma lucidum*, *Lignosus rhinocerotis*, *Pleurotus giganteus*, and *Grifola frondosa* and ethanol extract of *Cordyceps militaris* have been observed to promote the neurite outgrowth of differentiating N2a cells. It was also found that various mushroom extracts had no embryotoxic and neurotoxic effects in 3T3 and N2a cells. Therefore, *Ganoderma lucidum*, *Lignosus rhinocerotis*, *Pleurotus giganteus*, *Grifola frondosa*, and *Cordyceps militaris* may be considered safe and healthy dietary supplements for brain and cognitive health as well.

22.5.2.2 Toxicity Assessment of *Cyathula prostrata*

Cyathula prostrata is an annual to perennial plant with prostrate or erect stems growing from a long taproot. The plant growing in the wild can be harvested for human use, especially as a medicine as well as a food and source of soap. An admixture of the whole plant is taken as a treatment for fever and dysentery. The sap of the plant is used as ear drops to treat otitis and headache. The roots are served as an abortifacient and also used in the treatment of abnormal and frequent urination. A decoction of the roots is an antidote for dysentery, colds and cough, rheumatism, and dropsy. The leaves, mashed with water, are a remedy for cholera. The leaves are used to ease irritations of the throat. In a study performed by Priya Kannappan and Krishnakumari Shanmuga Sundaram (2009), they tried to find out the toxicity of methanolic extracts of *Cyathula prostrata.* Different concentrations of methanolic extracts of *Cyathula prostrata* were evaluated on 10-week-old female Swiss albino strain mice. Treated mice were found to have any obvious toxic symptoms only after 150 mg/kg/BW. Based on their study, it can be concluded that *Cyathula prostrata* can be considered safe at 100 mg/kg/BW dosage range without any aftereffects. Since this toxicity assessment study has been confined to animals, it is strenuous to estimate the self-administered dose for traditional use. Therefore, it is obvious to carry out clinical toxicological evaluations to determine a safe dose.

22.5.2.3 Toxicity Assessment *Phragmanthera capitata*

It is an obligate parasite that is attached to the rubber tree through a structure called haustorium. Both of its leaves and stems have been utilized for various ailments like gastrointestinal disorders, hypertension, diabetes, cancer, arthritis, etc. No such report has been found that stated the potential toxicity of *Phragmanthera capitata.* Ohikhena et al. (2016) tried to examine the toxicity of different solvent extracts (methanol, ethanol, acetone) of *Phragmanthera capitata* by using a brine shrimp hatchability and lethality assay (BSH and BSLA). They found different solvent extracts to be non-toxic in the brine shrimp lethality assay. Further, it has been suggested that in vitro, in vivo, and cancer cell line tests have to be performed in order to ascertain its use in traditional medicine.

22.5.2.4 Toxicity Assessment of *Kedrostis africana* Cogn.

Kedrostis africana also known as 'Baboon's Cucumber' often looks like English ivy. It is predominantly found in Namibia and South Africa. Mainly the tuber portion is being used against dropsy, syphilis, etc. Unuofin et al. (2017) performed experiments in order to find out whether different solvent extract (ethanol, acetone, and aqueous) of *Kedrostis africana* is toxic or not. Brine shrimp lethality test (BSLT) was performed to screen cytotoxicity. It was observed that the aqueous and ethanolic extracts were moderately toxic whereas the acetonic extract was non-toxic.

These findings further need to be supported by in vitro and in vivo toxicological studies. To check whether it has any anticancer properties, isolation of cytotoxic compounds and cancer cell line toxicity tests need to be performed.

22.5.2.5 Toxicity Assessment of *Sida acuta* Burn f. and *Sida cordifolia* L. of Burkina Faso

Sida acuta Burn f. and *Sida cordifolia* L. are most commonly used to treat children suffering from various diseases such as fever, malaria, pain, etc. This plant also has antibacterial as well as anti-inflammatory properties. According to an experiment done by Konaté et al. (2012), aqueous acetone extracts of *Sida acuta* Burn f. and *Sida cordifolia* L. contain steroids, alkaloids, coumarins, phenolic compounds, and saponosides. In a further study, they tried to examine the toxic effects of aqueous acetone extracts of these two species. This study was carried out on Swiss mice. Acute toxicity test was performed by injecting the mice with different quantities of aqueous acetone extracts. Very low toxicity values found in this study can be considered as indicative of safe therapeutic use.

22.5.3 Use of **Artemia salina** *in Toxicity Assessment of Nanoparticles*

Nanoscience, which is the latest arrival in the field of science, deals with materials on an ultrasmall scale. The term nanotechnology was first coined by a Japanese scientist Norio Taniguchi. By using nanoscience, nanotechnology aims at designing nanoparticles (NPs) and nanomaterials (NMs). Although nanoparticles are found to have multiple functions, there has been a substantial amount of debate and discussions on potential side effects of these particles on the living system and their associated side effects. So far, the use of nanotechnology research on pharmacology studies has been very limited. Both in vitro and in vivo tests are important to predict and assess toxicity more efficiently. Nowadays, researchers are relying more on in vitro methods over in vivo for cytotoxicity assessment of nanoparticles because of its labour intensiveness and cost. Along with that animal rights activities are more concerned with the involvement of animals in nanotechnology research. For any in vivo study, proper approval should come from the Institutional Animal Care and Use Committee (IACUC) so that ethical treatment is ensured. Due to these reasons, there is an increasing tendency to carry out in vitro methods for cytotoxicity assessment of nanoparticles. Mostly, MTT assay is being performed for cytotoxicity testing of various NPs. In this assay solubilization steps with tetrazolium are required, which can be toxic to cells. *Artemia salina* assay could be an alternative to this and has the potential to identify appropriate extracts among various extracts for drug discovery in medicinal plants. In 1959 Michael and coworkers proposed this assay which was later adopted by many laboratories for preliminary toxicity estimation. In a study performed by Rajabi et al. (2015), it was found *Artemia*-based toxicity assay of NPs are cheap, continuously available, and simple and could be a potential candidate for regulatory purposes, industrial monitoring requirements, or toxicity screening and, therefore, may be considered as a way out to the in vitro cell culture assay.

Flowchart Showing Methods of Performing Toxicity Assessment Studies (Adyin et al. 2016)

Test models selection

↓

Determination of sample size (following guidelines of Organization of Economic Co-operation Development (OECD), Food and Drug Administration (FDA) or European Authorities)

↓

Selection of Administration route

↓

Determining the concentration of plant extract

↓

In vitro cytotoxicity tests

↓

Genotoxicity tests

↓

In vivo test for chromosomal damage using rodent hematopoietic cells (repeated dose toxicity test, subchronic toxicity test, chronic toxicity test, single-dose toxicity test, Carcinogenicity tests, Reproductive and developmental toxicity tests, Local tolerance tests)

↓

Performing Toxicokinetic studies

↓

Immunotoxicity and antigenicity test

↓

Evaluation of Gastro-intestinal toxicity

↓

Renal and hepatic functions tests

↓

Drug interaction studies

↓

Final recommendation

22.6 Remediation of Biological and Chemical Contaminants

Often medicinal herbs contain microbes that have potential toxicity. A wide range of species contains mycotoxins like nutmeg (*Myristica fragrans* Houtt), sweet pepper (*Capsicum* L. spp.), ginger (*Zingiber officinale*), turmeric (*Curcuma longa* L.), etc. Moulds are a type of fungus having multicellular filaments called *hyphae*. Moulds are present everywhere, and mould spores are quite common in household and workplace dust. If the moulds are consumed along with the human food in which they have grown, it will lead to various human and animal health hazards. It has also been observed that there are some species that can synthesize toxic secondary metabolites, named as mycotoxins including aflatoxins, ochratoxins, fumonisins, etc. Moulds could be found in leaves, flowers, rhizomes, roots, seeds, etc. Moulds are predominantly from two genera: *Penicillium* and *Aspergillus*.

Production of mycotoxins by moulds is dependent on different factors such as the presence of any fungicide or any other microbial agents, CO_2/O_2 ratio, humidity, amount of substrate present, etc. Some of the strategies by which plants try to cope up with mould growth and their associated mycotoxin production are the following:

1. Essential oil is being produced by some medicinal herbs that pose some antimicrobial properties that interfere with mould development and production of mycotoxin as well (Kosalec et al. 2009).
2. The occurrence of mould sometimes is specific. For example, *Aspergillus flavus* produces more aflatoxin B_1 on cereals as compared to other species (Kosalec et al. 2009).
3. Powdered herbal drugs have been considered as one of the common forms of commercial herbal drugs. Different herbal plant species are used to manufacture powdered herbal drugs. In a study conducted by Razak et al. (2009), it was found that fungal growth was inhibited by powdered mixed herbal drugs.
4. Pesticides are chemical substances that deter, incapacitate, kill, or otherwise discourage pests. It includes all of the following: herbicide (controlling herbs), insecticide (killing insects), fungicide, insect repellent, animal repellent, etc. On the other hand, it can also be potentially toxic to humans and other species. There are some compounds found naturally that also have some insecticidal properties such as pyrethrin extracted from the chrysanthemum (*Tanacetum cinerariifolium*). They are fat-soluble and easily degradable in humans. Moreover, like pyrethrins, they do not bioaccumulate in humans. Pyrethroids are slowly replacing organochlorines and organophosphates because of lower toxicity and higher insecticidal activity in mammals (Kosalec et al. 2009).

22.7 Conclusion and Future Perspective

Therapeutic efficiency of various herbal medicines and minimal adverse drug reactions have given the traditional medicinal system a strong boost in becoming an important part of alternative medicine. The wide acceptance of natural remedies and increasing the economic importance of herbal drugs has brought a rise in the popularity of herbal products and helped in the promotion of using nature as a remedial basket for mankind. It can be clearly said that a bright future awaits for herbal medicine in the global pharmaceutical market.

Since there has been the presence of various contaminants in the medicinal plants or the plant products, their proper use requires the assessment of these contaminants and various remediation strategies. Contaminants like microbes get easily accumulated either in the plant or in the plant products maybe during the preparation steps. Other contaminants like toxic elements are accumulated in the plants either from the soil in which they are growing or from the water they are irrigated with. There may be lots and lots of contaminants in the plant that would prove harmful to human consumption. So, their proper assessment and removal are highly demanded.

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Chapter 23
State-of-the-Art Technologies for Improving the Quality of Medicinal and Aromatic Plants

Aryadeep Roychoudhury and Rituparna Bhowmik

23.1 Introduction

Today over 50,000 plant species are utilized by us for their bioactive compounds (Chakraborty 2018). They may be correctly called "green chemical factories" for their huge variety of resources that supply food, feed, pharmaceuticals, aromatic compounds and industrial raw materials (Nogueira et al. 2018). Analgesics like morphine, cardiotonic like digoxin, anticancer drugs like vinblastine and paclitaxel, etc. are only a few of the wide diversity of medicines derived from plants (Nessler 1994). In the past 30 years, 25% of the newly discovered drugs were from plant resources, and about 50% of irreplaceable drugs were developed from the understanding of secondary metabolite biosynthesis.

Unfortunately, despite their various applications, medicinal plants were left out from the syndrome domestication when humans first transitioned from nomadic to settled life style. While the major food crops like maize, rice, wheat, soybean, etc. were domesticated and dynamically evolved over time to create more valuable plants from wild resources with immense increase in productivity, medicinal plants remained unperturbed (Doebley et al. 2006). Consequently, their supply of bioactive compounds is low compared to the plant biomass. With the improvement of civilization, our reliability on plant-derived pharmaceuticals is ever increasing, and often natural resources fall short to meet the excessive demands. This has also led to over-exploitation of wild plant resources driving many plant species towards extinction. Today, the plant breeders are trying to compensate for these shortages by modern biotechnological solutions that improve productivity of the medicinal and aromatic plants (MAPs).

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Traditionally, the pharmaceuticals, fragrances, food colours and other bioactive compounds are obtained from whole plants, which in some cases take 4–6 years to mature and accumulate very low concentrations of useful secondary metabolites (Zhang et al. 1996). The commercial production is also restricted by geographic and climatic factors. Convectional techniques of cultivation and plant breeding fall short to meet the requirement of these neglected wild-crafted plants. For a few of these bioactive compounds, plant tissue culture methods like micropropagation could serve as a substitute method besides traditional cultivation. The genetic reserves of valuable plants at the brink of extinction could also be conserved by cryopreservation and synthetic seed generation. Tissue culture also opens the opportunity to generate genetically transformed cell lines using *Agrobacterium*-mediated gene transfer. Stable cultures, with increased metabolite synthesis, which are independent of growth regulators, may be obtained using *Agrobacterium rhizogenes*-mediated hairy root generation (Giri and Narasu 2000). Bioreactors with optimized media, elicitors and precursors help to further scale up the commercial production.

Metabolic manipulation of medicinal plants like changing the flux for target molecule production, redirecting common precursor, compensating rate-limiting steps and compartmentalization is another biotechnological approach to increasing production. Antisense RNA or RNA interference (RNAi) techniques may also be used to block the pathways leading to undesirable products. The initial point of metabolic manipulation of medicinal and aromatic plants lies in the ability of cloning and expressing genes that may play a role in either catabolic or anabolic pathways (Capell et al. 1998; Capell et al. 2004). To counteract the reduction in productivity by feedback inhibition, compartmentalization of the biosynthetic product may be attempted. Genetic intervention that helps in developing storage tissues for the secondary metabolites and overexpression of the rate-limiting step enzymes could prove beneficial as well.

Techniques like next-generation sequencing (NGS) that deals with the whole genomic and transcriptome data could prove very useful in studying the yet newly discovered medicinal and aromatic plants (Zhao et al. 2018). The transcriptome data could be combined with proteomics and metabolomics to study and manipulate the pathways. DNA barcoding application of NGS could help in identification and proper classification at genus and species level (Techen et al. 2014). Targeted genome editing methods like ZFNs (zinc finger nucleases), TALENs (transcription activator-like effector nucleases) and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats-Cas9), followed by their analysis by NGS, may help to create a variety of new useful bioproducts (Pouvreau et al. 2018). The COSTREL (combinatorial supertransformation of transplastomic recipient lines) method based on the supertransformation of selected transplastomic lines may enhance the production (Fuentes et al. 2016). However, the CRISPR/Cas9 method of genome editing is better than COSTREL in terms of faster results. The quality of these bioactive medicinal and aromatic compounds may be assessed and standardized using the electronic nose (E-nose) technique. This chapter discusses all the modern approaches that may be considered for conserving the natural resources and improving the productivity of the medicinal and aromatic plants for use at an industrial level.

23.2 Methods for Conservation of Medicinal and Aromatic Plants

In the modern century, the medicinal and aromatic plants are industrialized, leading to an ever increasing demand. This exploitation may even endanger the plants in their native lands (Soltani Howyzeh et al. 2018). Environment plays a chief role in determining the quality and quantity of the metabolic products produced in the medicinal and aromatic plants (Ncube et al. 2012). Climate change, deforestation, fire, heavy metal stress and other environmental and anthropogenic factors cause a decline in natural population of these essential herbs in near future (Atanasov et al. 2015). Ex situ cultures provide an insurance policy against loss of genetic resources by extinction (Li and Pritchard 2009). Slow growth cultures may be maintained for a shorter duration of 1–15 years, while cryopreservation may provide long-term storage (Niazian 2019).

23.2.1 Cryopreservation by Vitrification

Cryopreservation is a long-term storage method, where plant parts like shoot tips, bulbs, seeds, rhizomes, etc. may be stored in liquid nitrogen at −196 °C. 0–20% of the shoot tips stored by cryopreservation method retains regeneration potential without intermediary callus stage (Sharma et al. 2017a, b). Five elite lines of *Hypericum perforatum* L. could be successfully cryopreserved via a root-based method (Zhao et al. 2018; Urbanová et al. 2006). Long-term cryopreservation protocols have also been developed for *Eruca sativa* Mill. (Xue et al. 2008) and *Dendrobium candidum* (Yin and Hong 2009).

Failure of cryopreservation may be due to two reasons: (i) formation of large ice crystals inside the cell, damaging the germplasm, and (ii) prefreezing dehydration leading to rise in solute concentrations to lethal levels. A method of cryopreservation to overcome these problems is vitrification (Kundu et al. 2018). The term “vitrification” may be defined as “the solidification of a liquid brought about not by crystallization but by an extreme elevation in viscosity during cooling” (Fahy et al. 1984). In this process, most of the freezable water is excluded before ultrarapid freezing by means of mechanical or osmotic dehydration. This has a higher recovery percentage and may even be applied to cold-susceptible plants. Some plants that show successful regeneration after vitrification are shown in Table 23.1.

23.2.2 Synthetic (Syn) Seeds

Synthetic seeds may be produced by alginate encapsulation of explants. This is one of the best strategies for ex situ conservation and micropropagation of medicinal and aromatic plants as it provides genetic stability and ease in handling and trans-

Table 23.1 Different medicinal plants that could be successfully stored by vitrification method and their regrowth potency

Scientific name	Explant	Regrowth (%)	References
Dioscorea deltoidea	ST	83	Mandal and Dixit-Sharma (2007)
Rauwolfia serpentina	NS	66	Ray and Bhattacharya (2008)
Astragalus membranaceus, *Gentiana macrophylla* pall. *and Eruca sativa* mill.	HR	82.9, 75.7 and 100	Xue et al. (2008)
Catharanthus roseus	ECa	88.9	Fatima et al. (2009)
Dioscorea bulbifera	Ca	60–70	Hong et al. (2009)
Dioscorea opposita	NS	77.1	Li et al. (2009)
Panax ginseng	AR	60	Oh et al. (2009)
Rubia akane	HR	95	Kim et al. (2009)
Hypericum perforatum	ST	71.3	Brunakova et al. (2011)
Kalopanax septemlobus	ECa	99.3	Kim et al. (2012)
Cleome rosea	AR	63.6	da Silva Cordeiro et al. (2015a, b)
Dioscorea opposita	NS	52	Zhao et al. (2016)
Petiveria alliacea	SE	12 SEs/SE	de Almeida et al. (2017)

AR Adventitious root, *Ca* callus, *ECa* embryogenic callus, *NS* nodal segment, *HR* hairy root, *SE* somatic embryo, *ST* shoot tip

portation with effective space utilization (Nyende et al. 2003). This process is particularly useful for plants like *Moringa oleifera* and *Camellia sinensis* which are hard to regenerate by conventional methods (Muslihatin et al. 2018). This method can serve as an alternative to cryopreservation in maintaining the germplasm of novel medicinal and aromatic plants which contain high level of secondary metabolites (Lata et al. 2009).

Encapsulation of early cotyledonary-stage embryo of *Arnebia euchroma* in 3% alginate and $Ca(NO_3)_2$ was reported to show 60.6% germination and about 72% survival rate (Manjkhola et al. 2005). *Gymnema sylvestre*, a medicinal plant known for its antidiabetic properties, showed 88.2% germination of syn seeds (Saeed et al. 2018), whereas 91% recovery was seen from encapsulated shoot tips of *Urginea altissima* (Baskaran et al. 2018).

23.2.3 Micropropagation

Micropropagation is a process of rapidly multiplying the novel stock plant, usually one with superior genetic properties. It may be done in the form of organogenesis (both direct and indirect) or in form of somatic embryogenesis. Somatic embryogenesis produces a bipolar structure in one step, compared to organogenesis which requires different growth regulators for shoot and root induction; thus somatic embryogenesis is more favourable (Shen et al. 2018). Efficiency of this process depends on plant genotype, size of the explant cultured, plant growth regulators used, age of explant and compositional and environmental factors of medium like temperature, light intensity, pH, carbon source, etc. (Nalawade and Tsay 2004).

This process has been successfully applied for *Carum copticum* L. (Niazian et al. 2017a), *Sapindus trifoliatus* (Asthana et al. 2017), *Swertia corymbosa* (Mahendran and Narmatha Bai 2017) and *Abutilon indicum* (Seth et al. 2016), which are important medicinal and aromatic plants. Silver nanoparticles soaked in *Ulva lactuca* extracts (UL-AgNPs) were successfully used by Mahendran et al. (2018) in rhizome explant cultures of *Gloriosa superba* L. using the process of micropropagation.

23.2.4 Suspension Culture

Obtaining sufficient secondary metabolites from fully grown plants is time-consuming (taking 4–6 years) and economically not feasible due to the low productivity (Zhang et al. 1996). Medicinal plant cell suspension culture (MPCSC) may provide an alternative to traditional and chemical synthesis for medicinal and aromatic plant extracts (Yue et al. 2016). MPCSC deals with free cells or small flocks of cells from medicinal plants grown in a liquid broth with suitable media composition for mass production of particular secondary metabolite (Moscatiello et al. 2013).

MPCSC is advantageous over traditional cultivation as it is not restricted to a particular season or geographic location. It is a stable platform, and the metabolites meet same quality parameter levels. It has an upper hand over solid callus culture in providing homogeneity and higher efficiency as the raw materials are equally available to every cell (Xu et al. 2011a, b). This platform is not only good for mass production but also for research to investigate the physiology of plant cells and eliciting the biochemical pathways of metabolite production and modify them to our advantage by metabolic engineering.

Anticancer medicine paclitaxel (Taxol) has been obtained from *Taxus* species by cell culture methods (Onrubia et al. 2012). Suspension culture of *Taxus chinensis* cells shows better accumulation of taxol by MPCSC (Malik et al. 2011). Antitumour drug saponin, from *Panax notoginseng* (Zhang et al. 1996), and resveratrol from *Vitis vinifera* (Upadhyay et al. 2008) have also been successfully grown in suspension culture. A number of secondary metabolites like vincristine, vinblastine, vindoline, cathranthine, etc. with medicinal properties have been produced in large scale from *Catharanthus roseus* cell suspension cultures (Zhao et al. 2001). Due to its economic value, *Mentha pulegium* L. has been grown in suspension culture for production of menthol (Darvishi et al. 2016).

23.2.5 Biotransformation

MPCSC may also be used for biotransformation, which includes transformation of exogenously supplied compounds to materials of medicinal potential. The process of bioconversion mainly utilizes the enzymatic ability of the plant cells that makes long chemical synthesis easier (Giri et al. 2001). Suspension culture of *Eucalyptus perriniana* can carry out conversion of aroma compounds like carvacrol, eugenol and thymol into glycosides (Shimoda et al. 2006). Hyoscyamine could be converted to scopolamine by cultured transgenic tobacco cell, while *Catharanthus roseus* cell cultures have been shown to glycosylate capsaicin and 8-nordihydrocapsaicin (Shimoda et al. 2007). A number of new compounds like 1β-hydroxyl desacetylcinobufagin from cultures of *Catharanthus roseus* and *Platycodon grandiflorum* and 2β,3β,21-trihydroxy-4-pregnen-20-one, 2β,3α,2-trihydroxy-4-pregnen-20-one and 3β,21-dihydroxy-5α-pregnan-20-one-3β-O-β-glucoside from suspension culture of *Digitalis lanata* have been investigated (Ye et al. 2003; de Padua et al. 2012).

23.3 Strategies to Increase Production

23.3.1 Selection of Superior Cell Lines

In this selection process, large number of cultured cells is exposed to a toxic or environmental stressor in increasing amounts. Only those with the suitable mutation to survive under such conditions are selected, and they often show resistance to the inhibitor and increased product accumulation (Ramachandra Rao and Ravishankar 2002). HPLC (high-performance liquid chromatography) and RIA (radioimmunoassay) techniques may be used to screen better cell lines to start the culture (Chen and Chen 2000). One of the *Euphorbia milii* cell lines that accumulated about sevenfold higher levels of anthocyanin was selected by cell cloning methods (Stafford 2002). *Vitis vinifera* lines with 2.3- to 4.0-fold increased anthocyanin production could also be isolated (Curtin et al. 2003; Yue et al. 2014) (Fig. 23.1).

23.3.2 Precursor Feeding

This strategy is based on the concept that when an intermediate metabolite is fed to a growing cell culture, it increases the accumulation of the final product (Dörnenburg and Knorr 1995). Table 23.2 provides some examples of positive increase in metabolite concentration by precursor feeding.

One of the limitations of precursor feeding is that certain levels of the precursor could be toxic to the cultured cells and lead to decrease in metabolite concentration rather than showing a positive effect. Negative feedback inhibition due to precursor

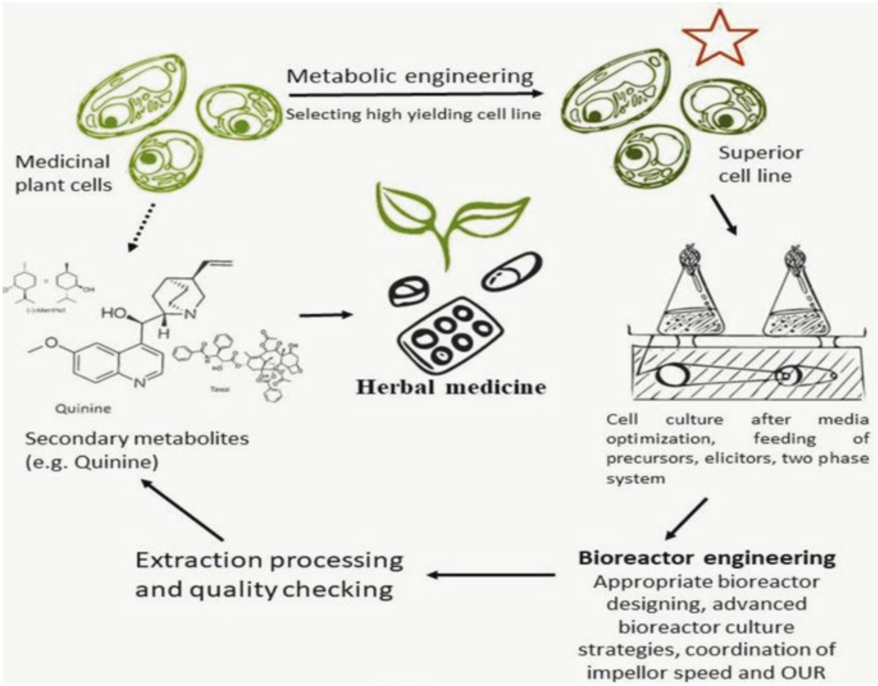

Fig. 23.1 Strategies to increase secondary metabolite production by MPCSC

accumulation could also reduce yield. Thus, screening the precursors for toxicity and determining their ideal concentration is very critical to the success of this technique (Yue et al. 2016).

23.3.3 Elicitors

Plants are believed to synthesize the secondary metabolites in nature, as a part of their defence mechanism against pathogens. Elicitors are the extracellular signalling chemicals, usually similar to the compounds produced by the pathogens, which trigger the plant defence responses and phytoalexin synthesis. Biotic elicitors such as bacteria, yeast, fungal polysaccharides, etc. may have a positive role in secondary metabolite accumulation. Reactive oxygen species, nitric oxide and jasmonic acid are few of the signal molecules that help elicit production of pharmaceutical compounds. Heavy metal ions, osmotic tress, ultraviolet rays and heat shock/cold shock are some of the abiotic elicitors known so far (Sharma and Shahzad 2013; Wang and Wu 2013).

Root colonization of *Centella asiatica* by *Piriformospora indica* has been shown to enhance asiaticoside production. Fungi isolated from *Piqueria trinervia* was used to induce antifungal monoterpene production in *Piqueria trinervia* (Saad et al. 2000). Bioinoculants such as growth-promoting bacteria and arbuscular mycorrhi-

Table 23.2 Some precursors and their roles in increasing production

Cell cultures	Product	Precursor	Improvement	Reference
Taxus chinensis	Paclitaxel	Phenylalanine and glycine	2.0-fold increase	Luo and He (2004)
Cistanche deserticola	Phenylethanoid glycosides (PeG)	Phenylalanine	Enhancement	Ouyang et al. (2005)
Pogostemon cablin	Patchouli alcohol	Cis-Farnesol	Increase from 19.5 to 25.5 mg/L	Bunrathep et al. (2006)
Cistanche Salsa	Phenylethanoid glycosides	Tyrosine, phenylalanine, caffeic acid and cucumber juice	Enhancement	Liu et al. (2007a, b)
Vitis vinifera	Anthocyanin content	Phenylalanine and methyl jasmonate	4.6- and 6.4-fold increase	Qu et al. (2011)
Larrea divaricata	Phenolic compounds	L-phenylalanine, cinnamic acid, ferulic acid and sinapic acid	Increased to varying degrees	Palacio et al. (2012)

zal fungi could enhance different medicinal and aromatic compounds. Bioinoculation with *Pseudomonas monteilii*, *Cronobacter dublinensis* and *Bacillus* spp. (in sequence) was shown to improve essential oil yield from *Ocimum basilicum* by 45–56% (Singh et al. 2013).

Plant growth-promoting rhizobacteria (PGPR) can elicit the production of secondary metabolites in *Mentha* × *piperita* plants. Similar increase in total phenol and monoterpene content may be seen on application of salicylic acid and methyl jasmonate which are important plant hormones needed in several stress-related signalling. Combination of PGPR and the phytohormones increases the efficiency of accumulation of aromatic compounds by *Mentha* × *piperita* (del Rosario Cappellari et al. 2020). Understanding the signal transduction and related gene expression mechanism of the elicitors would take us a step ahead in metabolic engineering of the cell lines for increased production (Belch et al. 2012; Mustafa et al. 2009; Nims et al. 2006).

23.3.4 Two-Phase Systems

Generally, the process of synthesis and accumulation of the secondary metabolites in plants takes place in different compartments. In cultured cells, the feedback inhibition due to the accumulated product often leads to reduction in yield. To overcome this problem, a two-phase system may be used, where the extracellular product is accumulated in a second, generally non-polar phase which helps to avoid the feedback inhibition (Collins-Pavao et al. 1996; Roberts 2007). It also makes product collection easier. Two-phase systems may also be used to enhance metabolite release or release the products stored within the cells.

Anthraquinone production in a *Morinda citrifolia* two-phase cell culture showed 2.0-fold higher product accumulation (Bassetti and Tramper 1995). Choi et al.

(2001), Raval et al. (2003) and Yamada et al. (2002) showed that the production of azadirachtin-like limonoids, β-thujaplicin and taxol could be increased by this system. Adsorbents like activated charcoal, cork tissues, liquid paraffin and Amberlite XAD-7HP resin have been used to accumulate these secondary metabolites and avoid feedback inhibition (Cai et al. 2012a, b; Zare et al. 2010). Taxol production from *Taxus baccata* could be increased by 2.2-fold using activated charcoal as adsorbent (Kajani et al. 2010). Addition of cork tissues for adsorbing sophoraflavanone G from suspension cultures of *Sophora flavescens* was shown by Chen et al. (2003); most of the product could later be purified from the cork tissues. Ajmalicine produced by *Catharanthus roseus* cultures could be effectively removed by Amberlite XAD-7HP resin, increasing the overall production of ajmalicine (Wong et al. 2004).

23.3.5 Bioreactors

Farm culture is not sufficient to meet the demand of plant-derived secondary materials because plant tissues making these metabolites are really slow-growing. These plants have never been domesticated and have low germination rate in addition to unknown ecological requisites (Canter et al. 2005). Use of in vitro systems for production of secondary metabolites in secondary metabolite synthesis has a number of advantages like faster recovery and better control on cellular environment along with easier and more economical recovery of product (Máthé et al. 2015). In vitro methods like suspension culture, micropropagation and hairy roots are mostly used for this purpose (Weathers et al. 2010). However, when it comes to large-scale production of valuable pharmaceuticals and aromatic compounds to meet commercial demands, bioreactors are the most eco-sustainable alternative (Werner et al. 2018). Bioreactors are constantly operating, usually closed, automated liquid culture systems which act like biological factories for producing high quality of secondary metabolites in large quantity (Máthé et al. 2015).

23.3.6 Co-Culture in Bioreactors

Bioreactors often make use of elicitors and co-culture strategies to improve production. Co-culture is the simultaneous cultivation of two related or unrelated species which affects growth or metabolism of each other. Often the co-culture of two hairy roots from different species, or suspension of a pre-grown cell culture in hairy root culture of a different species, leads to increased secondary metabolite synthesis (Mohagheghzadeh et al. 2008). Biosynthesis of monoterpenes in bioreactor cultivated microshoots of *Rhododendron tomentosum* was affected by the use of biotic and abiotic elicitors like nickel/copper salts, jasmonates, ethanol extract of aphid, chitosan and ergosterol (Jesionek et al. 2018). Higher production of ginsenoside compounds and caffeic acid has been observed in bioreactors with co-cultures of adventitious roots of *Panax ginseng* and *Echinacea purpurea* (Xue et al. 2008).

23.3.7 Medicinal Plant Cell Suspension Culture

MPCSC in bioreactors provides a large scale-up for obtaining valuable natural compounds from medicinal and aromatic plants in industries (Rodríguez-Monroy and Galindo 1999). Some drawbacks faced in case of culturing plant cells using bioreactors in comparison to microbial cells are less stable production rate, hindered growth rate and clumping of cells (Dörnenburg and Knorr 1995). While reducing the impellor speed can prevent any shearing strain on sensitive plant cells, gas bubbles are not effectively dispersed in the entire tank.

Compared to microorganisms like bacteria and fungi, plant cells often have less oxygen requirement due to their slower metabolic rates. High oxygen rates may even prove to be toxic and detrimental to the culture, and higher impellor speeds may not only damage cells but also strip volatile nutrients like CO_2 from the medium (Eibl and Eibl 2008). Some high-yielding cell lines do not continuously produce the products. Homogeneity of the cells and media needs to be essentially maintained in large cultures with high cell concentrations.

Pneumatically agitated bioreactors are perfect for moderate cell concentrations. Both chemostats and turbidostats may be used depending on the optimal culture parameters. There are different kinds of bioreactors used in plant tissue culture techniques like STR (stirred tank reactors), ALR (airlift reactors), gas-phase reactors, photobioreactors, etc. The choice of bioreactors depends on the starting materials and environmental conditions to be maintained. Different types of disposable bioreactors are also available which are much cheaper than conventional steel tanks (Weathers et al. 2010). The superiority of bioreactors over flask culture in production of phenolic acids like cinnamic, salicylic and caffeic acids and stilbenoids in *Scrophularia striata* has been established.

23.4 Different Types of Bioreactors and Their Use

Today, there are various types of bioreactors which are extensively used in microbial or mammalian cell cultures. With some modifications, they can be put to use in MPCSC as well for producing plant secondary metabolites (Eibl and Eibl 2008). The choice of suitable bioreactor depends on suitable oxygen supply, low shearing and sufficient supply of media components, with homogeneous distribution and product removal rates (Eibl and Eibl 2002, 2008).

23.4.1 Stirred Tank Reactors

One of the commonly used bioreactors is STR (Fig. 23.2). It is advantageous for its large capacity with facilitates for large-scale production. Impellors help in good oxygen mass transfer and good mixing of media. It may be used for highly viscous cell cultures (Huang and McDonald 2009). Prakash and Srivastava (2007) successfully established feasible industrial scale *Azadirachta indica* suspension culture in STR for production of azadirachtin. *Papaver somniferum* could be cultured in STR along with a plant fungi used as a bioinoculant to improve production. High concentration of sanguinarine, up to 300 mg/L under optimal condition, was achieved making it commercially applicable (Park et al. 1992). A 27% increase in productivity of podophyllotoxin was seen when high-yielding cell line of *Podophyllum hexandrum* was grown using bioreactor under optimal conditions in place of shake flask cultivation (Chattopadhyay et al. 2002).

However, STRs also have some disadvantages. They have high shearing force near the impellor and are very expensive to construct and maintain. There may be heating during operation (which may kill the sensitive cells), high energy requirement for mechanical agitation (especially in case of viscous cultures) and a risk of contamination with mechanical seal.

23.4.2 Bubble Column Bioreactors

Bubble column bioreactors (Fig. 23.3) are favourable for plant cells and easy to manipulate and scale up. They are also economically feasible and generate lower shearing strain on the cultured cells. Problems such as improper oxygen distribu-

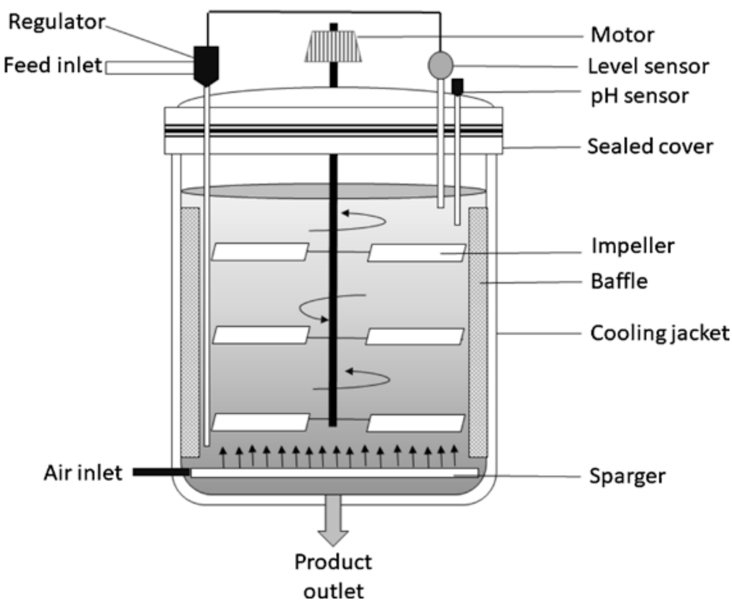

Fig. 23.2 Stirred tank reactor: The media is constantly stirred using the impellors which ensures homogenous distribution of media and cells; the level and pH sensors provide feedback control to maintain ideal conditions. Air is evenly bubbled through the sparger. The final product may be removed through pipes connected at the bottom

tion, foaming and imperfect mixing in high-density cultures are generally rectifiable (Smart and Fowler 1984).

Brown alga *Laminaria saccharina* exists in form of microscopic filaments in its gametophytic phase of life cycle. A novel cell line from this phase was to be isolated from suspension culture of *Laminaria saccharina* and grown successfully in illuminated bubble column reactor at 13 °C with carbon dioxide from air serving as the only source of carbon (Zhi and Rorrer 1996).

23.4.3 Airlift or Modified Airlift Bioreactors (ALR)

There are two types of airlift reactors, one with internal circulation using draft tube (Fig. 23.4) and the other with external circulation. This type of bioreactors provides good oxygen supply without the use of impellors. Periodically submerged airlift bioreactor or PSAB (2 L) was used by Yuan et al. (2004) to culture *Saussurea medusa* cell lines, and the total flavonoid production was investigated. The use of PSAB was proved beneficial to cell growth and led to a flavonoid concentration of 501 mg/L under optimal conditions. To a large extent, ALRs helped to improve the complication of reduced oxygen mass transfer faced in case of STRs.

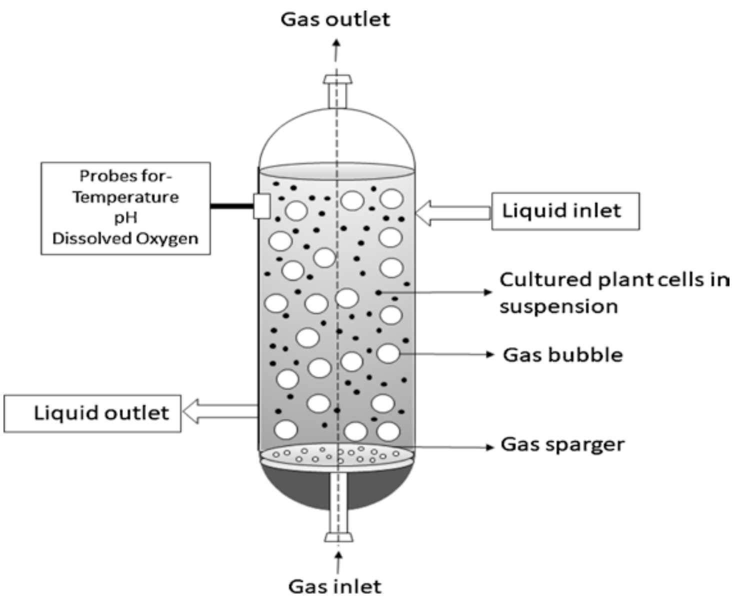

Fig. 23.3 Bubble column reactor: air is sparged from the bottom to agitate the media, impellors are absent, and probes are provided to check the culture conditions

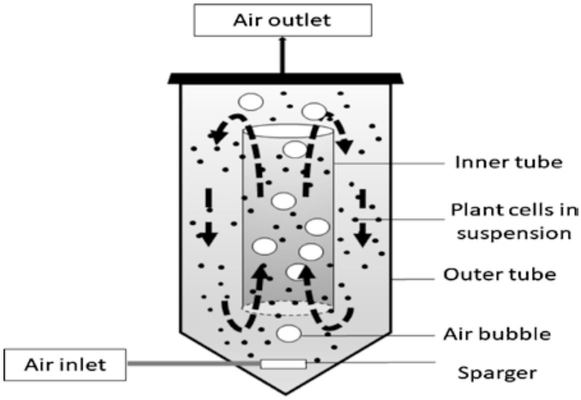

Fig. 23.4 Internal circulation ALR. Media circulates through the inner draft tube and the outer tube for good mixing and aeration

23.5 Genetic Manipulation of Medicinal and Aromatic Plants to Improve Yield

23.5.1 Agrobacterium Tumefaciens-Mediated Gene Transfer

Transgenic attempts for increasing yield and quality of specific herbal pharmaceuticals and aromatic compounds involve targeting the rate-limiting step by changing the expression of the key structural gene. Overexpression of this gene product or blocking of its competitive pathway may change the flux rate towards downstream products (Verpoorte et al. 1999; Gomez-Galera et al. 2007). Overexpressing the involved genes in biosynthetic pathways through *Agrobacterium*-mediated gene transfer could be one such approach. Two genes, tryptophan decarboxylase and strictosidine synthase, originating in *Catharanthus roseus* were transformed to *Bacopa monnieri* using *Agrobacterium tumefaciens*. This led to 25-fold increase in content of tryptophan in plant extract and an increase in content of terpenoid indole alkaloid metabolite (Sharma et al. 2017a, b; 2018). While *Agrobacterium* is a good vehicle for gene transfer, technical problems such as genotype dependency, recalcitrance to transformation and inherent problems in tissue culture procedure hinder its success. *In planta Agrobacterium*-mediated transfer, which bypasses the tissue culture procedure, is a good alternative.

23.5.2 Agrobacterium rhizogenes-Mediated Hairy Roots

Agrobacterium rhizogenes is an important tool in increasing pharmaceutical production from medicinal plants by inducing hairy roots, which are vehicles for metabolic engineering. These hairy root cultures of neoplastic tissues are well-known for their high growth rate, genetic stability, metabolic stability and growth in hormone-free medium over long term in in vitro cultures (Srivastava and Srivastava 2007; Grzegorczyk-Karolak et al. 2018). The Ri plasmid carrying *rol* genes, namely, *rol A*, *rol B*, *rol C* and *rol D*, are crucial for this root induction (Costantino et al. 1994; Nilsson and Olsson 1997). The efficiency of root induction by *A. rhizogenes* depends on the strain used; until now strains like A4, ATCC15834, LBA9402, MAFF03–01724, R-1601, R-1000 and TR105 have been applied in hairy root induction in many medicinal and aromatic herbs (Li et al. 2017; Thwe et al. 2016; Setamam et al. 2014). Nicotine can be directly produced from hairy root cultures of *Nicotiana tabacum*, and it also provides a massive scope for genetic engineering (Qian et al. 2005). *Trigonella foenum-graecum* of two genotypes, "Boshruyeh" and "Hamadan", were transformed using *A. rhizogenes* and showed greater efficiency of diosgenin production. However, the results also indicated that using a promising strain, with high yield like the Boshruyeh, led to better results (Zolfaghari et al. 2020).

Similar to *A. tumefaciens*-mediated transformation, different parameters like bacterial optical density (Pandey et al. 2013), in vitro regeneration parameters (plant growth regulators, additives, etc.) (Weremczuk-Jezyna et al. 2017), *Agrobacterium* killing antibiotics (Sivanandhan et al. 2016), inoculation duration (Rajesh et al. 2013), plant parameters (genotype, explants used, etc.) and use of chemical stimulants (acetosyringone, surfactant, etc.) (Fernando et al. 2016) should also be optimized for successful induction of hairy roots by *A. rhizogenes*. The AtPAP1 transcription factor has been overexpressed in *Leonurus sibiricus* through this method of hairy root induction leading to an increase in levels of five phenolic acids like chlorogenic acid, ferulic acid, neochlorogenic acid, caffeic acid and *p*-coumaric acid in the hairy root cultures (Sitarek et al. 2018).

23.5.3 Genome Editing Methods

TILLING (targeting induced local lesion in genomes) is a method of reverse genetic screening. It is used to evaluate the effect or function of an isogene which is synthesized by mutation of wild-type genes. These mutations may be natural or artificially targeted using mutagens. This helps us to compare the available polymorphic population with natural polymorphism without involving transgenic approaches (Varshney et al. 2009). This helps to avoid the ethical issues associated with transgenic plants and may be used to improve the productivity of specific bioactive metabolite.

Three targeted genome editing techniques are currently available, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and CRISPR/Cas9. These may be used to engineer specific portions of the DNA. In combination with the high-throughput technique like NGS, it may prove to be powerful tool for improving productivity. Three of these techniques have a DNA-recognizing domain and an endonuclease domain that helps to cleave and edit the recognized sequence (Gaj et al. 2013). ZFNs function as dimers of zinc-mediated DNA-binding finger and a Fok1 endonuclease. Recognition of a long stretch of specific DNA is possible by placing multiple fingers in sequence (Beerli and Barbas 3rd 2002; Segal et al. 2003). However, its use is limited by the low specificity and high toxicity of ZFNs (Ramirez et al. 2008; Cornu et al. 2008). In case of TALENS, DNA is recognized by the transcription activator like proteins of *Xanthomonas* sp., and fused Fok1 cleaves the recognized sequence (Li et al. 2011; Miller et al. 2011; Mahfouz et al. 2011). This recognizes 33–35 bp stretches of specific DNA and may be modified to recognize our target segment. Unlike ZFNs, TALENS are non-toxic and easier to engineer.

Clustered regularly interspaced palindromic repeat (CRISPR)/Cas9 is another powerful genome editing tool that was discovered 2 years after TALENs. It was initially discovered from bacteria, which used it to cleave the viral genome of bacteriophage, safeguarding itself from bacteriophage. It makes use of a single guide RNA (gRNA) which recognizes the target sequence and a nuclease Cas9 that makes a double-stranded cut. These cuts may be repaired by non-homologous end joining (NHEJ) which often introduces a mutation. Specific transgene with suitable fringing sequences may also be added to be incorporated in the cut sites (Zhou et al. 2018). This approach is very powerful as it may act on multiple parts of the genome and knock out every copy of the target segment. NHEJ TALENs make use of a similar approach.

CRISPR/Cas9 system has been used to knock out *rosmarinic acid synthase* gene (*SmRAS*) in the medicinal plant *Salvia miltiorrhiza* (Zhou et al. 2018). This helped to improve the production of salvianic acid A sodium (SAAS) and sodium salt form of DHPL. Li et al. (2017) applied CRISPR/Cas9 on *A. rhizogenes*-mediated knockout of *SmCPS1* gene hairy root cultures which is a vital gene of tanshinone biosynthesis pathway. Homozygous knockouts did not synthesize cryptotanshinone, tanshinone IIA and tanshinone I diterpenoids; however, the phenolic pathway remained intact. We may one day achieve the minimal plant cell genome, with no non-essential genes and pathways through CRISPR/Cas9 system, which would reduce the metabolite loss in all the competitive pathways and increase production of our target secondary metabolite (Noman et al. 2016).

23.5.4 RNA Interference (RNAi)

Antisense RNA is often used to knock down gene expression; however, it is not full proof in case of multigene families (Park et al. 2002, 2003). In RNAi technology, double-stranded RNA corresponding to our gene of interest is used to block the expression of that gene. The siRNAs (small interfering RNAs) are recognized by the RNA-induced silencing complex (RISC) which degrades one strand and guides the other strand of the dsRNA to its complementary RNA followed by its degradation. These may be expressed in all the tissues across all developmental stages to effectively block expression of specific enzyme even when they are encoded by multigene family.

This has been used to block the activity of codeinone reductase involved in the biosynthesis of morphine (an alkaloid) (Larkin et al. 2007). It was used by Inui et al. (2007) to knock down berberine bridge enzyme (BBE) in California poppy *(Eschscholzia californica)* cells, which led to the accumulation of (S)-reticuline, an important intermediate in isoquinoline alkaloid biosynthesis. RNAi nowadays is one of the prime technologies used for breeding of aromatic and medicinal plants, where traditional breeding attempts are not successful (Allen et al. 2004).

23.5.5 Polyploidy

Polyploids are generally characterized by superior vigour and performance in comparison to their diploid relatives, which are often beneficial in transgenic production (Bahmankar et al. 2017). It has also been seen that secondary metabolite content in polyploid plants is more than that in their diploid counterparts (Bahmankar et al. 2017), which led to the idea that artificial polyploidy induction may lead to the improvement of medicinal and aromatic plants (Salma et al. 2017). Colchicine, trifluralin and oryzalin are some of the frequently used antimitotic agents; however, the parameters such as minimum effective concentration of applied antimitotic agent need to be carefully optimized (Corrêa et al. 2016; Salma et al. 2017).

Polyploidy induction in *Pfaffia glomerata* led to a 31% increase in 20-hydroxyecdysone production in comparison to diploid counterparts (Corrêa et al. 2016). Colchicine is so far the most common antimitotic agent used in medicinal plants which showed polyploidy induction rates as high as 54.6% (Wang et al. 2017) in case of buckwheat (*Fagopyrum tataricum* L.). Bhringraj (*Eclipta alba* L.) polyploids developed using colchicine showed about 30.6% polyploidy induction efficiency and above 96.7% survival rate with increased production of secondary metabolite, wedelolactone (Kundu et al. 2018).

Polyploidization when combined with hairy root cultures could turn out to be a remarkable strategy for large-scale production of secondary metabolites in plants. Polyploids were induced in hairy root cultures of *Artemisia annua* L. which produces artemisnin, an antimalarial sesquiterpene lactone. While there were major differences in the growth and development, this attempt was successful in increasing artemisinin yields by 2–5 times (De Larry Jesus 2003).

23.5.6 Transformation of Regulatory Genes (Mostly Transcription Factors) for Complex Pathways

Biosynthetic pathways are very complex, and there has been inadequate success on overexpression of a single gene, which led to the thought of manipulating the regulatory genes that act as master switches for the entire pathway or its parts (Memelink et al. 2000). These regulatory genes are often transcription factors, viz., Myb-like (myeloblastosis-like) and bHLH (basic helix-loop-helix), which increase secondary metabolite production. Anthocyanin production could be turned on in tobacco and *Arabidopsis* using maize R (bHLH) and C1 (Myb-like) controlling genes (Lloyd et al. 1992). Overexpression of Lc and C1 transcription factors of maize in tomato resulted in a 20-fold increase in total flavonol production (Verhoeyen et al. 2002). Similarly, in *C. roseus*, two transcription factor genes, octadecanoid-responsive *Catharanthus* AP2-domain proteins ORCA2 and ORCA3, were cloned, which control a number of genes in the biosynthetic pathway of terpenoid indole alkaloid (TIA). ORCA3 is a master regulator whose overexpression led to increase in production of TIA (van der Fits and Memelink 2000). Dutta et al. (2007) suggested that a C-repeat binding TF (CrCbf) from *C. roseus* may have a regulatory role in TIA biosynthesis pathway during biotic stress. VlmybA2 (identified from grape) was overexpressed in tobacco resulting in anthocyanin production in tobacco (Geekiyanage et al. 2007). These regulatory genes not only have limited roles in complex biosynthetic pathways, but they also play important roles in controlling initiation and formation of storage tissues (e.g., glandular trichomes). Overexpression of two transcription factors, MIXTA and Myb in tobacco, has been shown to increase the number of supernumerary trichomes on the stems, leaves and cotyledons of the plant (Liu et al. 2005). This shows that regulation of the transcription factor genes could also bring about an increase in the secondary metabolite production by increasing the availability of storage tissues.

23.5.7 New Tools in Dissecting Metabolic Pathway

Mapping the unknown genes involved in biosynthetic pathway is now possible by the advancements made in molecular biology. These tools under systems biology include genomics, proteomics, transcriptomics and metabolomics (Chakraborty 2018). One such tool is cDNA-AFLP (cDNA-amplified fragment length polymorphism). It functions without any previously known sequence information making use of full-length cDNA. The use of full-length cDNA makes it better than the microarray analysis that needs pre-known sequences of the genes involved in particular secondary metabolite synthesis. In *C. roseus*, this method helped in deciphering the function of genes for nine metabolites that participate in the biosynthetic pathway of TIA compounds (Goossens et al. 2003).

Another tool, differential display analysis of gene expression, could be used to link metabolome profiling with the transcriptome profiling. This helped in dissecting biosynthetic pathway of taxol in *Taxus cuspidata* and anthocyanin in *Perilla frutescens* (Goossens and Rischer 2007). LC-TOF-MS offers highly accurate mass measurement for small molecules, charge state identification of multiple charged ions and greater differentiation of isobaric species. It can help detect nontarget compounds to extreme accuracy due to the following characteristics:

- It can collect data over a wide range of masses without hampering its sensitivity, thus achieving full spectral sensitivity.
- It can detect the interferences away from the target signal with very high resolution.
- It can detect elemental composition with its mass measurement accuracy (Ferrer and Thurman 2003).

QTL (quantitative trait locus) analysis of the relative differences of untargeted metabolites, measured by using LC-TOF-MS (liquid chromatography-time of flight-mass spectrometry), helped in uncovering new biosynthetic steps in *Arabidopsis* (Keurentjes et al. 2006).

23.5.8 Metabolic Engineering

The genetic network involved in the production of secondary metabolites is a puzzle, where the pieces need to be placed correctly to get the perfect picture (Goossens et al. 2003). The genes involved in the DXP (1-deoxy-D-xylulose 5-phosphate) pathway enzymes have been discovered, but their importance in regulating the metabolic flux is not yet known (Liu et al. 2005). The transcriptional factors that regulate biosynthesis of secondary metabolites in plants have been listed in recent years. For example, activators and repressors have been identified for *C. roseus* which controls genes involved in terpenoid indole alkaloid (TIA) biosynthesis (Liu et al. 2007a, b) that has been utilized in increasing secondary metabolite production.

23.5.9 Multigene Manipulation

The biosynthetic pathway of secondary metabolite may be in the form of a series of coordinated reactions, or in the form of a complex network of pathways controlled by multiple genes. Thus, desired metabolic alteration may be obtained by manipulating multiple genes at multiple regulating points. In the carotenoid biosynthetic pathway of rice, manipulation of the first committed enzyme in biosynthetic pathway, phytoene synthase, led to increase in phytoene accumulation; however, the ultimate goal, which was to increase carotenoid, was not achieved (Ye et al. 2003). Introduction of the entire carotenoid biosynthetic pathway by introducing additional genes, like *lycopene β-cyclase* (*LCY-β*) and *carotene desaturase* (*crt1*), resulted in transgenic seeds able to accumulate beta-carotene (Ye et al. 2003).

23.5.10 Complete Biosynthetic Pathway Engineering Using COSTREL Method

It is possible to transfer the entire biosynthetic pathway of valuable secondary metabolites from its source plant to a target crop which has higher biomass productivity like tobacco. Tobacco is an ideal vehicle for large-scale production of secondary metabolites by COSTREL method. The advantages of using chloroplast genome for metabolic engineering involve higher transplastome expression levels, multigene transformation in the form of operons, maternal inheritance and scope of supertransformation, where nuclear transformation can be performed on previously plastid-transformed lines. This helps immensely in increasing the target metabolite production (Fuentes et al. 2016, 2018). This was applied in case of artemisinin production, where chloroplast transformation of multiple genes involved in the artemisinin production, like genes encoding ADS (amorpha-4,11-diene synthase), CYP (cytochrome P450) and CPR (cytochrome P450 reductase) enzymes, was carried out in tobacco plant through gene gun method. The accessory genes which help to increase the production of artemisinin were transformed to the selected high productivity transplastomic lines. By this method, artemisinin production was significantly increased in supertransformed tobacco (Fuentes et al. 2016).

23.5.11 Redirecting Common Precursor

Increase in metabolic flux may also be achieved by redirecting the common precursor via blocking the competitive pathways or overexpressing the involved gene. This strategy was successfully applied for increasing the concentration of paclitaxel, one of the most effective anticancer drugs. Transgenic approach helped to transform the previously low-yielding *Taxus brevifolia* into commercially viable high-yielding form. Transformation of *taxadiene synthase* gene, which catalyses the conversion of geranylgeranyl pyrophosphate (GGPP) into taxadiene, helped to channelize the precursor in carotenoid pathway of tomato for increasing taxadiene production. This led to 660- to 20,000-fold increase in taxadiene levels in tomatoes (Kovacs et al. 2007).

23.5.12 Compartmentalization

Often the accumulation of the final product of biosynthetic pathway initiates a negative feedback loop which decreases the productivity. One strategy to overcome this is compartmentalization of the target metabolite to particular cellular organelle by single gene mutation. In plants, there are two pathways for terpenoid synthesis: the methylerythritol 4-phosphate (MEP) pathway occurring in plastid and mevalonate pathway occurring in cytosol. It has been seen that target gene overexpression in either of these compartments leads to a transport of sufficient pool of common precursors in the right direction, resulting in almost a sevenfold increase in total terpenoid biosynthesis. Results indicate up to a thousandfold increase in sesquiterpene concentration and 10- to 30-fold increase in monoterpene-like limonene compounds in transgenic tobacco (Wu et al. 2006).

At times, the plants may produce the secondary metabolite in sufficient amounts, but it may lack a proper subcellular compartment to store the same (Verpoorte et al. 1999). Li and Eck isolated the genes that are involved in subcellular compartment formation and characterized them. The cauliflowers expressing *Or* gene produced orange curd with increased β-carotene levels. On analysis, it was revealed that *Or*-expressing transgenic cauliflowers contain large membranous chromoplasts leading to this observation (Li and Eck 2007). Similar carotenoid sequestering chromoplasts are also found in granule-bound starch synthase (GBSS)-expressing transgenic potato that has increased carotenoid levels. These studies confirm that the presence of metabolic sinks for storing secondary metabolites helps in improving metabolite concentration (Li and Eck 2007).

23.5.13 Stress Tolerance

About 40% of the of the world's irrigated land is salt-affected. Salt stress harms the normal physiological process of plants reducing plant growth and secondary metabolite production (Tester and Davenport 2003). One solution to this problem could be organic farming, where soil microbes help to strengthen the plants against salt stress. Mycorrhizal fungi (AMF, arbuscular mycorrhizal fungi) form association with plant species like halophytes, xerophytes and glycophytes and often help in alleviating the salt stress (Al-Karaki and Hammad 2001; Feng et al. 2002; Talaat and Shawky 2011). Bharti et al. (2013) conducted a study on *Mentha arvensis* cv. Kosi, (a commercial source of menthol), whose productivity is reduced in presence of salt stress. The best organic farming solution for *Mentha* was found to be the use of two AMF *Glomus mosseae* and *Glomus intraradices*, which helped to improve its stress tolerance and product yield.

Most stresses in plants be it salt stress, frost stress, etc. finally affect the water availability to the plant cells. Thus, inducing drought tolerance in MAP could be a good way to make the plant sturdy against most other stresses as well. Drought stress often reduces the productivity of plants in terms of its secondary metabolites. Several attempts have been made to alleviate drought stress; some such strategies are using salicylic acid (Chen et al. 2014) and mycorrhizal fungus (Sanchez-Blanco et al. 2004). Salicylic acid is a signal molecule in plants that helps to improve plant resistances. It may perform its action by associating with the other signal molecules, bringing about a cascade which changes the levels of critical enzymes in metabolic network associated with plant stress (Singh 2003). Abbaszadeh et al. (2020) investigated the effect of salicylic acid on *Rosmarinus officinalis*. Most appropriate level of stress and salicylic acid was found to be 60% field capacity (FC) at 1 or 2 mM salicylic acid, which led to improved production of essential oil under drought stress conditions.

23.5.14 Systems Biology Approaches

For an effective breeding program, we need to assess the genetic variety of the yet unmapped medicinal and aromatic plants in nature (Niazian et al. 2017c). Molecular markers for medicinal and aromatic plants based on DNA like RAPD (random amplification of polymorphic DNA), RFLP (restriction fragment length polymorphism) and ISSR (inter-simple sequence repeat) may be used to explore the genetic diversity, for validating medicinal plant materials and for marker-assisted breeding (Joshi et al. 2004; Canter et al. 2005). Functional markers like expressed sequence tag (EST) databases can directly be used for identifying the characteristics of a genotype at a very early growth stage (Kumar and Gupta 2008). However, these methods are time-consuming and often have some specific limitations and problems.

DNA microarray is a much faster alternative, which has picomolar size DNA probes attached to a solid surface. This makes use of the hybridization property of the target DNA. The microarray technique is very useful in medicinal and aromatic plants as it allows us to compare the expression pattern between different species or from the same species at different growth stages. This helps us to track the gene fragments which are functional under specific in vitro or in field conditions. We can also evaluate the change in expression based on the environment (Ishkanian et al. 2004). For the unexplored plants, whose DNA sequences are not known, diversity array technology DART-TM (Direct Analysis in Real Time-Thermo-Mechanical method) and subtracted diversity array (SDA) may be used.

Another quick and cost-effective alternative to traditional molecular markers is next-generation sequencing (NGS). This may be used to sequence the genotype of a number of markers across the genome in just one step (Davey et al. 2011). NGS may also be used for evaluating the transcriptomics and metabolomics of the yet unexplored medicinal plants. Being more powerful than microarray technique, NGS may effectively distinguish expression form between homologous and paralogous gene copies. Soltani Howyzeh et al. (2018) made use of this method in case of *Trachyspermum ammi* to identify the unigenes participating in the metabolism of monoterpenoids. This helps in taking the functional breeding of these medicinal plants a step ahead.

NGS acts as a taxon identifier in case of DNA barcoding allowing fast analysis of genetic varieties of MAP at genus and species levels (Techen et al. 2014). DNA barcoding does not require full-genome scale data, but only needs short genomic sequences to identify and categorize the unknown MAP samples. In studying the wild populations of medicinal plants, the restriction site-associated DNA sequencing

(RAD-seq) data from NGS may be efficiently used for genotyping the unknown population (Davey et al. 2011; Zargar et al. 2015). NGS is used for generating genome-wide high-density single-nucleotide polymorphism (SNP) markers and for QTL detection. Thus, NGS also stands as a useful platform for genome-wide association studies (Edwards and Batley 2010).

23.6 Inheritance Relationship of Genes Involved in Secondary Metabolite Biosynthesis

There is sufficient variation among the chemical compositions of the different secondary metabolites produced in medicinal plants. A major chunk of the genes involved in specific steps of each variety of biosynthetic pathways are yet to be discovered (Keurentjes et al. 2006). Inheritance-based studies on a number of MAP showed that this variation could be qualitative or quantitative in nature, and the qualitative traits may in turn be simple or complex. Simple monogenic inheritance is seen in case of S-linalool synthesis in *Clarkia breweri*, where the main gene involved is *LIS* (encoding the enzyme LIS, linalool synthase). Similar dominant gene is also seen to play a role in linalool accumulation in *Mentha aquatica* (Raguso and Pichersky 1999). Other examples of simple monogenic inheritance include citronellal synthesis in *Cymbopogon nardus* var. *confertiflorus* (Rao 1997), dominant gene *C* in capsicum involved in capsaicin production (Blum et al. 2002) and *Frc* gene controlling fructan synthesis in *Allium cepa* (McCallum et al. 2006). Similar genetic inheritance was also seen in case of potato, where a single locus was considered to be involved in leptinine metabolism in *Solanum chacoense* (Ramakrishnan et al. 2015).

On the other hand, complex epistatic relationships have been observed in the biosynthetic pathways of some monoterpene in *Thymus vulgaris* and *M. piperita* (Vernet et al. 1986). The enzyme limonene synthase in *M. aquatica* involved in trans-carveol, trans-isopiperitenol and limonene production is bi-allelic in nature with epistatic relationship between the genes. Study of *Mentha* showed that there are many multiallelic loci, where some even show incomplete dominance (Croteau and Gershenzon 1994). *S. chacoense* (wild relative of potato) also showed the presence of bi-allelic interaction among the genes involved in biosynthesis of leptinide and acetyl-leptinide (Sagredo et al. 2006).

23.6.1 Use of Quantitative Trait Loci (QTLs) to Map Genes

Initially it was considered that the genes involved in secondary metabolite synthesis were quantitative in nature, obeying the rules of classical biometric techniques. However, it failed to account for the multigenic traits involving complex relationships between individual genes. NGS approach could be used in those circumstances to identify the genomic regions that regulate these quantitative trait variations, namely, QTLs or quantitative trait loci (Asins 2002; Collard et al. 2005; McCallum et al. 2006). QTL analysis was conducted in *Arabidopsis* and tomato, which showed that the content of plant metabolite has transgressive segregation, which may be used to develop better cultivars (Keurentjes et al. 2006; Schauer et al. 2006). Based on this genetic map, cloning of the QTLs could be carried out providing valuable information about the structure and function of the enzymes involved. This strategy was used for a QTL cap, in *Capsicum annuum*, responsible for the pungency trait which revealed that it was substantially different from the structural genes involved in capsaicinoid pathways (Blum et al. 2003). Thus, QTL analysis could be an important step in studying the genetics of metabolite biosynthesis.

23.6.2 Quality Assurance by E-Nose

Herbal medicines are often preferred due to fewer side effects, effectiveness and less chemical exposure (Chakraborty 2018). While these medicines make a part of home-made remedies, many people may collect or distribute it. This raises a question in their quality as there may be a mistake due to lack of expert understanding or adulteration. Also these compounds may not have passed through proper concentration checking and toxicity detection which hamper safe use (Zhao et al. 2006; Eloff et al. 2011). Most medicinal plants have an associated aromatic property due to presence of volatile organic compounds (VOCs). These VOCs may be analysed by gas chromatography and mass spectrometric methods. Another method based on signal processing and advanced data analysing algorithm is the E-nose or electric nose technique. Conventional flavour analysing techniques like gas chromatography, mass spectrometry and chemical detection find it difficult to characterize the complex mixture of aroma from medicinal plants. Analysing them by trained experts is both time-consuming and expensive process with room for errors (Perisa and Escuder-Gilabertb 2009). Advances in electronics, biochemistry and artificial intelligence made it possible to characterize VOCs using the E-nose technology which may have numerous applications (Wilson and Baietto 2009).

Human olfactory system has thousands of smell receptors, each of which relays signals to the brain; this helps us to detect odours at concentrations as low as parts per trillion (Breer et al. 1994). Brain next deduces the signal and tries to recognize the aroma, based on previous experiences. E-noses mimic the signalling processes involved in olfaction as well as its perceptional mechanisms. This instrument is constructed as an array of different electrochemical odour sensors along with a recognition system that helps to decipher complex smells (Reid et al. 2006). Similar to our primary neurons, E-noses respond to different odours with different sensitivities.

The interaction between the odour component and sensor gives rise to a change in physical or chemical property, generating electrical signals which may be recorded by computer systems. This recorded signal pattern fits in the signature of a unique aromatic compound, thus functioning as a chemometric tool for analysis. Comparing the signal patterns from a series of samples helps to correlate the differences with perceived sample smell (Ghasemi-Varnamkhasti et al. 2009). This instrument can also be used to detect the samples which are unfit for human consumption based on their complex odour. E-noses do not decipher individual sample components, but respond to the complete volatile sets of odour through a patterned digital algorithm (Zhang et al. 2008).

One of the main disadvantages of E-nose systems is that their efficiency is affected by environmental factors like air, moisture and temperature which can cause a drift in the signal. Internal calibration systems, with built-in algorithms, help to overcome these shortcomings (Baldwin et al. 2011). E-nose systems cannot provide complete information about the MAPs. However, combination of parameters like aroma, colour and taste helps to perform a real-time monitoring of product quality. It is expected in near future that approaches like E-nose, E-tongue and CVS (computer vision system) could be used together for complete evaluation of quality of medicinal and aromatic products. Smart feedback loops could also be used in industries through in-line monitoring which could help improve the overall product quality. Today, this idea is being used in the monitoring of saffron quality by a robust real-time technique (Sajad Kiani et al. 2016).

23.7 Conclusion and Future Perspectives

The world population is increasing almost exponentially, and technical interventions to feed and maintain this growing population are proving to be vital. However, in the long run, consumption of the synthetic medicines appears to have major side effects and health issues in both the developing and developed countries. Use of our immense natural reserves of herbal medicines could prove to be a promising solution to this problem. This calls for scaling up the productivity of wild species by using techniques like MPCSC, bioreactors, gene editing, metabolic engineering, etc. to meet the demand as well as prevent extinction of valuable herbs (Niazian et al. 2017c). MPCSC is a sustainable system for in vitro production of secondary metabolites, as well as for scientific research to improve the yield. It may be carried out independent of geographic and climatic conditions to secure steady production rate. Few MPCSCs that were already used in commercial-scale production are that of rosmarinic acid, *Echinacea* polysaccharides, ginsenoside, saponins, scopolamine, protoberberines and taxol using high-yielding cell lines (Wu and Zhong 1999). Membrane permeabilization, immobilization of plant cells in suspension by encapsulation and in situ product removal and elicitors are often used with MPCSC for better results.

Plant tissue culture lies at the centre of biotechnology-mediated breeding methods like polyploidy induction, bioreactor-facilitated production of compounds and genetic transformation using *Agrobacterium*. Today, many attempts are being made to develop transgenic plants through metabolic engineering for improved yield of pharmaceutical products. However, like every technological endeavour, it faces its own challenges. One of the major limitations of currently available techniques is transgene silencing. The results of time-consuming gene manipulation of complex metabolic networks are unpredictable, and often the network rigidity prevents worthwhile increase in target metabolite concentration.

However, new techniques of gene editing and manipulation of the regulatory genes in place of structural genes may help in overcoming such limitations. The biosafety and ethical issues associated with transgenic crops may prove to be another major setback. Development of cisgenic crops, by overexpressing "cis"-genes in our cultivated varieties from its wild relatives, could be a possible solution to this problem. Genetic modification and free targeted mutation using TILLING is another possible approach of increasing valuable secondary metabolite production (Nogueira et al. 2018).

With the success of many gene editing approaches, more transgenic cell lines could be generated and put to commercial application. Better understanding of the secondary metabolite synthetic pathways could help us to modify them according to our needs. MPCSC cell lines could be modified to secrete the product into the extracellular media for ease of product recovery. Bioreactor design could be perfected, with the proper combination of precursors and elicitors for ideal yield. Another

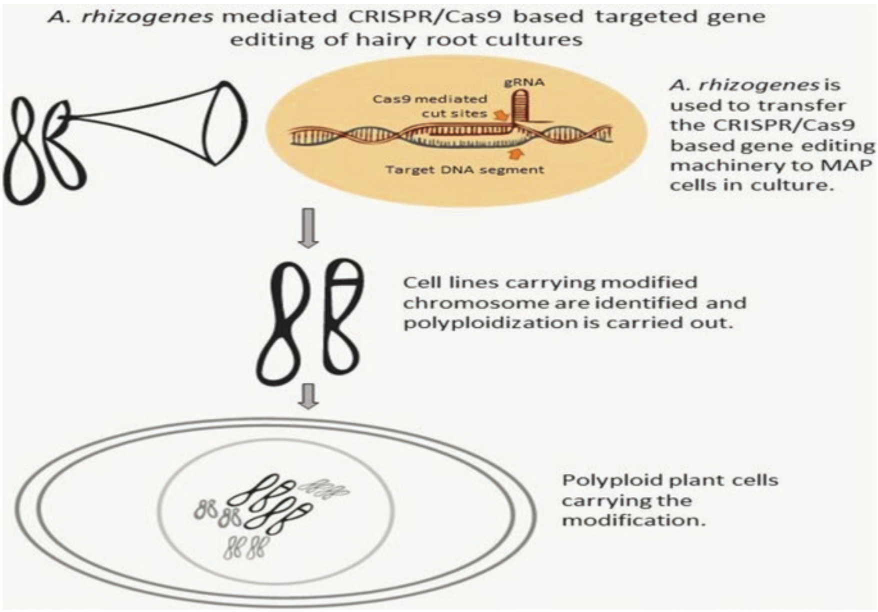

Fig. 23.5 Superior cell lines are generated through *A. rhizogenes*-mediated hairy root generation. On these cell lines, CRISPR/Cas9 system may be used to specifically recognize and cleave target sequence for introducing mutation/genetic knockout/knock-in. The cell line carrying the desired modification may be subjected to polyploidization to further improve productivity

plausible gene editing strategy (Niazian 2019) could be polyploidy generation of CRISPR/Cas9-armed *A. rhizogenes*-facilitated hairy root systems (Fig. 23.5).

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Chapter 24
System Biology Approach for Functional Analysis of Medicinal and Aromatic Plants

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24.1 Introduction

The organic compounds synthesized by plants are categorized as primary and secondary types of metabolites. An essential role is played by primary metabolites during growth and development of plants (Seigler 1995). The expression of secondary metabolites in plants is highly specific and depends upon plant type, stage of development, and plant organ. Despite of advancements in synthetic drugs and antibiotics, plants are still considered to be the major source of raw materials for treatment of various human diseases. During modern times a shift has been seen in the trend for use of herbal medicines as compared to the synthetic drugs (Jain et al. 2019). Medicinal and aromatic plants (MAP) serve as rich stock of secondary metabolites, which gave these plants great commercial and industrial importance. From centuries these secondary metabolites find numerous applications in healthcare for the use as medicines, flavoring agents, dyes for clothes, and insecticides. MAP plants find its use in both tradition and modern system of medicine (Chopra and Ananda 2003). These plants carry a variety of secondary molecules such as terpenoids, alkaloids, and flavonoids, saponins, lipids, carbohydrates, etc. which make an active ingredient of different drugs. Secondary metabolites possess various biological effects which form for the scientific base for use of herb in therapeutics. They have antibiotic antiviral and anti-cancerous properties. Recent use of "omic" workflow and bioinformatics plays a major role in discovery of natural products from plants, which identify and characterize biosynthetic gene clusters involved

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during the biosynthesis of secondary metabolites. In this chapter we will give a brief overview about the use of these omic approaches in medicinal and aromatic plants and role of secondary metabolites, their chemical structure, and pharmacological importance along with some selected examples. The various classes of secondary metabolites are described below.

24.2 Phenolics

Phenolics makes one of the largest groups among the secondary metabolites produced by plants. They are made up of a single aromatic phenolic or made up of polymeric structures. These phenolics give plants their specific flavor, color, or particular odor in case of aromatic plants. Most of these phenolics particularly flavonoids play significant role as an antioxidant and scavenger of free radicals.

24.2.1 Flavonoids

Flavonoids consist of a big group of polyphenolic secondary metabolites. Flavonoids structure contains a chroman ring having an aromatic ring at 2, 3, or 4 positions. Flavonoids are classified into different types based on level of oxidation of the central ring. Mostly different types of flavonoids include flavonols (quercetin, kaempferol, and isorhamnetin) present in some of the plants like onions, leeks, endives, and broccoli and flavones (apigenin, luteolin, and chrysoeriol). More than 2000 of flavonoid are known out of which 500 occurs as free state (Evans 2009). In a recent study, few traditional Tunisian medicinal plants were selected to be tested for bioactive compounds particularly phenolics in *Cymbopogon schoenanthus* (L.) Spreng, *Crithmum maritimum* (L.) Spreng, *Hammada scoparia* (Pomel) Iljin, *Retama raetam* (Forssk.), and *Ziziphus lotus* (L.) Desf. plants. The extracts obtained from these plants were checked for their antioxidant capacities (Najja et al. 2020), and also their antiproliferative role was explored by using breast cancer cell lines (MCF-7). Some examples of herbs containing flavonoids are *Betula pendula*, *Calendula officinalis*, *Sambucus nigra*, and *Leonurus cardiaca*. These plants are known for their anti-inflammatory and antiallergic, antithrombotic, and vasoprotective properties and role in tumor inhibition and protect gastric mucosa (Montanher et al. 2007; Serafini et al. 2010).

24.2.2 Terpenes

Terpenes make largest group among the plant secondary metabolites. “Terpene” name is derived from “turpentine” word, which means “resin.” All terpenes are derived from 5-carbon isoprene units arranged in different ways (Hoffman 2003).

Terpenes are branched into monoterpenes, sesquiterpene, diterpene, triterpene, and tetraterpene depending upon the number of isoprene units present (Thoppil and Bishayee 2011). The biosynthesis of terpenes occurs by two pathways in plants, namely, mevalonic acid and MEP pathway. Terpenes perform a variety of functions which includes to attract pollinators, as a signaling compound, for plant-pathogen interactions, defense of plants against environmental stresses (Chen et al. 2011). Monoterpenes made up of two isoprenes make an important component in essential oils or volatile oils from plants. Geraniol is one such ubiquitous compound found in small amounts in the volatile secretions from plants. Camphor and menthol are examples of monoterpenes which have been used as analgesics, anti-itching, and anthelmintics.

24.2.3 Tannins

Tannins are polyphenolic compounds which have the ability for protein precipitation. Based on this property, they are used in leather industry. Two main types of hydrolysable proteins are gallotannins and ellagitannins made up of gallic acid and ellagic acid units. Ellagitanins are known to be found in some of medicinal plants like *Geranium robertianum* and *Geranium maculatum* (Catarino et al. 2017). Some plants contain both hydrolyzable and condensed tannins as found in *Camellia sinensis* and *Hamamelis virginiana* (Puneet et al. 2013). Drugs which contains tannins work as antidiarrheals and used to work as antidotes when there is poisoning with the alkaloids and heavy metals. Epigallocatechin-3-gallate one of the active compound present in tea had shown its antiangiotenic properties in mice. Juice of *Vaccinium oxycoccos* found its use as antiseptic for urinary infections which is an example of hydrolysable tannins (Jepson and Craig 2008).

24.2.4 Coumarins

Coumarins are derived from benzo-α-pyrone, the lactone of O-hydroxycinnamic acid, coumarin. Coumarins had been found in more than 150 species of plants which belong to 30 different families. The sweet clover or melilot, *Dipteryx odorata*, is a rich source of coumarins (Hoffman 2003). Plants like *Atropa belladonna*, *Datura stramonium*, and *Daphne mezereum* are also rich in coumarins (Evans 2009). Coumarin-containing plants show many medicinal properties and used as anti-inflammatory, anticoagulant, and anti-cancerous compounds (Xu et al. 2015).

24.3 Alkaloids

Alkaloids are a type of organic compounds which have one nitrogen in their heterocyclic ring structure. Alkaloids are classifieds on the basis of their chemical structure into various types. Few examples of alkaloids are acridones, aromatics, carbolines, ephedras, ergots, imidazoles, indoles, pyrroloindoles, and pyridines (Tadeusz 2015). Alkaloids are not evenly distributed in the angiospermic plants. Alkaloid shows a number of therapeutic properties like analgesic, used for local anesthesia and also used for vasoconstriction. Some of these alkaloids are toxic to animals also (Seigler 1995). Nicotine found in tobacco plant is an example of alkaloids which has tranquilizing property and is main addictive component found in *Nicotiana* species. Nicotine is a cholinergic receptor which binds to receptors in the autonomic ganglia, in the adrenal medulla, and in the brain (Benowitz 2009).

24.4 Saponins

Saponins have a polycyclic glycone with a steroid or triterpenoid attached with a carbohydrate moiety. These sugars may be pentoses, hexoses, and uronic acids. They have both hydrophobic and hydrophilic asymmetry in their structure which helps these compounds to lower surface tension and shows soap-like characteristics. During in vitro conditions, they cause erythrocytes hemolysis. The saponins are found from about 500 different plants from 90 plant families. Saponins are found in all plant parts although their level is more concentrated in the roots of plants like *Digitalis purpurea*, *Eleutherococcus senticosus*, *Gentiana lutea* (gentian), and *Glycyrrhiza* spp. (Assa et al. 1973).

24.5 Identification and Characterization of Secondary Metabolite Pathway Through System Biology Approaches

The new evolution in the plant genomics research of medicinal plants together with advancement in other omics technologies helps for easy and quick disclosure of unidentified metabolic pathways and secondary metabolites. This chapter will provide brief overview about the application of whole genome and chloroplast genome sequencing in medicinal plants.

24.5.1 Genomics Studies Involving Medicinal and Aromatic Plants

Genomics involves study of an organism's complete genome, which refers to all DNA sequences in an organism. Few reports feature about signification of genomics research which leads to huge augmentation in understanding for plant systematic biology and will be useful for better understanding of new compounds to be used as a potential pharmaceutical product. Despite so much progress in genomic technologies, because of their complexity, unluckily there had been only a few well-assembled genomes of medicinal plants which had been released so far. In the *Catharanthus roseus* plants, belong to the family Apocynaceae, and known for synthesis of the bis-indole monoterpene indole alkaloids (MIAs). These alkaloid have an anti-cancerous property and used for this purpose (Aslam et al. 2010). MIAs belongs to type of terpenoid indole alkaloids (TIAs). These alkaloids make beneficial effects on the health of humans (Almagro et al. 2015). The genome assembly of *C. roseus* provides comprehensive picture of the genomic background of key notch in the MIA biosynthetic pathway which includes genes clusters, expression, and sub-functionalization (Kellner et al. 2015). Another study involves *Z. jujuba* plants which usually are known for its medicinal value, fruits of this plants carry rich source of vitamin C and sugar, and many important alkaloids, flavonoids, and phenolics are also present which are of importance for therapeutics. De novo genome assembly along with transcriptomics analysis (Li et al. 2014) proves that L-galactose is required during biosynthesis pathway for vitamin C. In the *Z. jujuba* plants, high-quality sequence genome is deciphered by Liu et al. in 2014. The genome size spans to 437.65 Mb, out of which 321.45 Mb is present on the 12 pseudo-chromosomes and 32,808 genes are present on them. Further transcriptomics studies of jujube-specific genes from 15 tissues reveal the mechanisms responsible for its high vitamin C content.

Azadirachta indica A. Juss. belongs to Meliaceae family and is popular for its pharmacological, insecticidal, and cosmetics properties. Krishnan et al. in 2012 had proposed the draft genome sequence and transcriptomes data for five different organs in neem tree (Krishnan et al. 2011, 2012). Another study involves its annotation and analysis for gene prediction, repeat elements, expression studies, and study of its phylogenetic relationship (Krishnan et al. 2012). An improved genome assembly was suggested by Krishnan et al. (2016) using long-insert libraries from Illumina HiSeq, by the use of using long reads sequencer from Pacific Biosciences (PacBio) and use of LoRDEC (Salmela and Rivals 2014). Assembling of genome was done with the help of "Platanus" pipeline, which is considered ideal tool used for assembly of heterozygous genomes. Reassembly of five organ-specific RNA libraries was pooled and by using Trinity complete genome assemblies (v2.0) was made based on this transcriptome analysis.

Recent advancement in the next-generation sequencing had made possible sequencing of complete chloroplast genomes. In most of the angiospermic plants, chloroplast genomes are small circular DNA which have a quadripartite structure made up of two inverted repeats along with two single copy regions large single-copy region (LSC) and small single-copy region (SSC). Mostly genomes of chloroplast in angiosperms are quite conserved and encode four rRNAs, about 30 tRNAs, and near 80 single-copy proteins (Kikuchi et al. 2013).

In this context study in *Pogostemon* was done, which belongs to Lamiaceae *family*. *Pogostemon cablin* is a native of Philippines. The detailed chemical analysis of *P. cablin* indicates that patchouli contains about 40 active components, like monoterpenoids and sesquiterpenoids, triterpenoids, flavonoids, and alkaloids. The patchouli alcohol, a tricyclic sesquiterpene is widely used in manufacturing of perfumes, soaps, and cosmetics. It also exert some pharmacological effects like inhibition of platelet aggregation, astringent, anti-inflammatory, antidepressant, febrifuge, carminative, diuretic, aphrodisiac sedative, and tonic properties. *P. cablin sequencing of chloroplast genome* was performed by Illumina HiSeq platform. Annotation of its genome when compared reveals conservation of genome sequence with several other members of *Lamiaceae* (He et al. 2016).

Forsythia suspensa plant finds its use for the treatment of inflammation, pyrexia, gonorrhea, and diabetes. In *F. suspensa* complete chloroplast genome sequence was elaborated by using Illumina sequencing (Wang et al. 2017). Its chloroplast genome size is 156,404 bp, which showed a region of large single-copy (87,159 bp) and a region of small single-copy (17,811 bp) interspersed between inverted repeat (25,717 bp). The total number of 114 unique genes was present which includes 80 genes which codes for the proteins, 30 genes for tRNA, and rest of the 4 codes for four rRNA. In *S. miltiorrhiza*, draft whole genome shows that size of plant genome is ~600 MB from which 30,478 are classified as protein coding genes, 1620 codes for different transcription factors; TF have a crucial role to play during the biosynthesis of tanshinone and phenolic acids which are of use for treatment of cardiovascular, cerebrovascular, and hyperlipidemia.

Artemisia lavandulaefolia YC is a member of family Asteraceae (Taleghani et al. 2020) found in China, Mongolia, Japan, and Korea. Mostly it is found on the side of roads, edge of forest, hills, and grassland, and in Europe it is popularly known as "wormwood" and used in cold treatment to dampness and to stop bleeding. Nangong et al. (2020) had deciphered the chloroplast genome sequence of *A. lavandulaefolia* YC. Its genome has 117 genes which include 82 protein-coding genes, 31tRNA genes, and 4 rRNA genes.

Plants of *P. polyphylla* have known medicinal properties present in southwest China. In that region, the roots of these plants are used as the local medicine, due to its analgesic, hemostatic, anti-tumor, and anti-inflammatory properties. The size of its chloroplast genome is 163,533 bp. In their genome 114 genes were annotated, from which 79 were protein coding genes, 30 tRNA and 4 rRNA genes (Song et al. 2017). The genome sequencing of *P. polyphylla* chloroplast was performed (Zhaoa et al. 2019) to understand its phylogenetic relationship which indicates that *polyphylla* is more closely resembled to *P. marmorata*. In the herbgenomics study, chloroplast genomes of *S. przewalskii* and *S. bulleyana* were sequenced and compared with two other species *S. miltiorrhiza* and *S. japonica*. The results indicated that both types of *Salvia* plants have a total 114 unique genes, which include 80 were protein-coding, 30 tRNA, and 4 rRNA genes (Liang et al. 2019).

Lycium chinense Mill, is a Chinese herbal medicine also find its use for dietary supplements. In the *L. chinense* sequencing of chloroplast genome proves its size to be around 155,756 bp (Yang et al. 2019). *Polygonum cuspidatum* (Japanese knotweed) is a Chinese herbal medicine which produces bioactive compounds like stilbenes and quinones. In *P. cuspidatum*, draft genome was explored by Illumina sequencing technology. Its genome size is ca. 2.56 Gb long, in which about 71.54% of the genome annotated to transposable elements only (Zhang et al. 2019). Gene prediction studies proved that its genome codes for a total of 55,075 functional genes which conclude to about 6776 gene families which are conserved among the 5 eudicot species and 2386 gene uniquely belongs to *P. cuspidatum*.

The four species of *Meconopsis genus* namely *racemosa, M. integrifolia* (Maxim.) Franch, *M. horridula* and *M. punicea*. These plants grow in the wild have important medicinal and ornamental properties. The chloroplast genome sequencing (Li et al. 2019b) in *Meconopsis* showed that its genome size ranges from 151864 to 153816 bp which codes for 127 genes from which 90 are protein-coding genes and 37 tRNA and 8 rRNA genes are present in the genomes of all four species.

24.5.2 Proteomics Approach

Proteomics is a tool which is used to identify and characterize all the proteins expressed in an organism or cell (Wilkins et al. 1996). Proteomics studies also involve post-translational modifications of proteins which include methylation, acetylation, glycosylation, and oxidation (Blackstock and Weir 1999). *Cannabis sativa* is used as a reliever for chronic pains. A study was performed by comparative proteomics approach for identification of expressed proteins in a specific tissue, because cannabinoids, which is the main active compounds in these plant, are found in different plant parts like glands, leaves, and flowers (Raharjo et al. 2004). Comparison of flower and gland proteomes revealed that near about half of the expressed proteins in flowers were also expressed in glands. The proteomics studies were performed on *Panax ginseng* with the use of two-dimensional gel electrophoresis (2DE) which resulted in 83 differentially expressed spots, from which about 62 of these spots were up-regulated whereas 21 were downregulated during slow-growth period; characterization of these proteins leads to interpretation that ginseng plants conserve energy during fast-growth period which will be required for promoting root elongation, while it spends stored energy for the secondary metabolites synthesis during slow-growth period; level of many proteins was changed during shift from fast to slow growth which helps us to gain more insight into ginseng proteome evolution (Ma et al. 2013). *Calotropis gigantea* belongs to the family Apocynaceae which have large number of medicinal properties for curing a large number of diseases. The peptide mass fingerprinting (PMF) technique results in the identification of proteins (Rehman et al. 2020) by comparing amino acid sequence with the reference protein database. The purpose of this was the identification of peptides having anti-cancerous properties by use of in silico approach.

From the last several years, a lot of research had been going in different plants for detailed proteomic studies to unravel pathways involved in biosynthesis of different metabolites, for example, proteomics studies performed in *Catharanthus roseus* and *Papaver somniferum* for identification of the molecules involved during alkaloid and morphine biosynthesis (Decker et al. 2000; Jacobs et al. 2005). In *Panax ginseng* plants, proteomic analysis had been carried for ginsenoside biosynthesis, proteins involved for hairy root formation, and marker protein identification (Lum et al. 2002; Kim et al. 2003; Ma et al. 2013, 2016). In *Mahonia bealei*, for proteins involved in production of benzyl isoquinoline alkaloids (Zhang et al. 2014). *Camellia sinensis* is also a popular medicinal plant which has many types of flavonoids including flavonols and isoflavones. Leaves' proteomic analysis of this plants was carried out by using different stages of development (Li et al. 2011). Various studies were performed in different medicinal plants to find out the effect of different embryo development stages on propagation of plants in *Coffea arabica*, *Cyclamen persicum*, *Crocus sativa*, *Araucaria angustifolia*, *and Dianthus caryophyllus* (Sharifi et al. 2012; Mwangi et al. 2013; Campos et al. 2016; DosSantos et al. 2016; Muneer et al. 2016). This analysis helps to elucidate the role of expression of proteins during different stages of embryo development.

24.5.3 Metabolomics Approaches

Metabolomics is a expeditiously growing technique to make an exhaustive quantitative and qualitative analysis for all the metabolites present in an organism. These studies resulted in generation of comprehensive metabolite profiles that ultimately helps to understand changes in metabolites levels in different samples, organ extracts, or biofluids and generate metabolic phenotypes (Gavaghan et al. 2002). Metabolomics is analysis of metabolites under some particular conditions by using different herbal extracts (Nicholson et al. 1999; Fiehn 2002; Beger et al. 2010).

The two different types of metabolomics analysis are by targeted, and another one is by global analysis. In global analysis non-discriminatory approach is used for metabolites profiling of all unknown metabolites whereas metabolite analysis using targeted approach involves some of already known metabolites profiles (Shyur and Yang 2008). GC-MS is most pronounced avenue for analytical studies of metabolites for generating precise and selective profiles of the volatile primary and secondary metabolites when working with crude extracts of plants (Fiehn 2001). Metabolome analysis by LC-MS is a persuasive and exclusive tool for generating metabolic plant secondary metabolites like alkaloids, glycosides, tannins, flavonoids, and carotenoids (Moco et al. 2009; Hou et al. 2010).

Metabolomic profiling in *Salvia miltiorrhiza* Bunge, a perennial herb, revealed secondary metabolite profiles of several different genotypes by using combined NMR and LC-DAD-MS based approach (Dai et al. 2010). *Coptis deltoidea* had been in use for medicinal properties and rich in benzylisoquinoline alkaloids. Metabolic profiles of ten BIAs from different plant were performed by ultraperformance liquid chromatography-electrospray ionization tandem mass spectrometry (UHPLC-ESI-MS/MS) and sequencing platforms (Zhong et al. 2020) which were used to explore BIA alkaloids biosynthesis pathway and detailed transcriptome analysis of *C. deltoidea*. Transcriptome sequencing used single-molecule real-time (SMRT) sequencing and results in generation of about 75,438 full-length transcripts.

In plants of *Valeriana officinalis* L., *Melissa officinalis* L., *Hypericum perforatum* L., and *Passiflora incarnata* L., both metabolic and proteomic analyses was carried out, which are used as a sedative anxiolytic for the treatment of sleep disorders. Integrated omics-based studies was performed to analyze the effects of extracts (Gonulalan et al. 2019) of these plants on the brain-derived neurotrophic factor (BDNF) expression levels on the SH-SY5Y cell line. Metabolome profiling of these plants was performed with the GC–MS and LC-qTOF-MS technique, whereas proteomics analysis was performed with LC-qTOF-MS system.

Picrorhiza kurroa found in the Northwestern Himalayans has reached near extinction level due to excess harvesting which has enormous medicinal and therapeutic properties. The active constituents present in *P. kurroa* are picroside-I and picroside-II, in a ratio of (1: 1.5) which are responsible for its medicinal properties. For identification of proteins responsible for picroside biosynthesis, protein expression (Sud et al. 2014) was performed in picroside accumulating and non-accumulating conditions with SDS-PAGE. By using the approach of MALDI-TOF/TOF MS followed by MASCOT analysis, 19 differentially expressed proteins were identified.

24.5.4 Transcriptomics Data

Dendrobium huoshanense C.Z. Tang and S.J. Cheng stems were used as a medicinal plant. In leaves, stems, and roots, identification of potential genes responsible for biosynthesis of active compounds was analyzed by transcriptomic analysis (Zhou et al. 2020). A comparative analysis revealed differential expression of genes involved with polysaccharides biosynthesis, genes related to fructose and mannose, and glycosyltransferase genes and flavonoids biosynthesis. *Ferula asafoetida* belongs to Apiaceae family used traditionally for its medicinal importance. Terpenoids and phenylpropanoid metabolites are the major active compounds of the root-derived resins that show anti-inflammatory and cytotoxic activities. *The c*omparative de novo transcriptome analysis of roots, leaves, stems, and flowers (Amini et al. 2019) was performed for identification of genes which have a role for terpenoid and coumarin biosynthesis.

Zanthoxylum planispinum medicinal plant is obtained majorly from many parts of East Asia. In this plant, transcriptome analysis was performed on different parts like leaf, early fruit, and mature fruit stage by using PacBio RSII platform. A total of 51,402 unigenes were identified 1781 bp from 82.473 Mb gene lengths. A total of 76 cytochrome P450 (CYP450) and related isoforms were identified (Kim et al. 2018) which are present in high diversity.

In *Ophiorrhiza pumila* plants, deep transcriptome studies along with untargeted metabolic profiling depicted those transcripts which possibly are involved during the biosynthesis of the anti-cancerous compound camptothecin (Yamazaki et al. 2013). Deep transcriptome analysis from *Papaver somniferum* helps in search of candidate genes for flavoprotein oxidase which is related to papaverine biosynthesis (Hagel et al. 2015). A comparative transcriptomic study was performed in *O. basilicum* and *O. sanctum* by Rastogi et al. in 2014 where many differential transcripts of phenylpropanoids in *O. basilicum* and of terpenoid biosynthesis in *O. sanctum* were inscribed. Recently CSIR-CIMAP, Lucknow, achieved success with whole genome sequence (Rastogi et al. 2015) of *O. sanctum*. In this plant, size of genome is 386 Mb in *O. sanctum*. Two libraries of long and short reads were of Illumina HiSeq 2000 (Liu et al. 2011); one of SOLiD 550 XL and one of 454 GS FLX were united for generating draft genome assembly.

Qian et al. (2013) who described the *Salvia miltiorrhiza* chloroplast genome is smallest when compared with other members of the family Lamiaceae with chloroplast genome of length 151,328 bp. A recent draft of neem (*Azadirachta indica*) has been published (Krishnan et al. 2012). The *Azadirachta indica (*neem) tree is rich source of large number of natural products, which includes the potent biopesticide azadirachtin. In spite of its huge applications in agriculture and pharmaceuticals, still the molecular studies for the biosynthesis of neem terpenoids are unexplored partially.

In the *P. cyrtonema*, leaf, root, and rhizome tissue RNA sequencing analysis (Wang et al. 2019) helps to identify a total of 164,573 unigenes from all three types of tissues, and from them 86,063 were annotated by getting information from public databases. Expression profile analysis reveals level of differentially expressed gene levels from rhizome tissues which was then compared with leaf and root tissues. Multiple genes which code for important enzymes, like UDP glycosyltransferases (UGTs), or transcription factors involved in polysaccharide biosynthesis were identified validation of few of these genes that codes for key enzymes were done by quantitative real-time PCR. *Cirsium japonicum*, a medicinal plant which belongs to Asteraceae family found in Asia, causes inhibition of tumors, improved immunity, and antidiabetic and hepatoprotective effects. By using RNA sequencing approach, expression analysis of genes (Roy et al. 2018) related with biosynthesis of silymarin from different tissues like flowers, leaves, and roots was performed. A total of 51,133 unigenes were obtained by using de novo assembly. The gene expression analysis revealed that gene expression in relation to the flavonoid pathway is more in flowers, while phenylpropanoid pathway expression was high in the roots. *Valeriana jatamansi* Jones is used for its medicinal properties in China. The active compounds found in this plant are generally called as valepotriates that belong to iridoid compounds. The transcriptome analysis (Zhao and Wang 2020) was performed on leaf and root tissues, and de novo sequence assembly was generated to understand the iridoid biosynthesis pathway; a total of 183,524,060 transcripts and 61,876 unigenes were obtained from 13.28 Gb clean reads.

24.5.5 Functional Validation via Other Molecular Approaches

Furthermore, to support finding out of paramount metabolic pathways in plants involves different techniques like the use of gene cloning, RNA interference (RNA), and virus-induced gene silencing (VIGS) techniques. RNA interference silences expression of mRNAs through homology-dependent binding which had been transformed into plants and which interferes with the targeted gene expression. RNAi approach had been used for generating transgenic poppies which stack up the benzylisoquinoline alkaloid reticuline instead of narcotic morphine (Allen et al. 2004). VIGS is used for a number of plants which are otherwise considered difficult for transformation (Mutthapa et al. 2008). In opium poppy, VIGS technology had helped for identification of genes responsible for benzylisoquinoline alkaloid (BIA) biosynthesis and predicted reactions involved in O-demethylation of thebaine to codeine and codeine to morphine (Hagel and Facchini 2010). In *Hyoscyamus niger*, although tropane alkaloids biosynthetic pathways had already been characterized very well, validating the role of candidate genes that codes for cytochrome P450s which represent tropane alkaloid pathway-enriched transcripts by using VIGS approach identified a CYP80F1 family member which when silenced resulted in buildup of littorine instead of hyoscyamine (Li et al. 2006). In *Catharanthus roseus*, VIGS-mediated suppression of 16-methoxy-2,3-dihydro-3-hydroxytabersonine N-methyltransferase (NMT) was performed, along with the generation of targeted metabolic profile of NMT-VIGS-silenced plants (Liscombe and Connor 2011).

24.6 Conclusions

Integrating system biology approach by the use of genomic, transcriptomics, proteomics, and metabolomics studies in medicinal and aromatic plants helps us for the prediction of gene functions involved in biosynthetic pathways of those compounds which make the bioactive component of these plants. By the use of an integrated approach, novel biomarkers can be designed based on functional and genomic data. These studies will further enable us to modify the structure and function of regulatory molecules involved in biosynthesis of desired metabolites by the use of few genetic engineering tools.

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Chapter 25
Synthetic Seed-Mediated Synchronized Multiplication Under In Vitro Conditions: An Efficient Technique for Conservation, Multiplication and Storage of Medicinal Plants

Shamshad Ahmad Khan, Priyanka Verma, Laiq Ur Rahman, and Varsha Parasharami

25.1 Introduction

The synthetic seed technology holds the potential for a commercial scale plant production which can be proven as an alternative source of producing plants without the help of true seeds. The first synthetic seeds were prepared by Kitto and Janick in 1982 by using somatic embryos of carrot and further desiccating them. The synthetic seeds can be described as structures most commonly somatic embryo or explants of cotyledonary stage covered with an artificial layer of sodium alginate.

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The advantages of synthetic seeds are immense which includes true to type clonal propagation, conservation of germplasm of elite plants, longer storage of explants and rapid and efficient multiplication of plants (Gantait et al. 2015). Apart from these points, an economical cost of production of plants and handling easiness are also important factor which makes synthetic seeds a viable option of conserving plants (Fig. 25.1). It is needless to state that continuing research attention towards the basic need of cost reduction of such techniques can not only broaden its commercial application but can also ensure the conservation of genetic resources.

Initially somatic embryos were used for synthetic seed preparation and propagation with an artificial seed coat (Gray et al. 1991), but nowadays most of the explants are used for propagation through synthetic seeds which may include nodal explants, roots, apical or axillary buds (Gantait et al. 2015). The most common gelling agent which is used for synthetic seed preparations are sodium alginate although there are many other gelling agents which are used which may include ethylene glycol, hydrogel, DMSO and various other chemicals. The gelling material provides the nourishment to the plants and also prevents the mechanical damage during the handling of explants (Gantait et al. 2015). The synthetic seeds have different implications in the field of biotechnology as this technique provides true to type clonal plants which is an efficacious technique for rapid propagation of plants. The recalcitrant plants which are hard to grow by seeds can be grown by somatic embryogenesis, and other tropical trees which produce recalcitrant seed can be grown and stored by synthetic seed technology as these seeds provide desiccation less atmospheres for suitable growth of plants. It is though desirable to produce hybrid seed species with low cost for industrial level production in species like cotton (*Gossypium hirsutum* L.) and soybean (*Glycine max* Merril.), but the problems of abscission and

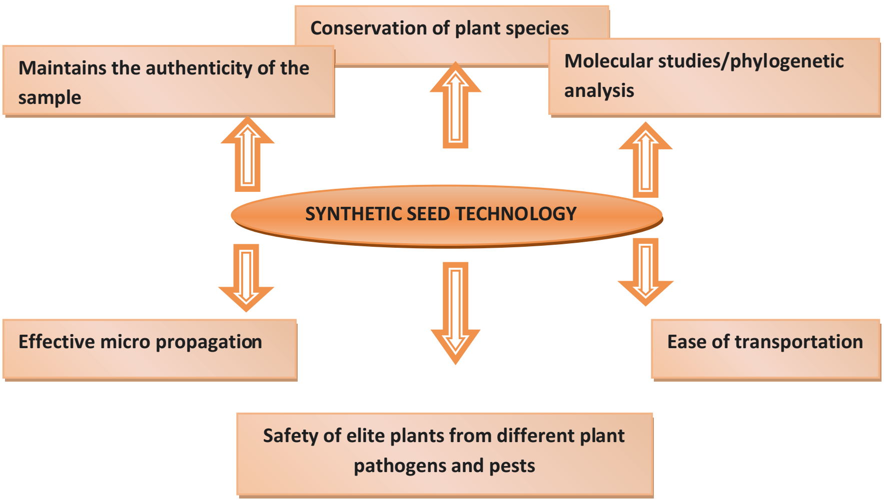

Fig. 25.1 Various advantages of the use of synthetic seed technology in micropropagation techniques

cleistogamous flowers pose serious threat, but through the mediation of synthetic seed, the small hybrid of these plants can be grown which reduces the high cost affair (Ravi and Anand 2012). The lack of accessory tissue from the somatic embryo such as protective coverings, endosperms, is the major drawback in utilizing synthetic seed technology, and it makes the storage of these explants a hard task to handle. Initial stages of synthetic seed technology was therefore focused on producing somatic embryos which carry resemblance to seed embryos in storage and handling characteristics in a way they can be further used as a unit for plant multiplication and conservation of the germplasm. During these times it was aimed that synthetic seed production of different plants can be done in a cost-effective manner. In the meanwhile it was also thought to add plant growth regulators, other growth enhancing components and growth nutrients in the encapsulated synthetic seed for their efficient differentiation and growth (Sharma et al. 2013).

Vastly synthetic seeds can be defined as encapsulation of the propagules which are raised under test tube conditions. To cover other aspects of in vitro raised plant tissues, the synseed can be explained as encapsulation of different plant parts such as auxiliary buds, shoot buds, somatic embryos or cell aggregates. The selection of these tissue types for synseed can only be considered potent when these tissue types have the ability to regenerate in to whole plants when sown as a seed. The concept of synseed was developed with the basic idea to conserve plants in a way that they can be regenerated when required. Apart from it low contamination rate and long-term storage of these seed was also considered major factor for using this technique. Later on fast growth of synseed as compared to the normal growth on basic medium and single-step shoot/root generation from synseed-derived plants also came in to foray. Alongside these advantages, the molecular profiling of synseed-mediated plant regeneration and its comparative analysis to the mother plant through ISSR or RAPD-based profiling also proved important tools to assess the genetic fidelity of synseed derived plants. One of the less investigated areas in synthetic seed application is the restoration of forest, grasslands, mine lands which are abandoned and rangelands in which the rehabilitation process can be achieved through this potential technique.

Conceptually, synseeds can be used as normal conventional seed which has two parts, one the zygote part and other in the endosperm part where the explant used for study works as embryo parts normally and the coating or capsule which is a gelling agent works as an endosperm part. The gelling agent sometimes is used alone or in many occasions used with plant growth hormones, biofertilizers, nutrients as well as bio controllers. Under in vitro synthetic seed propagation, normally sodium alginate is used as the gelling agent and is added drop by drop with the small cut explants to calcium chloride solution, and a chemical reaction occurs. For the proper synthesis of synseeds, the calcium chloride solution is constantly stirred at the time of dropwise addition of sodium alginate with explants. The chemical reaction results in the formation of sodium chloride and calcium alginate which is solidified. The selection of explant material for synthetic seed-mediated propagation is also one of the important factors on which the growth of synthetic seed depends and normally somatic embryo is the preferred explant material for such approaches because somatic embryos have the ability of a zygotic embryo with somatic cell properties. Apart from somatic embryos, other plant parts such as nodal segments, root explants and axillary shoot tips can also be used.

There are two kinds of synthetic seeds: (A) desiccated synthetic seeds and (B) hydrated synthetic seeds (Sharma et al. 2013).The former one is desiccated after their encapsulation with gelling agents such as poly ethylene glycol, and further the desiccation is attained by using the low relative humidity chambers in a slow process or by rapid process by keeping the synthetic seed containing plates open on the bench top for overnight. The desiccated synthetic seeds are prepared only of those plant species whose somatic embryos are desiccation resistant. In an opposite manner, those plant species whose somatic embryos are recalcitrant and sensitive to desiccation are grown by hydrated synthetic seed technology. For hydration of the seeds, the hydrogel is used for encapsulating the somatic embryo.

This chapter deals with hydrated synseeds and describes authors own work on synseed preparation and their assessments in important medicinal plants, namely, *Stevia rebaudiana*, *Acorus calamus*, *Bacopa monnieri and Hypericum perforatum*. The studies were performed both on semi-solid medium and liquid medium-based temporary immersion system. The TIS work on *S. rebaudiana and A. calamus* was performed in CSIR-CIMAP laboratory, while *B. monnieri and H. perforatum* work has been done in CSIR-NCL lab. Moreover, long-term storage of synseeds for conservation as one of the future prospects has also been discussed.

25.2 Synseed Preparation and Molecular Profiling of Synseed-Derived Plants

The gelling mixture, consisting of 3.0 and 4.0% (w/v) of sodium alginate (Sigma, St. Louis, MO, USA), was prepared by using full-strength/half-strength MS medium, supplemented with 3.0% sucrose. The complexation solution was prepared with 3% calcium chloride solution ($CaCl_2$), and both the ingredients were autoclaved at 120 °C under 15 lbs pressure for 20 min. The dissected explants were suspended in the sterile sodium alginate gelling mixture, dispensed dropwise into the $CaCl_2$ solution under continuous shaking and were kept in the solution for 20 min to complete the ionotrophic gelation of alginate. The resultant beads were consequently washed thoroughly for 3–4 times with sterile distilled water to remove any traces of $CaCl_2$ and were placed on hormone-free, MS semi-solid medium as well as in the fabricated glass vessel containing the same medium in liquid form for further growth and development. The cultures were incubated at 25 ± 2 °C under 16 h photo period, and observations were recorded at weekly intervals. Parameters, such as percentage of regrowth and regeneration in terms of shoot (single/multiple), root and complete plantlet (bearing both shoots and roots) formation, were used to evaluate the suitability of the employed procedures after 2 and 4 weeks of plating, respectively. Synseed-derived plants from all the four medicinal plants were subjected to ISSR analysis to evaluate the genetic fidelity. DNA was isolated from the leaves of the selected plants. DNA yield was determined using a NanoDrop spectrophotometer (NanoDrop®, ND-1000, NanoDrop Technologies, Wilmington, Delaware, USA). A total of 19 ISSR primers, namely, UBC-807, UBC-810, UBC-811, UBC-823, UBC-826, UBC-828, UBC-834, UBC-835, UBC-836, UBC-841, UBC-843, UBC-844, UBC-845, UBC-848, UBC-855, UBC-860, UBC-861, UBC-868 and UBC-891(University of British Columbia, Vancouver, Canada), were used to conduct genetic fidelity studies in four medicinal herbs, viz. *Stevia rebaudiana*, *Acorus calamus*, *Bacopa monnieri and Hypericum perforatum*. The 25 μl PCR mixture was composed of 10 x taq buffer (2.5 μl), 10 mM dNTPs mix (1.0 μl), taq DNA polymerase (1.25 unit) and1 μl primer (6 pmol) with 25 ng of DNA. The PCR reaction was constituted of denaturation (at 94 °C for 5 min) followed by 45 cycles of 1 min denaturation at 94 °C, annealing at 55 °C for 1 min and extension at 72 °C for 2 min with the final extension of 5 min at 72 °C. PCR reactions were conducted in a 90 well thermocycler (Eppendorf, Germany). PCR amplified products were separated on 1.2% agarose gel stained with 0.5 mg/l ethidium bromide in 1X TAE buffer using a Minipack-250 electrophoresis system (GeNei™) at 50 V for 2–3 h and visualized by fluorescence under UV light. The standard molecular weight marker used was double digested λ DNA with EcoRI and HindIII. The Gel Documentation System XR-Quantity One, Bio-Rad Laboratories and Thermal Imaging System, FTI-500 (Amersham Pharmacia Biotech, Piscataway, New Jersey, USA), were used to score and photographed the bands. By recording the presence (1) and absence (0) of apparent and reproducible bands on gels, the results of ISSR were assessed in a binary system. The monomorphic and polymorphic amplicon numbers generated by each primer were determined. Similarity indices were calculated (Nei and Li 1979), and cluster analysis was performed using the UPGMA (unweighted pair group method with arithmetic mean) method via software NTSYS 2.1 (Numerical Taxonomy and Multivariate Analysis System).

25.3 Synthetic Seed Production in *Bacopa Monnieri* and *Hypericum Perforatum*: Germination, Acclimatization and Genetic Fidelity Evaluation

The search for new routes of producing plants as an alternative source due to over exploitation of natural habitat and mass multiplication for further utilization is thus a priority in current and future scenarios for sustainable conservation and rational utilization of medicinal plant biodiversity. In such a search for alternatives production platforms, biotechnological approaches specifically and synseed technology assumes significance (Rao and Ravishankar 2002).

The synseeds were produced in both the plant systems, namely, *H. perforatum* and *B monnieri*. The 4% alginate is found to produce ideal beads that can be easily handled as well as showed trouble-free breakage during the germination process. At higher concentration, the beads were found to be hard, and germination was hindered. In *B. monnieri* 100% germination was observed after 25 days on MS basal media with single-step shoot/root generation (Fig. 25.2), while *H. perforatum* showed 70% germination (Table 25.1). Single-step shoot/root formation was

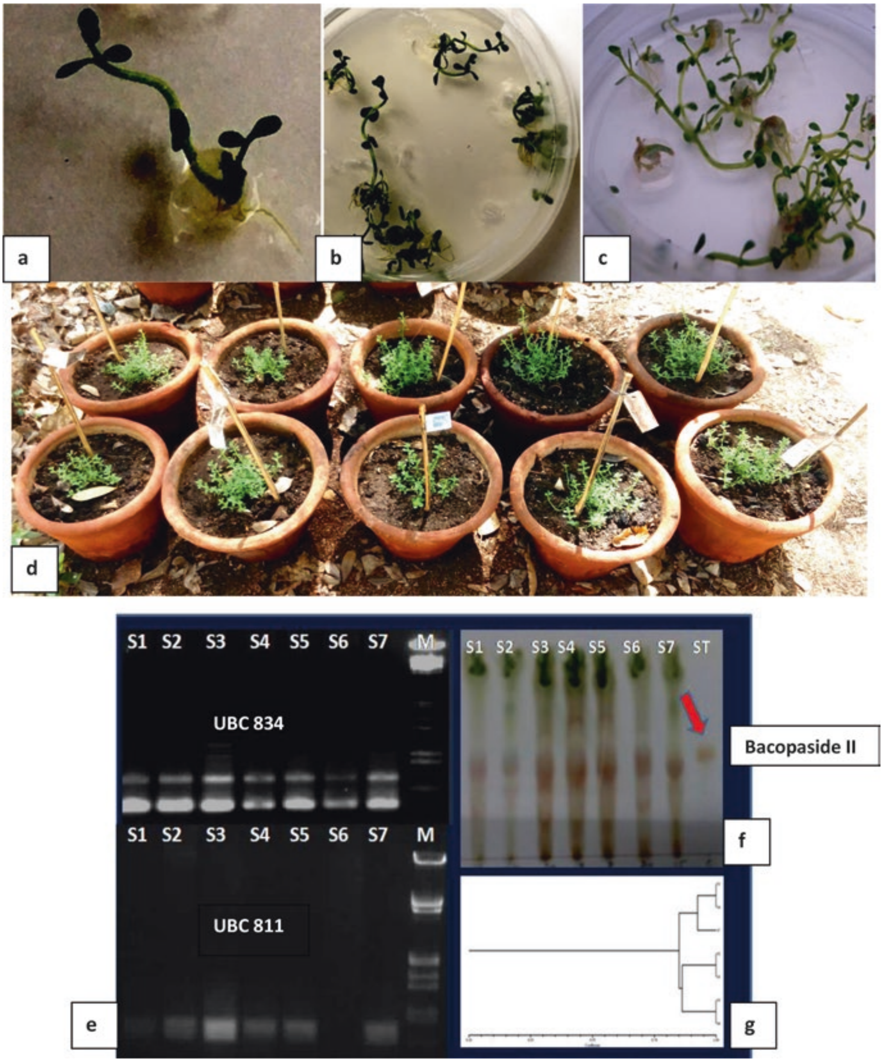

Fig. 25.2 Synseed-derived plants of *B. monnieri*, their acclimatization and assessment (**a**–**c**) regrowth of plantlets of *B. monnieri* plants from synseed; (**d**) glasshouse acclimatized *B. monnieri* plants; (**e**) the band patterns of the gels using ISSR primers in glasshouse acclimatized synseed-derived plants of *B. monnieri*; (**f**) the TLC analysis showing the BACOPASIDE 2 presence in glasshouse shifted *B. monnieri* plants; (**g**) the dendogram analysis of ISSR profiled *B. monnieri* plants

Table 25.1 Percent germination and glasshouse acclimatization of synseeds of *H. perforatum* (*Hp*) and *B monnieri* (*Bm*)

% Germination				Bud formation		Rooting		Green house acclimatization	
MS basal		WPM basal							
Hp	*Bm*	*Hp*	*Bm*	*Hp*	*Bm*	*Hp*	*Bm*	*Hp*	*Bm*
70 ± 5	100 ± 9	05 ± 1	30 ± 5	6thday	4th day	9thday	5th day	60%	100%

Table 25.2 ISSR profiles of *B. monnieri* and *H. perforatum* synseed-derived plants generated by ten primers

Primer code	Sequences (5″→3″)	TB		PB		MB	
		Hp	*Bm*	*Hp*	*Bm*	*Hp*	*Bm*
UBC 834	AGA GAG AGA GAG AGA GCT	16	14	0	0	16	14
UBC 891	HVH TGT GTG TGT GTG TG	15	14	3	0	12	14
UBC 836	AGA GAG AGA GAG AGA GYA	8	9	0	2	8	7
UBC 844	CTC TCT CTC TCT CTC TAC	16	7	0	0	16	7
UBC 811	GAG AGA GAG AGA GAG AC	11	10	1	3	10	7
UBC 843	CTC TCT CTC TCT CTC TAA	10	10	0	3	10	7
UBC 860	TGT GTG TGT GTG TGT GRA	3	7	0	0	3	7
UBC 807	AGA GAG AGA GAG AGA GT	11	7	3	0	8	7
UBC 868	GAA GAAGAAGAAGAAGAA	8	8	0	1	8	7
UBC 861	ACC ACCACCACCACCACC	6	14	2	0	4	14
	Total no. of bands	**104**	**100**	**9**	**9**	**95**	**91**

observed in *H. perforatum* only when 0.1 mg/l NAA was added into the gelling complex; otherwise only shoots were formed. Germination was recorded only on MS basal medium, while seeds turned black with the formation of callus on (woody plant basal medium) WPM. After 30 days, well-rooted plants of *B. monnieri* and *H. perforatum* were shifted to glasshouse. Plantlets were thoroughly washed with running tap water to remove the adhered phytagel. Finally, they were planted in a mixture of sterilized soil, sterilized sand and vermicompost (2:2:1). The hardened plants were covered with plastic cups to maintain humidity for 10 days and showed 100% survival rate. The hardened plants were shifted to earthen pots after 50 days and were watered daily. Glasshouse acclimatized randomly selected plants of *B. monnieri* and *H. perforatum* were tested molecularly on the basis of ISSR profiling and chemically on the TLC basis. The ISSR profile showed total of 100 and 104 bands, out of which 91 and 95 were found to be monomorphic in *B monnieri* and *H. perforatum*, respectively, indicating the clonal propagation (Table 25.2 and Figs. 25.2 and 25.3). Dendrograms showed more than 88% similarity in the raised plants. TLC profiling showed the presence of hypericin (Fig. 25.3) and bacopaside II (Fig. 25.2) in all the synseed raised plants of *H. perforatum* and *B. monnieri*, respectively.

Although in vitro propagation of *H. perforatum* and *B. monnieri* is a feasible option, its multiplication has been explored earlier, but very less (*B. monnieri*) or no attention (*H. perforatum*) has so far been focused towards the practical applicability

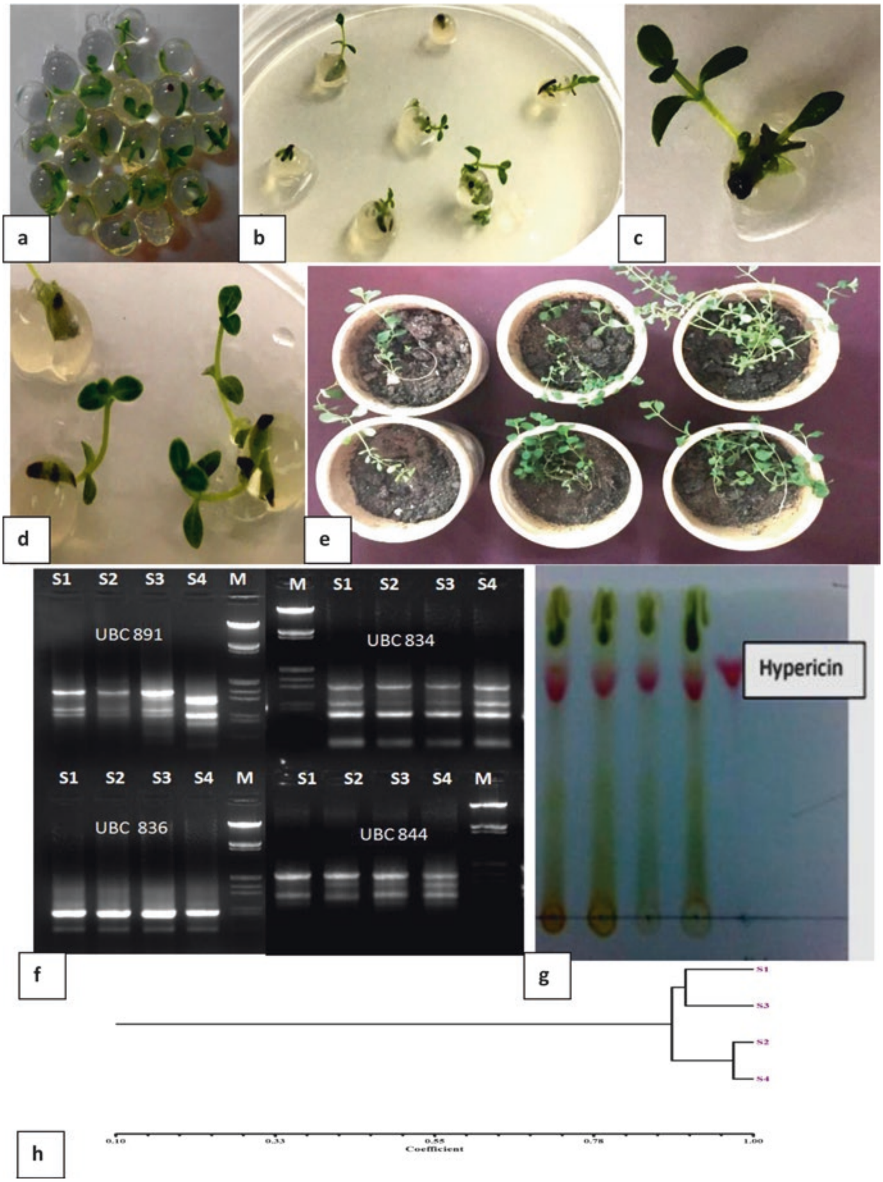

Fig. 25.3 Synseed-derived plants of *H. perforatum*, their acclimatization and assessment (**a**–**d**) regrowth of plantlets of *H. perforatum* plants from synseed; (**e**) glasshouse acclimatized *H. perforatum* plants; (**f**) the band patterns of the gels using ISSR primers in glasshouse acclimatized synseed-derived plants of *H. perforatum*; (**g**) the TLC analysis showing the HYPERICIN presence in glasshouse shifted *H. perforatum* plants; (**h**) the dendogram analysis of ISSR profiled *H. perforatum* plants

of synthetic seed technology as a quick regeneration tool for mass multiplication of these prized medicinal plant species as evidenced elsewhere. Successful utilization of synthetic seed technology as rapid mass propagation tool of choice for any specific plant system requires optimization of protocol for establishing an inexpensive, efficient and reproducible production system with high conversion percentage depicting complete plantlet formation in a single step without intervening subculturing for separate shoot/root formation. It is needless to state that continuing research attention towards the basic need of cost reduction of such techniques can not only broaden its commercial application but can also ensure conservation of genetic resources (Pati et al. 2011).

25.4 Synthetic Seeds and Temporary Immersion System (TIS): Cost-Effective High-Throughput In Vitro Tool

As the commercial efficacy of any optimized tissue culture protocol depends on its cost-effectiveness and rapidity, the use of liquid culture has gained significant attention in recent years as it not only overcomes the uneconomical burden of the cost of agar (5.2-fold cost reduction – Pati et al. 2011) but also proved efficacious for better multiplication rate, faster growth potential and greater ease of ex vitro transferring (Savio et al. 2012), conceivably through maintenance of higher water potentials, uniform nutrient supply and increased oxygen diffusion coefficient (Gupta and Prasad 2010). The use of liquid system, however, necessitates in-depth precision and refinement in terms of contact between cells/tissues with the liquid medium in order to abbreviate hyperhydricity and mechanical stress on plant tissues caused by the applied agitation/aeration to the liquid system (Gupta and Prasad 2010; Savio et al. 2012). Apart from this, continuous media supply with recycling facilities was also important aspect which was to be considered in liquid cultures. Accordingly, various strategies have been formulated by different authors for eliminating the problems yet sustaining the benefits of liquid culture, which predominantly involved temporary immersion system (Yan et al. 2010; Quiala et al. 2011), with/without support system (Gupta and Prasad 2010; Pati et al. 2011), and agitated or non-aerated thin-film liquid system (Pati et al. 2011). Compared to submerged cultures, temporary contact between propagules and liquid medium has proven advantageous in many plant systems (Prasad and Gupta 2006; Yan et al. 2010; Savio et al. 2012) and a variety of support materials such as paper bridge, cotton fibre, cellulose, florialite, glass beads, polyurethane/duroplast foam, rock wool and sugarcane bagasse has been tested to optimize the benefits and to circumvent the asphyxiation/hyperhydricity-related problem of liquid cultures for micropropagation of diverse plant systems (Prasad and Gupta 2006; Savio et al. 2012). However, the credibility of the direct use of liquid medium for regrowth of synthetic seed-based propagules has never been evaluated. Authors in their own lab successfully implemented the combination of synseed technology along with TIS system on two of the important medicinal plants, namely, *Acorus calamus* and *Stevia rebaudiana*. The successful in vitro propagation of elite clones of above plants through combining the advantageous potentials of synthetic seed technology and liquid culture system in the form of improvising a designer pored glass vessel to comply with the requirements of temporary immersion system (TIS) has been executed. Utilization of a above vessel with media recycling facilities and in-built perforated support plate to ensure adequate contact between the liquid medium and the synthetic seeds demonstrated 1.5- and 2.1-fold higher regrowth and 2.0 and 7.14 times better frequency of complete plantlet formation in *S. rebaudiana* and *A. calamus*, respectively, as compared to that on semi-solid PGR-free MS medium. Description of detailed study is as follows.

25.4.1 Conservation of Elite Clones of **Stevia rebaudiana** *and* **Acorus calamus** *(L.) via Synthetic Seed-Mediated Synchronized Regeneration and Utilization of TIS*

Stevia rebaudiana has been in the centre of research and industry due to production of low-calorie high sweetening properties and expected to replace artificial sweetener available in the market. One of the major constituents of this plant named Stevioside has been considered as a natural sweetener with sound safety for human consumption provided it taken in a limited amount and used in several formulations. Due to these low-calorie high sweetening properties, this plant system is targeted for in vitro studies which may overcome the problem of genetic variation through seed and germination percentage-related problems as its low. Alongside it, the in vitro studies are also preferred in this plant system because the vegetative propagation results in low individual plant generation from the parent plant (Sivaram and Mukundan 2003). *Acorus calamus* (L.) or sweet flag (family Acoraceae) is a semi-aquatic, perennial, rhizomatous herb that grows in the temperate and sub-temperate zones of Eastern Europe, Central Asia and North America (Gilani et al. 2006). The rhizomes of *A. calamus* produce aromatic oil that has been used for centuries in the traditional system of medicine for the cure of various ailments like asthma, epilepsy, mental ailments, dyspepsia, diabetes, gastrointestinal disorders, diarrhoea, etc. and has been harvested indiscriminately for commercial purposes (Si et al. 2010). The sweet flag oil has been reported to possess diverse pharmacological activities including antimicrobial, anti-anxiety, anti-asthmatic, antispasmodic, antiulcer, anti-anginal, anti-inflammatory, etc. (Raina et al. 2002). Consequently, confirmation of the anti-proliferative, immuno-suppressive and anti-carcinogenic activity of its active principles on human carcinoma cells has further heightened the demand of this plant to pharmaceutical industries (Mehrotra et al. 2003).The sweet flag oil also finds substantial applications as an important aromatic substance in the food and beverage industry. Other merits of this plant include pronounced insecticidal, larvicidal, antitermite and insect-repellent properties (Ganjewala and Srivastava 2011). Nevertheless, the use of *A. calamus* varieties finds ample applications for both pharmaceutical and flavouring purposes because of its otherwise incomparable credibility (Widmer et al. 2005). This situation clearly explains the need for biotechnological intervention not only for rapid multiplication of this normally vegetatively propagated medicinal plant to meet the quality and safety norms but also to conserve its dwindling genetic resources that is already threatened because of its irrepressible harvesting without adequate cultivation practices and habitat loss (Ginwal and Mittal 2010; Ginwal et al. 2011).

In the background of this information, in authors own lab, synseeds were prepared in above two systems using liquid culture system in a modified designer vessel with in-built perforated support plate that complies with the basic principle of temporary immersion system (TIS) for its efficient and cost-effective in vitro multiplication.

A modified culture vessel has been fabricated by the glass-blowing section by using glass tubes of about 6 cm diameter and 20 cm length in which a pored glass plate has been incorporated at the lower middle of the culture flask with media recycling duct at the bottom of the vessel connected to a filter sterilization unit at the tip for media removal with minimum contamination probability (Fig. 25.4). The idea was to provide an in-built support to the explants so that a temporary but adequate contact between the medium and synthetic seed-containing explants can be ensured to comply with the basic principle of temporary immersion system (TIS) through subsequent avoidance of submergence while incorporating liquid medium (in the lower portion) for economizing the culture procedure. Two vessels or series of vessels can be connected for mass multiplication of two or more plants together.

Shoot meristems from *S. rebaudiana* plant and rhizomes of *A. calamus* produced synthetic seeds upon encapsulation with different concentrations of sodium alginate devoid of any plant growth regulators. Among the tested concentrations of Na-alginate, beads of ideal morphology, in terms of isodiametric shape, texture and rigidity, could be obtained with the 3% concentration (Figs. 25.5 and 25.6), which corroborates the findings of several earlier reports involving diverse plant systems (Ali et al. 2012; Alatar and Faisal 2012). The higher and lower concentrations failed to deliver the desired results as the acute fragility/delicateness of the beads with low concentration significantly impaired their subsequent handling and transfer practices, and conversely, the extreme rigidity/ solidity of the beads with high concentration completely hindered the regrowth potential of the encapsulated propagules. These findings are in agreement with several earlier reports where importance of optimization of sodium alginate concentration for maximizing the regrowth frequencies of encapsulated explants had specifically been exemplified involving diverse plant systems (Swamy et al. 2009; Sharma and Shahzad 2012). The encapsulation and germination of *S. rebaudiana* under liquid culture system has been proven to be highly favourable for mass multiplication of elite somaclone (Fig. 25.6), as callusing was totally eliminated from the shoot bases or shoot/root interphase, which was found to be bottleneck in semi solid cultures. Moreover, the regrowth and complete plantlet formation frequency was about 1.5 and 2.0 times better than the seeds growing on semi-solid medium.

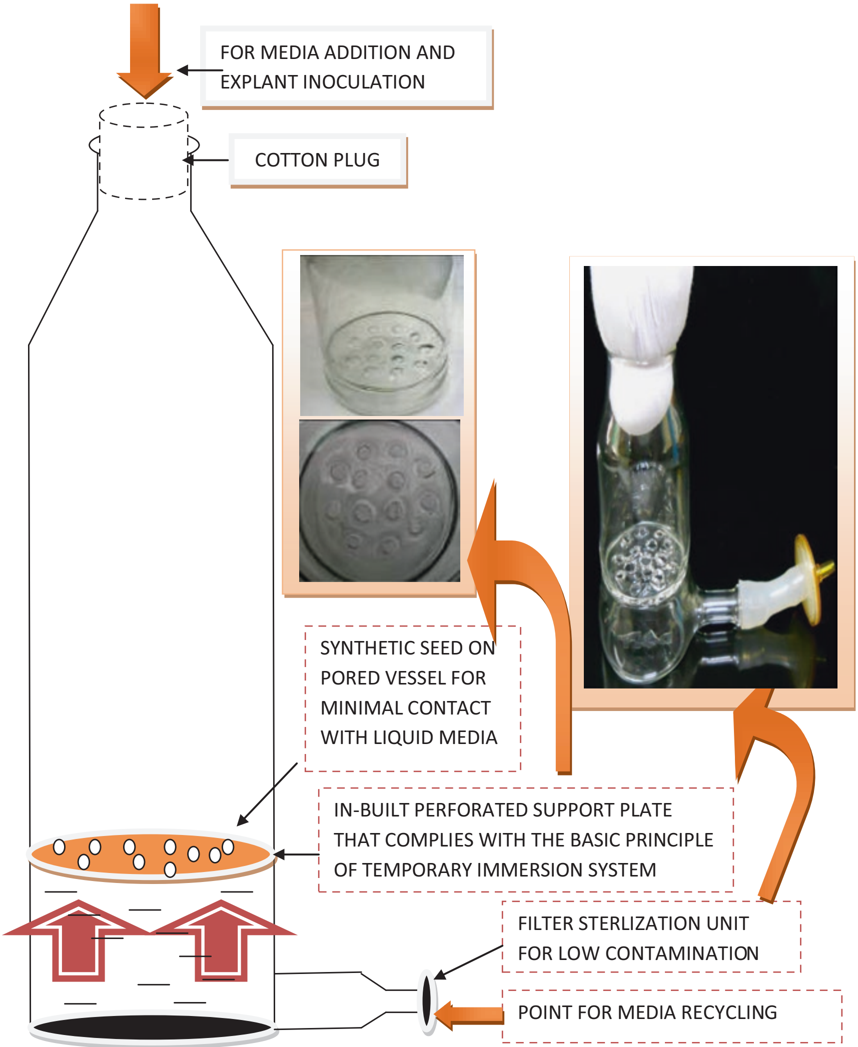

Fig. 25.4 A general layout of the design of modified pored glass vessel with an efficient temporary immersion system for conservation of elite clones of *A. calamus* and *S. rebaudiana*

The most ideal attribute of the encapsulated vegetative propagules of *A. calamus* would be their maximum regrowth potential in term of synchronous maturation exempting the consequential encumbrances of sub-culturing and hormonal supplementations. An almost 2.1 times higher regrowth could be achieved through the use of the former culture system (87.3%) as compared to that (37.7%) on semi-solid PGR-free MS medium (Fig. 25.5). Positive impact of liquid culture over that of solid culture has also been evidenced in two preliminary reports involving *A. calamus* micropropagation (Hettiarachchi et al. 1997; Sandhyarani et al. 2011). However,

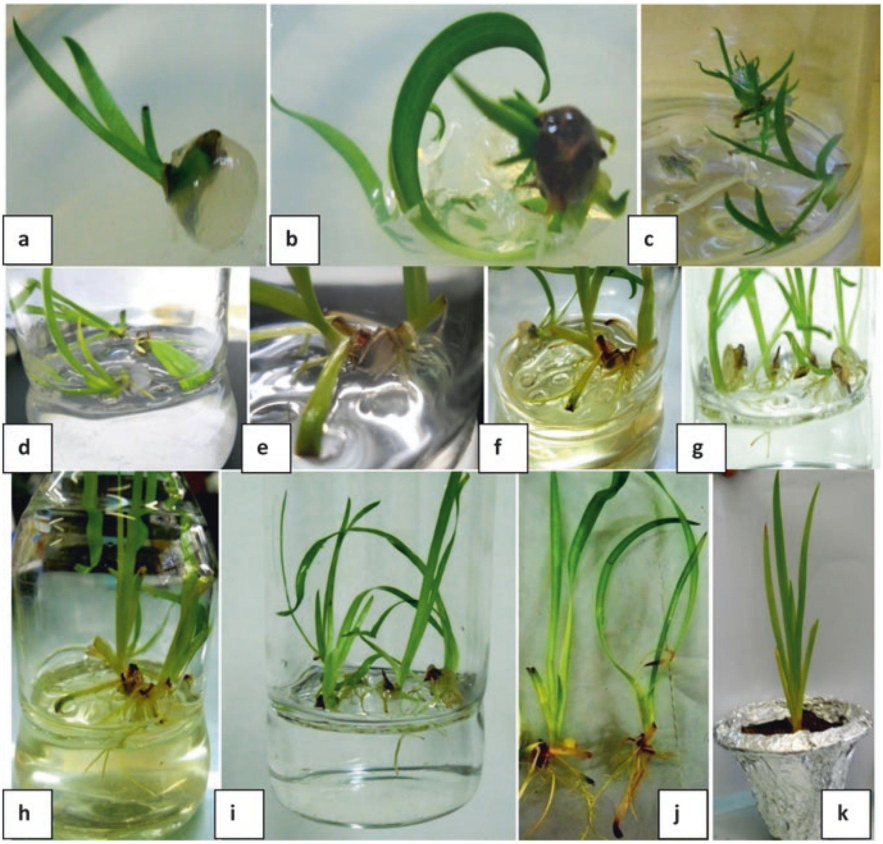

Fig. 25.5 Synseed production on MS basal semi-solid medium and TIS in *A. calamus.* (**a**, **b**) synseeds plated on semi-solid MS basal medium; (**c**–**i**) simultaneous shoot and root proliferation in synseed plated on liquid MS basal medium in pored glass vessel; (**j**) rooted plantlet derived from synseed plated in pored glass vessel; (**k**) synseed derived glasshouse acclimatized hardened plant

neither any attention has so far been focused towards the in-depth precision of the liquid culture system for mass multiplications of *A. calamus* in terms of its commercial relevance nor synthetic-seed-mediated multiplication has ever been attempted in this plant system. Significantly meaningful relations could be found between the type of media used and frequency of recovery of complete plantlets from synthetic seeds through concurrent induction of healthy shoots and roots without any intervening callus phase. When compared to semi-solid medium, liquid media in the modified vessel demonstrated a 7.14 times higher frequency of complete plantlet formation (73.25%) than that in the semi-solid (10.25%) counterpart (Fig. 25.7). The use of liquid medium confirming the TIS principle also demonstrated significant improvements in the root morphologies in terms of number, length, branching components and natural appearances (Table 25.3) as evidenced by several authors in earlier studies (Yan et al. 2010). The rooting percentage, root

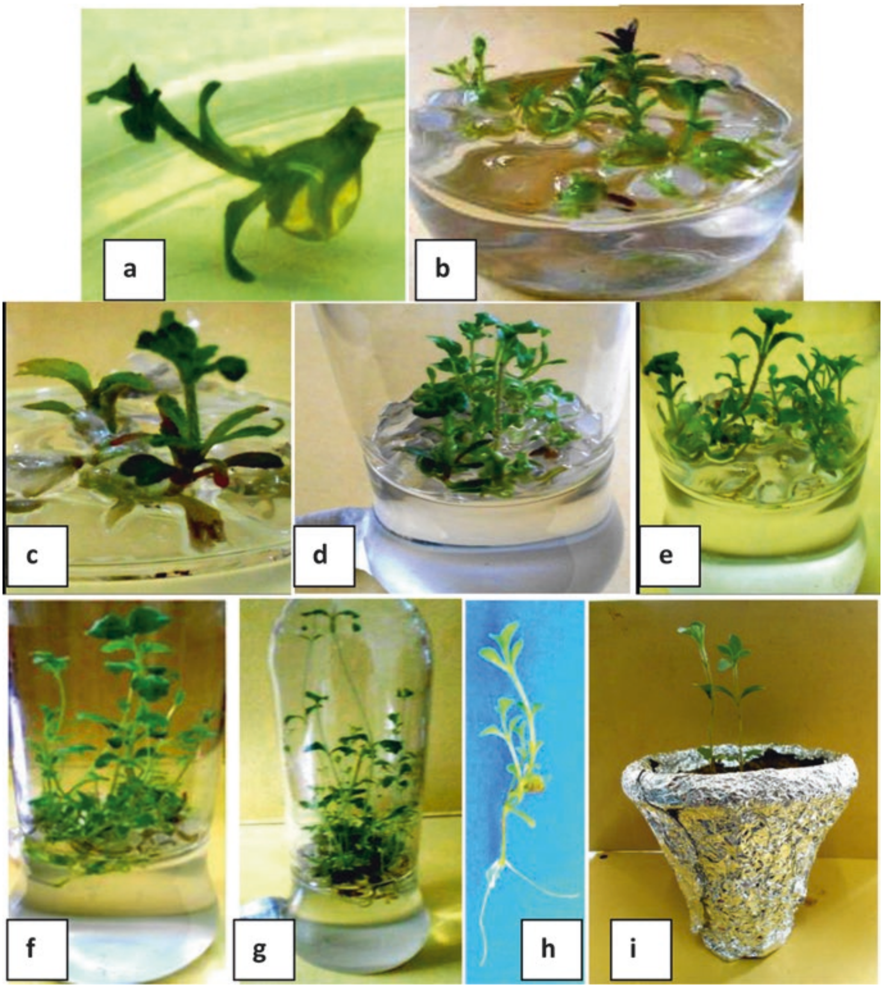

Fig. 25.6 **S**ynseed production on MS basal semi-solid medium and TIS in *S. rebaudiana.* (**a**) synseeds plated on semi-solid MS basal medium; (**b**–**g**) simultaneous shoot and root proliferation in synseed plated on liquid MS basal medium in pored glass vessel; (**h**) rooted plantlet derived from synseed plated in pored glass vessel; (**i**) synseed-derived glasshouse acclimatized hardened plant

number and root length in liquid culture were found to be 73.25 ± 2.5, 7.5 ± 2.38 and 3.07 ± 0.30 and 92.11 ± 2.2, 6.5 ± 2.33 and 3.44 ± 0.21 in *A. calamus* and *S. rebaudiana*, respectively.

The improved efficiency of the TIS system might be attributed to its ability to put forward the advantages of both liquid culture in terms of increased nutrient uptake and gelled culture for better gas exchange potentials, as corroborated earlier (Etienne and Berthouly 2002). On the contrary, the root formation ability of the semi-solid culture-based synthetic seeds of the present study was predominantly hampered

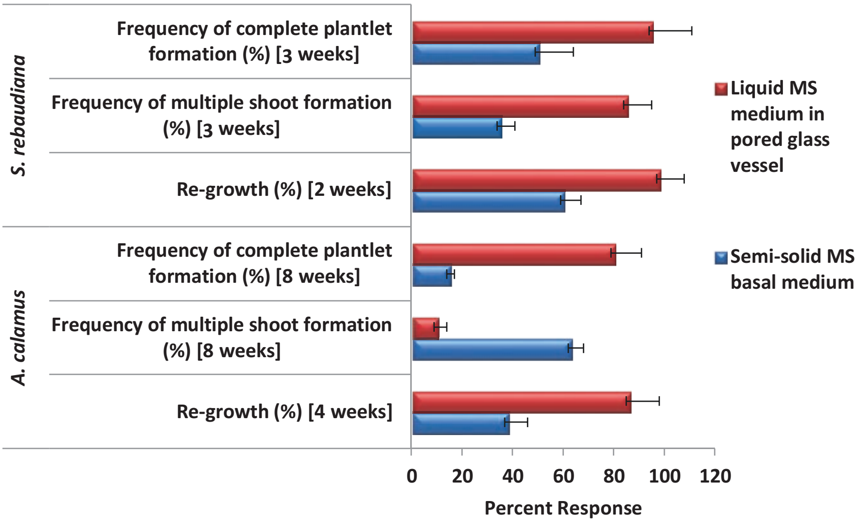

Fig. 25.7 Regrowth responses of *S. rebaudiana* and *A. calamus* synthetic seeds in semi-solid and liquid MS in pored glass vessel

Table 25.3 Comparative rooting response of synthetic seed-derived plantlets in semi-solid and liquid TIS system after 4 weeks of culture

	Rooting (%)		Root number		Root length (cm)	
	A. calamus	*S. rebaudiana*	*A. calamus*	*S. rebaudiana*	*A. calamus*	*S. rebaudiana*
Semi-solid MS basal medium	10.25 ± 2.22	89.55 ± 1.33	2.0 ± 1.29 (un-branched)	4.0 ± 1.35 (un-branched)	0.63 ± 0.15	1.85 ± 0.10
Liquid MS medium in pored vessel	73.25 ± 2.5	92.11 ± 2.2	7.5 ± 2.38 (branched)	6.5 ± 2.33 (un-branched)	3.07 ± 0.30	3.44 ± 0.21

Data are expressed as mean ± SD of 10 replicates

with the formation of shallow, frail and unbranched non-functional roots. Unpredictably, an almost 6.43 times higher potential of multiple shoot formation could be obtained on the semi-solid culture based responding encapsulated propagules of *A. calamus* as compared to that on the liquid medium, which however failed to demonstrate spontaneous or induced rooting response on half-strength MS medium containing either naphthalene acetic acid (NAA) or indole 3-butyric acid (IBA) at 1.0 mg/l, necessitating further optimizations.

It is pertinent to mention here that the use of liquid medium for regrowth of synthetic seeds is not a conventional practice, and apart from a recent report relating to filter paper bridge for growing synthetic seeds of *Pogostemon cablin* in liquid culture (Swamy et al. 2009), the credibility of direct use of liquid medium for regrowth of synthetic seed-based propagules has not been evaluated. However, as plantlet formation from synthetic seeds has been noticed to be highly sensitive to the planting substrate, considerable efforts have so far been made for optimizing the substrate for maximum plantlet development from synthetic seeds of diverse medicinal plants, where application of liquid media in combination with different substrates, such as cotton, filter paper, solerite, peatmoss, sand, etc. has been experimented with limited success (Ganapathi et al. 2001; Hassan 2003; Lata et al. 2011). It is obvious to state that high percentage of single-step post-planting and conversion of synthetic seeds into complete plants through optimization of regrowth conditions is a vital prerequisite for cost-effective, successful commercial utilization of this technique. But, disappointingly, majority of the reported studies have demonstrated serious constraints with regard to single-step shoot/root formation during synthetic seed regrowth, which alternatively necessitated multi-step culturing involving escalation of overall cost and time involvement (Etienne and Berthouly 2002). The observed data, therefore, adds preferentiality to the study of using liquid medium conforming the concept of TIS with lowest possible complexities to retrieve complete plantlets of *S. rebaudiana* and *A. calamus* through minimum time and labour. The liquid culture grown synthetic seed-derived healthy plantlets of *S. rebaudiana* and *A. calamus* demonstrated a total of 70–80% survival after 4 weeks of transfer to glasshouse following initial hardening as hydroponic culture, while only 15% the solid culture raised plantlets could be established in vivo (Figs. 25.5 and 25.6). This substantiates earlier reports where the temporary immersion system (TIS) has proved efficacious for micropropagation of diverse plant systems with proven record of quantitative advantages over that on gelled media, in terms of higher proliferation rate, improved morphological characteristics, greater potential of ex vitro transferring and reduced production cost (Yan et al. 2010; Etienne and Berthouly 2002).

The synseed-derived plants were subjected to genetic fidelity testing using 13 ISSR primers. Out of these, only eight produced specific and scorable amplification products by two randomly selected synthetic seed-derived liquid-medium grown *A. calamus* plants (A and B) in combination with that parent plant (P) (Table 25.4 and Fig. 25.8). A total of 102 bands were obtained, out of which 95 were found to be monomorphic (93.13%) and 7 were polymorphic (6.87%). The number of bands for each primer varied from 05 (UBC 848) to 18 (UBC 835 and 855) and ranged from 200 bp to 2.0 kb in size. The banding pattern of responded primers and its statistical analysis has been shown in Fig. 25.8 and Table 25.4, respectively. The similarity coefficients were calculated and used to generate cluster analysis using UPGMA clustering method. The clone A and B showed the maximum genetic similarity coefficient of 1.00 among each other, while they showed a similarity coefficient of 0.97 with the parent plant in the clad two. Similarly, in *S. rebaudiana* (SA and SB with parent SP), profile generated by eight responded ISSR markers (Table 25.4) showed total of 88 bands, out of which 78 were monomorphic and 10 were polymorphic. Band size ranges between 2027 and 300 bp. The randomly selected synthetic seed-derived plantlets of *S. rebaudiana* and *A. calamus* showed 93 and 97% similarity, respectively, with the parent plant which corroborates several earlier reports that have exhibited similar kind of genetic resemblance among

Table 25.4 Band analysis of synseed derived plantlets of *A. calamus* (AC) and *S. rebaudiana* (SR) generated by 13 ISSR primers

S. No.	Primer code	Sequence	Total bands		Band size (range in base pairs)		Monomorphic bands		Polymorphic bands	
			AC	SR	AC	SR	AC	SR	AC	SR
1	UBC-807	5’AGAGAGAGAGAGAGAGT 3’	17	12	1375–500	1375–300	16	12	1	0
2	UBC-810	5’GAGAGAGAGAGAGAGAT 3’	NR	NR	–	–	–	–	–	–
3	UBC-823	5’TCTCTCTCTCTCTCTCC 3’	06	06	947–500	1000–564	06	06	0	0
4	UBC-826	5’ACACACACACACACACC 3’	NR	16	–	1584–500	–	15	–	1
5	UBC-828	5’TGTGTGTGT GTG TGT GA 3’	NR	08	–	1904–450	–	06	–	2
6	UBC-834	5’AGAGAGAGAGAGAGAGCT 3’	15	NR	3500–600	–	15	–	0	–
7	UBC-835	5’AGAGAGAGAGAGAGAGCC 3’	18	11	1584–600	2027–947	18	9	0	2
8	UBC-841	5’GAGAGAGAGAGAGAGACC 3’	NR	10	–	1375–650	–	10	–	0
9	UBC-843	5’CTCTCTCTCTCTCTCTAA 3’	10	12	1700–450	2027–500	09	10	0	2
10	UBC-844	5’CTCTCTCTCTCTCTCTAC 3’	NR	NR	–	–	–	–	–	–
11	UBC-845	5’CTCTCTCTCTCTCTCTAG 3’	13	NR	2027–564	–	09	–	4	–
12	UBC-848	5’CACACACACACACACAAG 3’	05	NR	1584–1000	–	03	–	2	–
13	UBC-855	5’ACACACACACACACACCT 3’	18	14	1700–831	1504–400	18	12	0	2
		Total	102	88			95	78	7	10

NR not responded

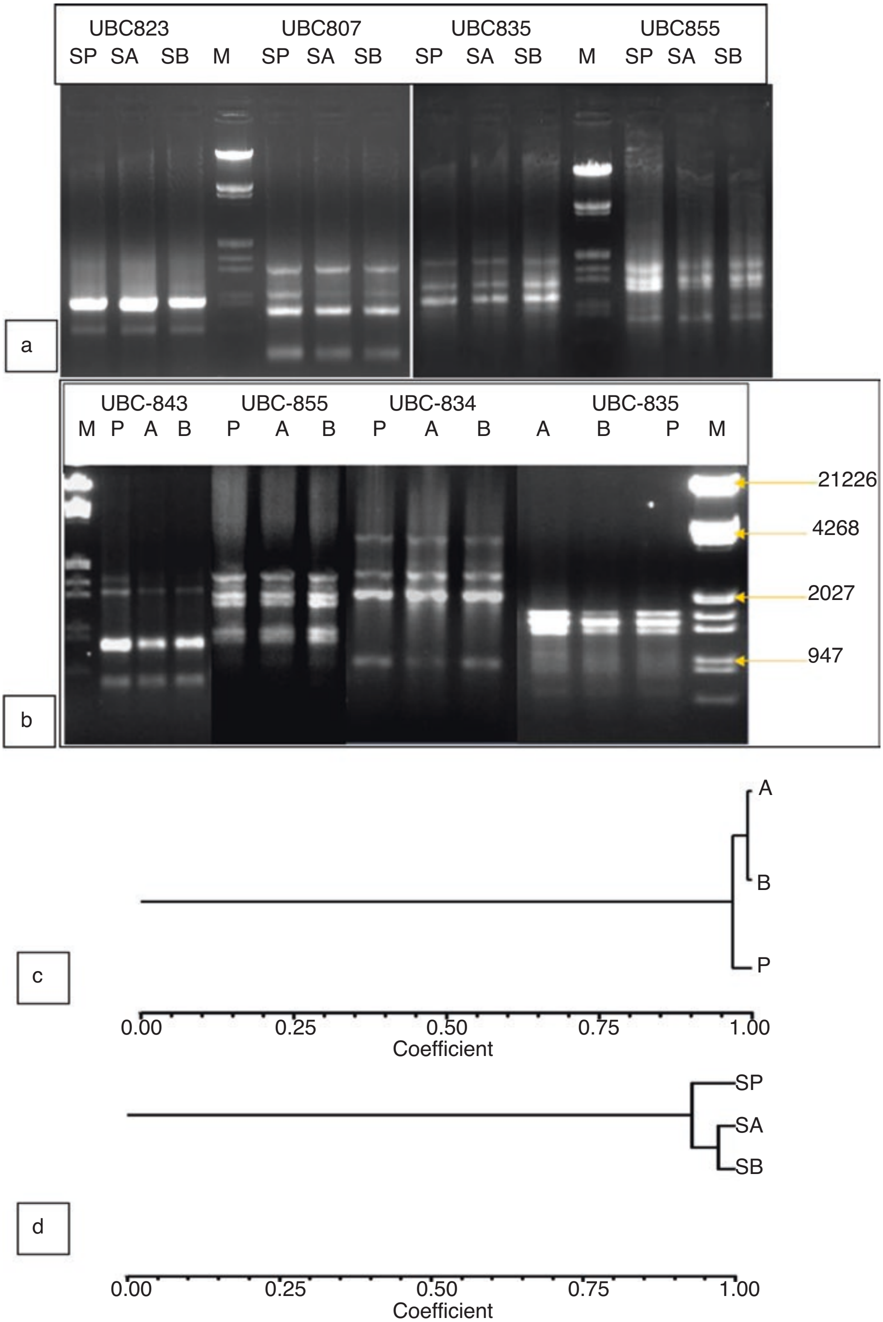

Fig. 25.8 ISSR proofing of *S. rebaudiana* and *A. calamus* in vitro and synseed-derived plants with different primers and their dendogram representations. Banding pattern generated by *S. rebaudiana* (**a**) and *A. calamus* (**b**); dendograms generated (**c**) *A. calamus* (**d**) *S. rebaudiana*

the parent plant and the synthetic seed derived (Lata et al. 2011). The overall observations evidently denoted the scientific relevance and economical significance of the authors study for ideal, single-step rapid multiplication of genetically uniform germplasm of *A. calamus* and *S. rebaudiana* plants in compliance with its commercial feasibility.

25.5 Synseed Technology as a Conservational Strategy for Medicinal Crops

The influence of research in synthetic seed is considered more impactful if protocols can be established for recovery of plants from synthetic seeds directly in the non-sterile conditions. One of the commonest and handy aspects that synthetic seeds cover is the transportability because through multiple shoot production or embryogenesis large number of plants can be generated but their transport from place to other is always at risk of contamination. This kind of in vitro raised cultures requires the shooted plant to induce rooting with a proper ratio followed by their greenhouse establishment after which the plants can be delivered or transported. No such above discussed problems come into foray with synthetic seed production as acclimatization can easily be achieved when the seeds are directly sown in to the soil, thus overcoming the complications of transport handling as well as delivery of plants.

Storing the synthetic seed is seen as a prime factor in the generating the whole plant from seeds, and many aspects such as light period, sucrose and agar concentration, temperature and moisture play important role in it. The synthetic seeds of different plant system respond differently as generally lower temperatures up to 4 °C are considered suitable for storing the seeds. But again, many tropical and subtropical species show better storing response on higher temperatures up to in between the range of 10 to 25° centigrade. Same way sucrose concentration in the media is also seen as a factor which can be altered to enhance the efficiency of storage of these synthetic seeds. Generally, 3% sucrose has been used in alginate mixture for storing purpose, but in some plants such as *E. alba*, the lower concentration from 3% to 1% has been used which yielded improved storage duration (Ray and Bhattacharya 2010). Same way sugar-free media were also tested to long-term storage in plants such as *C. fissilis* which resulted in enhanced storage provided the agar concentration is also varied (Nunes et al. 2003). As previously described, the basic media component changes helps in improving the storage properties of synthetic seeds of different plant species and agar variations in the MS media is can also help in improved storing properties. From 0.8 % agar which is commonly used to a higher concentration up to 1% and even a lower concentration of 0.4% (Nunes et al. 2003) can be seen effective depending upon the plant species used. Except these factors the duration of darkness period and irradiance has also been taken into consideration for an increase in storage property of synthetic seeds. The ultra-low temperature is a very useful approach for preservation since long time now and synthetic seed technique utilizes this approach to the maximum for long-term preservation. The synthetic seed technique can be seen as a future emerging technology provided that its applicability is more towards commercial applications. The single-step shoot/root generation protocols in plants can be optimized in plants so that commercially propagules can be produced. The lower temperature in combination with synthetic seed technique is also seen an effective way for long-term storage. The cryopreservation of embryo of coffee and carrots has been successfully achieved with this kind of approach where the lower temperature up to -20 degree centigrade can be used in combination with high molarity sucrose (Tessereau et al. 1994). The liquid nitrogen is used in this cryopreservation-mediated synthetic seed technique where embryos are first desiccated at a set humidity (75%) and temperature (24°) for at least 1-week duration followed by quick dipping in liquid nitrogen. The important factor in regeneration of whole plantlet form these cryopreserved embryos is the strain of embryo used and their respective size.

The lower temperature up to −196 degree centigrade is applied in liquid nitrogen in cryopreservation techniques which help in avoiding the crystal formation inside the cell which damage the semi permeable membrane of the cells. Various approaches of cryopreservation applied in synthetic seed techniques are desiccation, vitrification, two-step freezing, encapsulation-vitrification and encapsulation-dehydration, namely. Among them the most effective approach is encapsulation- dehydration in which encapsulated propagules are subjected to stepwise extraction of water through osmotic/evaporative dehydration (Sharma et al. 2013). More importantly this approach is less complicated and avoids the use of cryoprotectants which damage the encapsulated material, and there is no use of programmable freezers with helps in cost-effectiveness.

The regeneration potential of these approaches is also reported to be impressive as survived plants have a regeneration percent of up to 80%. For storage of synthetic seeds, different short- and long-term strategies are applied so that it can be preserved for longer periods of time, and this will help in easier transportation. Added advantages with stored synthetic seeds are they can easily be transported from one country to other without going for quarantine process as is the case with normal plant material transportation. For this purpose, short-, mid- and long-term strategies are taken into account which depend upon the plant species used.

The short-term strategies prefer the addition of growth retardants or some reduced amount of plant growth hormone in the polymer matrix used so that the growth of the plant is slow. The low temperature range helps for this purpose which is in the range of 4 to 14 °C depending upon plant material and the purpose of storage (Padilla et al. 2020). Though short- and long-term storage are useful strategies for successful storage of synthetic seeds, but it is important to note here that the regeneration potential of synthetic seeds decreases with the increase in the duration of storage. Many plant species have shown higher regeneration on initial weeks of their transfer for sowing, but the cold storage for longer periods of time decreases the regrowth potential.

However synthetic seed technique protocol is not as simple as complications are faced using this technique. One of the major constraints in these kinds of seeds is the germination percentage when soil transfer of these seeds is attempted because germination percent is low. Alongside it the green house transfer of synthetic seed-mediated plants is also not very efficient.

The synseeds produced from medicinal plants, namely, *Stevia rebaudiana*, *Acorus calamus*, *Bacopa monnieri and Hypericum perforatum*, have shown high percentage of greenhouse acclimatization, and the protocol and techniques discussed in the present chapter can be highly utilized in other medicinal plants also. To enhance the efficiency of transfer to the green house and field conditions, a better understanding at the genetic level is required. A comprehensive knowledge of the genes which are involved for desiccation, dormancy and regeneration of seed are needed for an improved transfer strategy of these synthetic seed-mediated plants.

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Chapter 26
Use of Secondary Metabolites from Medicinal and Aromatic Plants in the Fragrance Industry

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26.1 Introduction

The cosmetics, perfume, toiletries and hygiene industry have a significant weight in the population's consumption. According to the financial indicators of the Fragrance Foundation of New York, the size of the perfume industry amounts to US$ 6 billion, while globally the industry is valued at US$ 20 billion. This sector produces

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aromatic compounds for food and fragrances for perfumery and cosmetics, where essential oils are a fundamental ingredient. The industry manufactures essential oils and natural extracts that are used as inputs in other industries, while also producing chemicals or synthetic aromas. The analysis of the medium-term potential of this sector involves an examination of the lines of action that drive demand. It is important to consider that the key link between these products and the consumer is the processing industry itself (Bakkali et al. 2007). The trade in essential oils and aromatic extracts depends significantly on the strategies driven by an industry that invests huge amounts of money in research and development programmes associated with consumer preferences. Perfume is an aromatic substance manufactured to give a pleasant smell. It is a mixture that contains different aromatic substances, including natural essential oils (plant and animal ingredients) or synthetic essences, as well as a solid or liquid solvent, which can be alcohol in most cases, along with a fixative. Natural essences are mainly composed of alcohols, esters, phenols, aldehydes, ketones, acids and hydrocarbons, as well as small amounts of other chemical functions. Essential oils, on the other hand, are very complex natural mixtures that can contain from 20 to 60 different chemical compounds (Bakkali et al. 2008). The main group is made up of terpenes and terpenoids, while the second group consists of aromatics and aliphatic compounds, all characterized by their low molecular weight. The aromatic components present in essential oils are aldehydes (cinnamaldehyde), alcohols (benzyl alcohol, cinnamic alcohol, phenylethyl alcohol, phenylpropyl alcohol), phenols (chavicol, eugenol), derivatives of the methoxy group (anefole, elemicine, estragole, methyleugenol) and components with methylene dioxide (apiole, myristicin, safrole).

Among the most representative terpenes, present in essential oils, are acyclic, monocyclic and bicyclic monoterpenes (geraniol, limonene and terpineol); hydrocarbons (myrcene, ocimene, terpenes, p-cymene, phellandrenes, pinene, 3-carene, camphene, sabinene, among others); and alcohols (geraniol, linalol, citronellol, lavandulol, nerol, menthol, α-terpineol, carveol, borneol, fenchol, crysantenol, thujan-3-ol). Also included are aldehydes (geranial, neral, citronellal, citral); ketones (tegetone, chin, carvone, pulegone, piperitone, camphor, fenchone, fuyone, ombelulon, pinocamphone, pinocarvone); esters (propionate, citronellil acetate, menthyl, acetate, isobornyl acetate); and ethers (eucalyptol, menthofuran), along with peroxides (ascaridole) and phenols (thymol and carvacrol). For example, limonene, a hydrocarbon monoterpene, is the most abundant component in the essential oil of orange peel with a presence of 76.7%, followed by linalool, an oxygenated monoterpene present in 3.1% (Ferhat et al. 2006). 1.8-cineole (73.3%) is the main component of eucalyptus essential oil (Baranska et al. 2006; Silvestre et al. 1997; Cheng et al. 2009). The main compounds in the essential oil of the leaves of *Lippia alba* are carvone (25%), limonene (22%) and citral (21%) (Stashenko and Jaramillo 2004; Argyropoulou et al. 2007). On the other hand, geranium (38.7%), neral (24.5%) and limonene (5.8%) are the main compounds in the essential oil of the leaves of cidron (*Lippia citriodora*) (Argyropoulou et al. 2007). The components of the essential oils constitute mainly two groups of different biosynthetic origin. Depending on the quantity of essence contained in a perfume (between two and four tons of roses, for example, may be necessary to obtain one kilo of absolute essence from that flower), it may be called differently: cologne (between 2% and 4% essence), eau de toilette (between 5% and 12%), eau de parfum (between 13% and 20%), and finally perfume (between 21% and 25% essence). The demand for essential oils is growing in its two main uses: "aroma" and "fragrance". In the modality "aroma" also a growing tendency towards the "natural" is verified. A large part of aroma consumption is materialized in products that are or can be ingested by the population, so, potentially, the demand for natural aromas could reach sales of US$3.5 billion annually for the global industry. In cosmetics, studies carried out in the United Kingdom detect an increasing degree of consumer sophistication, but they still do not make a judgement on the medium-term trends in the market for essential oil-based cosmetics. Plant extracts and their derivatives constitute a very wide and heterogeneous group of products. The large number of plant species, their botanical diversity, the various products derived from them and the multiplicity of uses make them a complex and poorly defined sector, to which the derived products are added (Bakkali et al. 2007). For this reason, it has limited economic importance compared to food or textile crops. Nevertheless, the world market is in clear expansion. This is due to a significant increase in the level of demand, especially in the industrialized countries, where there are favourable prospects in the medium term. This situation raises possibilities for economic development in activities related to this productive sector, which is an alternative to surplus products. Overall, the plant extract sector, which lacks clarity and transparency, currently faces many difficulties of access for medium-sized enterprises in the agricultural sector. In addition, there is a lack of published or easily accessible information on production and markets (Bakkali et al. 2007). In contrast, there are comparative advantages of national production against foreign competition. This situation is due to the favourable characteristics of our country – not only ecological but also productive and of proximity to certain markets – for the cultivation and use of certain species of medicinal and aromatic plants, traditionally valued abroad for their high quality or due to the fact that there is little competition in markets. The existence of specific market segments with high development potential – essential biological oils and fresh seasoning plants – presents various opportunities for the perfume industry. From the point of view of production, one of the main aspects holding back cultivation is the absence or difficulty in finding plant propagation material that meets the requirements of the markets and profitable production. On the other hand, modern and industrial production of plant extracts must be linked to the development of mechanized crops. This condition is only possible on agricultural soils, which are susceptible to conventional mechanization, on plots of adequate size, and using crop varieties that can be selected in a morphological way. These must then be adapted for use in mechanical harvesting, while having the potential to act homogeneously at the ripening stage.

Therefore, this chapter aims to inform about the use of secondary metabolites extracted from some medicinal and aromatic plants of economic importance in the perfume industry, the importance of essences and about natural fragrances and enfleurage (extraction of aroma from flowers).

26.2 Perfumery Industry

Perfumes are mixtures of odoriferous substances of natural origin (essential oils) or synthetic (organic products), in order to achieve an aesthetic composition capable of impressing our sense of smell (Anne-Dominique 2004). The word perfume is derived from the Latin fumare (to produce smoke) and means smelting. Perfumery is the art of producing aromas by combining odoriferous substances. This can be done when fresh and dry petals are recycled separately, applying the extraction process, to obtain the pleasant and characteristic aromas (fragrances) of the flowers. The fragrance (perfume) of the flowers is due to the presence of volatile oils contained in the small vesicles of the plants, which, to remove them, requires the use of extraction processes such as distillation, absorption and maceration. The perfume as it is known today is divided into extracts (the one that lasts the longest on the skin), while the eau de toilette is a softer version. The cologne is more delicate in smell and very refreshing. Today the perfume industry is a powerful appendage of the chemical industry, with high sales volumes. Perfumes occupy an important position in the cosmetic market, becoming high value-added products with significant demand in many countries.

Currently, there are approximately 3000 commercial products in the fragrance industry, including both natural and laboratory-synthesized products. This gives an idea of the importance of this industry worldwide. Natural products derived from medicinal and aromatic plants used to produce perfumes are becoming increasingly expensive due to limited production and increased labour costs during harvesting and other production costs.

Germany is the leading European market in the perfume industry with 18.67 billion euros in sales in 2013; France is the second European market in the perfume industry with 17.07 billion euros in sales in 2013. The United Kingdom is competing strongly in the European perfume industry with a value of 14.46 billion euros in sales in 2013. It is Europe's third largest market by value. The fourth European market in the European sector is Italy, with 12.91 billion euros in sales in 2013, i.e. a 4% market share worldwide. The last key European country is Spain with a perfume sector that represents 9.70 billion euros in sales in 2013, according to euromonitor. The perfume industry is a large sector with great opportunities for the future. Between 400 and 500 new fragrances are created every year. Perfumery is not simply an industry or a science; it is also the art of subtly and precisely combining natural, vegetable, animal and synthetic aromas in an alcoholic solution. Its main objective is to achieve a persistent and homogeneous aromatic blend that is pleasant for the consumer (Sikora et al. 2018).

The fragrance industry is dedicated to the production of certain cosmetics, colognes, perfumes and similar and related products. Most perfumes are composed of a base of pure ethyl alcohol, to which a mixture of aromatic bodies or essences of animal, vegetable or synthetic origin is added, accompanied by stabilizers and fixatives. The creation of a perfume is currently very different from what it was in ancient times, due to scientific and technical advances that have allowed the creation of increasingly complex formulas, the choice and obtaining of quality raw materials and the work of perfumers which are the keys to success (Sikora et al. 2018).

26.3 Perfume Composition

The perfumes with flower aromas have basic ingredients such as jasmine and rose, although it is also produced with gardenias, violets, daffodils and lilacs. Some are made with critical fragrances such as lemon and orange, both from their flowers and from their own fruit (Anne-Dominique 2004). The oriental aromas are the most sensual and are composed of patchouli and musk. They have a perfect balance between flowers and spices and impart a mystical air. Some people say that knowing how to perfume oneself is an art and recommend that it be poured on those areas of the body where the heartbeat is most intense, such as the wrists, ankles, temples, earlobes and breasts, as the body's heat activates the fragrance and makes it last longer. In addition, some essential oils come from slow-growing trees, such as cedar and sandalwood.

Fragrance industry is in convergence with the pharmaceutical and petrochemical industry, in terms of production it is close to the pharmaceutical industry and the cost per kilogram of product its prices are similar to those of the bulk chemical industry. The major fragrance companies run two businesses: production of ingredients and sale of fragrances. In fact, the major companies sell ingredients to each other.

Each fragrance company's ingredient business competes for internal business with its rival companies. It is noted that perfume ingredients are produced by companies that do not produce fragrances, and these sources also compete for the ingredient business. The three main reasons why chemical companies outside the fragrance industry produce perfume ingredients are (1) availability of raw materials, (2) common intermediaries and (3) technology.

26.4 Perfume Ingredients Derived from Terpenoids

Terpenoids are the largest group of natural odorants and form the largest group of modern fragrance ingredients. Below are some examples related to the type of smell of some of the most important ingredients of the terpenoid fragrance. Menthol offers mint and coolant scent; linalyl acetate offers fruit and floral scent; carvone offers spearmint scent; citronellol and esters offer rose scent; dihydromyrcenol offers citrus and floral scent; geraniol/nerol and esters offer rose scent; hydroxycitronellal offers lily of the valley scent; borneol/isoborneol and acetate offer pine scent; linalool offers floral and wood scent; (methyl)ions offer violet scent; alpha-terpineol and acetate offer pine scent; acetylated cedarwood offers cedar scent. The volumes of these materials range from around 5000 tonnes per year for materials such as linalool and citronellol, which together are produced to around 10–20 tonnes per year (Sell 2006).

Menthol is extracted from several species of mint. Corn mint (*Mentha arvensis*) contains the highest proportion of l-menthol and is therefore the main variety grown for the production of menthol. Peppermint is grown in China, India, Brazil and the United States. Due to the climate and competition for land from other agricultural products, the supply of natural menthol is not stable. Price and availability fluctuate and, these movements have a major impact on the economics of fragrances (Sell 2006).

Diterpenoids have 20 carbon atoms in their structure, which means that very few are volatile enough to possess an odour. A diterpenoid is used in perfumery because it and its derivatives are odourless. That is, they are used as solvents. In view of their hydrophobicity and low volatility, these solvents also have fixing properties. Perfume manufacturers use ethanol as the solvent of choice because of its low odour. The low boiling point of ethanol means that the solvent evaporates quickly and does not remain on the skin. Among the various natural aromatic components, essential oils are the main therapeutic agents. They are a highly concentrated, volatile and complex mixture of aromatic components obtained from different organs of the plant (Sell 2003; Ashour et al. 2010).

There are 17,500 species of aromatic plants from different angiospermic families that produce essential oils, in particular Lamiaceae, Rutaceae, Myrtaceae, Zingiberaceae and Asteraceae. The essential oils contain approximately 20 to 60 different components in various concentrations. They are characterized by two or three main components at relatively high levels (20–70%) with several other minor components (trace amounts).

In general, these main components are responsible for the biological potentials of essential oils. The components of essential oils are classified into two major groups (terpenes and aromatic compounds) according to their biosynthetic origin.

26.5 Perfumery Supplies

Raw materials used in perfumery are traditionally divided, according to their origin, into natural and synthetic (Fratér et al. 1998).

Naturals. We call natural all those raw materials obtained from natural sources by applying physical separation techniques such as distillation or extraction. Natural products have been used for millennia as raw materials for perfumery: flowers, fruits, seeds, leaves, whole plants, woods, roots and resins have been, and are, primordial sources for obtaining aromatic materials (Fratér et al. 1998). The odoriferous glands of certain animals, such as the civet cat and the musk deer, have also been used to make perfumes for humans since the earliest moments of civilization.

Synthetics. Today there are several thousand synthetically manufactured aromatic chemicals that can be useful to the perfumer. Many of them, for example, vanilla, oxide roses and damask, were first discovered in nature and then synthesized.

Others, however, are purely the fruit of the imagination of chemists and have never been found in nature. Of course, not all are of equal value to a perfumer, and so the number of those often used in perfumery is closer to several hundred than to several thousand (Fratér et al. 1998).

26.5.1 Constituents of Natural Perfumes

As a result of the development of analytical methods, our knowledge of natural fragrances has evolved dramatically, so today we know a few hundred constituents of the most important essential oils, resinoids and absolute essences. The compounds present in fragrances vary from considerable magnitudes, to very small quantities at the level of parts per million (ppm). In order to simplify the presentation of this topic, the compositions will be given in three ranges:

1. >1% main constituents
2. 0.1–1% minor constituents
3. <0.1% trace components

The perfume of a natural product is usually the result of a complex interaction of the different constituents, so each and every one of the components contributes to the total olfactory perception. The role played by minor components and even those found only in trace amounts would seem irrelevant at first glance, since they are present in very small quantities. However, if correlated with their odourific values, it soon becomes clear that these compounds are equally important, or perhaps crucial, to the reproduction of the aromatic pattern of a specific natural fragrance. The most characteristic constituents are mentioned below, although it is known that there are hundreds of them.

Constituents Group 1 A representative fragrance of this category is the absolute essence of lily, obtained by steam distillation of the rhizomes of *Iris pallida* (flowering lily) or *Iris germanica*, which are grown in Italy and Morocco, respectively. Three years after planting, the rhizomes are harvested, peeled and dried. Finally, in order to release the irones, compounds that constitute the aromatic principle of the lily, the rhizomes have to be matured for about 3 years (Calkin (1994). The product of steam distillation, called lily "butter", contains 70% of various fatty acids, the most abundant of which is myristic. After further treatment, the fatty acids are removed, leaving the absolute essence of lily, which contains numerous linear hydrocarbons (2.5%), methyl and ethyl esters of fatty acids (4%), aldehydes, ketones, alcohols and pyrazines (diazines) in minute quantities and of course the irons (>80%). In addition, 60 trace components have been identified in the lily fragrance. Another example where a few constituents practically determine the complete aromatic character of a natural fragrance is the case of sandalwood oil (*Santalum album*). Here the two sesquiterpene alcohols a-santalol and beta-santalol make up 60%. The other important compounds are given and serve essentially as balancing components. The various phenols present in traces contribute to the "smoky" aroma of sandalwood oil.

Similarly, vetiver oil is composed mainly of sesquiterpenes or their derivatives. Some 50 constituents have been identified in total in this oil, the most important. Although the main constituents identified represent only 30% of the extract, they contribute decisively to the aromatic pattern of this fragrance.

Finally, in the absolute essence of oak moss, obtained from lichen (*Evernia prunastri*), many minor constituents and trace components serve to amplify the aromatic impression of a single key constituent, present in 35% of the total, methyl beta-orcinolcarboxylate.

Constituents Group 2 The absolute essences and essential oils studied in this part are the main derivatives of precious flowers such as rose, jasmine, spikenard and violet and orange blossom. In contrast to the fragrances belonging to Group 1, their aromatic profile is not only based on a few main constituents, but they are also critically important with minor constituents and trace components.

Bulgarian rose oil (*Rosa damascena*) has been thoroughly researched during the previous years, and 300 constituents have been identified. It has been shown that the minor and trace components (rose oxide, B-damascene, p-damascone, 1-p-menth-9-al, furan rose, p-ionne) play a major role in the aromatic profile of this precious essence, the most important in fine perfumery of all floral fragrances, of which about 15 metric tons per year are produced in the form of concrete and absolute essence.

Along with rose oil, the fragrances of tuberose (*Polianthes tuberosa*) and jasmine (*Jasminum grandiflorum*) are the classic flower essences in fine perfumery. Relatively few studies have been carried out on the fragrance of spikenard. The main constituents account for almost 70%. In contrast to the spikenard fragrance, the absolute essence of jasmine has been subjected to numerous extensive studies. One hundred and seventy constituents have been found, 15 of which represent 90% of the absolute essence.

Until 1980, only 20 constituents of the violet flower fragrance (*Violata adore*) were known. A systematic study of violet concrete has led to the identification of 80 new constituents.

At present, due to its high price, violet concrete and even more its absolute essence are used less frequently being replaced by synthetic components such as a and p-ionones. The last representative fragrance of this category of natural aromatic products is the well-known orange blossom absolute essence, which is obtained by hexane extraction from the flowers of the *Citrus aurantium*, bitter orange tree. This fragrance is characterized by the presence of various nitrogenous compounds, such as indol (2%), methyl anthranilate (3%) and phenylacetonitrile (0.8%).

Constituent Group 3 As an example of this class of floral fragrances, there is the absolute essence of mimosa (*Acacia dealbata/floribunda*). The olfactory part of this extract, which represents about 15% of the absolute essence, is characterized by a rich floral-woody note. Before 1985, very little was known about its composition. At present, 130 constituents have been identified.

The main constituents (hydrocarbons, fatty acids and esters) have virtually no impact on the overall fragrance. On the contrary, the minor constituents, mainly aldehydes (octanal, 2E-nonena1, 2E, 6Z-nonadienal), esters and alcohols, form the central part of the fragrance; however the complexity and richness of this fragrance are due to the trace components, among which several diethylacetals stand out.

The fragrance of daffodil (*Narcissus poeticus*) has sweet-floral, spicy, balsamic and slightly moss-wood notes, which contains about 280 constituents. The basis of this absolute essence is given by the constituents mainly representing 90% and is responsible for the notes, sweet, spicy and balsamic. However the most interesting aspect of this fragrance is due to the presence of a large number of minor constituents and trace components.

26.5.2 Scents

Plants contain the structures that have the greatest amount of volatile and non-volatile compounds related to aroma; many of them are synthesized by well-known biochemical transformations, while for others their origin or genesis is unknown. These substances are not homogeneously distributed in the tissue; this heterogeneity is not only quantitative but also qualitative.

Many are the factors that affect the biochemical cycles and consequently the production of aromas. Firstly, the genetics of each fruit or plant establishes a different mechanism for the production of aromatic substances; for this reason each fruit presents characteristic sensory properties due to the qualitative and quantitative proportion of these active molecules.

Besides the genetic aspects, the climatic conditions like (humidity, temperature, sunshine) the type of soil (pH, availability of nutrients, etc.) and natural practices (crop rotation, irrigation, fertilization, addition of hormones to the soil, etc.) also have a decisive influence on the production of aromatic substances, as well as their quantity and quality.

The aroma of a medicinal plant also depends on the conditions under which it is ripened, since it is not the same if it ends up on the plant as if the fruit is harvested unripe and stored in a chamber that has been conditioned for ripening. Among the plants that can be chosen as the main sources for obtaining raw material (essential oil) used in the fragrance industry are:

Aromatic plants: lavender, lemon balm, sage, rosemary, lavender, thyme, marjoram
Flowers: rose, jasmine, carnation, hyacinth, orange blossom, daffodil, spikenard, violet.
Algae and lichens: moss and seaweed
Spices: vanilla, cardamom, coriander, cloves
Citrus: orange (sweet or bitter), lemon, tangerine, bergamot
Grains and seeds: aniseed, dill, caraway

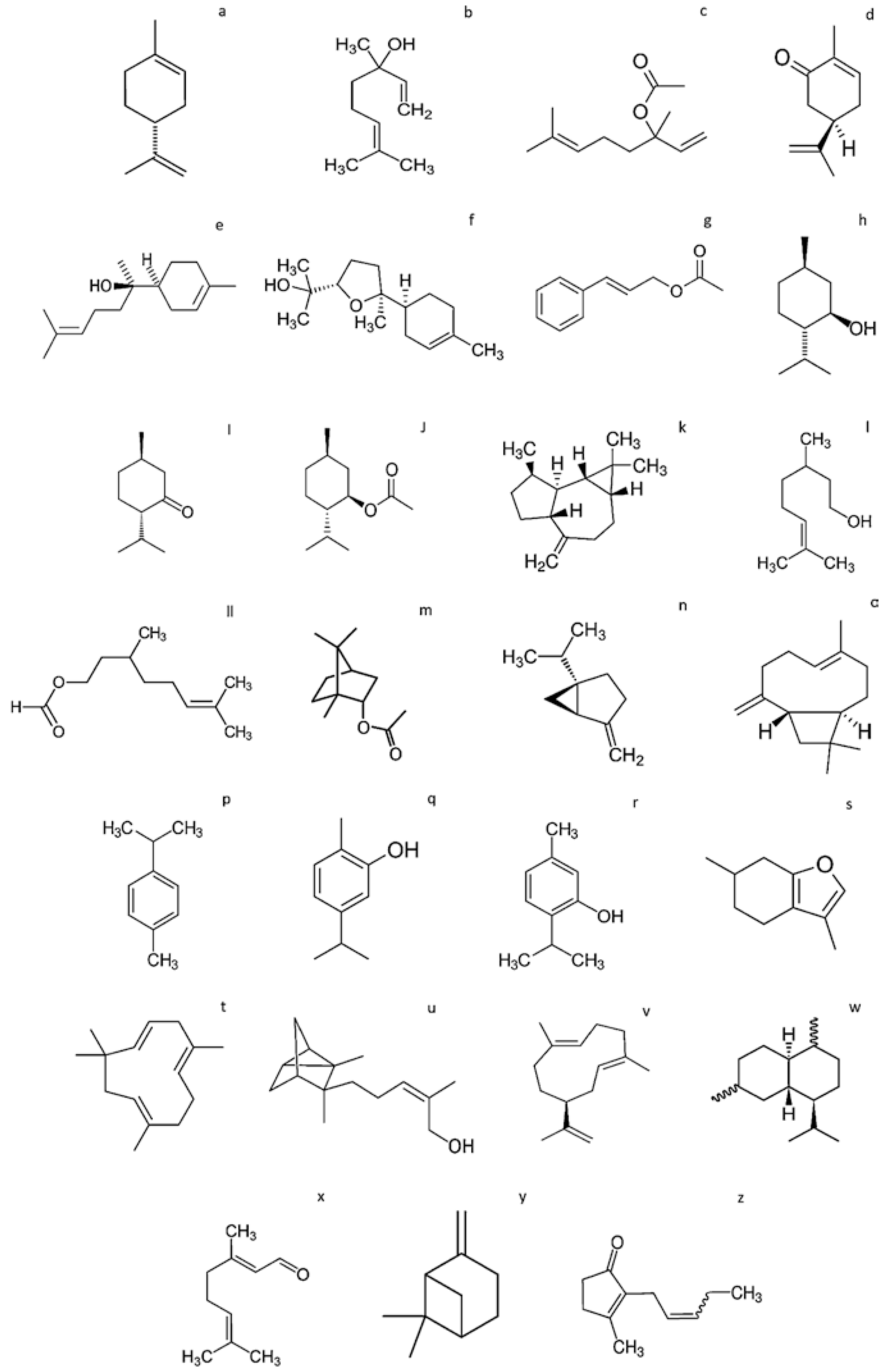

Fig. 26.1 Main components of the essential oils of various medicinal plants. (**a**) Limonene (136.23 g/mol, $C_{10}H_{16}$); (**b**) linalool (154.25 g/mol; $C_{10}H_{18}O$); (**c**) linalyl acetate (196.29 g/mol, $C_{12}H_{20}O_2$); (**d**) carvone (150.22 g/mol, $C_{10}H_{14}O$); (**e**) α-bisabolol (222.372 g/mol, $C_{15}H_{26}O$);

Balsam and resins: estoraque incense, myrrh, galbanum
Bark and roots: cinnamon, ginger, vetiver, angelica, calamus
Woods: birch, cedar, cypress, pine, sandalwood, laurel, patchouli, caneta
Other aromas (tobacco, camomile, verbena, mugwort)

Some examples of the main secondary metabolites used in the fragrance industry that come from plants are bergamot (*Citrus bergamia*) son el limonene, linalool, linalyl acetate; in caraway (*Carum carvi*) are carvone and limonene; in chamomile (*Matricaria chamomilla*) are alpha-bisabolol, bisabolol oxide B, (E)-beta-farnesene, alpha-bisabolone oxide cinnamaldehyde and cinnamyl acetate; in cinnamon (*Cinnamomum zeylanicum*) are cinnamaldehyde and cinnamyl acetate; in cornmint (*Mentha arvensis*) are menthol, menthone, isomenthone and menthyl acetate; in eucalyptus (*Eucalyptus* spp.) are 1,8-cineole (eucalyptol), limonene and aromadendrene; in geranium (*Pelargonium graveolens*) are citronelol, geraniol, citronellyl formate and linalool; in jasmine (*Jasminum* spp.) are benzyl alcohol, linalool, benzyl acetate jasmone and geraniol; in juniper (*Juniperus* spp.) are bornyl acetate, sabinene, alpha-pinene and limonene; in lavender (*Lavandula angustifolia*) are geraniol, linalool, linalyl acetate and beta-caryophyllene; in lemon (*Citrus limon*) are limonene, beta-pinene, gamma-terpinene and p-cymene; in lemongrass (*Cymbopogon citratus*) are citral (geranial), neral and myrcene; in oregano (*Origanum vulgare*) are carvacrol, thymol and cymene; in palmarosa (*Cymbopogon martinii*) are geraniol, geranyl acetate and linalool; in peppermint (*Mentha piperita*) are menthol, menthone, 1,8-cineole and menthofuran; in pine (*Pinus* spp.) alpha-humulene, caryophyllene, beta-pinene and beta-cadinene; in rose (Rosa damascena) are citronelol, geraniol, beta-pinene and beta-cadinene; in Rosemary (*Rosmarinus officinalis*) are camphor, 1,8-cineole, alpha-pinene, borneol, camphene and beta-phellandrene; in sandalwood (*Santalum album*) are alpha-santalol, beta-santalol and beta-curcumen-12-ol; in spearmint (*Mentha spicata*) are carvone, 1,8-cineole and limonene; in sweet basil (*Ocimum basilicum*) are linalool, alpha-cadinol, alpha-bergamotene and gamma-cadinene; in thyme (*Thymus vulgaris*) are thymol, carvacrol, terpinene and cymene; and in ylang-ylang (*Cananga odorata*) are geranyl acetate, benzyl benzoate, eugenol, germacrene-D and geraniol. Figure 26.1 shows the chemical structures, molecular weight and chemical formula of the secondary metabolites mentioned in these examples.

Fig. 26.1 (continued) (**f**) bisabolol oxide (238.37 g/mol, $C_{15}H_{26}O_2$); (**g**) cinnamyl acetate (176.215 g/mol, $C_{11}H_{12}O_2$); (**h**) menthol (156.15 g/mol, $C_{10}H_{20}O$); (**i**) menthone (154.25 g/mol, $C_{10}H_{18}O$); (**j**) menthyl acetate (198.30 g/mol; $C_{12}H_{22}O_2$); (**k**) aromadendrene (204.35 g/mol, $C_{15}H_{24}$); (**l**) citronellol (156.27 g/mol, $C_{10}H_{20}O$); (**ll**) citronellyl formate (184.27 g/mol; $C_{11}H_{20}O_2$); (**m**) bornyl acetate, $C_{12}H_{20}O_2$ 196.29 g/mol); (**n**) sabinene (136.23 g/mol, $C_{10}H_{16}$); (**o**) β-caryophyllene (204.36 g/mol, $C_{15}H_{24}$); (**p**) p-cymene (134.21 g/mol, $C_{10}H_{14}$); (**q**) carvacrol (150.22 g/mol, $C_{10}H_{14}O$); (**r**) thymol (150.22 g/mol, $C_{10}H_{14}O$); (**s**) menthofuran (C10H14O 150.22 g/mol); (**t**) humulene (204.35 g/mol, C15H24); (**u**) α-santalol (220.36 g/mol, $C_{15}H_{24}O$); (**v**) germacrene (204.35 g/mol, $C_{15}H_{24}$); (**w**) γ-cadinene (204.35 g/mol, $C_{15}H_{24}$); (**x**) neral (152.24 g/mol, $C_{10}H_{16}O$); (**y**) β-pinene (136.23 g/mol, $C_{10}H_{16}$); and (**z**) jasmone (164.24 g/mol, $C_{11}H_{16}O$)

26.5.3 Natural Fragrances

Primary qualitative phytochemical tests showed in the Lilium family steroidal saponins, phenolic glycosides, flavonoid alkaloids and pyrroline-pyrrolidine alkaloids (Farsam et al. 2003). In the absolute essence of lily (belonging to group 1), the percentage of the main constituents are cis-γ-irone (42.0%), cis-α-irone (32.0%), trans-α-irone (3.6%) and β-irone (1.4%), the majority percentage being 79.0%. Among the important trace components that correspond to percentage values lower than <0.1%, the following stand out: n-nonanal, n-decanal, linalol, geraniol, benzyl alcohol and fatty acid esters (Farsam et al. 2003). Figure 26.2 shows the main secondary metabolites isolated from the absolute essence of lily.

Trade oils from *S. album* mainly contain santalols (50–70%) associated with close metabolites (Howes et al. 2004), with α-santalol ranging from 40% to 55% and β-santalol from 17% to 27% of total oil (Verghese et al. 1990).

From the two molecules, β-santalol is the main source of the odour profile, while α-santalol has a very weak odour. Degradative alcohols such as α-santalal and other molecules also contribute to the odour profile described by Anonis (Anonis 1998).

In sandalwood oil (belonging to group 1), the percentage of the main constituents are α-santalol (46.0%) and beta-santalol (20.0%), corresponding to the majority percentage of 66%. Among the important minor constituents that are in a range of 0.1–1% belong the following metabolites: α-santalene and β-santalene; among the important trace components that correspond to percentage values lower than <0.1% stand out the ortho- and para-cresol, para-vinifenol, guaiacol, para-methylguaiacol and 6-methoxy-4-allylguaiacol. Figure 26.3 shows the main secondary metabolites isolated from sandalwood oil.

Vetiver oil is commonly used as a main odour contributor in the fragrance industry and as a flavour agent in the food industry (Martínez et al. 2004). Despite its special aroma, vetiver oil possesses various biological activities, such as anti-inflammatory (Balasankar et al. 2013), antibacterial (Luqman et al. 2005) and antioxidant (Luqman et al. 2009), making it beneficial in aromatherapy (Devprakash et al. 2011). The annual market demand of vetiver oil was estimated at up to 250 tons, worth approximately $200 million per year (Burger et al. 2017), while the worldwide herbal cultivating area has reached nearly 10,000 hectares.

In vetiver oil (belonging to group 1), the percentage of the main constituents are Kusimone (9.0%), khusimol (5.0%), α-vetivone (4.0%), β-vetivenene (4.0%), β-vetivone (3.0%), vetiselinelol (3.0%), cyclocopacamphenol (2.0%) and zizaene (2.0%), corresponding to the majority percentage of 32.0%. Among the important minor constituents that are found in a range of 0.1–1% belong the following metabolites: 10-epi-γ-eudesmol, elemol, junenol and α-y-langene; among the important trace components that correspond to percentage values less than <0.1% are α-vetispirene, acoradiene, prezizaene, α-funebrene and kusinol (Andreea et al. 2019). Figure 26.4 shows the main secondary metabolites isolated from vetiver oil.

In Bulgarian rose oil (belonging to group 2), the percentage of the main constituents are citronellol (31.0%), geraniol (12.0%), nerol (8.0%), linalol (2.0%) and

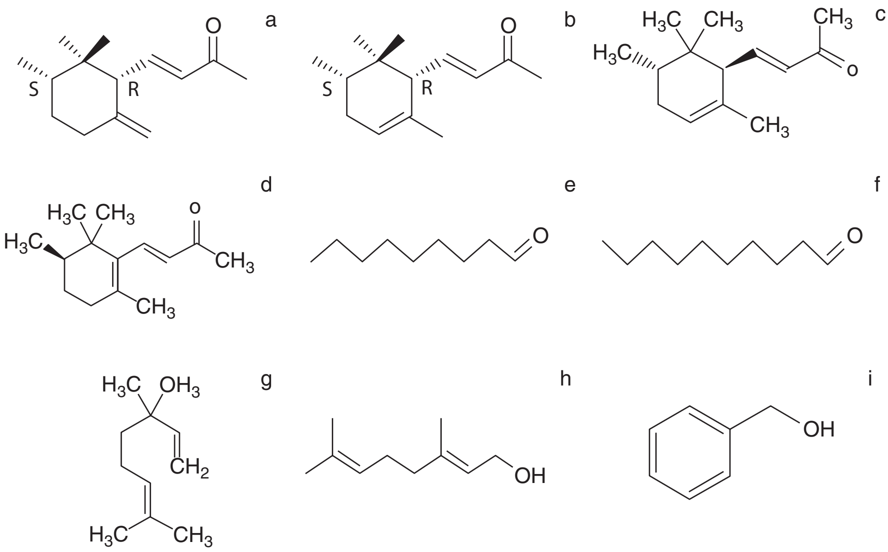

Fig. 26.2 Main secondary metabolites isolated from lily absolute essence. (**a**) Cis-γ-irone (206.32 g/mol, $C_{14}H_{22}O$); (**b**) cis-α-irone (206.32 g/mol, $C_{14}H_{22}O$); (**c**) trans α irone (206.32 g/mol, $C_{14}H_{22}O$); (**d**) β irone (206.32 g/mol, $C_{14}H_{22}O$); (**e**) n-nonanal (142.24 g/mol, $C_9H_{18}O$); (**f**) n-decanal (156.20 g/mol, $C_{10}H_{20}O$); (**g**) linalool (154.25 g/mol, $C_{10}H_{18}O$); (**h**) geraniol (154.25 g/mol, $C_{10}H_{18}O$); and (**i**) benzyl alcohol (108,14 g/mol, C_7H_8O)

phenylethyl alcohol (2.0%), corresponding to the majority percentage of 55.0%. Important minor constituents in the range of 0.1–1% include the following metabolites: phenylethyl acetate, phenylethyl tiglate, citronellulose acetate, caryophyllene and rose oxide, important trace components in the range of <0 percent. 1% include ethyl diethyl acetal, n-pentanal diethyl acetal, n-hexanal diethyl acetal, ethyl citronellil acetal, ethyl neryl acetal, ethyl geranium acetal, beta-damascene, beta-damascone, rose furan and nerol oxide (Mohaddese 2016). Figure 26.5 shows the main secondary metabolites isolated from Bulgarian rose oil.

In the absolute essence of nard (belonging to group 2), the percentage of the main constituents are methyl palmitate (14.5%) and methyl benzoate (12.2%). Among the important minor constituents found in a range of 0.1–1% belong the following metabolites: 5-decanolide and jasmine lactone, among the important trace components that correspond to percentage values lower than <0.1% include massoia lactone, tuberolactone, methyl dihydrocinnamate, hexyl methyl ether, phenylacetonitrile, 1-nonen-4-ol, alpha-damascone, anethole, 6-methyl-alpha-pyrone, p-methoxyphenol acetonitrile and epoxy heugenol (Kumar and Singh 2011). The main secondary metabolites isolated from nard absolute are shown in Fig. 26.6.

In the odourific part (5%) of the violet flower concrete (belonging to group 2), the percentage of the main constituents are alpha-ionone (14.1%), dihydro-beta-

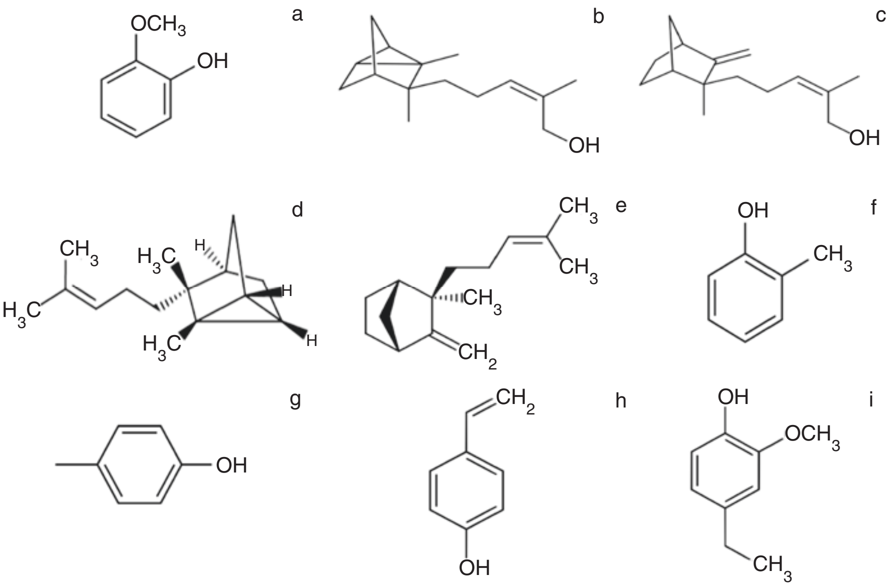

Fig. 26.3 Main secondary metabolites isolated from sandalwood oil. (**a**) Guaiacol (124.14 g/mol, $C_7H_8O_2$); (**b**) α-santalol (220.36 g/mol, $C_{15}H_{24}O$); (**c**) β-santalol (220.36 g/mol, $C_{15}H_{24}O$); (**d**) α-santalene (204.35 g/mol, $C_{15}H_{24}$); (**e**) β-santalene (204.35 g/mol, $C_{15}H_{24}$); (**f**) O-cresol (108.140 g/mol, C_7H_8O); (**g**) para-cresol (108.14 g/mol, C_7H_8O); (**h**) p-vinylphenol (120.15 g/mol, C_8H_8O); and (**i**) 6-methoxy-4-metilguayacol

ionone (11.4%), 2E,6Z-Nonadienal (7.2%), 1-octen-3-ol (5.2%), 1,4-dimethoxybenzene (4.5%), cis-3-hexen-1-ol (4.0%), dihydroxy-alpha-ionone (3.9%), cis-3-hexeline acetate (2.3%), n-nonanal (2.2%), linalol (2.0%), 2E-Nonen-1-al (1.7%) and 2E,6z-nonadiel-1-ol (1.5%), the majority percentage being 60%. Among the important minor constituents which are found in a range of 0.1–1% belong to the following metabolites: 1,2,4-trimethylbenzene, n-hexanal, n-heptanal, ethyl amyl ketone, 2,5-dimethyl-2-vinyl-4-hexanal, cis-jasmone, beta-ionone, 1,8 cineol, benzyl alcohol, butyl butyrate, benzyl benzoate and borneol, among the important trace components that correspond to percentage values lower than <0.1% include camphor, cis-3-hexenyl formate, hexyl isobutyrate and α-terpineol. The main secondary metabolites isolated from violet flower concrete are shown in Fig. 26.7.

In the odourific part (70%) of the orange blossom absolute essence (belonging to group 2), the percentage of the main constituents are: linalool (30.0%), linalyl acetate (7.5%), farnesol (5.6%), nerolidic acetate (5.5%), methyl anthranilate (3.0%), indol (2.0%), ocimet (1.2%), alpha-terpineol (1.2%) and limonene (1.0%), the majority percentage being 57.0%. Important minor constituents in the range of 0.1–1% include the following metabolites: linalool oxide, phenylethyl acetate, phenylacetonitrile, cis-jasmone, methyl linoleate, ethyl palmate and methyl jasmonate; important trace components in the range of less than <0 percent. 1% include

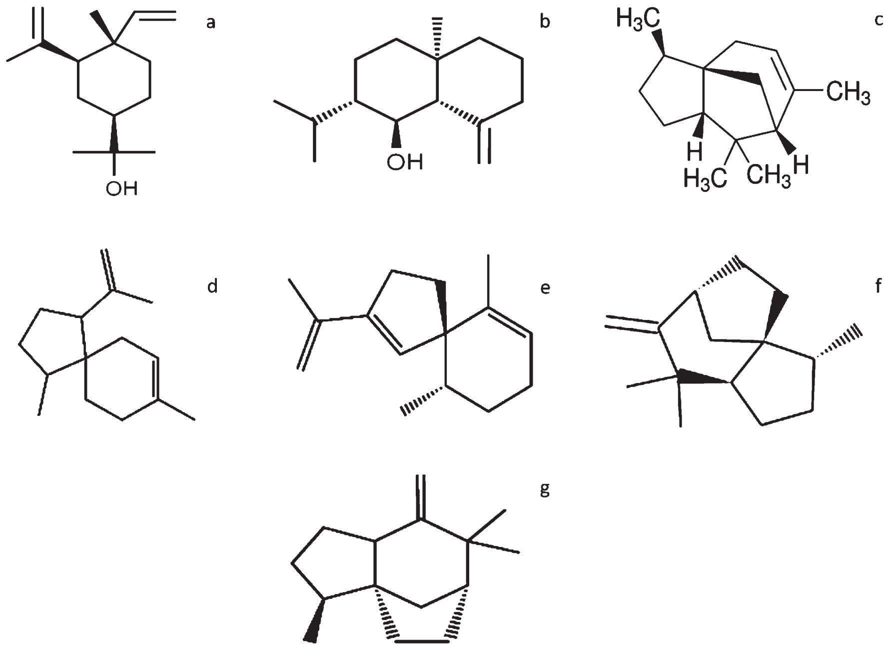

Fig. 26.4 Main secondary metabolites isolated from vetiver oil. (**a**) Elemol (222.36 g/mol, $C_{15}H_{26}O$); (**b**) junenol (222.37 g/mol, $C_{15}H_{26}O$); (**c**) (+)-α-funebrene (204.35 g/mol, $C_{15}H_{24}$); (**d**) α-acoradiene (204.35 g/mol, $C_{15}H_{24}$); (**e**) α-vetispirine (202.34 g/mol, $C_{15}H_{22}$); (**f**) prezizaene (204.35 g/mol, $C_{15}H_{24}$); and (**g**) zizaene (204.35 g/mol, $C_{15}H_{24}$)

cis-3-hexenyl formate, 2-aminobenzyl alcohol, quinazoline, 6.7-epoxy acetate and linalyl (Jeannot et al. 2005). The main secondary metabolites isolated from orange blossom absolute essence are shown in Fig. 26.8.

In the odourific part (15%) of the absolute essence of mimosa (belonging to group 3), the percentage of the main constituents are heptadecene (45.0%), nonadecene (13%), ethyl palmitate (10%), ethyl linoleate (2.5%), phenylethyl alcohol (1.5%), nonanal (1.0%) and isofitol (1.0%), the majority percentage being 74.0%. Important minor constituents in the range 0.1–1% include the following metabolites: octanal, decanal, cis-3-hexenyl acetate, n-hexanol, benzaldehyde, linalol, n-heptanol, n-octanol, ethyl benzoate, anethole, benzyl alcohol, benzyl benzoate, 2E-nonenal and anisaldehyde; important trace components in the range less than <0.1% include beta-damascone, cis-jasmone, ethyl 2-ethylbutyrate, acetal diethyl butane, acetal diethyl penta, acetal diethyl hexane and acetal diethyl octane (Gandhiraja et al. 2009). The main secondary metabolites isolated from absolute essence of mimosa are shown in Fig. 26.9.

In the odourific part (20%) of the daffodil absolute essence (belonging to group 3), the percentage of the main constituents are alpha-terpineol (23.7%), trans-isoeugenol methyl ether (20.0%), benzyl benzoate (19.4%), coumarin (6.9%), benzyl alcohol (5.0%) and delta-carene (3.4%). Important minor constituents in the

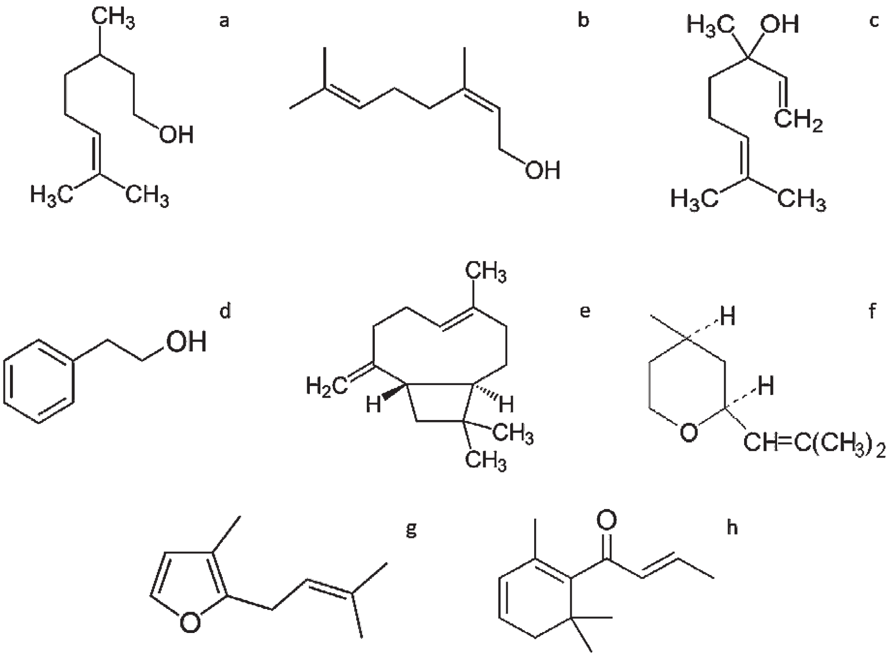

Fig. 26.5 Main secondary metabolites isolated from Bulgarian rose oil. (**a**) Citronellol (156.27 g/mol, $C_{10}H_{20}O$); (**b**) nerol (154.25 g/mol, $C_{10}H_{18}O$); (**c**) linalol (154.25 g/mol, $C_{10}H_{18}O$); (**d**) phenylethyl alcohol (122.16 g/mol, $C_8H_{10}O$); (**e**) caryophyllene (204,36 g/mol, $C_{15}H_{24}$); (**f**) oxido rose (154 g/mol, $C_{10}H_{18}O$); (**g**) rosefuran (150.22 g/mol, $C_{10}H_{14}O$); and (**h**) *β*-damascenone (190.2814 g/mol, $C_{13}H_{18}O$)

range 0.1–1% include the following metabolites: methyl benzoate, ethyl benzoate, cis-3-hexenile benzoate, cis-3-hexeline acetate, benzyl valerate and phenyl propyl acetate; important trace components in the range less than <0.1% include 2-amylfuran, 2E-dodecenal, 2E,6Z-nonadienal, 2E-nonenal, 1-p-menth-9-al and beta-ionone (Komienko and Evidente 2010). The main secondary metabolites isolated from absolute essence of mimosa are shown in Fig. 26.10.

26.5.4 Enfleurage

Enfleurage is an extractive method for obtaining aromatic essential oils from certain flowers. Enfleurage consists of applying an odourless lipid base, usually animal fat, to the flower from which the aroma is to be extracted. The essential oils are then dispersed through the base, acquiring most of the fragrant particles of the raw material (D'antuono et al. 2000).

The process takes about 3 days and can be done by overlapping on a solid butter or by immersing the flowers in the slightly heated fat to liquefy it; with the latter

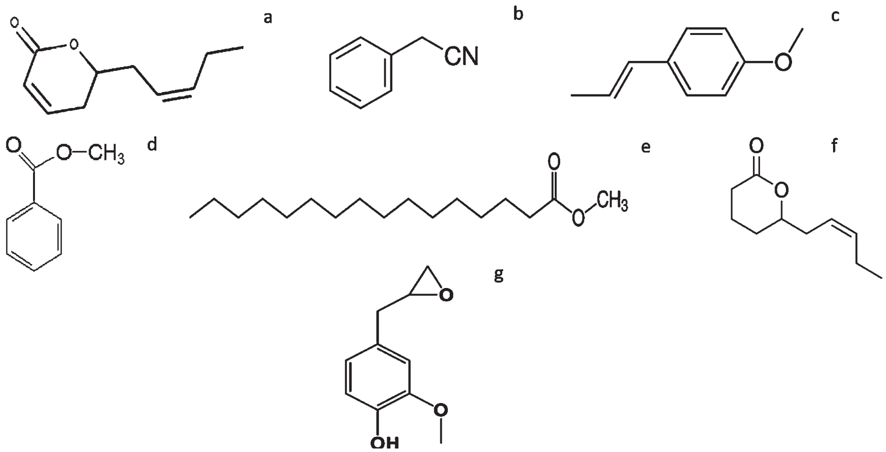

Fig. 26.6 Main secondary metabolites isolated from nard absolute essence. (**a**) Tuberolactone (166.22 g/mol, $C_{10}H_{14}O_2$); (**b**) phenylacetonitrile (117.15 g/mol, $C_6H_5CH_2CN$); (**c**) anetol (148.20 g/mol, $C_{10}H_{12}O$); (**d**) methyl benzoate (136.00 g/mol, $C_8H_8O_2$): (**e**) methyl palmitate (270.46 g/mol, $C_{17}H_{34}O_2$); (**f**) jasmine lactone (168.24 g/mol, $C_{10}H_{16}O_2$); and (**g**) epoxy eugenol

method, the fat can be liquefied several times, strain it and change the flowers, so that it becomes more and more perfumed. For example, the extraction of aromas from the orange blossom by enfleurage gives rise to the orange blossom essence, which has a sweet and mild smell, whereas if more modern methods are used, Neroli is obtained, a 100% pure oil, with a much more intense and complex aroma. With the process described above, an aromatic butter is obtained, good for use in cosmetics and cleaning products; however, there is a final step that allows the extraction of the essence called "absolute". The process to obtain the absolute is basically to mix the fat with alcohol so that the fragrance is transferred to it, then separate fat from alcohol and make it evaporate leaving only the concentrated and pure essence (D'antuono et al. 2000).

Nowadays enfleurage is only used with flowers that do not tolerate other methods, the best known case being jasmine, from which the absolute is obtained after an enfleurage process. For the majority of flowers, it has been replaced by methods that obtain the oil directly and at the same time are delicate. The most natural and used method is steam distillation (D'antuono et al. 2000).

26.5.5 Physiology of Smell

Fragrances for the sense of smell play an important role in the physiological effects of mood, stress and work capacity. Fragrance is a volatile chemical component with a molecular weight of <300 Da, which humans perceive through the olfactory system. In the olfactory process, fragrance molecules from the air bind to the cilia of

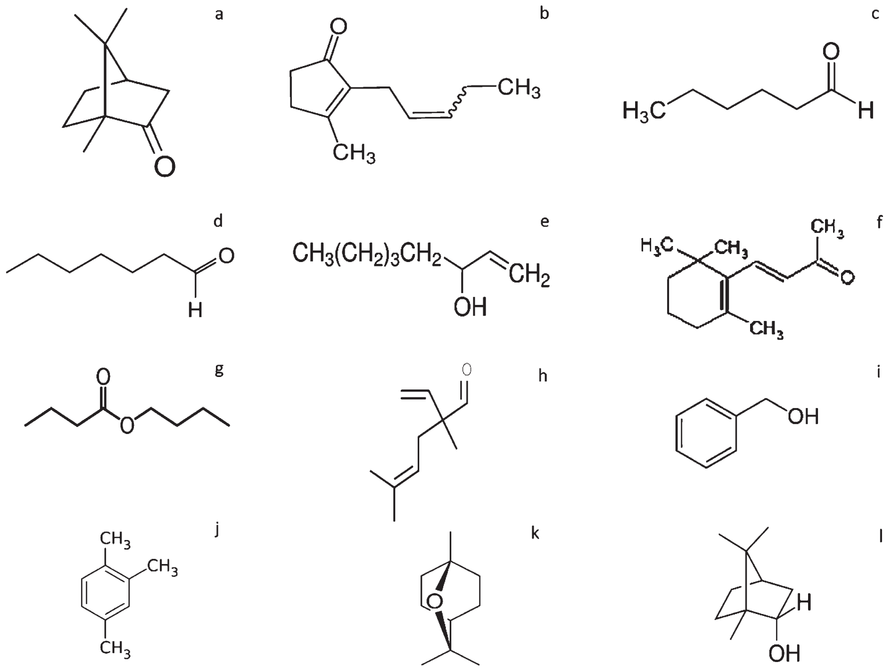

Fig. 26.7 Main secondary metabolites isolated from violet flower concrete. (**a**) Alcanfor (152.12 g/mol, $C_{10}H_{16}O$); (**b**) cis-jasmone (164.246 g/mol; $C_{11}H_{16}O$); (**c**) n-hexanal (100.16 g/mol, $C_6H_{12}O$); (**d**) n-heptanal (114.18 g/mol, $C_7H_{14}O$): (**e**) 1-octen-3-ol (128.21 g/mol; $C_8H_{16}O$); (**f**) β-ionone (192.3 g/mol, $C_{13}H_{20}O$); (**g**) butyl butyrate (144.21 g/mol, $C_8H_{16}O_2$); (**h**) 2,5-dimethyl-2-vinyl-4-hexenal (152.23 g/mol; $C_{10}H_{16}O$); (**i**) alcohol bencílico (108.14 g/mol, C_7H_8O); (**j**) 1,2,4-trimethylbenzene (120.19 g/mol, C_9H_{12}); (**k**) 1,8-cineol (154.25 g/mol, $C_{10}H_{18}O$); and (**l**) borneol (154.25 g/mol, $C_{10}H_{18}O$)

the olfactory receptors in the olfactory epithelium, located in the nasal cavity. The receptors coupled to the guanine nucleotide binding protein (G-protein) (GPCR) are activated and electrical signals are generated. The electrical signals are then transmitted to the brain by olfactory sensory neurons via the olfactory bulb and the upper olfactory cortex (Angelucci et al. 2014; Sell 2006). Finally, these electrical signals modulate brain functions, including memory, thoughts and emotions (Kandhasamy and Songmum 2016).

Several studies explain that fragrance inhalation greatly affects brain function, because fragrance compounds are able to cross the blood-brain barrier and interact with central nervous system receptors (Kutlu et al. 2008; Touhara and Vosshall 2009). Many studies suggest that olfactory stimulation of fragrances produces immediate changes in physiological parameters such as blood pressure, muscle tension, pupil dilation, skin temperature, pulse rate and brain activity (Diego et al. 1998; Field et al. 2005).

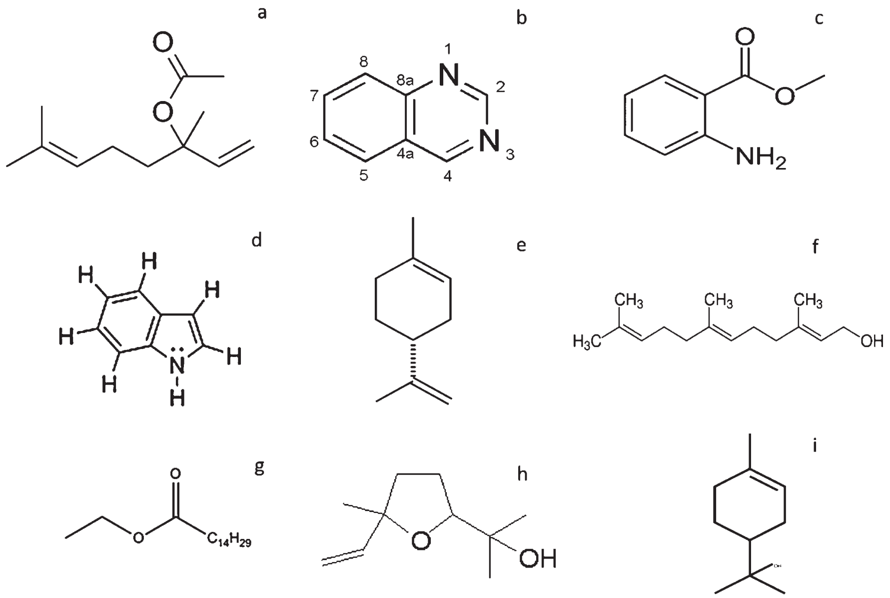

Fig. 26.8 Main secondary metabolites isolated from orange blossom absolute essence. (**a**) linalool acetate (196.29 g/mol, $C_{12}H_{20}O_2$); (**b**) quinazoline (130.15 g/mol, $C_8H_6N_2$); (**c**) methyl anthranilate (151.165 g/mol, $C_8H_9NO_2$); (**d**) indol (117.15 g/mol, C_8H_7N): (**e**) limonene (136.23 g/mol, $C_{10}H_{16}$); (**f**) farnesol (222.37 g/mol, $C_{15}H_{26}O$); (**g**) ethyl palmate (284,48 g/mol, $C_{18}H_{36}O_2$); (**h**) linalool oxide (170.00 g/mol, $C_{10}H_{18}O_2$); and (**i**) α-terpineol (154.25 g/mol, $C_{10}H_{18}O$)

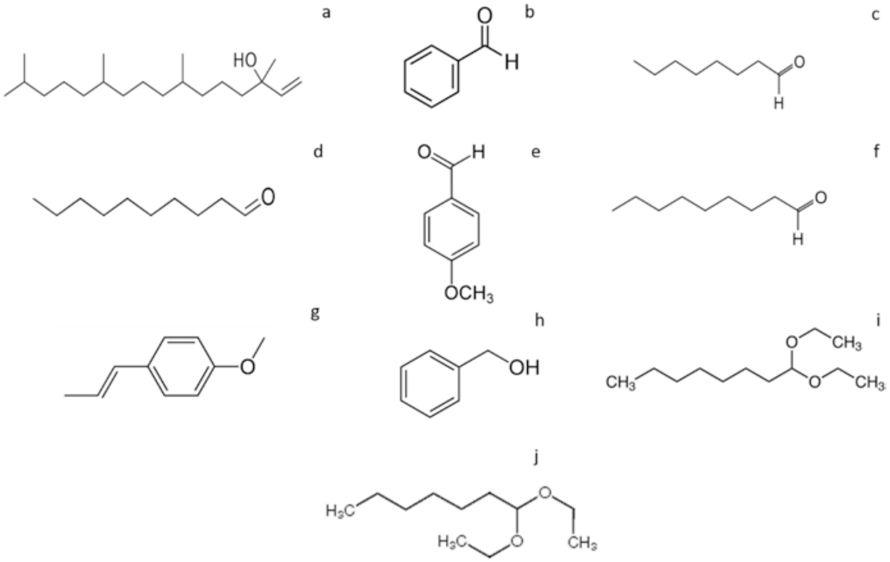

Fig. 26.9 Main secondary metabolites isolated from the absolute essence of mimosa. (**a**) Isophytol (296.54 g/mol, $C_{20}H_{40}O$); (**b**) benzaldehyde (106.13 g/mol, C_6H_5CHO); (**c**) octanal (128.21 g/mol, $C_8H_{16}O$); (**d**) decanal (156.20 g/mol, $C_{10}H_{20}O$); (**e**) 4-anisaldehyde (136.15 g/mol, $C_8H_8O_2$); (**f**) nonanal (142.14 g/mol, $C_9H_{18}O$); (**g**) anetol (148.20 g/mol, $C_{10}H_{12}O$); (**h**) benzyl alcohol (108.13 g/mol, C_7H_8O); and (**i**) octanal diethyl acetal (202.34 g/mol, $C_{12}H_{26}O_2$)

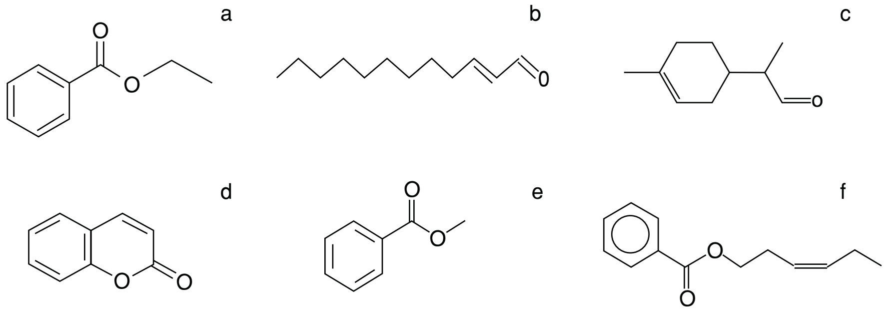

Fig. 26.10 Main secondary metabolites isolated from daffodil absolute essence. (**a**) Ethyl benzoate (150.18 g/mol, $C_9H_{10}O_2$); (**b**) 2-dodecenal (182.30 g/mol, $C_{12}H_{22}O$); (**c**) 1-p-menthen-9-al (152.23 g/mol, $C_{10}H_{16}O$); (**d**) coumarin (146.03 g/mol, $C_9H_6O_2$); (**e**) methyl benzoate (136.15 g/mol, $C_8H_8O_2$); and (**f**) cis-3-hexenile benzoate (204.00 g/mol, $C_{13}H_{16}O_2$)

26.6 Conclusions

A large number of medicinal and aromatic plant extracts with development potential require experimentation to obtain and generate new odour fragrances. Natural secondary metabolites allow to obtain fragrances that are harmless to humans. The cultivation of medicinal and aromatic plants is a viable alternative or complement in some areas and farms, although the need for a certain specialisation of the farms should not be ignored due to the production and quality demands that these crops usually have. Demand requirements mean that organic or biological production must sometimes be chosen in order to obtain sufficiently remunerative prices in the fragrance industry.

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Chapter 27
The Conservation and Utilization of Medicinal Plant Resources

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27.1 Introduction

India is floristically rich and is globally recognized as one of the 12 megadiversity countries (Singh and Chowdhery 2002) accompanied by 10 biogeographic regions (Rodgers and Panwar 1990) along with more than 40 sites that are well-known for their genetic diversity and high endemism, in their updated list of Earth's biodiversity hotspots, involved 2 from India. India enjoys different climatic and altitudinal variations, incorporated with diverse physiography, undulating topography, and ecological habitats which eventually lead to the development of an extremely rich vegetation with a unique diversity in medicinal plants that provide an invaluable source of raw material for traditional medicine systems along with pharmaceutical industries both in India and abroad. With the growing pressure for medicinal plants, most of which is still experienced by wild collection, consistent pressure is generated on the existing resources which finally leads to the reduction of some forest species and also at the same time the forest land suffers the loss of its natural flora at an alarming rate (Shivarajan and Balachandran 1999). In order to control this situation, various steps have been taken such as commercial cultivation and habitat conservation, to set up the natural reserves and to implement the laws for exporting the plants which should be kept under control (Rao et al. 2003). The development of plant resources and the preservation of useful medicinal plants is, therefore, a very important issue.

Globally medicinal plants are considered as important sources of new drugs (Nalawade et al. 2003). In addition, up to 80% of the people rely entirely on herbal drugs in developing countries in their primary health care, while over 25% of the medicines specified in developed countries are obtained from plant species grown in

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the wild (Hamilton 2004). The use of medicinal plants is growing speedily globally with the growing pressure for natural health products, herbal drugs, and secondary metabolites of medicinal plants (Nalawade et al. 2003; Cole et al. 2007). There are between 50,000 and 80,000 flowering plants that are used worldwide for medicinal purposes as reported by the International Union for Conservation of Nature and the World Wildlife Fund. Due to habitat destruction and overharvesting, approximately 15,000 species are at risk of extinction, and with the growing human population and plant utilization, around 20% of the wild resources have almost already been depleted (Ross 2005; Bentley 2010). With the rapid loss of biodiversity and habitat destruction, the threat of vanishing of medicinal plants has been increased globally, and this menace has been well-known for decades particularly in India, Nepal, Tanzania, Uganda, and China (Nalawade et al. 2003; Hamilton 2008; Zerabruk and Yirga 2012). Medicinal plant conservation and their sustainable use have been widely studied (Larsen and Olsen 2007; Uprety et al. 2012). A set of different recommendations have been compiled in relation to their conservation, including the formation of systems for inventorying species and monitoring their status and the demand for integrated conservation groups based on both ex situ and in situ methods and techniques (Hamilton 2004). For medicinal plants with additional resources available, sustainable use of wildlife can be another effective alternative for the conservation. This situation is especially acute due to the high demands of huge populations particularly in South Africa and China.

Medicinal and aromatic plants constitute a compatible component of the natural biodiversity establishment around the globe. Medicinal plants contain ingredients that are effective for treating disease or relieving pain. On the other hand, aromatic plants have a distinctive fragrance or smell (King 1992). Plants may generate over 10,000 distinct compounds as they represent a vast storehouse of drugs and defend them from the predators; further these compounds may also act as the potential drugs (King 1992; Izuakor 2005). Biodiversity is the variety and variability of plant and animal life on the Earth in which they live and is typically a measure of variation in the ecological and evolutionary processes that keep them functioning. On the other hand, conservation includes careful protection and preservation of species from extinction particularly systematic management of the natural resources to prevent them from destruction or even overexploitation. Plant medicines were discovered historically by trial and error. Just as how people have learned to make use of plants for food is the same way how they have learned to utilize plants as medicine (UNESCO 1994). For instance, our ancestors realized that that the bark of a willow tree is used to cure various pains and aches when taken in the form of a tea. Later, willow bark is known to contain the salicylic acid as discovered by the scientists, an active ingredient in aspirin. Further plant medicines like aloe, belladona, cinchona, and opium poppy were selected for use based on strong evidence as collected by indigenous practitioners (Okigbo and Mmeka 2006).

27.2 Need for Conservation of Medicinal Plants

Skillful arguments and public awareness have been raised many years ago in order to understand the destruction of tropical rain forests and seasonally dry monsoon forest. Issues have been largely ignored in the past, but today, remarkable efforts are being made to conserve biodiversity (Azimahtol and Soliman 1998). The present-day cry of environmental degeneration, fragmentation, ecological genocide, genetic erosion, and extinction as well as destruction of our biological heritage is the result of inaction (Krikorian 1998). Medicinal plants and their natural habitats are at the risk of overexploitation due to the continuous destruction of the forests than ever before (Walter and Gillett 1998). The conservation of the endangered medicinal plant species in their natural habitats and to achieve their sustainable exploitation in less vulnerable areas is our utmost goal (Cunningham 1993).

Huge amounts of medicinal plants from the forests are being harvested in order to meet the requirements of the increasing populations and also the needs of the expanding regional and international health-care market products. A large number of medicinal plants in India are released from the wild to meet the growing demand for the use of raw materials needed for domestic consumption and export, thereby declining the natural resources rapidly. Medicinal plants have an impact on health, income, cultural identity, agroforestry, and health security. There is therefore a demand for the maintenance, conservation, cultivation, and evaluation of germplasm for future use. The conservation of biodiversity includes the conservation, rehabilitation and improvement of biodiversity in the area so that the abundance and distribution of species and communities contribute to sustainable development. To conserve the evolutionary potential of species is the utmost aim of the biodiversity conservation by maintaining natural levels of diversity that are important to species and populations to respond to short-term and long-term environmental changes in order to overcome critical factors that can lead to extinction.

The purpose of conservation is to support sustainable development by protecting and utilizing biodiversity resources in ways that do not diminish biodiversity or damage the important habitats and habitats. Generally, it includes activities like evaluation, characterization, collection, disease indexing, propagation and storage, elimination, and distribution. Conservation of genetic resources has long been achieved as an integral part of biodiversity conservation. There are two ways to conserve plant genetic resources, i.e., in situ and ex situ conservation.

27.2.1 Conservation Strategies for Medicinal Plants

For medicinal plants effective conservation strategies should be undertaken within two areas, i.e., in situ and ex situ conservation. Based on these strategies, there is a need for integrated conservation efforts. Further information is required for medicinal plant production, use, trade, recognition of medicinal plant stock, development of sustainable harvesting methods, preservation of traditional knowledge, and intellectual property rights.

27.2.1.1 In Situ Conservation

In situ conservation is on-site conservation or the continued maintenance of plant populations or species in their natural habitats to which it is adapted. As most of the medicinal plant species are endemic so their medicinal properties are principally being there due to the presence of the secondary metabolites that respond to the environment and that may not be manifested under culture conditions (Coley et al. 2003; Figueiredo and Grelle 2009). In situ conservation of all communities allows us to protect indigenous plants and preserve natural communities, as well as their complex network of relationships. In addition, in situ conservation increases the amount of diversity that can be conserved (Forest et al. 2007) and also strengthens the link between resource conservation and sustainable use (Long et al. 2003). Throughout the globe in situ conservation has concentrated on building protected areas and adopting an ecosystem-based approach, rather than on species (Ma et al. 2012). In situ conservation is successfully dependent on the regulations, rules, and possible compliance of medicinal plants in growing habitats (Soule et al. 2005; Volis and Blecher 2010). In order to ensure that wildlife representatives of endangered species are preserved, core conservation areas or other protected areas that will allow natural processes to remain undisturbed should be selected (Cunningham 1997).

27.2.1.2 Natural Reserves

One of the major causes for the loss of medicinal plant resources is the degradation and habitat destruction (Rodriguez et al. 2007). These reserves are important wildlife protected areas created to protect and restore biodiversity (Chiarucci et al. 2001; Rodriguez et al. 2007). Around 12,700 protected areas have been established globally, covering 13.2 million km2 or 8.81% of the Earth's surface (Huang et al. 2002). Protecting native habitats requires the assessment of the contribution of individual habitats and ecosystem functions to plant conservation (Liu et al. 2001).

27.2.1.3 Wild Nurseries

It is impossible for wild nurseries to designate each natural forest plant habitat as a protected area, facing costs and land use (Soule et al. 2005; Kramer and Havens 2009). These nurseries are developed for the species oriented domesticating and cultivating of endangered medicinal plants in a protected area, natural habitats, or areas within a short distance from where plants grow naturally (Hamilton 2004; Schippmann et al. 2005; Strandby and Olsen 2008). Due to habitat degradation, invasive species, and overexploitation, many wild species populations are under severe stress; these wild nurseries can provide successful approach for medicinal plant in situ conservation that are endangered, endemic, and in demand (Li and Chen 2007; Liu et al. 2011).

27.2.1.4 National Parks

National parks (Table 27.1) are areas that are strictly reserved for wildlife well-being and do not allow activities such as forestry and farming. No private ownership is allowed in these parks. Their boundaries are well marked and functioning. They are usually small reserves spread over from 100 square meters to 500 sq. Km. Priority is given to the conservation of a single plant or animal species in these parks.

27.2.1.5 Wildlife Sanctuaries

The sanctuary is a protected area only reserved for human activities and animal conservation such as timber harvesting, collecting small forest products, and allowing private ownership as long as it does not interfere with the well-being of the animal (Table 27.2). The boundaries of sanctuaries are poorly defined and allow for controlled biotic interferences like tourism activities.

27.2.1.6 Reserves

It is a separate category of protected areas, where human populations are also a part of the system. These are usually large protected areas over 5000 square kilometers. Biosphere reserves represent "biological laboratories for sustainable development" and learning centers for human and environmental compliance (Table 27.3). These reserves are the only sites in the UN system that specifically call for mutually

Table 27.1 List of some major national parks in India

Name	Established	Area (in km^2)	State
Corbett national park	1921	1318.5	Uttarakhand
Kanha national park	1955	940	Madhya Pradesh
Sariska national park	1955	866	Rajasthan
Gir national park	1965	258.71	Gujarat
Kaziranga national park	1974	471.71	Assam
Dudhwa national park	1977	490.29	Uttar Pradesh
Silent Valley National Park	1980	237	Kerala
Kanger Ghati National Park (Kanger Valley)	1982	200	Chhattisgarh
Nanda Devi national park	1982	630.33	Uttarakhand
Sundarbans national park	1984	1330.12	West Bengal

Table 27.2 List of some major wildlife sanctuaries in India

Name	Established	Area (in km²)	State
Mudumalai wildlife sanctuary	1940	321.55	Tamil Nadu
Hazaribagh wildlife sanctuary	1954	183.89	Jharkhand
Mount Abu wildlife sanctuary	1960	288.84	Rajasthan
Ghana bird sanctuary	1982	28.73	Rajasthan
Anamalai wildlife sanctuary (Indira Gandhi wildlife sanctuary and National Park)	1989	117.10	Tamil Nadu
Jaldapara wildlife sanctuary	2012	216	West Bengal

Table 27.3 List of some major biosphere reserves in India

Name	Established	Area (in km²)	State
Gulf of Mannar	1980	10,500	Tamil Nadu
Nanda Devi	1982	5860.69	Uttarakhand
Great Nicobar	1989	885	Andaman and Nicobar Islands
Manas	1990	2837	Assam
Pachmarhi	1999	4926.28	Madhya Pradesh

supportive avenues for conservation and sustainable development. Such interaction requires consensus-driven policy, decision-making cultural sensitivity, and scientific expertise (UNESCO 2010).

Biosphere reserves are examples of how people and nature are unique institutions (sites) and how humans and nature respect each other's needs. These reserves contain genetic elements that have developed over millions of years that carry the means for future survival and adaptation. These sites are of global importance, with significant potential for future economic growth, particularly as a result of new trends in biotechnology (Ministry of Environment and Forests, Government of India 2007).

Structure and Design of Biosphere Reserves

The biosphere reserve has been identified to have three interrelated areas to manage activities that are complementary to the development of biodiversity conservation and sustainable management issues (Özyavuz 2012). The biosphere reserve consists of three parts, viz., core, buffer, and transition zone.

Core Zone The inner zone is the core zone. It is an undivided and legally protected area. This zone stays completely peaceful or undisturbed. It must have a satisfactory habitat for many plant and animal species together with higher-order predators and should carry centers of endemism. These regions often preserve the wild relatives of economic species and also constitute major genetic reservoirs. There are also areas of extraordinary scientific interest in the core zone. A core zone secures legal protection and management and research activities that do not affect natural processes and allow wildlife. The core zone must be freed from all human pressures external to the system.

Buffer Zone The buffer zone is between the core and the transition zone. Some educational and research activities are permitted. These uses and activities include resources, limited entertainment, tourism, fishing, and grazing, as well as demonstration sites that allow for renewal of value addition, reducing its impact on the core zone. Natural activities in the biosphere reserves are allowed to continue if they do not adversely influence the environment.

Transition Zone The outermost part of biosphere reserves is the transition zone. The activities such as forestry, fishery, recreation, and cropping are allowed here. This is generally not a delimitation and is a collaborative area where conservation, knowledge, and management skills are applied and used in accordance with the purpose of the biosphere reserve. Manipulative macro-management techniques are used both in the buffer and the transition zones. In order to understand the patterns and processes in the ecosystem, experimental research fields are used. Modified or degraded landscapes are incorporated as rehabilitation areas to restore the environment, thereby returning to sustainable productivity.

27.2.2 Ex Situ Conservation

Ex situ conservation is the preservation or protection of species of biological diversity outside their natural habitats. These include the establishment of forests and the maintenance of collections of farmlands, home gardens, botanical gardens, and an arboreta in an area outside their natural habitat (Roche 1992). The core of ex situ conservation is the rapid development of alternative sources of medicinal plants by growing at a sufficient quantity through cultivation and at a low enough price to compete with the prices achieved by wild medicinal stock collectors (Cunningham 1997). This will satisfy market demands, lead to more secure jobs, and provide fewer incentives to gather in the wild. If this does not happen, naturally occurring species will disappear in the wild, thus compromising the local medical resource base.

27.2.2.1 Zoos

Zoological gardens or zoos or zoological parks are facilities where the animals are housed within enclosures or seminatural and open areas, cared and displayed to the public, and where they may breed in some cases also. Zoological parks are considered as important means of biodiversity conservation by many universal thinkers and environmentalists (Balcombe 2009; Melfi 2012). The zoos attract approximately 450 million visitors each year and are therefore uniquely positioned to have the highest educational and economic value (Carrizo et al. 2013). Zoos serve not only as places for entertainment and animal activity but also as centers, museums, research centers, and informal animal banks (Lacy 2013). Although some people do not like zoos, most people enjoy them. Over the last few decades, zoos have made great strides in their management through the cooperation of people from diverse ecosystems (Leus 2011.).

Zoos breed endangered species to increase their numbers. Several species in the zoos have been saved from the extinction through captive breeding (Melfi 2012). Animal management in zoos includes identification of animals, housing, breeding, health, feeding and dealing with social networks (Ratledge 2001). In order to determine whether a species or taxon is included in the zoo's collection plan, several procedures and mechanisms have been implemented. The most commonly used methods include how this species is valued, by its uniqueness, contribution to education or research and the nature of conservation (Melfi 2012).

Zoos assist the animal to protect food, shelter, social networks and spouses, and are motivated by desire (moral behavior), strengthened by happiness (Balcombe 2009). Some zoos have regard for the welfare of their animals in the past, but today's zoos have a poor environment for their animals (Eaton 1981). Many zoo animals also became endangered or disappeared due to tourist disturbances, poor weather, and inadequate space (Ratledge 2001). From this perspective, many scholars point to the negative aspects of keeping animals in zoos as they cause pain, stress, misery, and suffering and the animals experience the consequences of evolution (Balcombe 2009). The main purposes of the modern zoos are the welfare of animals, conservation, education, research, and entertainment, but these can be controversial. For example, tourists enjoy learning by watching how captive animals behave, but tourists often want to observe and interact with animals in close proximity. Unfortunately, intimacy and interaction with humans involves a variety of forms of oppression (Fernandez et al. 2009).

However, developing zoos are involved in education, research, and conservation, with the goal of keeping healthy animals, behaving as if they are in their natural environment (Eaton 1981). The current prototype for population control is important to reduce the rate of genetic deterioration and slow adaptation to the captive environment and to maintain the general behavior (Dickie 2009; Lacy 2013). Therefore, population management is aimed at not perfecting the resources immediately available to founders (Lacy 2013). Therefore, in order to maintain species certification for purposes of conservation, demonstration, education, and research, regular human recycling is required (Leus 2011; Lacy 2013). Most current breeding programs are based on genetic manipulation by analysis of individual pedigrees in order to minimize cohesion (Lacy 2013).

27.2.2.2 Captive Breeding

Captive breeding is a fundamental part of the gross conservation program that helps to protect species, subspecies, or populations from extinction. It is a major management practice for vulnerable populations, people, and man-made and natural factors of the species (Leus 2011). In small and fragmented populations, even if human caused threats could be magically transformed, species will still have a higher probability of extinction by random population growth and genetic events, biodiversity, and disasters. In order to enhance the physical and mental welfares of captive animals, environmental enrichment programs are being used which can be obtained by increasing the manifestation of natural behavior and the reduction of abnormal behavior patterns. Effective environmental enhancement involves the development of comprehensive design and provision of feeder devices, novel materials, relevant public groups, and other stimulants (Claxton 2011). For successful ex situ management, the lowest demand particularly in the captive populations is the incorporation of genetic diversity that exists in wild populations. However, there are several biological and ecological barriers that are limited in achieving the goal of breeding in exile of many species (Melfi 2012). One of the crucial ultimata is the circular effect of the management of small numbers of genetic and human genetic disorders due to the loss of genetic diversity and population density (Melfi 2012). In addition, adaptive populations in captivity may also adapt well to wild conditions and may exhibit little fitness upon reintroduction (Williams and Hoffman 2009). Most remarkably, within captive areas, housing and livestock will also have dominant consequences on birth and death rates (Melfi 2012).

27.2.2.3 Aquarium

The aquarium is an artificial environment for aquatic animals. It can also be used to include amphibians or large marine mammals and plant species to attract visitors. They are generally found in zoos or marine habitats of different sizes. 15,750 defined species of freshwater fish make up about 25% of the biodiversity and the key to economic and nutritional resources where more than 11% are threatened (60, extinct; 8, disappeared in the wild; and 1679, threatened) (Carrizo et al. 2013). Freshwater (0.3%) is available that supports 47–53% of the world's total water resources that are at risk of overfishing, pollution, habitat loss, animal husbandry, invasive species, and climate change. The aquarium is used to compliment home by entertainers, presenting as public exhibitions, to provide a large number of human and animal feed (Reid et al. 2013). Fish are frequently overlooked when developing conservation priorities. This leads to a lower focus on reasonable conservation efforts rather than giving more attention to their importance in food and health. However, in addition to the clear diversity of freshwater fish, wetland habitats and their associated aquatic fish species continue to be lost or destroyed at an alarming rate (Dudgeon et al. 2006). One advice is that aquariums establish a sustainable ecosystem program that prioritize endangered species (VU, EN and CR) and those considered as EW to support species conservation in situ and facilitate the restoration of species through reintroduction or translocation efforts where appropriate (Carrizo et al. 2013).

27.2.2.4 Botanical Gardens

Botanical gardens are comprised of living plants, grown under glass in greenhouses and conservatories or out of doors, and are used to display or grow plants mainly for scientific and educational purposes. It can be taxonomic collection of a specific family, genus or group of species, indigenous plants, wild relatives, and medicinal, aromatic, or textile plants (Institute of Biodiversity Conservation 2005). There are more than 2000 botanic gardens, which carry over 80,000 plant species in their living collections and receive hundreds of millions of visitors annually (Dudgeon et al. 2006; Brutting et al. 2013). In addition, they have precious and unique combinations of officers dedicated to botanical research, systems, conservation education, and public awareness (Dudgeon et al. 2006). They still have excellent connections between themselves and other professionals, conservation organizations, and non-governmental organizations (Blackmore et al. 2011). They come up with a variety of services for sectors that employ and maintain biodiversity such as forestry, agriculture, biofuel and pharmaceutical industries, ecotourism, and protected area management. They possess a distinctive opportunity as a tourist attraction and scientific institution for the conservation and documentation of biodiversity by connecting and shaping citizens to the current environmental challenges (Blackmore et al. 2011). In order to achieve the target of the Global Strategy for Plant Conservation 2020, they also play a major role to develop 75% of the world's endangered species ex situ (Brutting et al. 2013). Botanical gardens provide an opportunity for crop plants to grow under changed environmental conditions (Brutting et al. 2013).

Botanical gardens not only play major role in conservation by ex situ method, but they are able to maintain the ecology to promote the survival of rare and endangered plant species (Huang et al. 2002; Havens et al. 2006). By the development of propagation and cultivation protocols in the botanic gardens, they further play an important role in the conservation of medicinal plants along with undertaking programs of variety breeding and domestication (Maunder et al. 2001).

27.2.2.5 Gene Banks

Another management technique used for the conservation of biodiversity is the genome resource banking. Due to the shortage of biodiversity, various types of gene banks have been developed which entirely depend on the type of the materials being conserved. These include for seeds (seed banks), for plant tissues and cells (in vitro gene banks), for live plants (field gene banks), and for living embryos, sperm, chromosomes, eggs, tissues, and DNA (chromosome, pollen, and deoxyribonucleic acid (DNA) banks) that are held in short- or long-term laboratory storage; they are usually freeze-dried or cryopreserved (Clarke 2009). Approximately 7.4 million PGRFA accessions have been conserved currently in over 1750 gene banks (Dulloo et al. 2010). The main purpose of the conservation in gene bank is to maintain the flourishing genetic diversity as long as possible and also to decrease the frequency of regeneration globally that may result in loss of genetic diversity (Dulloo et al.

2010). With the rapid advancement in the field of genomics and molecular genetics, the only material which is becoming increasingly more and more in demand is the DNA which is ultimately being utilized in the molecular studies and is one of the most important materials from the gene banks. The establishment of a repository of DNA storage facility as a complementary "backup" for the ex situ collections in traditional has been suggested (Dulloo et al. 2010). In order to establish DNA banks for endangered animals, special efforts have been made, and in various parts of the world, few plant DNA banks like Australian Plant DNA Bank, Kew Royal Botanic Garden, and Missouri Botanical Garden have been developed (Ryder et al. 2000). The archives of extracted genomic DNA have been developed already by many research groups. A network of DNA banks in Germany has been recently established by the Global Biodiversity Information Facility which provides DNA samples of complementary collections (protists, microorganisms, fungi, algae, animals, and plants) (Dulloo et al. 2010).

Seeds are usually the easiest and convenient material to collect and to maintain in a viable state for long periods of time, thus making them preferable to be conserved in gene banks (Vertuccj 1993; Brutting et al. 2013). The techniques of seed banking depend on the storage at low temperatures of dried seeds of threatened or other plants as the most prerequisite factors influencing seed longevity are the relative humidity, temperature and seed moisture content (Ellis and Roberts 1980; Dickie et al. 1990; Brutting et al. 2013). For short-term conservation, seeds are generally conserved at moisture content between 3 and 7% and are stored at 4 degrees Celsius, while for long-term conservation, it is between −18 and − 20 degrees Celsius (Vertuccj 1993; Dulloo et al. 2010). The present study shows that there are variations in seed longevity of different species stored under similar conditions. In addition, it has been found that seed type such as endospermic or non-endospermic and intraspecific variation may also affect accession longevity (Dulloo et al. 2010). In addition, for ensuring seed longevity in seed banks, high initial quality seeds are a major prerequisite.

Plants that cannot be conserved as seeds such as cold sensitive and or seeds that are desiccation because of their recalcitrant nature or are clonally propagated are traditionally conserved as live plants in ex situ field gene banks. But, field gene banks present real logistical challenges; they need larger and more expensive areas, and they are more vulnerable to natural disasters, fire, pests and diseases, extreme weather, vandalism, political unrest, and theft and are often at risk due to policy changes on land use (Dulloo et al. 2010).

In vitro conservation refers to a single type of gene bank known as slow-growth conservation method. It includes culturing of various parts of the plant like tissues, cells, and meristem with growth retardants into pathogen-free sterile culture in a synthetic medium, which has been identified as a good way to complete and deliver backup to field collections (Dulloo et al. 2010).

Another technique for genome conservation is cryopreservation, in which living tissues are stored in liquid nitrogen at very low temperatures (−196° C) to prevent metabolic mitotic activity (Dulloo et al. 2010). It is now recognized that cryopreservation methods provide greater protection against the long-term, cost-effective conservation of genetic resources as well as traditional seeds. Storing in liquid nitrogen clearly predicts the long life span of lettuce seeds with half-lives calculated as 500 and 3400 years, respectively, for fresh lettuce seeds stored in liquid phases of nitrogen and in the vapor phases (Dulloo et al. 2010).

27.3 Benefits and Differences of in Situ and Ex Situ Conservation

27.3.1 Benefits of In Situ Conservation

1. Flora and fauna live in natural habitats without human intervention.
2. The life cycle of living things and their development takes place in a natural way.
3. It bestows the necessary green cover and corresponding convenience that our environment needs.
4. It is less expensive and easier to maintain.
5. The interests of the indigenous people are also protected.

27.3.2 Benefits of Ex Situ Protection

1. It is useful for the population decline of the species.
2. Endangered species are successfully bred on the verge of extinction.
3. The endangered species are kept in captivity and then released into natural habitats.
4. Ex-situ centers provide opportunities to observe wildlife, otherwise it would not be possible.
5. It is very useful for research and scientific work of different species

27.3.3 Difference between "In Situ Protection" and "Ex Situ Protection"

Some major differences between in situ and ex situ protection are as follows:

In Situ Protection

1. It is the conservation of the natural habitats of endangered species.
2. Endangered species are protected from predators.

3. The depleting resources are augmented.
4. The population recovers in the natural environment.

Ex Situ Conservation

1. It is conservation outside the natural habitats of endangered species.
2. Endangered species are protected from all adverse factors.
3. They are placed under human supervision and provided with all necessary items.
4. Offspring born in captive breeding are released for storage in natural habitats for acclimatization.

27.4 Utilization of Medicinal Plant Resources

27.4.1 Concept of Medicinal Plant Resources

In the plant systems of economic botany, medicinal plants represent an independent entity (Simpson and Conner-Ogorzaly 1986). However, plants with pleasant taste and odor (i.e., aromatic plants) are not usually present in these systems. Medicinal plants are often known to contain the biologically active substances with alleged medicinal properties which have health effects or prove to be applicable as drugs according to Western standards (Farnsworth and Solejarto 1991). However, in the simplest way, one can differentiate between therapeutic (pharmacological) and medicinal and miscellaneous applications (cosmetic, culinary, food, etc.) (Agri-Food Canada 2013).

The history of aromatic and medicinal plants dates back to the times of our ancestors who used natural ingredients found in nature to cure their suffering, diseases, and wounds. Medical plants (sensu stricto) are also largely being used for the self-medication and are also widely used in national health services. Being an important part of our natural wealth, medicinal plants may serve as both raw materials and therapeutic agents for producing a number of diverse products. Similarly, their products from aromatic and medicinal plants also grow many times over. Traditional and modern medicines include the products which are used in the medicinal (health care), through the food industry (daily culinary uses) to cosmetic uses (Hornok 1992). Medicinal plants have become industrial products for worldwide use for the first time with new concepts, e.g., cosmeceuticals, nutraceuticals, aromatherapy, phytotherapy, etc., and their use in functional foods and animal husbandry is expanding into new applications according to Baser (2005). In the conventional medicine, both national and international legislative and regulatory authorities seem to have an ambitious approach to the increased use of medicinal and aromatic plants.

27.4.2 *Medicinal and Pharmacological Applications of Medicinal Plants*

Successful diagnosis, prevention and treatment of mental and physical illness, improvement in the symptoms of the diseases, and beneficial changes or control of the mental and physical state of the body refer to the therapeutic activity (WHO 2003).

27.4.2.1 Traditional Medicine

Sum total of the knowledge, practices, and skills based on the principles, beliefs, and experiences of various cultures is the traditional medicine. According to Magner (1992) bush medicine, traditional medicine in India, Mesoamerican medicine, traditional African medicine, traditional Chinese medicine, South Asian traditional medicine, Islamic medicine, South American traditional medicine, etc. are historically the main centers of traditional medicines. The traditional use of herbal remedies reflects the long historical use of these medicines. National authorities have accepted that their use is effectively accepted and broadly recognized to be safe and effective. For primary health care, up to 80% of the population in Africa uses the traditional medicine. In order to meet their primary health-care needs, some countries in Asia, Latin America, and Africa also make use of traditional medicine. Adaptation to traditional medicine in high-income, industrialized countries is known as “complementary“ and “alternative” medicine (CAM) and is used by up to 65% of the population (Baser 2005). In the industrialized countries, traditional medicine (TM) has continued its popularity, and also its use is fast growing (Bagozzi 2003). WHO has prepared a traditional medicine strategy in the years 1998, 2002, and 2013 in order to stimulate the proper use of traditional medicine (TM)/ “complementary” and “alternative” medicine (CAM) which focuses on the policy, safety/quality/effectiveness, accessibility, and rational use of TM/CAM.

According to Barnes et al. (2007), herbal medicines are also known as herbal products, phytotherapeutic agents, herbal remedies, phytopharmaceuticals, and phytomedicines. Phytotherapy is the use of herbal medicines as evidence- or science-based approach for the treatment and prevention of disease. This approach differs from traditional medical herbalism in that it relies heavily on herbal medicines and mainly on their empirical and traditional uses (Barnes et al. 2007). Aromatherapy, traditional medicine, balneology, phytotherapy, and homeopathy are the most frequent forms of therapeutic uses of medicinal and aromatic plants.

27.4.2.2 Phytotherapy

The concept of phytotherapy, in a simple way, is the healing with various forms of plant-derived medicines (herbal medicines). Although it is interpreted differently in different countries, it contains elements of both modern and traditional (ethnomedicine) medicines (Mills and Bone 2000). Phytotherapy (herbal medicine) includes the use of dried plant matter or plant part extracts of natural origin or health-promoting agents as medicines to treat the symptoms manifested. In this latter case, it is similar to traditional medicine (Barnes et al. 2007).

Herbal Medicinal Products (HMP)

Herbal medicinal products according to the European legal definition are medicinal products, particularly comprising one or more herbal substances or one or more herbal preparations as active ingredients or one or more such herbal substances in combination with one or more such herbal preparations (DIRECTIVE 2001/83/EC of the European Parliament and of the Council (2001). Herbal medicine sales are booming. This is especially true in the United States, where the herbal ingredient market now reaches $ 4 billion a year. Sales for the herbal antidepressant, in St. John's wort, rose by 2800% and have the fastest growth recorded in 1 year. Herbal pharmaceuticals are subject to clinical trials before regulatory authorities register as herbal medicinal products. In a preliminary survey, the global ca.there were 748 clinical trials on 96 plant species or their assemblies and 823 written papers on the results of these clinical trials were published as suggested by Baser (2005). As a common rule, phytopharmaceuticals and plant drugs used by phytomedicine must be generated and proven effective under strict quality requirements (Màthé and Màthé 2008). Similarly, the safety of application should be guaranteed along with their reliable good quality (good laboratory practice¼GLP, good manufacturing practice¼GMP, good agricultural practice¼GAP, etc.). Regardless of the regulatory pathway to reach the market, the quality of the herbal medicinal product should be illustrated. The European Scientific Cooperative on Phytotherapy (ESCOP) provides an umbrella organization for the evaluation of herbal medicinal products established in 1989 by six EU national scientific bodies. It contributes to the acceptance of phytotherapy and assists scientific research in Europe. According to Steinhoff (1999) and Barnes et al. (2007), European Scientific Cooperative on Phytotherapy currently publishes 80 monographs on individual herbal drugs. Herbal remedies are a kind of dietary supplement, and by the use of these herbal medicines, people try to maintain or improve their health.

27.4.2.3 Aromatherapy

Since ancient times, aromatherapy has been known as a therapeutic tool. It is a natural therapeutic approach aimed at improving a person's mood, cognitive functions, or health status based on the effectiveness of aromatic plants and plant extracts. It is used by personal care, hygiene products and wellness manufacturers, furthermore practitioners, including chiropractors, massage therapists, doctors and nurses. It is also useful in the treatment of infectious diseases because of the presence of most essential oils being powerful antimicrobials.

Aromatherapy can be put in combination with other types of alternative complementary therapies. There is increasing scientific evidence such as expectorant, antibacterial, sedative, anti-inflammatory, etc. on the pharmacological effects of aromachemicals and essential oils (Krings and Berger 1998; Rowe 2004).

Renaissance of Essential Oils

The effects of aroma chemicals and essential oils on human and animal organism and also their antifungal and antimicrobial activities are also being fully investigated such as gastrointestinal system, nervous system, respiratory system, immune system, etc. These studies, as currently intensively surveyed by Baser (2005), Bakkali et al. (2008), Màthé (2009), Baser and Buchbauer (2009), Franz et al. (2010), and Weiss (1997), and the growing number of proofs on the effectiveness of essential oils and their components in traditional therapy have already been suppied by others in the following fields such as animal repellent activity, skin penetration enhancing activity, plant growth-promoting activity, plant growth-inhibiting activity, anticancer and antimicrobial effects, pest control, enhancement of soil respiration, etc. (Baser 2005).

27.4.2.4 Balneology and Mineral Therapy

In alternative medicine, balneology refers to the use of various types of baths to relieve pain, muscle aches, or stress and thus promote healing as an aspect of hydrotherapy (Routh et al. 1996). Medicinal and aromatic plants have been increasingly used to add up advantageous consequences of hot and cold water treatments (Ghersetic et al. 2000; Varga 2010).

27.4.2.5 Homeopathy

Since the early nineteenth century, homeopathic medicine or homeopathy is a comprehensive medical system that was practiced in the United States and developed in Germany over 200 years ago. Homeopathy is used for the treatment of many diseases and also for wellness and prevention (Dantas and Rampes 2000; Dantas et al. 2007). In accordance with its basic laws (by Christian Samuel Honeyman), homeopathy seeks to promote the body's ability to heal itself by giving more dilution in very small doses. Homeopathy uses a full range of medicinal and aromatic plants for this purpose (Der Marderosian 1996; Jonas et al. 2003). So far it is still a disputable field of alternative and complementary medicine, as many of its main ideas are incompatible with established laws of science, essentially biochemical, physics, and chemical sciences.

27.4.2.6 Veterinary Medicine and Animal Welfare

There is a long record of the utilization of medicinal and aromatic in the curing/treatment of many animal diseases almost as old as human medicine. Interest in herbal products has increased notably around the globe. The importance of medicinal and aromatic plants in animal welfare, veterinary medicinal applications, or animal husbandry has been carried out by many researchers in this field and has increased as many scientific publications account for their significance (Laudato and Capasso 2013; Wallace et al. 2010; Russo et al. 2009; Sabaldica 2011). Around 70% of the animals are being treated with the herbal medicines like sheep, cattle, pigs, horses, and goats followed by 5.3% of dogs, 9.1% of poultry, and 4.3% of rabbits according to Viegi et al. (2003). This is not only due to a widespread tendency with regard to the use of natural products for the treatment of diseases but also because of the possibility of sufficient evidence with reference to the effectiveness of herbal remedies (Franz et al. 2010; Laudato and Capasso 2013).

27.5 Industrial Utilizations

From the sources of the plants, many useful drugs have been developed. Many specific substances can be obtained from plants like colorants, biocides, cosmetics, essential oils, dyes, and pharmaceuticals. According to Lubbe and Verpoorte (2011), numerous aromatic and medicinal plants are cultivated for such industrial uses, although most botanicals are still collected in the wild. After the discovery of penicillins, drugs were discovered from microbial sources, and diving technology also opened the seas in the 1970s. In the late 1980s, combinatorial chemistry shifted the focus of drug discovery efforts from nature to the laboratory bench (Cragg and Newman 2013). Natural product drug discovery has already made significant pharmaceuticals from natural sources that have revolutionized the treatment of acute diseases. Nature continues to be a major source of development for new constructive and effective drug development based on multidisciplinary cooperations (Wermuth 2011).

27.5.1 Pharmaceutical Industry

The dominant role of medicinal and aromatic plants for the pharmaceutical industry is described by a number of factors. These plants are the source of many biologically active substances due to their healing effects, which cannot be ignored by modern medical instruments (Màthé 2015). Due to their often unknown characteristics and complexity, they are not yet synthesized. Often, their synthetic production of cardiac glycosides, ergot alkaloids, etc. is unreasonably high priced. For the semisynthetic manufacturing of substances like corticosteroids from *Solanum* alkaloids or for segregating so-called model compounds like secologanin, the pharmaceutical industry often uses the active principles of medicinal and aromatic plants as starting materials. For the production of many health-care products and environment-friendly plant protection products like pyrethroids and phyto-cosmetics, medicinal and aromatic plants serve as raw materials.

27.5.2 Food and Feed Additives

Food additives can be incorporated into the diet to maintain flavor or enhance its taste and appearance. From the centuries some additives have been used such as salting with bacon, preserving sweets, or using sulfur dioxide as with wines. Medicinal and aromatic plants are often being used by the food industry in the form of condiments and spices besides being also used as culinary herbs in the second half of the twentieth century with the origin of the processed foods (Màthé 2015). Both artificial and natural additives have been introduced with their official list in Codex Alimentarius (2014). The importance of medicinal and aromatic plants in food additives is also enhanced by their antimicrobial, fungicidal, and bactericidal properties, which make them the most valuable preservatives both for canned products and meat preparations and fresh vegetables (Davidson et al. 2005). For the safety of humans and livestock, medicinal and aromatic plants in recent times have been used to build up and enhance the healthy feed based partially on their traditional uses in animal care and to discover successful substitutes to direct the removal from regular use of antimicrobials in animal feeds Codex Veterinary Drugs (Codex Alimentarius 2014).

27.5.3 Dietary Supplements Versus Nutraceuticals

The quality of the food products has been improved from the centuries by the use of food additives. There were about 2500 different chemicals in the United States including aromatic and medicinal plants by the early 1960s. Most of the plants are widely used in the daily diet which contains both nutraceutical and medicinal values (Burdock 2010). Food items comprise minerals, vitamins, and lesser known substances like plant foods, enzymes, amino acids, and herbs. They are also sold in the form of hard- or soft-shell capsules and tablets. While some foods are well understood, others require further study. Unlike medicines, supplements are not planned to diagnose, treat, cure, or prevent diseases (Enna and Norton 2012). The government does not accept for safety and effectiveness before marketing foods in the United States. Before they go off to the market, food manufacturers and distributors must first ensure that their products are safe. According to Paul et al. (2004), manufacturers are needed to generate food products to highest-quality standards and make sure that they do not carry impurities or contaminants and they are strictly labeled. Foodstuffs can be taken off from the market if they are found to be adulterated and unsafe or if claims about products are false and misleading (FDA 2014).

Over the past decade, the nutraceutical industry has grown quickly. According to the *Nutrition Business Journal* (NBJ), Boulder, CO, US sales reached $ 32.5 billion in 2012. Minerals ($2.4 billion), herbs and botanicals ($5.6 billion), specialty/others ($6.1 billion), and vitamins ($10.6 billion) are included in the top supplement categories. In the meantime, the beverage market and functional food is acquiring remarkable progress when consumers focus on disease prevention (Moloughney 2013). Bagchi (2014) in the recently published volume provides an updated awareness into the importance, scope, and sustainable growth opportunities in the functional food and nutraceutical industries. The newest regulatory changes and their effects will also be surveyed. In the quality of these products, public confidence is built on a wide variety of safety studies and GRAS (generally recognized as safe), sophisticated quality control, peer-reviewed publications, and cutting-edge human clinical studies. Globally the growing population has now begun to realize the effectiveness and functions of functional foods and nutraceuticals (Màthé 2015).

27.5.4 Cosmetic Industry

Herbal preparations are popular and are of primary and personal importance in home and health care. The popularity of health-care plant-derived products has been discovered to their growing acceptance and use in the cosmetic industry in addition to increasing public costs in the daily maintenance of personal health and well-being (Aburjai and Natsheh 2003). According to a recent survey of European Union member states, over 1400 herbal preparations are being extensively used. Cosmetic products should be safe undergoing normal or suitable conditions of use. Most importantly, the risk-benefit argument should not justify a risk to human health (Màthé 2015).

The herbal extracts are essentially added to cosmetics mainly due to their antioxidant components such as polyphenols, carotenoids, and flavonoids. In addition, they have also been used for their topical anti-inflammatory properties (Kole et al. 2005). Anti-wrinkling, skin tissue regenerators, and antiaging creams are the examples of such beauty-based treatments. The skincare products derived from the medicinal plants like skin tonics, skin creams, etc. are assembled together as dermaceuticals (Hoarea and Da Silva 1999).

27.6 Drug Discovery

In spite of the exhaustive research in medicinal chemistry, nature has always been a profitable source of drugs and proceeds to bring lead compounds (Màthé 2015). In fact, research on natural sources of chemicals focused on microorganisms and terrestrial plants. Moreover recently, however, organisms of marine origin have also been investigated (Grabley and Thiericke 1999; Tulp and Bohlin 2004).

27.7 Conclusion

Biodiversity plays a considerable role in human survival and the healthy functioning of natural systems, although it is largely deficient due to human activities. It is necessary to conserve biodiversity either in ex situ or in situ or both practices in amalgamation, depending on the state of conservation and its purpose. Although in situ conservation is increasingly being used for biodiversity conservation, ex situ conservation is recommended as it is complemented by various technologies like aquariums, captive breeding, gene banks, zoos, and botanical gardens. Although India is rich with biodiversity resources, more and more people depend on it directly or indirectly for their livelihood. Despite the good advances in the protection of gene bank, it has yet to be improved. Likewise, awareness should be given for developing a Botanical Garden and a National Zoological Park.

As a result of the growing demand for plants, most of which are still adapted to forest procurement, there is a constant pressure on existing resources, which leads to the continued degradation of some species in the forests, and the forest land is losing its natural flora at the same time at a rate that makes forests vulnerable. Medicinal plants constitute an important national resource and inhabit a key sector of health-care system. Directive 2001/83/EC of the European Parliament it is present in the text and has been highlighted by yellow colour. Initiating and supporting the conservation, management and sustainable use of medicinal plants for human and livestock health care, and promoting the conservation, i.e., in situ and sustainable utilization of medicinal plants in and around the world, are of importance. It encourages the conservation of threatened species of medicinal plants and their habitat for livelihood security through sustainable breeding and conservation of wild medicinal plants based on various conservation practices. For the sustainable utilization and conservation of medicinal plants, there are however a variety of suggestions; only an insignificant fragment of them have accomplished adequate preservation for medicinal plant resources through traditional conservation in botanical gardens or natural reserves.

In the industrialized countries, the revival of interest in medicinal and aromatic plants has considerably increased the market demand on these natural resources that have been used since the early times of human civilizations. Likewise with the adaptability of medicinal and aromatic plant species, their use is also many times greater. In fact, however industrial and therapeutic uses can be well-known. In the developed parts around the globe, the herbal medicines have become popular, whereas in the health-care systems of low-income countries, the traditional medicine continues to be a priority. To produce good quality of medicinal and aromatic plants, they should be used safely and with effectiveness that have become a prerequisite both for the different forms of uses and the conservation of our endangered natural resources. For the survival of these old natural resources, the sustainable utilization has become a necessity as well as a prerequisite.

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Chapter 28
Medicinal Plant Resources: Threat to Its Biodiversity and Conservation Strategies

Tawseef Ahmad Mir, Muatasim Jan, Rakesh Kumar Khare, and Musadiq Hussain Bhat

28.1 Introduction

From the beginning of human civilization, plants had been biological weapons to fight diseases and damages. Early man lived at the mercy of nature in persistent threat of diseases. Archives from the early civilization of world give an idea that a significant number of drugs being used in modern medicine were in use even in ancient times (Ugaonkar 2002). Plants used for curing various ailments were documented even in the manuscripts like Bible, Rigveda and Iliad (Posey 1990). The use of wild species for the treatment of diseases is nothing new now. In recent years the medicinal plants residing in natural areas have gained increasing scientific and commercial attention (Marinelli 2005).

In countries like China and India, plants have been used as the basis of traditional medicine system for hundreds of years (Gurib-Fakim 2006). Such plant-based system of medicine plays a vital role in primary healthcare facilities. Taxol an anticancer agent from *Taxus brevifolia* reveals the value of plants as a source of novel drugs and also emphasises the need of conserving these valuable plant resources (Gurib-Fakim 2006). There are number of areas in the world where a huge knowledge on plant use for curing various diseases exists. Medicinally important plants have been a part of cultural, social and religious aspect of early civilization (Folke 2004). According to World Health Organization, about 80% of the world population, especially those living in the rural and backward areas of developing countries, mainly depend on traditional medicines for their primary healthcare needs (WHO 2003).

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Plants have proved the source of traditional medicine systems like Ayurvedic, Chinese and Unani.

Medicinal plants have been used by ancient Chinese some 6000 years ago (Marden 1999). The Romans, Babylonians, Greeks, Egyptians and Sumerians developed their particular Materia Medica from medicinal plants. On the other side, some (Aztecs, Incas and Mayans) developed primitive medicines (Calixto 2005). Al-Razi and Ibn Sina (ninth to twelfth century A.D.), the Arabian Muslim physicians, brought new drugs from plants into general use that revolutionized the history of medicines. Rig Veda (4500–1600 B.C) the oldest book in the history of mankind provides the most primitive mention of medicinal plant use in the Indian subcontinent as far as the South Asian data is concerned. South Asia harbours more than 8000 plant species with known medicinal values (Switzer et al. 2003). So, it is apparent that South Asia is a land of many rich traditional systems of medicine. The Ayurvedic, Unani, Siddha and Tibetan system of medicine remains a vital source of day-to-day health and livelihood for hundreds of thousands of people (Switzer et al. 2003). Medicinal plants comprising trees, grasses, herbs, shrubs and vines are principle source for the raw materials being used in traditional healthcare system. Medicinal plant extracts are also being used in modern allopathic medicines. Until 1930, most of the medicines were of plant origin (Swain 1972).

Due to the growing recognition of natural products to be cheaper and without any side effects, herbal medicines are becoming popular worldwide, and in both developing and developed countries, the demand for medicinal plants is increasing. Plant products are not only used in traditional healthcare but also as raw material in the formulation of modern and herbal medicine (Alves and Rosa 2007). Due to increasing demand for medicinal plants and the growth of human population, a constant pressure is created on existing resources which lead to continuous depletion and extinction of some of the species in the forests, and on the other hand, natural forest flora is being lost at an alarming rate (Shivarajan and Balachandran 1999). Therefore discussions on traditional medicine and biodiversity are becoming vital (Alves and Rosa 2007).

Medicinal plant conservation problems are mainly regarded as part of larger biodiversity strategy conservation, even though they may require a greater attention as many of these plants are exploited by indigenous communities for their sustenance (Jha 1995). For the conservation of medicinal plant species, several proposals have been put forward in different parts of the world. This includes the in situ conservation by establishing preservation areas and cultivation, as well as projects including botanical gardens and genome banks in ex situ conservation (Cunningham and Mbenkum 1993; Kala 2000; Kala et al. 2004). However, all these conservation proposals must include the direct participation of indigenous communities in sustainable use programs which will help not only because of their tremendous knowledge regarding the use of these resources but will also prove to be helpful in the protection and management of these species in their natural environment and to save the cultural heritage accrued over the time (Van Staden 1999).

For the assessment of plant resources having medicinal and economical value, a worldwide movement and researches are being carried out. To fulfil the increasing demand of herbal products many researchers across the world are focussing on ethnobotanical investigations. Due to the extreme collection and exploitation, medicinal plants are under a great threat (Laloo et al. 2006). Over the years, the population of many high value medicinal plants has declined due to constant exploitation and significant loss of their natural habitats (Kala and Sajwan 2007). According to a highly conservative estimation, the existing loss of medicinal plant species is between hundred and thousand times greater than the expected natural extinction rate and at least one major potential drug is lost every two years (Pimm et al. 1995).

28.2 Diversity of Medicinal Plants Used Worldwide

It has been estimated that more than 50,000 plant species are being used worldwide that makes more than one tenth of the total drugs and health products. Nevertheless, medicinal plants are not distributed uniformly across the world (Huang 2011; Rafieian-Kopaei 2013). For instance, the highest number of medicinal plants used is found in China and India with 11,146 and 7500 species, respectively, followed by countries like Columbia, South Africa, the United States and other 16 countries with percentage of medicinal plants (Fig. 28.1) ranging from 7% in Malaysia to 44% in India against the total number of plant species (Rafieian-kopaei 2013; Marcy et al. 2005).

28.3 Role and Significance of Medicinal Plants

The herbal era with the use of medicinal plants is making a comeback in modern world. Nowadays, in comparison to the synthetics, the herbal products are considered safe to the life and environment. For centuries, herbs had been valued for their

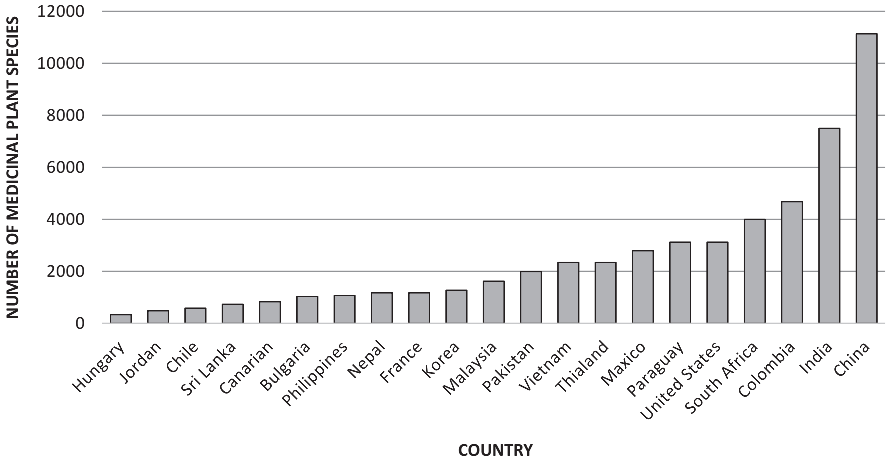

Fig. 28.1 The number of medicinal plant species in different countries

medicinal, nutritional, flavouring and other qualities, but their importance has been surpassed by modern age synthetic products for a while. Therefore, for the safety, security and other benefits, our blind dependence on synthetics is over, and we are returning towards the herbal products (Bodeker 1997). Besides, in developing countries, we can still find some medicine men and women expert in herbal lore in industrialized countries as well, who collect medicinal herbs for their primary healthcare needs and also for their families and local use by walking through woods and local forest areas (Hamilton 1997). Medicinal plant collection and other important forest products provide income to the forest local inhabitants for their sustenance, who collect medicinal plants while gathering firewood and fodder from these forests (Dutta and Dutta 2005; Bhat et al. 2018). Ayurveda, Siddha and Unani system of medicine whose therapeutic agents are derived from plants contributes to public healthcare not only in developing but also in developed countries as well (Kolata 1996). This increased demand of medicinal herbs provides a source of income to small farmers (Act No. 22 of 1992). Thus, medicinal plants not only contribute to the healthcare but also to the cultural identity, financial income and livelihood security.

28.3.1 Plants in Traditional Medicine

To meet the primary healthcare needs, 70–80% of the people worldwide chiefly rely on traditional medicinal system (Farnsworth and Soejarto 1991; Pei 2001). The demand for traditional medicine across the globe is not only large but is growing as well (Srivastava 2000). In India, it has been estimated that the market for Ayurvedic medicine is expanding at 20% yearly (Subrat 2002), whereas in the last 10 years, the amount of medicinal plants has grown by ten times from just one province of China, Yunnan (Pei 2002). Growing human population across the globe plus insufficient facilities of allopathic medicine in developing countries has led to the increased demand for herbal medicine.

As a source of therapeutic agents and contributors of primary healthcare needs as well as economies, the importance of medicinal plants is well established in both developing and industrialized countries (Wheelright 1974). Plants contribute to major part of traditional therapy. Medicinal plants play a fundamental role in the well-being of billions of people around the world because of less or no access to modern pharmaceuticals and also due to the accepted cultural preference for traditional medicine. Besides developing countries, demand for traditional therapies is also growing in developed countries because of the increasing environmental awareness and desire for natural healing through herbal resources.

28.3.2 Plants in Herbal Medicine and Botanicals

Richer countries are becoming more fashionable in context of herbal medicine, which in Europe and North America has increased the market sector by 10–20% annually. Prescription of herbal preparations within conventional medicine fluctuates greatly between countries, for example, being much in Germany compared to other countries. Moreover, a number of botanical products like health food, food supplements and herbal teas are being sold for the purpose of health and personal care (ten Kate and Laird 1999). For commercial purposes, medicinal plants have been grouped in a broader category of ‘medicinal and aromatic plants’ (MAPs) that not only includes the plants used medicinally but also includes foods, condiments and cosmetics (Schippmann et al. 2002).

28.3.3 Plants in Pharmaceutical Medicine

Plants have played a central role in drug discovery by providing ingredients, thus contributing hugely to western medicine. Several drugs having botanical origin are either formulated by directly extracted from plants or by the transformation of various chemical compounds found within them, while others are synthesized from inorganic compounds having plant origins. There are many more hidden secrets in the plant world that are yet to be unveiled (Mendelsohn and Balick 1995).

28.3.4 Medicinal Plants: Socioeconomic and Cultural Factors

Apart from the important global healthcare resource, medicinal plants play a vital role as an economic resource, traded widely from local to international scale. Information of medicinal plants and their associated knowledge are an integral part of indigenous communities, playing a vital role for health and well-being of these communities (Karki 2001). Moreover, it also provides an essential source of income to these people, as a large number of people depend on medicinal plants for their survival. Since a huge proportion of medicinal plants is collected from the wild which can put extreme pressure on these valuable resources. As this practice is not sustainable, the cultivation of species that are in large demand may offer an alternative source of livelihood to these communities (Agarwal and Narain 1990). Medicinal plants are largely collected by shepherds, herders or other economically downgraded sections of the community (Olsen 1997).

28.3.5 Medicinal Plant-Based Market Place

Medicinal plants have always got a large market place specifically at international level. Well it is not easy to analyse data related to medicinal plant market because companies ranging from large to medium size hesitate to share this data (Laird and Pierce 2002). Same is the case with wholesalers; they also hesitate to share this valuable data as they fear that companies may bypass them. Furthermore, due to the process of cross-trading between companies, it becomes more difficult to understand the trade (Dennis 1998). At most of the times, the manufacturers are unaware about the sources of their medicinal plants. In Europe and North America, most of the manufacturers buy medicinal plants from large wholesalers, which include Germany.

For medicinal plants, the largest global markets are China, France, Germany, Italy, Japan, Spain, the United Kingdom and the United States, among which Japan is the country with highest rate of medicinal plant-related drug consumption in the world (Laird 1999). With this ever-increasing medicinal plant demand, the global medicinal plant trade is expected to grow to five trillion by 2050 (FLRHT 1996). It has been estimated that China's total medicinal plant output from cultivated and wild sources is about 1.6 million tonnes (Kuipers 1997). India exports major raw medicinal plant resources and processed plant-based drugs besides China (Lambert et al. 1997). Europe on the other hand is the large trader of medicinal plants with at least 2000 medicinal plant species being traded, in which 1200–1300 are native to the continent (Lange 1998). About a quarter of global imports of medicinal plants are into Europe each year. Among the European countries, Germany is the leading medicinal plant importer, while France, Italy, Spain and the United Kingdom are among the 12 other countries that depend on medicinal plants from Asia and Africa (Lange 1998).

28.4 Threats to Medicinal Plant Biodiversity

Global stock of medicinal plants has been under continuous threat, a concern for conservationists as well as resource users. Demand of medicinal plants in pharmaceutical industries, for herbal remedies and for other natural products, has increased to a greater extent during the recent years, due to which plants are being collected from the wild and are exploited in an unsustainable manner (Anon 1994; Ayensu 1996). As a result of overexploitation, habitat destruction, land use changes and other similar factors, threats to genetic diversity and species survival have also increased in case of medicinal plant resources (Arora and Engels 1993). In addition medicinal plants are also being traded for economic basis in an unsustainable manner (Natesh 1997).

The conversion or destruction of habitat not only threatens the loss of plant resources but is indeed a great threat to traditional community life, cultural diversity and the associated knowledge of medicinal importance of some endemic species (UNESCO 1994). Nearly 20% of the wild medicinal plants have been exhausted with the increasing human population and plant consumption (Ross 2005). However, the nature, extent and impact of these factors as well as the degree of threat vary in different countries. Therefore it is important to delve out the geographical distribution and biological characteristics of medicinal plants, so as to use feasible conservation technique to preserve them.

28.4.1 Habitat Destruction

For living species including global medicinal plants, habitat destruction is considered as a big threat that can render them to extinction. Everywhere in today's world (high alpine regions as well as coastlines, rainforests as well as deserts), natural habitats of animals and plants are under the influence of humans (TRAFFIC 1999). This threat (habitat destruction) may affect medicinal plant diversity in many different ways. One of the most important concerns is the speedy extinction of valuable plant species by the destruction of ecosystem. One of the best example is increasing number of slash and burn clearings in the South America, Africa and Southeast Asia rainforests (Touchell and Dixon 1997). In addition, if the soil in such areas which has lost its natural vegetation is not allowed to regenerate back to its normal position becomes prone to uncontrollable soil erosions. Therefore the natural vegetation along with its medicinal plants disappears irreversibly. For the purpose of aquaculture, cultivation and other such purposes in many developing countries, mangrove forests are destructed that lead to wetlands and coastal plant species loss (UNEP 2007).

From the last hundred years due to various anthropogenic activities and climate change, alterations in habitat structures eventually affected the plant species to have inadequate range of tolerance to changing climatic factors (UNEP 2007). Himalayan region which is one of the 34 biodiversity hotspots of the world has lost more than 70% of its original habitat (Hachfeld and Shippmann 2000). Conversion of forests, wetlands and grasslands to agricultural land and human settlements has been increased due to increased population in these regions. Construction of roads through natural habitats has eventually contributed to fragmentation of habitats and also enhances the spread of invasive species, insects and deadly diseases and in turn leads to the loss of valuable medicinal flora (WWF and IUCN 1997). Thus, there is a dire need for better understanding and threat assessment of several plant species along with their habitats with major focus on their adaptability range.

28.4.2 Overexploitation

Due to anthropogenic overexploitation, medicinal plant diversity is under substantial threat. In this world of humans where everyone is unaware about the bankruptcy of earth's resource capital, overexploitation of medicinal plant resources has become a great threat to the persistence of global medicinal plant diversity (Peres 2010). Humans use the resources present on earth's surface and also peeps into inner strata which is totally an unsustainable practice. With overharvesting of medicinal plant species, the access to traditional remedies is being lost and poses a major threat to some of the valuable wild species as well as their habitats. As according to World Conservation Union (IUCN), 50,000 to 80,000 medicinal plants are used globally in traditional healthcare system among which nearly 15,000 medicinal plant species may be threatened by overharvesting (Bentley 2010). Increasing pressure on wild medicinal plants due to overharvesting has placed a number of species at the verge of extinction. Many of the medicinal plant species are not sensitive to harvesting pressure, but others can be driven to extinction even with lightest levels of harvest (Peres 2010). Humans are therefore depleting the earth's valuable resources, thereby posing impacts on future generations.

28.4.3 Genetic Erosion

Besides other threats, genetic erosion is a major threat to medicinal plant species. In addition to the natural genetic process of erosion, humans too induce genetic changes by various mutations. Genetic changes induced in medicinal plant species by cultivation and improvement of different varieties often exaggerate the concentration of certain compounds. These changes take place very quickly in contrast to the natural changes within a few years. These changes provide desired results in the beginning but disturb the natural equilibrium of the plants. These desired compounds can be isolated to treat several diseases, but in the long run, the therapeutic properties of these plants remain questionable (WWF and IUCN 1997). Furthermore, genetic erosion is poorly documented in wild plants. There seems a little doubt from theoretical considerations that many medicinal plant species not listed as threatened might be suffering from genetic erosion now or will face in near future (Holsinger and Gotllieb 1991). Medicinal plant species that are threatened are calculated to 4160 or 10,000 (Vorhies 2000).

28.4.4 Lack of Effective Regulation

Due to the weakening of customary laws that have been traditionally regulating the use of natural resources, medicinal plants have become increasingly threatened (Pant 2002). Such laws have been easily weakened by modern socioeconomic forces. Besides obliged to follow the Convention on International Trade on Endangered Species of flora and fauna (CITES) and Convention on Biological Diversity (CBD), many European countries have developed regional and national laws in order to overcome various conservation issues related to medicinal plants. For example, the uprooting of any species of wild plant except by landowners or other authorized people is prohibited by the UK Wildlife and Countryside Act (1981). Moreover, countries like Poland have listed species of medicinal plants that cannot be collected without proper permission and Italy has passed a law in 1931 that regulated the medicinal plant collection (Lange 1998). This effective regulation is however absent in most of the developing countries making the effective protection of endangered species difficult. Some of the community standards that traditionally safeguarded the medicinal plants have been replaced by forest laws, which remain weak in terms of implementation and enforcement especially in the remote areas of greater Himalayas. The population of some valuable aromatic plants like *Nardostachys grandiflora* and *Picrorhiza kurroa* has declined due to illegal trade in Nepal and Indian borders even being listed in Appendix II of CITES because of difficulty of enforcement of laws. Besides this species speciation is also not done properly in a number of developing countries (Mulliken 2000). Furthermore, there is no mention that what kind of threat is prevalent in what kind of social perspective and why.

28.5 Need for Conservation

Medicinal plant diversity along with their natural habitats will remain under the continuous threat of overexploitation as long as the destruction of forest resources continues (Walter and Gillett 1998). Therefore to preserve the natural habitats of vulnerable medicinal plant species and to attain the sustainable exploitation in less vulnerable areas, conservation is of utmost importance (Cunningham 1993). With the disappearance of forests, numerous valuable medicinal plant species are being lost at an alarming rate, and already some of the medicinal raw materials are running short in different pharmaceutical industries across the world (Tewari 2000). If deforestation continues at the same rate, mass extinction is likely to take place, and it is felt that significant amount of genetic diversity may be lost. Fragmentation of habitats in addition to deforestation harmfully affects the diversity of rare species (Shyam et al. 2002). To preserve the medicinal plant diversity, various conservation measures, like commercial cultivation, habitat conservation, natural reserve establishment and implementation of laws, should be enforced (Rao et al. 2002). Recent studies have shown that many plant species are in danger of extinction, whereas some have already become extinct from their habitats. Presently various initiatives have been put forward by government and non-government organizations to conserve degrading medicinal plant diversity (Shyam et al. 2002).

Medicinal plant existence is threatened in their natural habitats by the destruction of their natural ecosystems despite their recognized importance and value. This problem is even worsening with the opening of forest areas for farming and transmigration (Bapedal 2001). A number of medicinal plants have been depleted, and a number of other novel species identities and benefits would remain unknown to human kind as they have been lost without being properly explored and documented (Rifai et al. 1991). To protect the medicinal plant resources, there is a need to conserve and document these plants with their traditional uses.

28.6 Conservation Issues

Owing to the threats and much growing importance of medicinal plant resources, conservation of these resources is receiving much attention across the globe for traditional healthcare. The interest in natural herbal medicines in healthcare system is renewing since mid-1980 due to the limitations of modern allopathic medicines (Tempesta and King 1994). So the interest towards the promotion of traditional healthcare system to meet primary healthcare is increasing across the globe. For example in South Asia, due to the high and unaffordable prices of pharmaceutical drugs, there is strong and growing resurgence towards the protection and promotion of traditional herbal drugs (Switzer et al. 2003). This has brought many conservation issues regarding the medicinal plants.

28.6.1 Biological and Ecological Conservation Issues

These types of issues related to conservation of medicinal plants are considered as primary issues about which conservationists are much concerned. Due to population increase, overexploitation, environmental unfriendly harvesting techniques, habitat destruction and illegal trade of medicinal plants, the medicinal plant resources are under continuous threat of extinction. This made conservationists to come with strong conservation policies for this eroding biodiversity (Switzer et al. 2003). A number of plant species are on the verge of extinction and a good number has already attained extinction, so it is urgent to play a role so as to resist resource depletion.

28.6.2 Protection of Traditional Knowledge

The knowledge of use of medicinal plants is a burning issue that is disappearing with time and needs proper attention. As this knowledge is passing on from one generation to another, it is facing a great threat of getting vanished once for all. The traditional knowledge regarding types, management, distribution and methods of extraction and useful properties of medicinal plants is declining rapidly (Hamilton 2001). For hundreds of years, people and traditional herbal healers in rural areas of South Asia are practising traditional medicines. If this traditional medicinal knowledge and subsistence-oriented medicinal plant applications are getting proper values, this valuable knowledge can not only be save, but a good number of can be created in rural areas as well. Therefore safeguarding this traditional knowledge can add to livelihood and economic opportunities to the people and communities and will promote traditional medicinal practices in families, where this knowledge is vanishing due to the financial problem (Karki 2001).

28.6.3 Quality Healthcare Provision

Since the decline in traditional medicinal knowledge and plant resources, suitable provisions for healthcare attention are arousing in health policy makers. Nearly all countries until recently promoted western medicine, but this has now been changed especially in countries like China and India. Lacking official recognition has impacts on conservation, as these recognition policies can elevate the village level practitioners because these practitioners are the most knowledgeable persons in their local communities and have internal faith for the conservation of medicinal plant resources. An increased recognition and authority to these practitioners can play a vital role in management of plant resources (Hamilton 1997). So according to some of the conservationists, integration of indigenous medicinal knowledge into the formal healthcare system is a better option (Craig 2002).

28.6.4 *Building Knowledge Systems*

The process of building knowledge systems, information and learning is always considered as a major issue by conservationists to provide opportunities for the sharing of knowledge in order to encourage learning. To accomplish this type of conservation objective, the documentation of information, conservation status and medicinal plant use in healthcare is an essential exercise needed. Many of the countries like India don't have any estimation of present consumption and future demands of medicinal plants used locally. Conservation and improved production of medicinally important plants are impaired in the absence of such information (Hamilton 1997).

28.6.5 *Indigenous Intellectual Property Rights*

As traditional uses of medicinal plants can be a guide for the new drug development, intellectual property rights have become a serious concern because many consider this kind of theft is taking place in indigenous intellectual property. In this regard a large number of patents have been provided on genetic resources and other such type of knowledge obtained from developing countries (Balick and Cox 1996). In UN Convention of Biological Diversity 1992, the idea of benefit sharing evolved which adopted that the exploitation of the right of knowledge holder will be ensured through fair and equitable benefit sharing. The prevention of bio-piracy, the misappropriation of traditional knowledge systems and the fair and equitable sharing of benefits coming out of the utilization of biological resources and related traditional knowledge are some of the most relevant issues related to the conservation of biodiversity and other associated traditional knowledge (Karki 2009). It has been observed that, in most of the countries, issues related to the development and conservation have the least priority, and there is always lack of government budget for this sector as compared to other similar sectors (Vedanand 2002).

28.7 Conservation Strategies for Medicinal Plant Resources

Increased demand for wild resources in recent years has led to the over harvesting of medicinal plant resources largely from wild populations (Bentley 2010; Ross 2005). It has been estimated that the present loss of plant species is higher than the expected extinction rate and at least one potential drug is lost every two years (Pim et al. 2005).

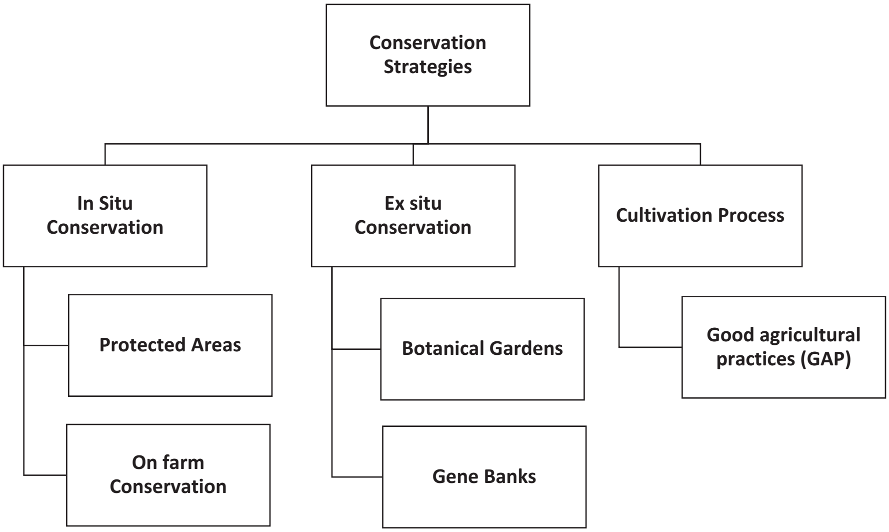

Fig. 28.2 Strategies for the conservation of medicinal plants

According to the International Union for Conservation of Nature and the World Wild Life Fund, about 50,000–80,000 plants are being used for medicinal purposes globally among which about 15,000 species are threatened by various factors (Bentley 2010). Due to these threats, the risk of extinction of medicinal plants has increased worldwide, especially in China, India, Kenya, Nepal and Uganda (Heywood and Iriondo 2003; Zerabruk and Yirga 2012). Therefore, conservation of medicinal plants has been studied properly (Larsen and Olsen 2007). For the conservation of medicinal plants, various recommendations have been put forward (Fig. 28.2), including the formation of systems for species inventory, the enhancement of cultivation efforts, improved management of wild populations, public awareness, law enforcement and the need for conservation practices based on in situ and ex situ strategies (Hamilton 2004). Wild nurseries and natural habitats, for example, are typical examples to maintain the medical efficiency of plants in their natural habitats, whereas botanical gardens and seed banks are important patterns of ex situ conservation and sustainable development (Sheikh et al. 2002; Coley et al. 2003).

28.7.1 In Situ Conservation Approach

In situ conservation approach of conservation is adopted to ensure that plant species continue to grow in their natural habitat in wild. This type of conservation involves the protection and establishment of plant resources in the location of their natural occurrence, where wild populations are protected (Cunningham 1997). In situ conservation is provided in certain protected areas where they grow and evolve naturally and by ensuring that as many as plant species continue to grow in managed habitats (IUCN-WHO-WWF 2003). Due to high market demand and poverty as some of the communities are totally dependent on the sale of medicinal plant species, these resources are exploited at a very high rate without any proper control. A strategy for the sustenance of medicinal plants involves a range of actions such as identification and protection of areas where a high density of vulnerable species are present and commercial exploitation is taking place at a high rate (Srivastava 2000).

In situ conservation not only ensures the protection to traditional medicinal plants but also maintains the integrity of natural communities as well as their complex network relationships (Gepts 2006). Besides making the link between resource conservation and sustainable use, in situ conservation can increase the diversity of species to be conserved (Long et al. 2003). In situ conservation efforts are focused on establishing protected areas, and this approach is ecosystem oriented rather than species oriented. This type of conservation is dependent on the rules, regulations and potential agreement of medicinal plant species with growth habitats (Ma et al. 2012). This type of conservation encourages genetic variability and adaptability of species to varying environmental conditions and allows ecological and evolutionary processes to take place. Hence, natural ecosystems are preferred for the conservation of biodiversity (Heywood and Baste 1995).

28.7.1.1 Protected Areas

Most often the biodiversity conservation is achieved by establishing protected area systems. These are geographically defined areas that are maintained and regulated for obtaining specific conservation objectives (Miller et al. 1995). Protected areas play a vital role in conservation of medicinal plant resources at their natural place of occurrence. To get the effectiveness of protected areas for the maintenance of plant resources, it is necessary to provide sufficient human and financial resources in place. Benefits derived from the conservation and sustainable use of plant resources should be equally shared with local people. It has been seen in many of the countries (e.g. African countries) that they fail to meet the objectives of conservation only because of exclusion of local people from participation (Zegeye 2004; Jeffries 1997).

These include areas such as natural reserves and wild nurseries. Natural reserves are protected areas created to conserve and restore degrading biodiversity. About 12,700 have been established worldwide accounting 13.2 million km^2 (Huang et al. 2002). It requires the assessment of the contributions and ecosystem functions of individual habitats to conserve medicinal plant species by protecting key natural habitats (Liu et al. 2001). In wild nurseries, endangered medicinal plant species are conserved by cultivating and domesticating in natural habitat or at place only a small distance from natural habitat (Hamilton 2004; Schippmann et al. 2005). As wild medicinal plants are under heavy pressure due to over harvesting, habitat degradation and exotic species, these endemic and endangered medicinal plant species can be best conserved by wild nurseries approach of in situ conservation (Liu et al. 2011).

28.7.1.2 On-Farm Conservation

On-farm conservation is the conservation of crops and their wild relatives and the agroecosystems in which they occur. Home gardens, crop fields, agroforestry systems, grazing lands and fallow fields all are included in agroecosystems. On-farm in situ conservation is important for maintenance of medicinal plant diversity. For the maintenance and diversification of domestic medicinal plants, indigenous resource management systems and agricultural practices are of immense value (McNeely et al. 1995). Farmers and pastoralists around the world maintain a tremendous diversity of medicinal plant varieties. In addition, indigenous knowledge, skills and practices associated with farmers play a vital role in management and conservation of agrobiodiversity (Deribe et al. 2002). Therefore, farmer-based on-farm conservation of medicinal plant biodiversity has been found to be a more successful approach.

28.7.2 Ex Situ Conservation Approach

The technique of conserving the biological diversity outside the zone of their natural occurrence is termed ex situ conservation (Borokini et al. 2010; Antofie 2011). It ensures the conservation of overexploited and endangered medicinal plants having slow growth, low abundance and those vulnerable to diseases (Hamilton 2004; Havens et al. 2006; Yu et al. 2010). The aim of ex situ conservation is to cultivate and naturalize the threatened species to safeguard their continuous survival and to produce necessary amount of material used in the preparation of potential drugs. This technique is often an immediate action taken to sustain medicinal plant resources (Swarts and Dixon 2009).

This approach of conservation encompasses the establishment of plantations, maintenance of living collections in farm fields, home gardens and botanical gardens outside their natural habitats (Roche 1992). Medicinal plant species when grown far away from their natural habitats may help not only to retain their high potency, but their reproductive materials can also be selected and stored in gene banks for future replanting. Ex situ conservation also acts as a source of material for research purposes and ecosystem restoration (Hamilton 2004). It is the type of rapid development of alternative supply of medicinal plant resources through cultivation in huge quantities at lower prices in order to compete with prices obtained by wild medicinal plant gatherers. Hence, the market demands will be satisfied and in addition will result in more secure jobs (Cunningham 1997).

28.7.2.1 Botanical Gardens

Botanical gardens include herbarium, lecture rooms, laboratories, libraries, museum and experimental or research paintings. It can be a collection of particular family, genus, medicinal or aromatic plant species (Institute of Biodiversity Conservation

2005). In ex situ conservation, botanical gardens have a significant role as they sustain the ecosystem to enhance the survival of rare and endangered plant species (Christie 1998). Botanical gardens often harbours taxonomically and ecologically diverse flora and includes a wide variety of plant species grown together under common conditions (Primack and Miller-Rushing 2009). Botanical gardens are of tremendous importance for the conservation of medicinal flora through the development of propagation and cultivation protocols and undertaking programs of domestication and variety breeding as well (Maunder et al. 2001).

Botanical gardens provide a platform where arable medicinal plants are grown under controlled and modified environmental conditions. Being considered as living laboratories, botanical gardens promote and undertake scientific research on medicinal plants in particular and biological diversity in general (Brutting et al. 2013). Botanical gardens are therefore the institutions that hold the documented collections of living plants for the purposes of scientific research, conservation and for display and education (Wyse Jackson 1999). Thus, botanical gardens should be provided necessary resources so that they can play a vital role in the assessment and conservation of medicinal resources.

28.7.2.2 Gene Banks

Genome banking is another technique used for the conservation of medicinal plant resources. Genome banking comprises of seed banks for seeds; field gene banks for live plants; in vitro gene banks for plant tissues and cells and pollen, chromosome and deoxyribonucleic acid banks for the plant resource conservation held in laboratory storage for short or long term (Clarke 2009). Gene bank aims to keep the genetic diversity alive so as to conserve endangered medicinal plant species and to reduce the frequency of regeneration. The demand of DNA for molecular studies is rapidly increasing from gene banks with the development in the field of molecular genetics and genomics (Dulloo et al. 2010). The utmost easiest and convenient material to maintain in a viable form for long periods of time is seeds making it an ideal material for conservation in gene banks. In seed banks, dried seeds of threatened and other medicinal plants are stored at moisture content between 3 and 7 percent at the temperature of 4 degree Celsius for short-term conservation and in between −18 and −28 °C for long-term conservation (Brutting et al. 2013; Dulloo et al. 2010).

Seed banks play a vital role for rapid access to plant samples for the evaluation of their properties, which provides possible information for the conservation of remaining natural populations (Li and Pritchard 2009). Recalcitrant seed bearing or clonally propagated plants are traditionally preserved as live plants in field gene banks as these plants cannot be conserved as seeds. Another important technique used for the conservation of medicinal plant resources in the form of living tissues at very low temperatures is cryopreservation, where tissues to be preserved are kept in liquid nitrogen at −196 °C temperature to arrest the metabolic activities. It has been believed that cryopreservation method of genome conservation is cost-effective and offers greater security for long-term preservation (Dulloo et al. 2010).

28.7.3 Cultivation Practice

Even though wild medicinal plant resources are widely considered more effective than those cultivated, domestication is generally widely used and accepted practice (Gepts 2006; Joshi and Joshi 2014). For solving the problems faced in production of medicinal plants such as pesticide contamination, toxic components, low contents of active ingredients and misidentification of botanical origin, cultivation provides the opportunity to use new techniques (Raina et al. 2011). Under controlled growth conditions, cultivation can enhance the production of active secondary metabolite compounds and ensures stability as well. As such cultivation practices are so designed to provide optimum levels of nutrients, water, additives and environmental factors like temperature, light and humidity to obtain improved yields of medicinal products (Liu et al. 2011; Wong et al. 2014). Furthermore, harvest volume of medicinal plant resource is decreased with the increase in cultivation that in turn benefits the recovery of their wild relatives and also decreases the prices to a reasonable range (Uprety et al. 2012; Larsen and Olsen 2007; Gepts 2006).

28.7.3.1 Good Agricultural Practices (GAP)

To regulate production and to facilitate the standardization of herbal medicines, good agricultural practices have been formulated for medicinal plants, which ensures good quality, harmless and pollution-free herbal medicines (Chan et al. 2012). Good agricultural practices include wide range of items, like the ecological environment of production sites, germplasm cultivation, collection and quality aspect of pesticide detection, macroscopic or microscopic authentication, chemical characterization of bioactive compounds and assessment of metal elements (Makunga et al. 2008). Many countries like China have actively promoted the implementation of GAP for the growth of commonly used herbal medicines in areas where these medicinal plants are being traditionally cultivated (Liu et al. 2011; Ma et al. 2012). For its ability to create economically and environmentally sustainable production systems, organic farming has received much attention. Constant use of organic fertilizers not only supplies soil nutrients but also improves soil stability, thereby affecting the growth of medicinal plants. Therefore, for the growth and sustainability of medicinal plants, organic farming is receiving more attention (Macilwain 2004).

28.8 Sustainable Use

Destructive harvesting of medicinal plant species having limited abundance and slow growth may lead to resource exhaustion and even species extinction (Larsen and Olsen 2007; Baker et al. 2007). Therefore good harvesting practices should be formulated so as to promote the sustainable use of medicinal plant species. Root and whole plant collecting is considered destructive to medicinal plants (e.g. herbs, shrubs and trees) than the collection of their leaves and other aerial parts. Using leaves or other aerial parts of medicinal plants in place of roots as a remedy can be a best alternative (Wang et al. 2009). Therefore, the need of the hour is to promote and practice sustainable use of medicinal plants so as to preserve them for future generations.

28.9 Conclusion

Human existence and health function of natural ecosystems are dependent on the role played by biodiversity. Medicinal plants, a part of this biodiversity, form the back bone of healthcare system as well as the economy of the nation. Herbal medicines play a crucial role in healthcare sector and environment and characterize a major natural resource which is on the verge of extinction. As most of the world's population is directly or indirectly dependent on medicinal plant resources for obtaining their primary healthcare and high profile pharmaceutical industries use these resources to get benefitted, our medicinal plant resources are getting overharvested that poses a serious threat of extinction. This threat is also due to the increasing demand of these resources in market. A number of medicinal plant species are being lost at an alarming speed due to habitat destruction and other harmful anthropogenic activities. Due to unsustainable exploitation of medicinal plant resources by different means such as grazing, fuels, food and medicine and due to unmanaged trade of these resources has put them in jeopardy. Medicinal plant resources need much attention in terms of protection, management, research and increasing level of public awareness so as to save this depleting heritage. There is a need to conserve these medicinal plant resources and their ecosystems as well. Government should give priority to these resources and should promote their sustainable and restricted use. Establishment of natural protected areas like wild nurseries, wild reserves and botanical gardens should be promoted. For conservation and mass production of medicinal plants, cultivation should be enhanced. Moreover, national and international non-government organisations in collaboration with government can play a crucial role to save the biotic diversity contributing towards a number of benefits to society. Future of the medicinal plant worldwide is based on the ability to solve the present havoc on these resources and to promote alternative agricultural cultivation of these resources. Many of the researchers stressed over the conservation of medicinal plant resources through sustainable management approaches as such these approaches need to delve out the present trend of medicinal plant collection, distribution and consumption. There is a dire need to investigate the factors which can work as preventive measures to redress these activities or provide the information about the extent they are seditious to sustainability of medicinal plants in a particular area. Therefore it's important to work for the conservation and sustainable utilization of medicinal plant resources so as to save this valuable heritage.

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Chapter 29
Phytoremediation Potential of Medicinal and Aromatic Plants

Irshad A. Lone and Masrat Gaffar

29.1 Introduction

Today the world is witnessing an unprecedented growth in population which is projected to reach 10.9 billion by 2050. In India alone, the current population is approximately 1.27 billion, and it is showing continuous upsurge with the passage of time. The general population is expected to face food crisis in the near future as the already burdened agricultural sector is confronting tremendous challenges to provide adequate food supply while maintaining high productivity and quality standards. Under these circumstances, feeding 1.27 billion mouths would indeed be a huge challenge. Furthermore, the soil quality has also degraded immensely by incorporation of heavy metal contaminations to the soil, atmosphere, and water bodies which is posing a severe threat to not only agriculture but ecology and environment too. Heavy metals encompass collection of metals (>20 gcm^{-3}) and metalloids (5 gcm^{-3}) with relatively higher atomic masses and atomic numbers (Alloway 2011). A portion of these metals are required in fulfilling various essential biochemical and structural features of plants such as growth and development, redox reactions, electron transport, and several other fundamental metabolic processes and as such are termed as "essential trace elements," for instance, chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), iron (Fe), molybdenum (Mo), and zinc (Zn) (Kabata-Pendias 2000), while a few other metals with unknown biological functions are found to be toxic for plants even at minimal concentrations. Such metals are termed as "nonessential metals," for instance, arsenic (As), lead (Pb),

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cadmium (Cd), and mercury (Hg) (Shahid et al. 2017). In recent past, the dramatic rise in heavy metal concentrations in various ecological units has been attributed to expansion in several anthropogenic activities such as mining, industry, metallurgy, fertilizer usage, and automobiles, which has resulted in buildup of heavy metals in natural environments such as water, air, and soil. Production of heavy metals into air, soil, and water bodies, in this manner, may get absorbed by plants where it could inflict various potential lethal effects on them.

29.2 Impact of Heavy Metals on Plants

Heavy metal pollution is today considered as the biggest challenge to agricultural sector because it has direct impact on the yield and quality of production through mediation of crop physiology. On exposure to heavy metal stress, plants undergo a series of changes ranging from leaf necrosis, chlorosis, stunted growth, decline in respiration and photosynthetic activities, and decrease in percentage and rate of germination to apoptosis of cell which ultimately leads to the death of the plant (Wang et al. 2018). Stress of heavy metals also disturbs the homeostasis of plants, for instance, disarray in transpiration, water uptake, nutrient uptake, and gated ion channels (Demidchik 2017).

Lead (Pb) is treated as a nonessential element for a plant, and coincidently there is absence of lead uptake channels in plants too. But still this noxious metal makes its entry into the plant. This is possible due to its association with sulfate and phosphate salts, which makes it relatively soluble and accessible to the plants. Pb also gets entrapped on root surfaces by means of organic acids particularly by mucilage uronic acid through its carboxylic group. Increased concentration of Pb facilitates accumulation of Pb inside root cells, and subsequently it acts like an innate barrier as it leads to primary obstruction of Pb for its uptake to the other parts of the plant. Accumulation of Pb inside a plant develops a plethora of lethal impact on its several functions such as growth, morphology, physiology, photosynthesis, and other biochemical assays. Usually enzyme activity is either switched off or altered by resorting to undesired bonding with sulfhydryl (-SH) groups. Tolerance of plants to Pb toxicity varies from plant to plant depending upon their ability of respective mechanism. Certain plants are very susceptible to Pb concentrations, whereas others tend to be tolerant and possess the ability to accumulate higher concentrations of Pb. Such plants are termed as “hyperaccumulators.” Buckwheat is regarded as an excellent hyperaccumulator on account of its tremendous potential to amass and tolerate higher absorption of Pb in its leaves without presenting any significant damage (Horbowicz et al. 2013). It has been estimated that buckwheat accumulates around 1 g/Kg of Pb in its shoots, 8 g/Kg in its leaves, 2 g/Kg in its stem, and 3.3 g/Kg in its roots on dry weight basis without showing any lethal damage (Tamura et al. 2005). In many plant species such as *Allium*, barley, and others, Pb has been found to be responsible for inhibition of root and stem length while showing predominant expansion in the leaf area. The intensity of elongation, however, is dependable upon various parameters such as pH, concentration gradient, and ionic composition.

Aluminum (Al) which is ranked as third most abundant element after oxygen and silicon constitutes about 7% of the Earth's crust. Under normal circumstances, a bulk amount of Al exists in the form of harmless oxide species and aluminosilicates; however, it gets solubilized into the lethal form of Al^{3+} once it gets exposed to acidic soil due to anthropogenic activities. The lethal form of this Al is toxic for the plant which hampers plant growth and as a result negatively impacts agricultural yield (Smirnov et al. 2014). Exposure of increased concentrations of Al not only inhibits elongation of roots and shoots but also results stunted growth and development of the plant besides declining the production of biomass. This hampers uptake of water and minerals. Al toxicity also hampers the stimulation of ferritin synthesis due to their impeded actions of proton pumps on endosomes. Despite being a non-redox metal, Al-mediated toxicity leads to oxidative stress of plants. Binding of Al to biomembranes due to its capacity to form electrostatic bonds preferably with oxygen donor ligands results in its rigidity which alternatively promotes the lipid peroxidation of biomembranes and other cells as is corroborated by the findings in various species of plants such as wheat, barley, green gram, and triticale. Higher concentrations of Al are also responsible for deficiency of magnesium and calcium. Toxicity of Al level differs from one species to another. While some plants have been found extremely prone to Al-induced stress even at micro-molar concentrations, others display higher tolerance. Buckwheat shows the large tendency to accumulate Al. As much as 1.5 g/Kg of Al in the form of Al oxalates has been reported mainly in the leaves without any lethal damage (Zhang et al. 1998).

Another toxic heavy metal having devastating impact upon agricultural production is mercury (Hg). Existing in several different forms such as mercury sulfide (HgS), mercuric ion (Hg^{2+}), mercuric oxide (HgO), and methylmercury (CH_3Hg^+), the predominant form of Hg in agricultural land is in its ionic form, i.e., Hg^{2+} (Pirzadah et al. 2018). Hg prefers to exist in the solid phase due to its binding capacity against the sulfides, clay particles, or other organic matter and as a result has the tendency to get accumulated in various parts of the plant. It inflicts there several harmful effects to the plant cells and its tissues such as closing of stomata and obstructing flow of water by means of binding with water channel proteins. Heavy concentrations of Hg metal also impede the activity of mitochondria by inducing oxidative stress that promotes lipid peroxidation and eventually inflicts damage to cellular biomembranes (Pirzadah et al. 2018).

29.3 Oxidative Stress and Antioxidant Defense Response in Plants

Toxicity of heavy metal is indeed a huge concern to agricultural sector as it leads to oxidative stress in plants and causes deleterious effects that result in the decline of production yield. A variety of physical, chemical, and morphological changes are observed in plants in its biochemical processes such respiration, photosynthesis, lipid metabolism, protein synthesis, growth, and energy productions. These changes of plant cells could be attributed possibly to three main rationales:

(i) Direct interaction between metal and proteins on account of their affinities against carboxyl, thionyl, and histidyl groups, as a result guiding the metal to aim catalytic, transport, and structural positions of the cellular units.
(ii) Induced reactive oxygen species (ROS) generation which triggers the antioxidant defense structure (both enzymatic and some non-enzymatic antioxidants).
(iii) Dislocation of essential cationic species from their specific binding sites, which enables total collapse of functions.

From the above three rationales, the most recurrently evidenced manifestation of heavy metal-mediated stress in plant living systems is the disproportionate production of ROS such as hydroxyl (HO•), alkoxy (RO•), peroxyl (RO_2•), superoxide anion (O_2•-), singlet oxygen (½O_2), hydrogen peroxide (H_2O_2), etc. Free metals which are redox-active such as Cr, Fe, and Cu are observed to be responsible for direct enhancement of ROS production through metal-dependent *Fenton-type* and *Haber-Weiss-type* reactions (Fig. 29.1) in comparison with the heavy metals which are physiologically non-redox-active (e.g., Mn, As, Ni, Co, Pb, Zn, Cd, Hg) (Kovacik and Backor 2008). Under normal growth circumstances, several metabolic processes such as respiration and photosynthesis generate ROS, and there always strikes equilibrium between the ROS generation and its detoxification inside the cellular surface. However, under many abiotic strain conditions like heavy metal toxicity, the equilibrium balance between the production of ROS and its detoxifica-

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\cdot}OH + OH^{-} \quad (1) \quad \text{..[Fenton Type Reaction]}$$

$$Fe^{3+} + O_2^{\cdot -} \longrightarrow Fe^{2+} + O_2 \quad (2) \quad \text{..[Haber-Weiss Type Reaction]}$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + {}^{\cdot}OH + OH^{-} \quad (3)$$

Overall

$$O_2^{\cdot -} + H_2O_2 \xrightarrow{Fe^{3+}/Fe^{2+}} O_2 + {}^{\cdot}OH + OH^{-} \quad (4)$$

Fig. 29.1 Schematic illustration of Fenton-type and Haber-Weiss-type reactions in Fe-mediated production of free radicals. *(In Fenton-type reaction, iron reacts with H_2O_2 to generate highly reactive hydroxyl radicals. Under stress conditions, $2O_2^{\cdot -}$ behaves as an oxidant of [4Fe-4S] cluster-containing enzymes and assists ·OH production from H_2O_2 by making Fe (II) available for Fenton reaction. In the Haber-Weiss reaction, the superoxide radical takes part as well. Similar to Fenton chemistry, Haber-Weiss-type reactions also participate in iron-induced free radical generation. In such reactions, an oxidized metal ion is reduced by $O_2^{\cdot -}$ and then combines with H_2O_2 to generate hydroxyl radical. The hydroxyl radical is extremely reactive with estimated half-life of < 1 ns in aqueous solution. Generation of ·OH in proximity to DNA could potentially make this radical to react with bases of DNA or its backbone to further release stranded breaks or damaged bases)* tion gets disrupted. This leads to the phenomenon of *oxidative stresses* in which in plant cells experience overproduction of ROS which affects the overall redox homeostasis of the plant cell. The chief cellular machinery vulnerable to smash up by oxidative stress-generated ROS includes proteins (denaturation), lipids (saturated fatty acid peroxidation inside membrane), nucleic acid (DNA plus RNA), and carbohydrates. Peroxidation of lipids (LPO) is considered one of the foremost fallout of oxidative damage to plant living systems which is being evaluated through the quantification of malondialdehyde (MDA), a three (03)-carbon, low-molecular-weight (LMW) aldehyde product formed during radical assault on polyunsaturated fatty acid (PUFA) systems. Nature has equipped plants to counter distinct abiotic and biotic stress environments and as a result acts as sinks for abhorrent chemicals. Plants in general serve as green regulators to defuse the toxicity of the heavy metals either within the plant cell matrix or in its immediate rhizosphere (Dalcorso et al. 2010). Plants utilize special defensive mechanisms to scavenge the excess generated ROS, which includes triggering of antioxidant arsenal like catalase (CAT), glutathione reductase (GR), glutathione peroxidase, glutathione S-transferase (GSTs), superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), dehydroascorbate reductase (DHAR), polyphenol oxidase (PPO), shikimate dehydrogenase (SKDH), phenylalanine ammonia-lyase (PAL), and cinnamyl alcohol dehydrogenase (CAD), non-enzyme antioxidant systems such as glutathione, proline, alpha-tocopherol, ascorbic acid, carotenoids, and terpenoids and numerous phenolic molecules such as anthocyanins, lignins, tannins, flavonoids, flavonols, coumarins, cinnamyl alcohols, cinnamyl aldehydes, cinnamyl acid, and many other related compounds, etc. (Blokhina et al. 2003; Nicholson and Vermeris 2011). Annulations of heavy metal toxicity can also be achieved by mode of "tolerance" when plants sustain under huge metal concentration or by the tactics of "evasion" when plants are able to obstruct metal uptake. In the process of tolerance, heavy metals are chelated intracellularly by discharging out organic acids or other ligands which bind to metals such as metallothioneins (MTs) and phytochelatins (PCs) (Seth et al. 2012). Such metal binding ligands offer the plant to take up heavy metal ions and sequester them within vacuoles. The capacity of vacuole sequestration decides the fate of heavy metal toxicity, and as a result it becomes extremely important to untangle its impact and mechanistic approach of haulage and sequestration. The process of evasion entails the precipitation or chelation of heavy metals in the rhizosphere consequently restricting its entrance and translocation above ground part.

29.4 Phytoremediation and Its Relevance for Sustainable Green Environment

Plants have the capability to extract lethal metals from the contaminated soils. This ability is nowadays been exploited to revitalize the health of the soil polluted with various toxic heavy metals by means of newly introduced technique of phytoremediation. "Phytoremediation is the appliance of plant-controlled interactions with groundwater and organic and inorganic molecules at contaminated sites to achieve site-specific remedial goals" (Landmeyer 2011). The main idea after the process of phytoremediation via plants is the extraction of noxious metals from the top soil, then translating them into forms which are least toxic that can't contaminate the string of food chain besides being economically viable. Plants have the potential to lessen the burden of environmental contamination by absorption of certain noxious elements and subsequently transporting them in different parts of the plant in least toxic structures. Remediation via plants is a wonderful sustainable tool in comparison with other known techniques as it does not afflict any damage to the biochemical and physical possessions of the soil while improving the quality of soil simultaneously. Conventional approaches for cleansing up of heavy metal-contaminated soils are not only laborious and time-consuming options but non-affordable too. As a result, the alternative viable green approach of phytoremediation technology is utilized for the elimination of toxic metals from contaminated sites. This technology is effective, affordable, and a sustainable tool to uphold the health of the soil. It maintains the natural conditions of the environment for sustainable and green environment by remodeling and reducing the load of pollution. The process of phytoremediation is achieved through several techniques which include phytoextraction, phytostabilization, rhizofiltration, phytovolatilization, and phytodegradation. All these techniques differ in their fundamental mode of purposes. For instance, some remedial processes involve detoxification and stabilization, while others resort to leaching and containment (Kamusoko and Jingura 2017). The most preferred strategies for the phytoremediation of soil are phytoextraction and phytostabilization. For having phytoremediation projects to be successful, selection of an appropriate plant is an essential prerequisite. So, choosing plants with ability to convert noxious elements into useful ones is considered as a landmark innovation in this aspect. One should consider always three basic things while choosing the plant: (a) economic plant with high value, (b) least or no threat of contagion in the final products, and (c) higher biomass and higher efficiency level for heavy metal extraction.

Several plants possess the capability to grow and endure stress in metalliferous soils. These plants can be categorized into three main categories: metallophytes, pseudometallophytes, or hyperaccumulators. Ecologically, the use of edible plants is not viable for phytoremediation because the toxic metals may enter into the food chain by human or animal consumption and attribute some lethal effects. Therefore, nonedible crops such as aromatic and medicinal plants are the suitable targets of interests to be used as potential alternatives as phytoremediation crops. Aromatic plants are considered as important sources of essential oils which exhibit potential market in cosmetic industry, perfumery, and aromatherapy. The essential oils from medicinal and aromatic plants are free from any threat of toxic heavy metal accumulation from plant biomass as heavy metals are not removed from the tissues during the extraction of essential oil through distillation process and leave them in the extracted residual plant biomass (Fig. 29.2). As a result, cultivating of such plants in metal-contaminated areas may restrict heavy metal access into the food chain (Khajanchi et al. 2013). Further, owing to their fragrance, animals are not able to inflict any damage to aromatic plants, and as such these can be cultivated on large

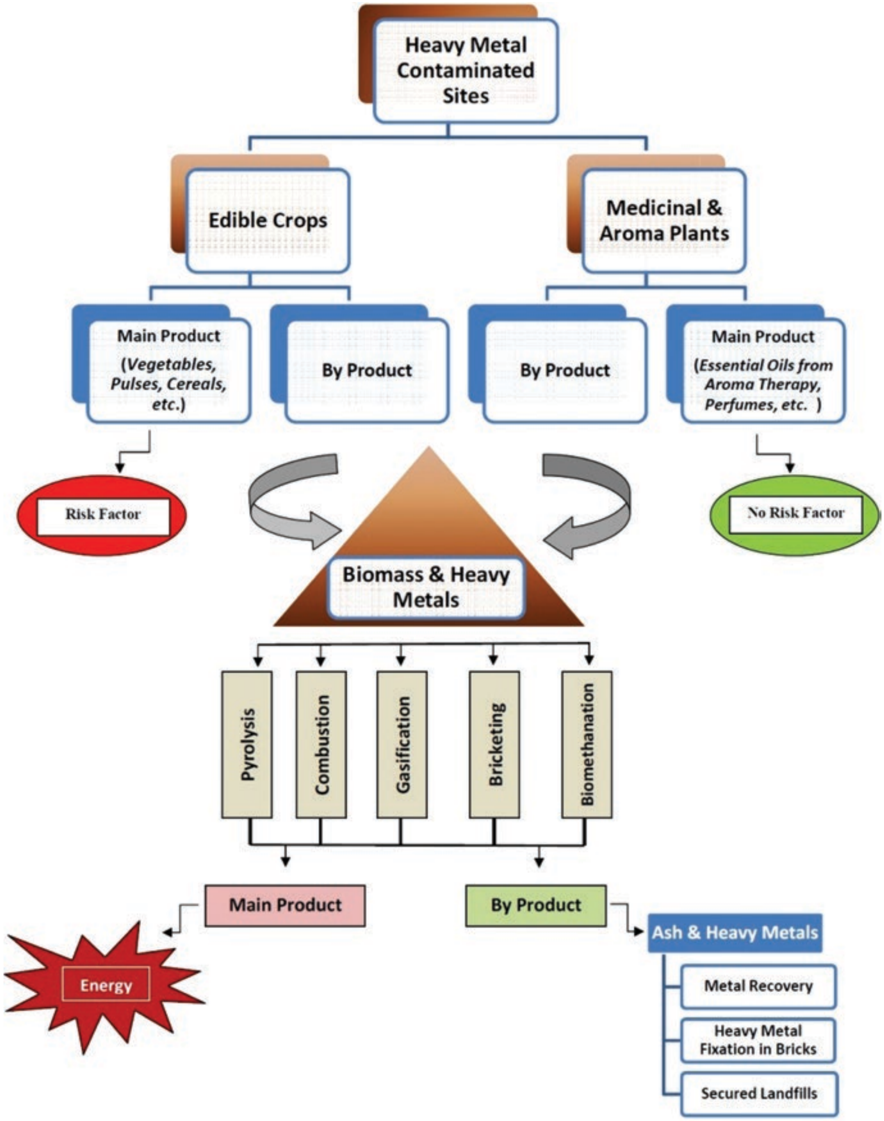

Fig. 29.2 Phytoremediation benefits of heavy metals by medicinal and aromatic plants against edible crops

scale. Several aromatic and medicinal plants have been recognized to have the potential to amass toxic metals and as a result are being used in phytoremediation technology. Cultivation of aromatic and medicinal plants, therefore, offers an innovative approach to remediate contaminated soils for sustainable development of agricultural yield.

29.5 Phytoremediation Potential of Some Medicinal and Aroma Plants: *A Mechanistic Approach*

In this section, attempts have been made to document the research performed so far on phytoremedial potential of some of the crucial aromatic and medicinal plants along with their possible mechanism of actions. Apart from the following plants, a few other medicinal and aromatic plants exhibiting phytoremediation capabilities have been briefly summarized in Table 29.1.

*29.5.1 Vetiver or Khus (*Family – *Poaceae)*

There are several species of this plant and the most widely known is *Chrysopogon zizanioides* or *Vetiveria zizanioides*. Popularly known as vetiver or khus, the average oil yield of the plant on the basis of dry weight is 1.00–1.50%. It is the most important medicinal plant used for phytoremediation of heavy metals such as Cd, Pb, Cr, Zn, etc. Having tolerance for wide ranges of heavy metals, saltiness, sodicity, and pH, apt use of vetiver plant is recommended in old landfills for heavy metal removal and reclamation of land. Regular pruning of shoots of vetiver plant augments the general uptake of heavy metals toward shoots and its other parts. Studies have shown that phytochelatins in vetiver leads to the formation of complexes when treated with the mixture of EDTA and Pb; and production of such complexes was

Table 29.1 List of some other promising medicinal and aromatic plants used as phytoremedial plants

Name of the plant	Heavy metal	References	Name of the plant	Heavy metal	References
Aloe vera	Cr	Pirzadah et al. (2019)	*Hypericum perforatum*	Ni, Cr, Cd	Lajayer et al. (2017); Pirzadah et al. (2019)
Artemisia annua	As	Rai et al. (2011)	*Matricaria chamomilla*	Ni	Kovacik et al. (2009)
Bacopa monnieri	Fe	Pirzadah et al. (2019)	*Panax ginseng* Meyer	Cu	Ali et al. (2006)
Brugmansia candida	Ag, Cd	Lajayer et al. (2017)	*Phyllanthus amarus* Schum and Thonn	Cd	Rai et al. (2005)
Catharanthus roseus	Cd, Pb, Ni, Cr	Ahmad and Misra (2014)	*Senecio coronatus*	Ni	Pirzadah et al. (2019)
Dioscorea bulbifera	Cu	Lajayer et al. (2017)	*Trigonella foenum-graecum*	Cd, Co, Cr, Ni	Lajayer et al. (2017)
Euphorbia hirta	Cd, radioactive waste	Hamzah et al. (2016); Pirzadah et al. (2019)	*Withania somnifera*	Cu	Khatun et al. (2008)

predicted to develop tolerance of Pb inside vetiver species. Further, the chelant-bound lead complexes were found to be less detrimental and effortless in terms of uptake by plant (Andra et al. 2009). The heavy metal Pb possesses the capacity to increase the essential oil quantity of vetiver species. Planting of vetiver grass in Pb-rich zone could offer as an essential tool for retrieval of soil polluted with Pb. Sequestration of Pb in roots makes vetiver species predominantly an ideal candidate for phytostabilization. For sites contaminated with Cr metal, vetiver species shows electrolyte leakage, and the percentage of this leakage gets enhanced with the increase in Cr concentration. It also establishes the substantial uptake of Cr in plant tissues (Pillai et al. 2013). Cultivation of *Vetiveria zizanioides* at heavy metal contamination sites of synthetic wastewater registered the removal of about 80–94% Pb and around 77–78% of Cr which amounts to 5–20 mg/L of Pb and Cr (Singh et al. 2014). Vetiver species acts a phytostabilizers for Cd. Studies have reported that vetiver species accumulates much more Cd than that of its counterpart hyperaccumulator *Thlaspi caerulescens*. An ideal edge of using vetiver species is that its waste is not presumed noxious once being harvested and could be exploited safely for generation of bioenergy, compost, or even as a matter for skilled works. The vetiver species have longer expectancy of life of about 50 years, which constitutes it a widely accepted contestant for the means of phytoremediation. Although vetiver are less powerful in substantial metal uptake in comparison with other species, still it is experienced highly defiant to inconsiderate weather and soil conditions, enabling it an ideal contender for contaminated zones. The shoots of vetiver could be harvested periodically without replanting amid reclamation of contaminated soil. Different mechanistic approaches geared up by vetiver species to show resistance against heavy metal oxidative stress encompass metal chelations and sequestrations of heavy metals by phenolics, glutathione S-transferase, and low-molecular-weight thiols (Pandey et al. 2019). Heavy concentrations of metals at contaminated sites also enhance activities of glutathione, peroxidase, catalase, and guaiacol (Pandey et al. 2019). The heavy metal threshold limit in vetiver grass on dry weight basis is projected to be higher than 0.8 g/Kg (Ng et al. 2017). All these distinctive features enable vetiver species ideal for phytoremediation.

*29.5.2 Lemon Grass (***Family –** *Poaceae)*

There are about more than 50 species of lemongrass, and the most widely known are *Cymbopogon flexuosus* and *Cymbopogon citratus*. Commonly known by the name of lemon grass, the average oil yield of the plant on the basis of dry weight is 0.36–1%. It finds wide applications as phytoremediation plant for heavy metal contaminants such as Cu, Pb, Cr, Fe, Mn, As, Ni, etc. Lemon grass has the ability to cope up with long-term stresses due to heavy metals. Applying chicken fertilizer to lemongrass at the percentile of five (5%) can assist in phytoremediation of Cu toxicity (Pandey et al. 2019). Studies conducted by Sobh et al. (2014) reported that stem of lemon grass (*Cymbopogon citratus*) has the potential to adsorb Pb^{2+} ions from aqueous solutions and as a result could be utilized as effective, eco-friendly, and low-cost adsorbent for the management of wastewater contaminated with Pb^{2+} ions (Sobh et al. 2014). It can work as an ideal biosorbent for the exclusion of Pb^{3+}, Cd^{2+}, Ni^{2+}, and Zn^{2+} from industrial effluents (Hassan 2016). Lemon grass (*C. citratus*) shows biosorption of Ni^{2+} by virtue of the presence of hydroxyl and carbonyl functionalities on its surface (Pandey et al. 2019). It has the capacity to phytostabilize Mn, Fe, and Cu besides accumulating Cr, Zn, As, Cd, Ni, Pb, and Al. This is corroborated by the parameters of bioconcentration and translocation (Gautam and Agrawal 2017). Lemon grass has been used for the reclamation of sites contaminated with Cr^{4+} and As^{3+} (Jha and Kumar 2017). Cadmium induces proline biosynthesis in lemon grass, and its accumulation was found highest in young leaves. A major impact on essential oil content of lemon grass was reported by inoculation of mycorrhizal fungi loaded with heavy metals (Pandey et al. 2019). Wastewater irrigations witnessed abundant growth of lemon grass herb with increased essential oil yield. From the points underlined above, it could be inferred that lemon grass acts a prospective means for phytoremediation of sites contaminated with heavy metals.

*29.5.3 Palmarosa (***Family –** *Poaceae)*

Palmarosa is the common name for the species of *Cymbopogon martini*. The average oil percentage of the plant on the basis of dry weight is 0.7–1.1%. Palmarosa species are used for phytoremediation of heavy metals such as Cr, Ni, and Cd and act as potential phytostabilizers. Such species when grown in tannery sludge profoundly contaminated with heavy metals take participation in amendment of the soil (Pandey et al. 2019). The heavy metal uptake for various metal contaminants both in shoots and roots of Palmarosa species have been registered in the following manner: Cr > Ni > Pb > Cd.

*29.5.4 Citronella (***Family –** *Poaceae)*

There are several species of citronella, and the widely known species of this plant is *Cymbopogon winterianus* Jowitt. Commonly named as citronella, the average oil percentage is about 0.4–1.1% on fresh weight basis. *C. winterianus* J. is used as a phytoremediation plant for heavy metals such as mainly Cd, As, Fe, Ni, and Cu. Although there is not a good deal of work done on this species, however, it has been suggested that *Cymbopogon* species could be used as prospective phytostabilizers in areas contaminated with heavy metals for the abovementioned metals (Jisha et al. 2017). The highest accumulation of cadmium in citronella when cultivated in contaminated sites was established in the following order: roots > stem > leaf sheath > leaves. Due to comparatively increased accumulation of Cd in root system, citronella at times experiences death also.

29.5.5 *Geranium* (Family – *Geraniaceae*)

There are many well-known species of this medicinal and aromatic plant which includes *Pelargonium hortorum*, *Pelargonium roseum*, *Pelargonium graveolens*, etc. *Geranium* is the common name used for *Pelargonium* species, and the average oil percentage is about 0.15–0.3% on fresh weight basis. This plant is widely used for the phytoremediation of heavy metals such as Ni, Pb, and Cd. Usually medicinal and aromatic plants are metal-specific for the accumulation of heavy metals; however, *Geranium* is a plant which acts as a multi-metal accumulator, and as a result it is unique from other metal accumulators. This is further corroborated by the findings of KrishnaRaj et al. 2001, where *Geranium* has been used as a successful hyperaccumulator in soils contaminated with multi-metals (KrishnaRaj et al. 2001). *Geranium* is more prone to chromium toxicity than Ni, Cd, and Pb (Chand et al. 2016). Fragrant geraniums when cultivated at heavy metal-contaminated sites possess the ability to tolerate uptake of Pb from its contaminated biomass by the formation of complexes (KrishnaRaj et al. 2001). Larger buildup of Ni and Cd in biomass of fragranted geranium is attributed to its vast defense machinery which shields the plant from getting damaged at its sites of high metabolic activity. The plant invokes proficient photosystem-II mechanism for taking care of metabolic activities while containing damage to the photosynthetic motorized system due to heavy metal particles (Dan et al. 2000). *Pelargonium* which is considered as a high biomass cultivator possesses the capacity to phytoremediate Pb in its roots together on acidic and calcareous soils through the formation of complexes with organic species (Arshad et al. 2016). Conspicuous presence of metallothioneins and phytochelatins was established by the development of bonds in the company of glutathione and cysteine, which played an essential part in metal buildup and the subsequent tolerance in such plants. In tannery sludge, *Geranium* offers the best option for better oil yield and as a result has the capability to act as a potential hyperaccumulator for certain heavy metals.

29.5.6 *Chamomile* (Family – *Asteraceae/Compositae*)

Chamomile or camomile is the common name for several daisy-type plants of the Asteraceae family. The notable varieties of *Chamomilla* species include *Matricaria chamomilla* (German chamomile) and *Chamaemelum nobile* (Roman chamomile). The average yield of essential oil on the basis of dry weight ranges from 0.24% to 1.9%. Heavy metal contaminants for which chamomile species are known for phytoremediation include Cd, Cr, and Ni. The order of metal content in chamomile plant has been observed highest in roots followed by leaves and flowers. Being tolerant to Cd, chamomile plant has recorded higher accumulation of Cd in anthopodia. The mobility of Cd in chamomile plants is recorded higher than Pb. Unlike a hyperaccumulator, chamomile plant preferably excludes metals. The ability of chamomile plant to tolerate Cd metal is established by the fact that there is slight impact on accumulation of lignin and phenolic structures. The tendency of chamomile plants to translocate Cd from root system to shoot system is attributed to soluble phenols. Chamomile plants possess dynamic protection against heavy metal contaminations, and this is ascribed to the potential antioxidant defense system, particularly antioxidant enzymes (Pandey et al. 2019). Phenolics of chamomile plant have the ability to act as a root barricade so as to prevent translocation of Ni metal to above the ground parts of a plant. Phytoremediation of Cr heavy metal is shown by chamomile plant through its roots. The plausible mechanism by which the plant exhibited its phytoremediation potential was due to sequestering of Cr metal and its subsequent reduction from Cr^{4+} to Cr^{3+} (Kovacik et al. 2014). Chamomile has also the potential to effectively endure nickel-mediated heavy metal stress (Ghanavatifard et al. 2018). Uptake of Ni metal was shown to be phenol-independent unlike Cd uptake which was attributed to phenols (Kovacik et al. 2014). Heavy metal toxicity due to Ni treatment has shown bioaccumulation of chlorogenic acid, an antioxidant phytochemical molecule (fourfold increase in 12 μM Ni treatment) produced as a defensive mechanistic response (Lajayer et al. 2017).

*29.5.7 Mint (***Family –** ***Lamiaceae)***

There are many species of mint, among which the mostly widely known species includes *Mentha crispa* and *Mentha pulegium*. These species are popularly known as mint. The average oil percentage of *Mentha* species on the basis of fresh weight ranges from 0.5% to 0.9%. Mint exhibits tremendous phytoremediation potential against heavy metal contaminants such as Cr, Cd, and Pb. As a phytostabilizer, *Mentha* species accumulates majority of the metals in the roots as compared to aerial portion. Comparatively less sequestration of Pb and Cr metals in the shoots as compared to root tissues, *Mentha* species demonstrates non-hyperaccumulatory nature for the allied metals. Heavy metal contamination of Pb toxicity was endured by *Mentha crispa* L. (Lajayer et al. 2017). Mint species accumulated with Cr and Pb metals displayed notable changes in the composition of their essential oil. Carvone fraction present in essential oil witnessed a significant upsurge after treatment with Pb. *Mentha pulegium* showed significant elicitation of pulegone, cis-isopulegone, alpha pinene, thymol, etc. in its essential after treatment with Cu and Zn. (Lajayer et al. 2017). By appliance of organic matter or vermicompost, *Mentha* species have the potential to grow at metal-contaminated sites without showing any toxic indications. Growing of *Mentha spicata* in sludge area at a ratio of 75:25 utilizes waste sludge for better quality of oil and its yield besides showing highest metal absorption capacity (Patel et al. 2016).

29.5.8 *Basil (*Family – *Lamiaceae)*

The notable species of basil for phytoremediation methods include *Ocimum tenuiflorum* and *Ocimum basilicum*. The average essential oil yield of such species on fresh weight basis ranges from 0.3% to 0.7%. Commonly known as the basil, the *Ocimum* species are used as phytostabilizers for Cr, Cd, and Pb metals as they have the potential to accumulate heavy metals. Such plants act as both food and medicinal crops. For avoiding contamination of food chain, such plants should be discouraged for food consumption if cultivated for the purpose of phytoremediation. *Ocimum tenuiflorum* possess the ability to sequester larger quantity of Cr in the root system compared to leaf system (Pandey et al. 2019). The tolerance to Cr-mediated oxidative stress in *Ocimum* species was attributed to hyperactivity of antioxidant defense system. Cr stress produces high amount of eugenol oil in *Ocimum tenuiflorum*. Studies have reported that Cd transport is reduced from roots to shoots due to its application of silicate fertilizers. This declines the concentration of heavy metals among the edible portions of *Ocimum* species. The use of Pb, Cd, and Cr as phytoremediated crop in soil results in decrease in linalool content of basil oil while showing increase in its methyl chavicol content (Lajayer et al. 2017). Arsenic stress was found to increase the production of essential oil in *Ocimum* species and the level of linalool in it, an important gradient to increase the commercial value of the plant (Pandey et al. 2019).

29.5.9 *Rosemary (*Family – *Lamiaceae)*

Rosmarinus officinalis is the well-known species of rosemary plant. The average oil percentage of the plant on fresh weight basis is about 1–1.5%. Commonly called as rosemary, *R. officinalis* is used for the phytoremediation of heavy metals such as Pb, Zn, Cu, Cd, etc. Different parts of rosemary plant are metal specific in terms of phytoremediation capability. The roots of rosemary plant are observed to accumulate metals in bulk quantity in comparison with shoot system (Boechat et al. 2016). In *R. officinalis* plant, the accumulation of contaminated metals like Cu, Zn, Cd, and Pb was concentrated highest in flowers followed by leaves and stems; however, Pb, Ni, and Fe were concentrated in the following order: leaves, flowers, and stems. Cultivation of *Rosmarinus officinalis* in metal-polluted sites demonstrates excellent amount of tolerance. Exploitation of this kind of plant could prove a reliable option for recuperation of contaminated soil as it has the capacity to immobilize heavy metals in the contaminated soil while observing meager transfer rate to shoot system. Rosemary plant has the capability to serve as effective phytostabilizer, potential biomonitor, and an efficient hyperaccumulator for nickel and other metal-contaminated zones.

29.5.10 *Sage (***Family –** *Lamiaceae)*

Sage is the common name used for *Salvia* species, and the average oil percentage for such species varies from 0.6% to 1.5% on fresh weight basis. Sage plants are generally utilized for the phytoremediation of Cd and Pb heavy metals. The accumulation capacity of sage plants depends largely upon heavy metal concentrations in the soil (Pandey et al. 2019). Clary sage is found to be the most encouraging crop in the sites contaminated with heavy metals. Besides acting as a potential metal hyperaccumulator, sage could be used for production of essential oil on large scale that could boost cosmetic and perfume industries. As hyperaccumulator, *Salvia* has the tendency to accumulate Zn, Cd, and Pb, and the order for metal accumulation is highest for leaves followed by roots, inflorescence, and stems (Angelova et al. 2016).

In clary sage, the concentration of heavy metals in shoot system follows the order as Cr > Ni > Fe > Pb (Chand et al. 2015).

29.5.11 *Lavender (***Family –** *Lamiaceae)*

The most notable species of lavender is *Lavandula vera*. The average oil percentage of this plant on fresh weight basis varies from 1% to 1.5%. Commonly known as lavender, *Lavandula* species are used for the phytoremediation of Zn, Cd, and Pb. Lavender is a very less known plant as per its phytoremediation is concerned; however, its species could be utilized for hyperaccumulation of Zn, Cd, and Pb (Angelova et al. 2016). The distribution of Cd accumulation was observed highest in the leaf system followed by root system. Stems and inflorescence were found to have identical Cd distribution. Similarly the distribution of Pb accumulation was observed highest in stems followed by roots and leaves. Inflorescence was, however, found to have same Pb concentrations as that of leaves.

29.6 Advantages of Using Medicinal and Aroma Plants for Phytoremediation

Usually the plants are grown on large scale as food crops; however, there are a variety of medicinal and aromatic plants that are grown for their tremendous health and economic benefits due to the presence of their secondary metabolites. Phytochemicals extracted from these plants are used as lead compounds today to design the synthesis of novel drugs for various health conditions such as cancer, diabetes, arthritis, heart ailments, and other disorders. Besides finding wide applicability in folklore and general medicine, such plants could be utilized for the production of essential oils, perfumery, and cosmetics (Pandey et al. 2019). Since medicinal and aromatic plants are not associated directly with food chain, such plants provide an additional edge for their safe usage as phytoremediation crops. Further, growing of nonedible medicinal and aromatic plants at zones contaminated with heavy metal is recommended as a lucrative and viable job. In the previous sections, we have learnt about a large number of medicinal and aromatic plants being utilized as phytoremediation plants for treatment of heavy metal contaminations. Majority of these plants are recognized from the families of Asteraceae, Geraniaceae, Poaceae, and Lamiaceae. Medicinal and aromatic plants of perennial origin are also used for the production of higher-grade essential oil and large amount of biomass, for instance, citronella (*Cymbopogon winterianus*), lemon grass (*Cymbopogon flexuosus*), vetiver (*Chrysopogon zizanioides*), and Palmarosa (*Cymbopogon martini*). The general paybacks of employing aromatic and medicinal plants for phytoremediation means could be broadly studied under two major aspects:

29.6.1 Environmental Aspect

One of the major advantages of using medicinal and aromatic plants as phytoremediation plants is their limited access into the general food chain (Verma et al. 2014). Further, the composition of the essential oil also remains unaltered with no considerable threat of metal pollution. The use of plants such as vetiver and lemon grass could tolerate toxic environments while simultaneously enhancing quality of soil and bringing down its erosion (Pandey et al. 2019). The accumulated heavy metals from contaminated sites by aromatic plants are then removed after harvesting. In the recent past, several methods have also been suggested for efficient reclamation of heavy metals from the collected biomass. Considering the multiple benefits as discussed above, medicinal and aromatic plants offer suitable mechanisms for phytoremediation of metal-contaminated soils in a natural and sustainable manner.

29.6.2 Economic Aspect (Availability and Demand)

There is an uninterrupted rise in universal requirement of essential oils, and as per estimates it is predicted to reach to the target of five trillion dollars by the culmination of year 2050 (Verma et al. 2014). Essential oils are considered as lofty worth products which find tremendous use as sweet-smelling agents in diverse industries such as cosmetics, aromatherapy, and perfumery. However, a mounting space is observed in the universal demand and production of the essential oils (Pandey et al. 2019). In order to fulfill the ever-increasing demand of essential oil, aromatic and medicinal plants come into play. Such plants can be cultivated on metal-polluted sites which besides acting as phytoremediation crops could prove helpful for restoration of essential oils. It has also been observed that steam distillation process of essential oil could exclusively reduce the hazard of heavy metals in essential oil extractions as these harmful metals are retained in the extracted residual mass of the plant. For this reason, the use of medicinal and aromatic plants for phytoremediation finds wide acceptability in the market.

29.7 Contaminated Biomass: *Threat Appraisal and Its Sustainable Use*

Phytoremediation process of heavy metals is accompanied by the generation of contaminated biomass which is being generated in large amount after each consecutive harvest. One of the chief concerns for everyone is the safe disposal of this heavy metal-contaminated biomass. Several methods have been adopted from time to time for the safe disposal of the polluted biomass, which entails phytomining, chemical extraction incineration, or simple burning. Many researchers have performed risk assessment of medicinal and aromatic plants for their potential as phytoremediation crops. Medicinal and aromatic plants yield essential oil as the major by-product that is utilized for nonedible reasons such as cosmetics and perfumes, soaps and detergents, insect repellents, etc.; hence, such plants are considered as important agents from keeping away of contamination of food chain (Lal et al. 2013). For safe consumption of food, the critical limits for certain heavy metals such as Pb, Cd, Cr, and Ni are 1.3, 1.5, 1.5, and 2.5 ppm, respectively (FSSAI 2011). In agreement with this, many heavy metals observed in essential oils that were phytoremediated by aromatic and medicinal plants were within the ambit of these critical limits (Lal et al. 2013). Similar results were observed by the experiments conducted on crops irrigated with municipal sewage, and the key rationale for low metal contamination of essential oil was attributed to general distillation method for extraction of essential oil (Pandey et al. 2019). Hence, aromatic and medicinal plants embrace a higher place above food crops for the purpose of phytoremediation for there is lower risk of contamination of food chain through such plants.

29.8 Conclusion

Heavy metal contamination is growing at an alarming rate majorly due to upsurge in numerous anthropogenic activities which affects plant and human health and ultimately shows decline in the agricultural produce. In this backdrop, aromatic and medicinal plants hold a great significance in containing this contamination by way of phytoremediation processes at sites polluted with heavy metals. Exposure of higher concentrations of heavy metals elicits a number of lethal effects on morphological, biochemical, and physiological phenomena of the plants and consequently disturbs overall redox homeostasis of their cells. Generally, plants have the innate ability to detoxify heavy metals by means of several ways like exclusion, chelation, and sequestration. The enzymatic and non-enzymatic detoxification mechanisms are also geared up to cope up with heavy metal stress. Such detoxifications mechanism have been exhaustively studied and reported. Nevertheless, there are several medicinal and aromatic plants in which defensive mechanisms against heavy metal stress are still poorly understood. Such plants merit further studies in order to recognize the fundamental mechanisms accountable for the allied responses to heavy metal stresses. A wide range of species of medicinal and aromatic plants possess the ability to thwart heavy metal contaminations by serving as hyperaccumulators, phytostabilizers, biomonitors, and facultative metallophytes, thereby resisting the oxidative stress; however, there are many other kinds of species which become susceptible to heavy metal stress. Uptake of heavy metals by roots and aerial parts of medicinal and aromatic plants gears up of detoxification mechanisms and witnesses significant rise in accumulation of secondary metabolites and the corresponding expression of genes, which subsequently leads to profound alterations in the quality and quantity of associated natural products. In view of paucity of comprehensive and reliable data with regard to access of heavy metal ions in the secondary metabolite processes and their corresponding expression of genes, further investigations deem to be needed. It is an established fact that percentage of essential oil gets enhanced in certain medicinal and aromatic plants due to stress of heavy metals. As a result, economic benefits could be exploited by cultivating such type of plants in zones of heavy metal stresses. Being a source of high-valued essential oils, non-food plants serve as ideal candidates for phytoremediation of heavy metal-polluted sites. Thorough research needs to be performed in this branch so as to make it cost-effective, feasible, and sustainable technology in coming future. Harvested biomass forms an essential by-product of decontaminations methods. Phytoremediation potential of perennial medicinal and aromatic plants must be investigated in experimental fields for their higher quantity of post multi-harvested biomass. In raw materials of medicinal and aromatic plants, the WHO/FAO has set highest tolerable limits for certain hazardous metals such as As, Cd, and Pb; however, there are several essential elements like Ni, Zn, Mo, Mn, and Cu which are considered as toxic at higher concentrations, yet their admissible toxicity limits are still not ascertained as on date. Besides elucidating the toxicity limit ranges, an interdisciplinary approach is necessary to unravel the molecular mechanisms involved in heavy metal stress. Plant-metal interaction is also indispensible for numerous purposes, for instance, bio-fortification, i.e., design plants in such a way that they can accumulate metals indispensable for human health. Extensive research must be focused on nonedible crops of medicinal and aromatic plants as they have great potential in remediating soils contaminated with toxic metals, besides essential oil production gets enhanced under heavy metal stress as these act as elucidating agents.

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Chapter 30
Medico-legal Perspectives of Usage, Commercialization, and Protection of Traditional Drug-Yielding and Essential Oil-Yielding Plants in India

Piyali Mukherjee and Soumya Mukherjee

30.1 Introduction

India is one among the 17 mega biodiversity countries of the world. India is rich in ecosystem biodiversity and possesses 15 agro-climatic zones (National Biodiversity Authority 2003). India is known to be a rich archive of medicinal plants (Selected Medicinal Plants 2009). Apart from various species of non-flowering plants, the zones harbor a pool of diverse traditional drug-yielding plants associated with their usage in folk and medicine (Ayurveda, Unani, Siddha, and Homoeopathy). The usage and commercialization of medicinal plants are regulated by various departments and governmental board. The knowledge of traditional medicine involves the skills and practices based on belief, experience, and reliability of different cultures, which are used to maintain health and diagnose various diseases. Traditional medicine comprises medical aspects of traditional knowledge, which developed over generations within various societies before the span of modern medicine (Medicinal Plant and Traditional Knowledge Study for Children 2018). The word "protection" in traditional knowledge has two perspectives that protection may be granted to exclude the unauthorized use by third parties of the protected information (Gopalakrishnan 1998). On the other hand, the "protection" also means to preserve traditional knowledge from uses that may have negative effect to the person's life or culture of the communities that have developed and also applied. Indigenous communities seek to gain economic benefits in traditional knowledge; geographical indications and trademark are the alternative tools. The potential value of geographical indications and trademarks is to protect the plants and germplasms, which are

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specified and unique to geographical regions. Plants possess a wide network of chemical defense molecules associated with various physiological adaptations, which imply their success rate in the course of evolution. Secondary metabolite accumulation (alkaloids, terpenes, steroids, glycosides, volatile or essential oils, and phenols) and their necessary effects protect the plants from various graziers, insects, or pathogen attack. However, these molecules also form a good source of pollinator attractants thus bringing about reproductive success. More than 90% of the plant-based food sources produced in the entire world yield as a result of pollination. In this context, it is worth mentioning that essential oils and volatile aromatic compounds present in different plant organs are of immense potential to nature in a holistic sense and to mankind as well. Humans have methodically exploited the natural reserve of aromatic and essential oil sources from different plants in various biodiversity-rich areas of the world. Ayurveda or Unani knowledge from ancient civilization reports the usage of more than 2000 plant extracts available in the form of essential oils or aromatic compounds having therapeutic, perfumery, or food additive effects.

The use of essential oils (EO) in form of perfumes and incenses dates back to, as old as, Greek, Roman, or Egyptian civilizations. Various rituals among roman empire in the ancient period involved worship of gods of natural forces (Fig. 30.1). Paintings illustrating the practice of aromatherapy and medicinal use of essential oil in Roman or Egyptian civilizations (A, B) are provided.The process of mummification among Egyptian communities involved the use of a lot of perfumes. Hence, the process of plant identification and its use for EO extraction has been prevalent in ancient civilizations. Greeks used different perfumes to adore different body parts. Various illustrations and historical documents reveal the use of such extracts in ancient times. Hindu rituals consider rose, camphor, and sandalwood as the major sources of EO to be used as offerings to god. EO popularization in India was prevalently brought by the advent of Mughal Empire. Babar during his reign popularized

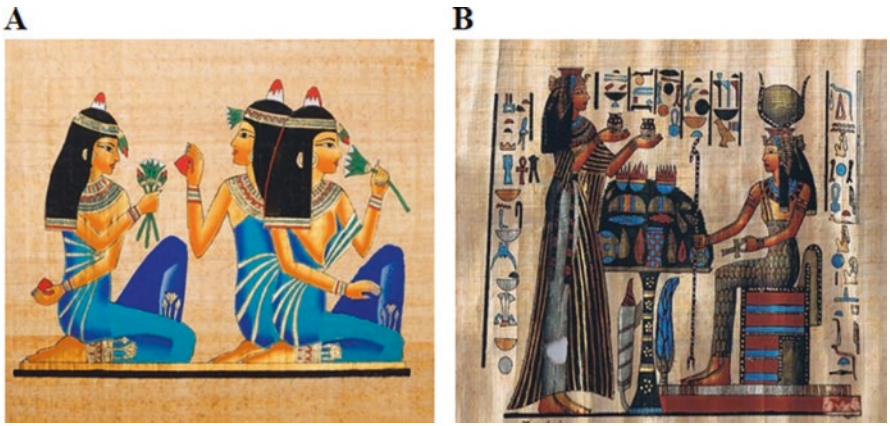

Fig. 30.1 Ancient paintings showing the use and popularization of essential oil products in Egyptian civilization

the use of various types of attar prepared out of EO extracts. Later on in the British reign, India exported varieties of EO extracts in form of perfumes. Queen Elizabeth I frequently used such Indian products. The production of natural EO faces competition with the synthetic compounds, which are acquiring market value in form of substitutes. Therefore, most importantly, the maintenance of elite clones of plant varieties and breeding for high yield of EO are necessary for optimum extractions.

30.2 The Indian System of Medicine

The traditions, culture, civilization, and religion of the people in India enable to classify the medicine systems into two categories – the classical and the traditional systems, respectively. The classical system of Indian medicine is comprised of Ayurveda, Siddha, Amchi, Unani, Yoga, and Homeopathy (Srinivasa Ragavan et al. 2012). On the other hand, the traditional system of Indian medicine is largely based on public or society. Figure 30.2 summarizes the percentage usage of plant species in various systems of Indian traditional medicine (Bhandari 2017). Specific usage, which is oral in character and involves folklore practices, is prevalent among some ethnic groups, families, or individuals. This system includes tribal practices, local health, and household remedies (Jamshidi-Kia et al. 2018). The Indian system of medicine is the pinnacle of Indian thought of medicine that represents the way of healthy living with a unique cultural history. In India, more than 15,000 medicinal plant species are available in wild of which only 3000 plant species have been codified in Indian medicine system. It has been reported that Ayurveda (900) system uses largest number of botanical species followed by Siddha (800), Unani (700), and Amchi (300) (Bhandari 2017).

The word “herb” has been derived from the Latin and French word “herba.” Apart from herbal medicine, there are various types of traditional medicine

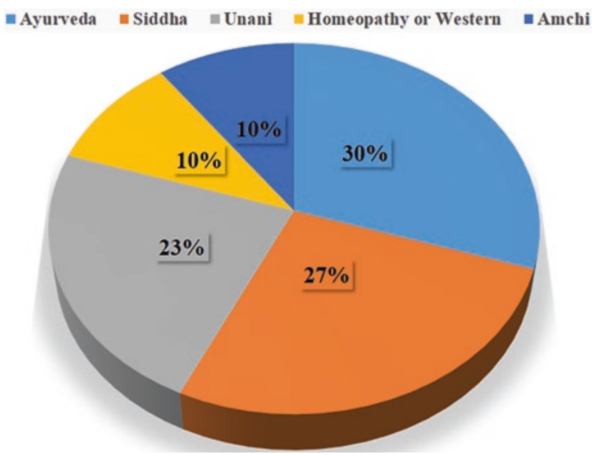

Fig. 30.2 Pie chart representing the percentage use of plant species in various systems of medicine in India

practices in India, namely, Ayurveda, Yoga, Siddha, Unani, and Homeopathy regulated by Ministry of AYUSH (Briggs 2002). The necessary bioactive compounds are required to be extracted from various plant parts for formulating various medicines. Around 8000 herbal remedies have been codified in the Ministry of Ayush Systems in India (Sandberg and Corrigan 2001). Ayurveda, Unani, and Homeopathy medicine include wide practices (AYUSH in India 2011).

Ayurveda The term "Ayurveda" has been derived from an Indian word "Ayur," which means life and "Veda" explains knowledge. Ayurveda is one of the oldest integrated healing systems and which is based upon belief, experiences, and practices that of mind, body, and spirit (Morgan 2002). A number of treatises have been obtained from various scholars of Ayurveda. In the era of 1000 B.C., Ayurveda was extensively documented in the compendia called *Charak Samhita* and *Sushruta Samhita* (Ayurvedic Medicine Summary 2018). The fundamental parts of Ayurveda include all objects and living bodies, which are composed of five basic elements called the *Pancha Mahabhootas*, namely, earth, water, fire, air, and ether. Ayurvedic medicines are generally safe if they are manufactured and consumed properly as prescribed by the physician. Ayurveda system of treatment is proven to be more effective and satisfactory in the treatment of chronic, metabolic, and life style diseases compared to conventional medicine (Mishra et al. 2001).

Unani Unani medicine is a system of alternative medicine that originated in ancient Greece. Hippocrates is known to have established the foundation of Unani system of medicine. In the eleventh century B.C., it was introduced to India by the Arabs and Persians (National Health Portal: Unani 2019).The basic theory of Unani system is based upon the well-known four humor theory of Hippocrates, which includes blood, phlegm, black bile, and blue bile. Unani system of medicine has been effective in cure of various diseases such as rheumatoid arthritis, jaundice, nervous debility, skin diseases such as vitiligo and eczema, sinusitis, and bronchial asthma (Husain et al. 2010). Since ancient time, Unani hakims, and European and Mediterranean cultures used to inculcate various herbs as medicine.

Siddha Siddha is an ancient system of medicine prevalent in South India and possesses close resemblance with Dravidian culture. The word Siddha appears from the Tamil word that means perfection. The treatment methods of siddha are to restore the balance of the mind-body system. The Siddha system of medicine emphasizes on the age of patient, environment, sex, race, habits, mental framework, habitat, diet, appetite, physical condition, and physiological constitution of the diseases. It is thus individualistic in nature. Diagnosis of diseases are commonly accomplished through examination of pulse, urine, eyes, study of voice, color of body, tongue, and status of the digestion of individual patients. Mercury, sulfur, iron, copper, gold, bitumen, white, yellow, red-arsenic, and other minerals as well as vegetable poisons, marine and animal products are extensively used in siddha tradition. In siddha medicine, the use of plants such as *Acalypha indica*, *Dichrostachys cinerea*, and *Mukia maderaspatana* are extensively used for a various purposes (Subbarayappa 1997).

Homeopathic Medicine The word Homeopathy, primarily appears from Greek word, through Latin into English, and literally means "like disease". Around 1810 A.D., homoeopathy was introduced into India by various European missionaries, and it received official recognition in 1948 by the Parliament. Dr. Christian Friedrich Samuel Hahnemann, a German physician, codified the fundamental principles of Homoeopathy. This treatment is based on the assumption that mainly relies upon the susceptibility or proneness of an individual to the incidence of the particular disease in addition to the action of external agents such as bacteria and viruses. Homoeopathic medicines are prepared mainly from natural substances such as plant products, minerals, and also from various animal sources. Homoeopathy has its own areas of strength in therapeutics, and it is particularly useful in treatment for allergies, autoimmune disorders, and viral infections. Homoeopathic medicines are also helpful as supplement of health care.

Amchi The Amchi system of medicine, also known as Tibetan System, is practiced in northern India and some other regions of the Himalayas particularly by the Buddhists. This system traces its origin to Ayurvedic system and includes treatments by herbs, minerals, animal organs, spring and mineral waters, puncturing of veins, mysticism, and spiritual powers (Che et al. 2017).

30.3 Usage of Traditional Medicine

The approach of indigenous herb–based traditional health care practices applicable to human health is known as ethno-medicine or traditional medicine (Scope and Importance of Traditional Medicine 2003). The knowledge of any herbs, animals, and minerals that have curative effects were gathered as an outcome of experimentation through trial and error method across several generations (The Natural Farmers 2019). Ethno-medicine is the mother of all other systems of medicine such as Ayurveda, Siddha, Unani, Herbal medicine, and also Homeopathy (Medicinal Plant 2009).

There are several kinds of usage implied for healing of various diseases.

(a) Herbs such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, and safflower are used to heal wounds, sores, and boils.
(b) Basil, fennel, chives, cilantro, apple mint, thyme, golden oregano, variegated lemon balm, rosemary, and variegated sage are some important medicinal herbs and can be planted in kitchen garden. These herbs are easy to grow, look good, taste, and smell amazing, and many of them attract bees and butterflies (Introduction and Importance of Medicinal Plants and Herbs 2019).
(c) Many herbs are used as blood purifiers by eliminating the metabolic toxins. These are also known as "blood cleansers." These few herbs can improve the immunity of the person as well as reduce fever.

(d) Some herbs have their antibiotic properties. Turmeric is useful to stop the growth of germs, microbes, and bacteria and also widely utilize as a home remedy to cure cut and wounds.
(e) To reduce fever and generate such heat, antipyretic herbs such as *Chirayta*, black pepper, sandalwood, and safflower are suggested by the Indian traditional practitioners.
(f) Sandalwood and cinnamon are used to impede the discharge of blood, mucus, and also serve as aromatic astringents.
(g) Some herbs such as marshmallow root and leaf are used to neutralize the acid generated by the stomach. These herbs are applicable as antacids to heal gastric and also provide proper digestion (Shaw 1998).
(h) Indian sages were reported to provide effective remedies from plants against poisons from snakebites and animals.
(i) Herbs such as cardamom and coriander are known to be effective in increasing appetite. Other aromatic herbs such as peppermint, cloves, and turmeric emit a soothing aroma and are used as culinary products and taste enhancers in various cuisines.
(j) Aloe, sandalwood, turmeric, and khare khasak are commonly used as antiseptic and are rich in medicinal values.
(k) Ginger and cloves are used in cough syrups. They also possess expectorant property, which promotes the thinning and ejection of mucus from the lungs, trachea, and bronchi.
(l) Chamomile, calamus, ajwain, basil, cardamom, chrysanthemum, coriander, fennel, peppermint, spearmint, cinnamon, ginger, and turmeric are helpful for blood circulation. They are also used as cardiac stimulants.
(m) Herbal medicine practitioners suggested calmative herbs, which provide a pleasant effect to the body and are also used as sedatives.
(n) Certain aromatic plants such as aloe, golden seal, barberry, and chirayata are helpful as mild tonics. These plants reduce toxins in blood for its bitter taste.
(o) Certain herbs such as cayenne (lal mirch, myrrh, camphor, and guggul) have also been reported as stimulants to increase the activity of an organ system. Table 30.1 summarizes the source of certain essential oil-yielding plants and their families.
(p) Golden seal, aloe vera, and barberry are used as tonics for health. For treating cut and wound, honey, turmeric, marshmallow, and liquorice are very much effective.

30.4 Documentation, Commercialization, and Market Share for Traditional Medicine

The World Health Organization (WHO) has been coordinating a network called the International Regulatory Cooperation for Herbal Medicines that improves the quality of medical products made from medicinal plants (World Health Organization

Table 30.1 Plant sources and families for various essential oils

Oil name	Plant source	Family
Ajwain seed oil	*Trachyspermum* spp.	Apiaceae
Aniseed oil	*Pimpinella anisum*	Apiaceae
Asafetida oil	*Ferula* spp.	Apiaceae
Basil oil	*Ocimum basilicum*	Lamiaceae
Bergamot oil	*Citrus* spp.	Rutaceae
Caraway seed oil	*Carum* spp.	Apiaceae
Cardamom oil	*Eletarria cardamomum*	Zingiberaceae
Celery oleoresins	*Apium graveolens*	Apiaceae
Chandan wood oil	*Santalum album*	Santalaceae
Cinnamon oil	*Cinnamomum* spp.	Lauraceae
Clove oil	*Syzygium aromaticum*	Zingiberaceae
Coriander seed oil	*Coriandrum* spp.	Apiaceae
Cumin seed oil	*Cuminum* spp.	Apiaceae
Dill seed oil	*Anethum graveolens*	Apiaceae
Fennel seed oil	*Foeniculum* spp.	Apiaceae
Garlic oil	*Allium sativum*	Amaryllidaceae
Ginger oil	*Zingiber officinale*	Zingiberaceae
Horseradish oil	*Amoracia rusticana*	Barssicaceae
Jasmine oil	*Jasminum* spp.	Oleaceae
Juniper oil	*Juniperus* spp.	Juniperaceae
Lavender oil	*Lavendula* spp.	Lamiaceae
Lemongrass oil	*Cymbopogon* spp.	Poaceae
Mandarin oil	*Citrus* spp.	Rutaceae
Nutmeg oil	*Myristica fragrans*	Myristicaceae
Paprika oil	*Capsicum* spp.	Solanaceae
Parsley oil	*Petroselinum*	Apiaceae
Pepper oil	*Piper nigrum*	Piperaceae
Peppermint oil	*Mentha piperata*	Lamiaceae
Rosemary oil	*Rosmarinus* spp.	Lamiaceae
Star anise oil	*Illicium* spp.	Schisandraceae
Tagetes oil	*Tagetes* spp.	Asteraceae
Thyme oil	*Thymus vulgaris*	Lamiaceae
Turmeric oil	*Curcuma longa*	Zingiberaceae

2006). WHO has established the strategy of four objectives for traditional medicines in order to integrate them as policy into national healthcare systems; to provide knowledge and guidance on their safety, efficacy, and quality; to increase their availability and affordability; and to promote their rational, therapeutically sound usage (World Health Organization 2013). WHO aims to develop and enforce various policies in integration with education and training, research, and development. In 1993, The Convention on Biological Diversity (CBD) was the first international environmental convention to develop the use and protection of traditional knowledge (Richard 2000) related to the conservation and sustainable use of biodiversity that was signed at the United Nations Conference on Environment and Development (UNCED) (Environment Issues 2019). In 2001, The Government of India established 1200 formulations of various systems of Indian medicine such as Ayurveda, Unani, Siddha, and 1500 Yoga that translated into five languages, namely, English, German, French, Spanish, and Japanese as repository of the Traditional Knowledge Digital Library (PIB 2010). India has signed various agreements with European Patent Office (EPO), United Kingdom Intellectual Property Office (UKIPO), and United States Patent and Trademark Office (USPTO) to prevent the grant of invalid patents for patent search and examination at International Patent Offices access to the TKDL database (Press Information Bureau 2010). In 2016, Dr. Shashi Tharoor, Member of Parliament from Thiruvananthapuram, introduced a Private Bill named The Protection of Traditional Knowledge Bill, 2016, for the protection, preservation, and promotion of traditional knowledge system in India (Tharoor 2016). Article 27 subsection 3(b) of the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPs) sets out such conditions under intellectual innovations may be excluded from patenting. In 2001, TRIPs-related Doha Declaration, the TRIPs agreement includes the relationship between the TRIPS Agreement, the 1992 Convention on Biological Diversity (CBD), and the protection of traditional knowledge and folklore (TRIPS Agreement Annex 1C 1994).

The term “Commercialization” is a process of introducing a new product or production methodology that deals with trading and its availability in the market. It includes several aspects necessary for commercial ability, namely, innovative management, quality control, profit motive, and innovation of with novel methodologies. It is also associated with legal aspects that include the process of manufacturing, sale, profit, and cost management. India is one among the various mega diversity countries, which produce diverse species of plants (Bonati 1980).There is a ubiquitous demand of herbal or traditional medicines worldwide. In India, nearly about 80% rural communities consume these medicinal plants for their personal uses (Ekor 2014). In the Indian herbal industry, around 960 plant species are used for turnover of industrial profitability into more than 80 billion rupees (Sahoo and Padmavati 2013). Exports of traditional or herbal medicine include medicines of AYUSH (Ayurveda, Unani, Siddha, and Homoeopathy) products, which hold a share of total 3% of Indian pharmaceutical export (Singh et al. 2005). Around 70% of the export from the herbal sector consists largely of raw materials, which are calculated to have values around 10 billion rupees per annum (Sahoo and Padmavati 2013). A total of 30% of the export items consists of finished products, including herbal extracts (Nirmal et al. 2013). Among the global herbal exportation, India’s share for herbal export market is less than 1% (Mishra 2010). However, the AYUSH industry represents one of the oldest traditional forms of medicine in India, which has attempts to provide opportunities to the emerging market. In order to evaluate the constraints, questionnaire-related surveys were undertaken, which revealed that Indian herbal drug industry is facing limitations in the production, commercialization, and regulation for traditional or herbal medicinal drugs (Calixto 2000). A questionnaire-based survey revealed that primary challenges were associated with production, commercialization, and marketing approval for traditional or herbal medicine in India and abroad (Food and Drug Administration 2019). The result of survey is the major impediment for exporting the different regulatory desideratum and the limited stock market in foreign countries. The major challenge for Indian herbal drug manufacturing firms emerged from its standardization, quality control of raw materials, and herbal formulations (Castot et al. 1997). Exiguous regulatory guidelines, non-implementation of good agricultural, bevy practices, and weak implementation of the Drugs and Cosmetics Act of 1940 are considered as a major snag for the Indian herbal industry or Indian traditional medicine industry.

30.5 Chemical Nature and Properties of Essential Oils

Essential oils (EOs) are chemically terpenoid (mono or sesquiterpenes) or phenyl propanoid compounds, which exhibit a considerable degree of lipophilicity in the nature of solubility. They, however, differ from the edible fatty oils both in structural and physicochemical properties. Although EOs may remain liquid at room temperature, they volatize much faster than fatty oils. Fatty oils possess long chains of aliphatic fatty acids composed of saturated and unsaturated carbon chains. This structural conformation renders them non-volatile in nature. Essential oils do not undergo rancidification or oxidation even after prolonged exposure to air. This decreases their possibility of degradation unlike fatty oils, which undergo conversion of lipidic or carboxylic groups to aldehyde. Furthermore, essential oils do not undergo saponification or soap formation with alkali. Essential oils are optically active and possess higher refractive index than fatty oils. Therefore, EOs possess specific properties of aromatic nature and are freely diffusible through biological membranes (nose and mucous linings) thus functioning as an effective chemical in various potential therapies, perfumeries, and flavor additives (Hyldgaard et al. 2012). Figure 30.3 summarizes the structural features of some EO derivatives (Adapted from Hyldgaard et al. 2012).

30.6 Ecological and Evolutionary Benefits of Essential Oils: Plant Defense (Allelopathy) and Communication

Essential oils are secondary metabolites synthesized from terpenoid and phenyl precursors in various plant parts such as leaves, flowers, fruits, and seeds and in secondary tissues of stems, roots, and rhizomes. Plants utilize these molecules in a variety of eco-physiological aspects involved with plant defense, microbial growth inhibition, and allelopathic effects. Most importantly, these volatile molecules are often secreted from specialized nectaries or oil glands present in the base of ovaries or glandular trichome heads in leaf epidermis. This process provides plants with a good networking system of communication with pollinators. The evolutionary benefit of various flowering species is achieved through entomophilous pollination,

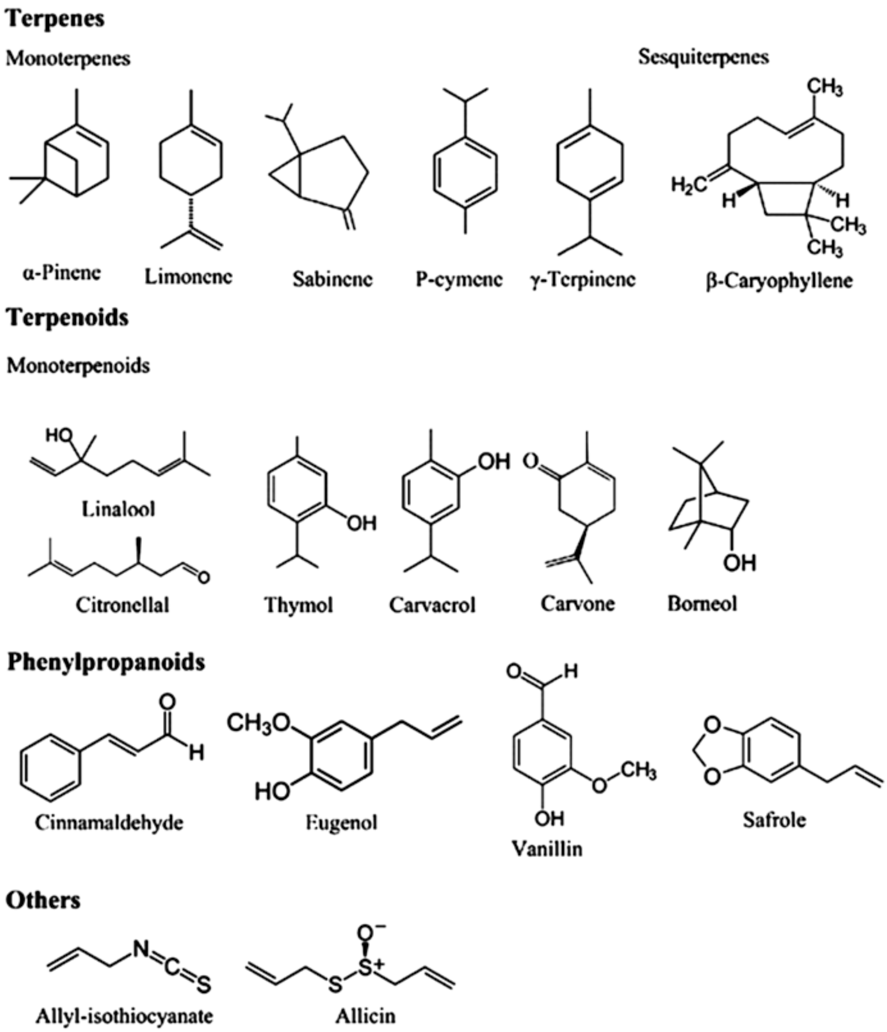

Fig. 30.3 Structure of various terpenoid compounds representing volatile essential oils in plants

which involves the liberation of these volatile aromatic oil compounds as chemoattractants. Intra-species networking and development of suitable defense mechanisms are also achievable by systemically dispersed volatile molecules of these categories. This phenomenon is relevant in cases of anti-herbivory or insect-deterrent aromatic essential oil liberation, which protects the plant from various predators. Essential oil like compounds in the root exudates of certain plants often inhibit seed germination of other plant species in its vicinity. This effect (allelopathy) is an important aspect of inter-species competition and success of various species, which strengthens their reproductive niche in a community. One of the common examples of pollen allelopathy is evident in a grass member of Asteraceae called

Parthenium hysterophoros. This species produces a sesquiterpene lactone parthenin present in the intine of dispersed pollens. This results in inhibition of pollen germination of other species in its vicinity (Hyldgaard et al. 2012).

30.6.1 Climatic Variability and EO Collection

Harvesting of plant parts for EO extraction is accomplished from various organs such as root, bark, rhizome, fruits, flowers, or young buds. In order to prevent microbial contamination, the plant parts thus collected undergo stringent extraction processes required for purification and collection of oleoresins or other aromatic compounds. Harvesting stages are important to optimize proper oil content, aroma, or flavor. Fruits such as berries in some plants are harvested in post fertilization stages or pre-ripening, which yields optimum oil content, for example, in black pepper. Followed by harvesting, proper sterilization or blanching in hot water is mandatory to remove all sorts of microbial contamination present in the plant parts. Collection of leaves or stems (basils, mint, or oregano) involves selection of the particular season in order to obtain suitable aroma from extracted essential oil. Stems and leaves are selected for harvesting at an elongated or matured stage but prior to complete flowering. Rhizomes such as turmeric or ginger require harvesting at the younger stages to ensure less fiber content. Post-harvesting stages involve careful drying of plant samples in order to remove water and make them suitable for storage, devoid of any microbial degradation. In this context, it is worth mentioning that the drying step is commonly termed as “curing” and involves dry oxidation or fermentation resulting due to the activity of some enzymes.

30.6.2 Extraction Methods for Essential Oils

The commonly used methods for extraction of essential oils vary in their efficiency and output and depend upon the type of compounds associated with the essential oil or aromatic terpenoids present in different plant organs. Thus, the choice of extraction process solely depends upon the type of compound desired to be extracted. Steam distillation is one of the convenient methods for EO extraction, which is cost-effective and less labor intensive. Water or dry steam distillation involves heating the plant material at specific temperature and pressure. However, prolonged exposure to high temperature may result in hydrolysis, isomerization, or alteration in the fragrance quality of the oil. Further fractionation steps may be required for isolation of associated compounds. Steam distillation may sometimes appear to be more advantageous over water-steam distillation in order to preserve the quality of oils. Solvent extraction is often used as a method of extraction using organic solvents such as petroleum or benzene. The solvents are passed through the plant materials followed by evaporation of the solvent in vacuum. This process leaves residual essential oils

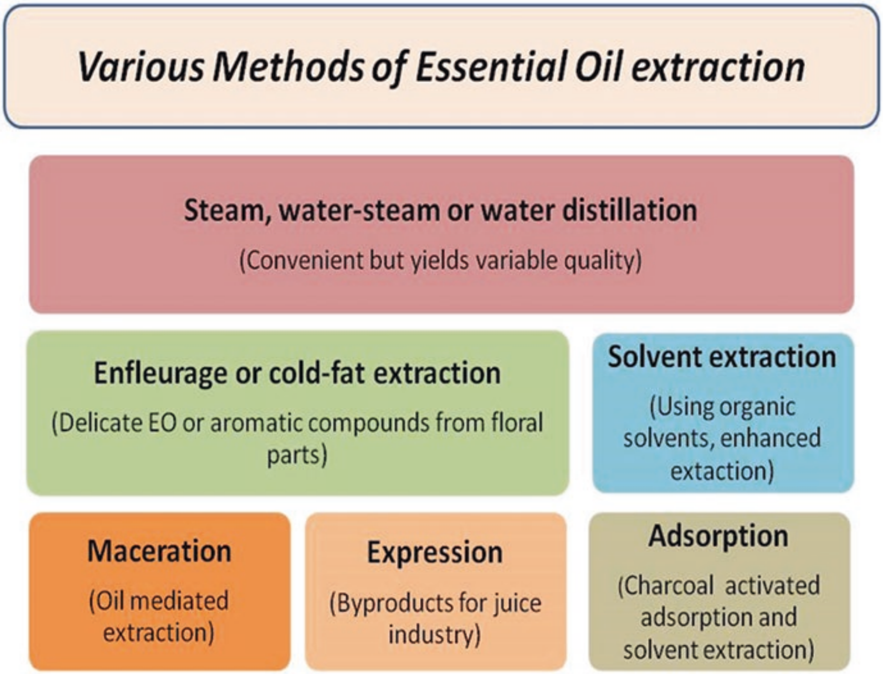

Fig. 30.4 Methods of EO extraction and their features

and wax compounds. Alcohol extraction and freezing method are used to separate oils from waxes. Nutmeg oleoresins are commonly extracted through this process. Delicate floral aromatic compounds or essential oils are heat sensitive and get diluted in water distillation. These compounds are therefore isolated by cold-fat extraction or enfleurage process (Kocchar 2009). Floral parts are spread over casted fat layers in dark cold rooms. Repeated replacement is done with freshly collected floral parts until the fat layer gets saturated with the floral EO. Waxes and resinous compounds are separated by alcoholic extraction at low temperatures. Other processes involve maceration and oil facilitated extraction, expression, and adsorption (Fig. 30.4).

30.7 Commercialization of Essential Oil

Climatic fluctuations in the crop growing seasons pose major hurdles in collection, optimization, and supply of good quality spices in different developing countries. High cost of crop maintenance and low production often bring reluctance in harvesting due to high processing costs involved with extraction methods. Good quality spice devoid of any contamination and adulteration again poses a challenging task for traders or processors. Export quality essential oils in India are ensured with fine extraction steps without any artificial flavoring and contamination. Thus, a high

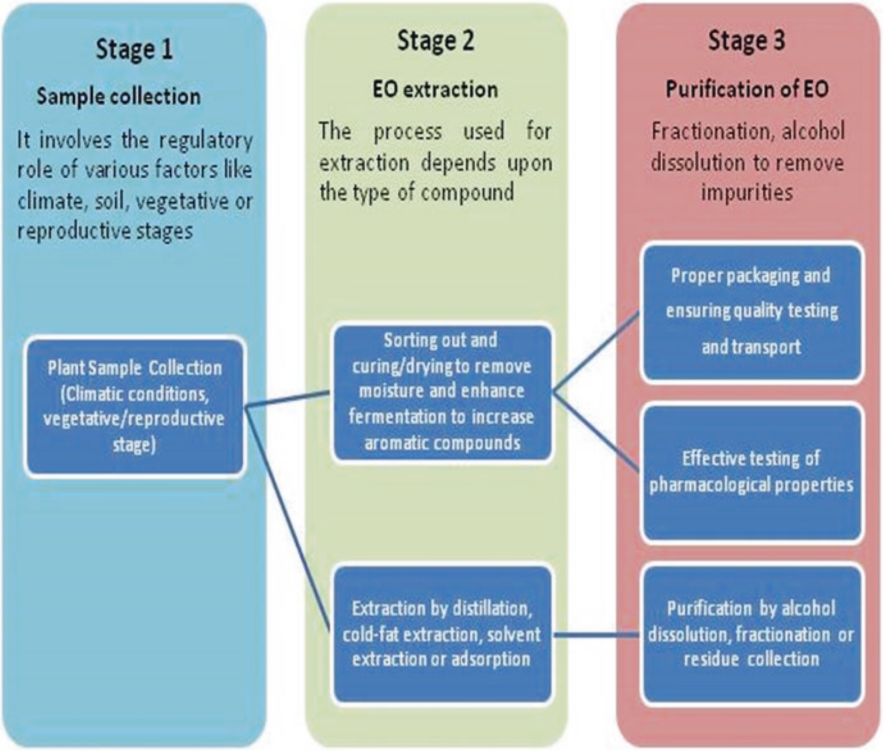

Fig. 30.5 Stages of EO extraction and preparation of products for commercialization

production cost gets associated with such protocols. Figure 30.5 depicts the various stages of EO processing. Cultivation of spices can prove beneficial in raising economy in several rural areas of developing countries in the form of niche crops. However, poor farmers with the concern of food security are often most likely to shift to food crops owing to the uncertainty associated with growing cash crops. The awareness of social and economic benefits associated with cultivation of EO-yielding plants and optimization of the extraction of the active principles require further improvement. Developing countries, however, have the awareness of the requirement of natural essential oils as a better mode for food industry and aroma therapy in comparison with artificially supplemented aromatic liquid. In order to improve sustainable use of the natural reserve of aromatic plants and their proper cultivation, knowledge of biodiversity and status of endemic and endangered plants are required to be implemented (Patel 2014). The hurdles or limitations faced by some of the countries involve the following:

- Improper agricultural practices and fluctuations in agro-climatic conditions
- Poor research in the breeding practices and scarcity of high-yielding varieties
- Adverse weather conditions including flood, low rain fall, or frost

- Poor optimization of extraction process leading to poor yield and high production cost
- Scarcity of financial assistance, loans, or governmental support

In view of overcoming the limitations and to provide technical support, United Nations Industrial and Developmental Organization (UNIDO) has been assisting the developing countries for more than three decades. The objectives of UNIDO are to cover wider aspects of plant selection, cultivation practices, quality improvement, and effective marketing. The significant efforts involve the implementation of new extraction plants to optimize yield from the plant samples. This leads to the coordination of university-industry efforts to set up proper R&D provisions for essential oil market. Rural areas have been supplied with good varieties of aromatic plants having optimum yield and tolerance to various abiotic factors. UNIDO has been successful in implementing trained personnel to various countries, which involve different fields such as chemistry, pharmacology, and agriculture. The upliftment of basic farming skills and the assistance from professional scientists have resulted in the establishment of essential oil industry in various regions of different countries (Tuley 1995). Proper quality maintenance is mandatory for the essential oil market to flourish. Among the various ways of UNIDO-assisted advancements, rectification of essential oil helps in quality improvement, which involves selective fractionation to remove unwanted substances from the essential oil extract. This process improves the stability and odor characteristics of various compounds. Training and hand practice workshops have been organized in different countries to promote the exploitation of these potentially economic aromatic plants. The countries producing large amount of cheap or poor quality oils have been assisted to produce various isolates and chemically modified aromatic products, which have turned useful for local perfumeries, cosmetics, or toiletries. UNIDO-assisted programs have proved effective to promote R&D efforts across various countries. This platform is necessary to provide scientific assistance to industrialization of aromatic plant products. R&D-mediated efforts have been constantly benefiting the production of EOs in terms of new formulations and competition with synthetic chemical substitutes. Marketing of essential oils and the economic output depend upon the price demands by buyers and substitution with synthetic products. The export quality production of EOs can provide foreign market value and de-popularize the usage of imported additives. A percentage of the product can be marketed for common usage, and the remaining consumption by user industries will effectively provide good market value. Proper development of entrepreneurship from developed countries and joint ventures can prove effective in raising the market value of EOs.

30.7.1 EOs as Potential Reserve for Drug and Pharmacological Sector in India and Abroad

India is one among the various mega-diversity countries, which harbor more than 17,000 species of angiosperms in wild habitats. The country has the prevalence of tropical climate with ambient temperature and humidity throughout the year. India is the center of origin for various spice-yielding plants such as black pepper, clove, cardamom, and mint. At present, India has been the highest producer of menthol, which occupies a good place in the world market of essential oils. Table 30.1 summarizes the source of certain essential oil-yielding plants and their families. The leading organization, which is involved in inter-disciplinary upliftment of EO research, trading, and marketing, is Essential Oil Association of India (EOAI) established in 1956. This organization aims to collaborate research on cultivation and preservation of aromatic plants, EO extraction, and trading in different countries. The prevalence of aromatic compounds and EO in food, flavoring additive, and agricultural sector has ever since increased in India. EOAI promotes training and research for cultivation and extraction processes of EO in the form of workshops held at regular intervals. This helps the entrepreneurs with enhanced skills to exploit such fields. India has various ISO certified companies such as Expo Essential Oil, which have huge client bases among reputed pharmacy companies such as Ranbaxy, Reckitt Benckiser, and Glaxo. The use of EO in drug or medicine manufacture remains as an invariable part of Indian companies. Companies provide EO in various forms such as natural EO, organic EO, rectified EO, or carrier EO. The composition and extraction steps regulate the purity, effectiveness, and cost of such EOs available with companies. The Spice Board of India also promotes the cultivation, extraction, purification, and export of potential quality EO to various countries. In the potential front, India has been taking an attempt to conserve the wide varieties of aromatic plants growing in different regions of Himalayas, Western Ghats, and other diversity-rich areas. Cryopreservation of seeds and other plant propagules have been successfully achieved in various institutes and departments financially assisted by DBT, DST, CSIR, and MOEF. Furthermore, potential research has progressed for in vitro multiplication of various essential oil-yielding aromatic plants.

30.8 Procedurals for Protection of Medicinal or Drug-Yielding Plants

There are three major procedures associated with the protection or conservation of genetic diversity of medicinal and drug-yielding plants (Ajazuddin 2012). The Indian Forest Act, 1927, describes the grant of legal recognition to the rights of traditional forest dwelling communities and plant and wildlife conservation.

1. *Use Rights:* The law provides rights to use and to collect the minor forest produce herbs, medicinal plants that have been traditionally collected, use of grazing grounds and water bodies, and use of traditional areas by nomadic communities.
2. *Right to protect and conserve:* The law provides rights to protect and manage the forests for people of village communities.

Legislation There are several regulations for protection of medicinal and various drug-yielding plant (Keller 1991). Various laws formulated for the conservation of forests and wild plants, which protect medicinal plants as well as traditional plants, are enlisted as follows:

(a) Forest Act, 1927
(b) Wildlife (Protection) Act 1972 and Wildlife (Protection) Amendment Act 1991
(c) Forest (Conservation) Act, 1980
(d) Environment Protection Act, 1986
(e) National Forest Policy, 1988
(f) National Biodiversity Act, 2002
(g) The Scheduled Tribes and Other Traditional Forest Dwellers Act, 2006

In Situ Conservation In situ conservation deals with the on-site conservation of the wild genetic resources or genetic diversity in natural habitat. In India, the conservation of forest areas preserves the plants through protected areas such as national parks, wildlife sanctuaries, and biosphere reserves. In situ conservation of medicinal plants is important to the AYUSH for their dependency on medicinal plants. It can be involved survey, inventorization, and documentation of essential medicinal plants. In situ conservation of medicinal plants is carried out through following activities: Medicinal Plants Conservation and Development Area's (MPCDA's) and In situ Resource Augmentation.

Ex Situ Conservation Ex situ conservation of medicinal plants is a complementary action to conserve the genetic diversity (Mills 2001). Various species of medicinal plants and their wild populations are better conserved in ex situ methods wherein the edaphic factors are favorable for plant growth and metabolism. It also helps to engage the number of stakeholders in production and regeneration of medicinal plants. Conservation of medicinal plants can be accomplished by the ex situ that outside natural habitat by cultivating and maintaining plants in botanic gardens, parks, other suitable sites, and through long-term preservation of plant propagules in gene banks and in plant tissue culture repositories (Patel 2014).

30.9 Rules and Regulations of Traditional Medicine and Drug-Yielding Plants

Medicinal plants are a major resource base for the traditional medicine and herbal industry and provide health security to a large segment of Indian population. These traditional medicine or drug-yielding plants are regulated by several Acts and controlled by various boards. The Government of India has set up the National Medicinal Plant Board (NMPB) on November 24, 2000. It is located under Ministry of AYUSH (Ayurveda, Yoga & Naturopathy, Unani, Siddha & Homoeopathy), Government of India. The basic function of NMPB is a proper mechanism for coordination to develop between various ministries, departments, organization and implementation of policies, and growth of medicinal plants in the following areas:

(a) To advise concerned ministries, departments, organizations, state, and governments on policy matters relating to schemes and programs for development of medicinal plants
(b) To provide better guidance in the formulation of proposals, schemes, and programs, which are undertaken by agencies having access to the land for cultivation, infrastructure for collection, storage, and transportation of medicinal plants
(c) To assess demand or supply position relating to medicinal plants both within the country and abroad (Jordan et al. 2010)
(d) To identify and perform qualitative and quantitative assessment of the efficacy of medicinal plants
(e) To organize training programs for capacity building of stakeholders on medicinal plants (cultivation, conservation, GAPs, storage, PHM, and market information)
(f) To organize globally the import and export of raw material, medicine, food supplements, and herbal cosmetics for marketing of product, which increase their reputation for quality and reliability
(g) To undertake and award scientific, technological, research, and cost-effectiveness studies
(h) To develop protocols for cultivation and quality control
(i) To encourage the protection of patent rights and intellectual property rights

30.10 Legal Protection of Medicinal Plant and Intellectual Property Rights

Medicinal plants are protected under several Acts and regulations such as Biological Diversity Act, 2002, The Drugs and Cosmetics Act, 1940 (Amendment 2005), and Forest Act. These Acts have been implemented to protect the use of traditional medicinal plants.

(a) *The National Biodiversity Authority (NBA)*: It was established in 2003 and to implement Biological Diversity Act, 2002. The NBA is a statutory body that performs regulatory and advisory function of conservation, sustainable use of biological resource, and equitable sharing of benefits of use for Government of India.
(b) *The Biological Diversity Act (2002)*: The Act provides implementation of the provisions of the Act with National Biodiversity Authority focusing on advice the Central Government relating to the conservation of biodiversity, sustainable use of its components, and equal sharing benefits of the utilization of biological resources through decentralized system. According to Section 37 Sub-Section (1), stated that the State Government in the selection of areas of biodiversity importance to be notified as heritage sites and measures for the management.

(c) *The State Biodiversity Board (SBBs)*: It focus on advising the State Government, as provided guidelines issued by the Central Government, relating to the conservation of biodiversity, sustainable use of its components, and equal share benefits for utilization of biological resources. According to Sections 3, 4, and 6 of the Act, the NBA is considered for requesting such granting approval. It can be regulated by granting of approvals for commercial utilization of any biological resource by people.
(d) *The Local Level Biodiversity Management Committees (BMCs)*: They are responsible for promoting conservation, sustainable use, and documentation of biological diversity including preservation of habitats, conservation of land races, folk varieties and cultivators, domesticated stocks, and breeds of animals and microorganisms. Intellectual Property Rights protect the use of traditional medicine through defensive protection and positive protection (IPR and Traditional Medical Knowledge 2009).
(e) *Defensive Protection*: It includes protection of sacred cultural manifestation that is symbol or words being registered as trademark and also standstill the people outside the community from acquiring intellectual property rights over traditional knowledge. In India, patent applications have led to the compilation of a searchable database of traditional medicine by patent examiners, which can be used as evidence (Traditional Medicine and Intellectual Property Rights: Indian Perspectives 2019).
(f) *Positive Protection*: It is the granting of rights that empower communities to promote their traditional knowledge, control its uses, and benefit from its commercial exploitation. It can be also protected through the existing intellectual property system. Under Intellectual Property Rights, patents are type of IP protection for medicines (Traditional Knowledge and Intellectual Property 2009). To obtain a patent, an invention must be novel, inventive, and industrially applicable. A patent can be granted to a set of exclusive rights for a limited time of 20 years. It allows the inventor without seeking permission to prevent others from making, using, selling, offering for sale, or importing the patented invention. Patents based on traditional medical knowledge include patents based on maca, a traditional Peruvian food and medicine first cultivated by the Incas, and a patent based on kava, a medicinal plant, which is domesticated first in Vanuatu.
(g) *Trade Secret*: It is the information of an IP holder, which possesses some economic advantage. They generally cease to provide protection when trade secrets become known. Traditional medical knowledge holders may not disclose their knowledge and set aside for secret. The traditional medical knowledge for specific formulations are known and also transmitted only to individual healers and not to the whole community.
(h) *Trademarks*: Trademarks are protected in various distinctive signs such as words, phrases, symbols, and designs to identify the source of a product. This helps the consumers to identify the products with preferred characteristics as a specific brand of herbal medicine (Xiaorui 1998). Trademark rights can be protected through registration or use in commerce. Trademarks have been used for market products on traditional medical knowledge. Trademarks can help to dis-

tinguish the authentic goods and also which are not supposed to prohibit third parties from using traditional knowledge without any different marks or the trademarks. It cannot be used to protect the traditional medical knowledge.

(i) *Geographical Indication (GI)*: It is another type of intellectual property right to identify the origin of goods. Geographical indications can be used to distinguish products based upon traditional medical knowledge to specify the location, which they cannot protect against the use of traditional medical knowledge. Geographical indications are protected by various countries that may require registration or use in commerce. Besides trademarks, geographical indications can also be used for the protection of products, which are based on traditional medical knowledge.

30.11 Case Studies on Traditional Medicine

There are several case studies related to intellectual property rights. These are some examples of the abuse of power as follows:

Turmeric Patent Case Study

There are two US-based Indians, namely, Suman K. Das and Hari Har P. Cohly who were granted a US Patent 5,40,504 on March 28, 1995, on uses of turmeric in wound healing. The patent was assigned to University of Mississippi Medical Center, USA. This patent claimed the administration of an effective amount of turmeric as a novel finding through local route to enhance the wound healing process. Before any patent is granted, it has to fulfil the basic requirements of novelty and utility. However, if the claims have been covered by relevant published art, then the patent becomes invalid. The Council of Scientific and Industrial Research (CSIR) could locate around 32 references, which state the use of turmeric in India for more than 100 years old in Sanskrit, Urdu, and Hindi, prior to filing of this patent. The formal request for re-examination of the patent was filed by CSIR at United States Patent and Trademark Office (USPTO) on October 28, 1996. The examiner was rejected all the claims as being anticipated. However, the re-examination certificate was issued on this case on April 21, 1998, and the re-examination proceedings was about to close.

Neem Oil Case Study

The United States has been taking the undue advantage of the low level of patent awareness and slipshod in enforcement of law. The neem tree is widely used in India in many areas of medicine, toiletries, contraception, timber, fuel, and agriculture. Since the mid-1980s, US corporations have taken out over a dozen patents on neem-based materials. When the US Patents Office (USPTO) granted patent for "Neem oil" for antiseptic use, the Council of Scientific and Industrial Research (CSIR) urged for re-examination of the case, notwithstanding without success. Indian researchers and indigenous communities possess collective local knowledge, which are often expropriated by outsiders.

Amazon Rain Forest Plant Case Study

In the indigenous community of the Amazon, many traditional healers and religious leaders used to collect a plant named *Banisteriopsis caapi* and also process it to produce a ceremonial drink "ayahausca" called "yage." They used this ayahausca for the purpose of religious and healing ceremonies. According to traditional values, ayahausca was formulated and administered only under the direction of traditional healers. A Plant Patent No. 5751, issued to Loren Miller on June 17, 1986, by The United States Patent and Trademark Office claimed rights over a supposed variety of *B. caapi*, which Miller dubbed "Da Vine." This challenging circumstance to this patent was made by the Center for International Environment Law (CIEL), which is on behalf of the Coordinating Body of Indigenous Organizations of the Amazon Basin (COICA) and the Coalition for Amazonian Peoples and Their Environment. COICA is a coordinating body of more than 400 indigenous communities.

The patent claimed to have identified a variety of the species with new or distinctive physical features, especially the color of the flower. According to Prof. William A. Anderson, University of Michigan, a leading expert on the plant family to which *B. caapi* belonged, the features described as "prior art" were already in the records of major herbaria. Further, the plant grew naturally throughout the Amazon Basin. Legally speaking, plant patents cannot be awarded to plants that are found in an uncultivated state. On re-examination, The United States Patent and Trademark Office (USPTO) revoked this patent on November 03, 1999. The inventor was be able to convince the US patent and Trademark office (USPTO) on April 17, 2001. Following the consideration of original claim, the innovator was assured with the restoration of patent rights.

30.12 Conclusion

There are nearly about half a million traditional medicinal plants in the world, which possess immense potential for treating the afflicted person, some of that have not yet been studied in medical practice on medical activities. The utilization of medicinal plants has a long history across generations. The use of the original traditional plant or raw material is prone to several drawbacks for treatment or experimentation. This includes changes associated with plants in different climates and simultaneous development of synergistic compounds. There are challenges associated with adverse effects of antagonists and losses of bioactivity due to the variability, accumulation, storage, and preparation of herbal raw materials. The isolation of compounds and the utilization of pure substances with bioactivity are beneficial. The modern procedure of unravelling natural drugs by appropriate examination of therapeutic effects and determination of toxic doses can control the quality of the therapeutic formulation. The inception of the development of herbal or traditional medicines was concurrent with the evolution of chemistry, isolation, purification, and determination of plant potential. It is necessary to further explore the repositories of native wild species present in various sacred grooves, reserve forest, and endemic flora in various parts of tropical India. The process of extraction and commercialization of EO involves many stringent steps including cultivation, climatic factor providence, and clonal propagation of elite quality plants. Present issues associated with environment management and sustainable development involves the preservation or protection of wild species in different regions of the world. Thus, commercialization of the EO-yielding varieties should be selected keeping in view the process of preservation and protection. Public concern and awareness should be developed among different sectors of rural and urban areas of the country depicting the medicinal and pharmacological properties of the EO extracts and the plants used for the same. Suitable market value and effective economy derived from commercialization of EO shall be maintained to compete with synthetic aromatic compounds.

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