Ajay Sharma
Gulzar Ahmad Nayik *Editors*

Immunity Boosting Medicinal Plants of the Western Himalayas

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This book is dedicated to
My Grandfather Late Pandit Ram Krishan Sharma
and
My Ph.D. Supervisor Late Prof. Damanjit Singh Cannoo

Preface

Medicinal plants are the gift of mother nature to humanity for fighting against a variety of ailments. Till date, various investigations on medicinal herbal plants are being carried out. The results of these studies revealed that the medicinal herbal plants have the ability to strengthen the immune system and to fight against the variety of pathogens. Currently, innumerable medicinal plants used in Ayurveda, Unani, Siddhi, Homeopathy, Romanian, Persian, and Chinese traditional medicinal systems are being evaluated to check their immunity boosting potential. Therefore, the main goal of this book is to draw on the rich culture, folklore, and biodiversity of immunity boosting medicinal plants of the Himalayas, with particular emphasis on the Indian Trans-Himalayan and Western Himalayan region. All the plants included in this book are extensively used by the local tribes and people for their health-promoting properties from ancient times. These traditional health-promoting activities of various plants were also supported by various scientific articles published in different reputed journals.

This book presents a comprehensive guide to traditional immunity boosting medicinal plants of Western Himalayas, their traditional uses, phytochemistry, pharmacology, conversation, toxicology as well as future prospective. The book offers a valuable asset for researchers and graduate students of chemistry, botany, biotechnology, microbiology, and the pharmaceutical sciences. This book will be substantial contribution to the knowledge of the region and countries. Also, the book will be very useful to scientists, graduates, undergraduates, along with researchers in the fields of natural products, herbal medicines, ethnobotany, pharmacology, chemistry, and biology. Further, it is an equally significant resource for people working in different traditional medicinal systems; doctors (especially those engaged in Ayurveda, Chinese traditional medicinal system, Amchi, and Allopathy); the pharmaceutical industry (for drug design and synthesis); biochemistry and biotechnology sciences; and the agricultural sciences.

Mohali, Punjab, India — Ajay Sharma
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About the Editors

Ajay Sharma is a Natural Product Chemist with strong Organic Chemistry, Phytochemistry, Biochemistry, and Ethnobotany background. Presently, he is working as an Assistant Professor at the University Centre for Research and Development, Department of Chemistry, Chandigarh University, Mohali, India. He completed his Graduation (B.Sc. Medical) from Govt. College Hoshiarpur (affiliated to Panjab University, Chandigarh), Master's Degree in Organic Chemistry (Natural Products) from Punjab Agriculture University Ludhiana and his PhD (Organic Chemistry—Natural product) from SLIET (Sant Longowal Institute of Engineering & Technology, Deemed University), Punjab. He has published over 51 peer-reviewed papers and book chapters in reputed international/national journals and books. Presently, his six edited books are under production in different international publication houses. He has various honors/Fellowship/Awards to his credit, i.e., Role of honor from PAU Ludhiana (2011), Merit-cum-topper scholarship during M.Sc. (PAU Ludhiana) (2009–10), MHRD fellowship Award for Ph.D. (2012), Award of INSPIRE fellowship (JRF, 2013) from DST, Award of INSPIRE fellowship (SRF, 2015) from DST, 1st Prize in Poster Award at National Conference (NICS-2016) and Best Poster Award in (TSFS-2016). He does research in Organic Chemistry, Natural Products Chemistry, Phytochemistry, and Food Chemistry. His research activities have focused on isolation and characterization of bioactive extracts from traditional medicinal plants and evaluation of their biological potential. Currently, his research group at Chandigarh University is investigating the field of more environmentally friendly synthesis of metal nanoparticles and carbon nanodots using plant extracts/plant-based biomass followed by assessing their biological, catalytic, and sensing potential. Till date, he has successfully guided six M.Sc. dissertation students.

Gulzar Ahmad Nayik completed his Master's Degree in Food Technology from Islamic University of Science and Technology, Awantipora, Jammu and Kashmir, India, and PhD from Sant Longowal Institute of Engineering and Technology,

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Chapter 1
Introduction to Plants of Western Himalayas

Gifty Sawhney, Gauri Sanjay Navgire, Suraj Parihar, Umer Farooq, and Mohammad Javed Ansari

1.1 Introduction

The Himalayan mountain range is one of the wonders of nature that is blessed with a variety of features, diversities, and reservoirs of fauna, flora, and landforms (Badola and Aitken 2003). The Western parts of the Himalayas include the areas of Badakhshan in northeastern Afghanistan/southern Tajikistan, Pakistan through north-western India. The western range has a higher altitude compared to its eastern counterpart. The climate of this region promotes the growth of a large repertoire of plants which are unique to the region. Majority of these plants are medicinally

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important in one way or the other which takes us to one such important group of plants which have been traditionally used and are famously known to improve human immunity in general (Badola and Aitken 2003).

Improving immunity has been a topic of discussion during the current pandemic, when the whole world is digging deep to find prophylactic as well as therapeutic natural/synthetic treatment against the virus. Hence, medicinal herbs have been in the limelight for their extraordinary properties of boosting the immune system through the consumption of their extracts or by applying simple formulations.

The local traditional medicinal system is a strong pillar in the survival and existence of the population where the knowledge is passed through generations keeping the practice and the system alive (Pandey 2007). The world is evolving every day, with modern medicines taking over other age-old medicinal practices, medicinal plants have always taken the driving seat when it comes to improving health and immunity. Use of the correct combination of plants in significant proportions will provide the right amount of phytochemicals and pharmacological properties alone will assist in recovering the immune system or boost it depending on the disorder (Pandey 2007).

India possesses one of the world's oldest traditional cultures, known as folk tradition, which is linked to the usage of medicinal herbs and is founded on indigenous beliefs, knowledge, and skill (Uniyal et al. 2011). The number of studies conducted on these "traditional use" is enormous and listing those results is outside of the scope of this chapter. Hence, an example for the same has been given as follows: the study methodology was of different types of surveys in all the communities present in the locations of Kangra district of Himachal Pradesh in the west Himalayas (Uniyal et al. 2011). The *Vaidyas* from these communities were the key people that answered all of these survey questions. The plants documented in this study are known but the unique ways in which they are used is of importance. Some plants were found to be useful to cure other disorders example: *Berginea cilita* which is used for kidney stones in one region is used for sexual dysfunctions in another which is a novel use of the plant. A unique treatment for curing joint pains which is practiced in one of the regions is documented in this chapter (Uniyal et al. 2011). The researchers termed it as medicated acupuncture. A dried pencil size stick of *Vitex nigunda* is sharpened by knife at one end and is dipped in a decoction preparation using *Syzygium aromatic, Calotropis gigantean*, and *Mentha longifolia.* This stick which is medicated is then pressed against the affected part of the body for 5–6 days in a row for 1 min thrice a day. This method of administering the drug is novel and the belief in its efficiency is widespread in the region (Uniyal et al. 2011).

1.2 Materials and Methods

Search tools like Google Scholar, PubMed, and Web of Science were used to obtain the prior art literature in the form of books, research articles, and review literature on the topic of interest. Google Patents was used to obtain patent literature data using

search keywords: Immunity booster, medicinal plants, Western Himalaya, and formulations.

1.3 Medicinal Plants

1.3.1 Aconitum heterophyllum

Aconitum Heterophyllum is indigenous to India and the Western Himalayas. It is commonly known as "atees" or "tivisa" and it favors sub-alpine and alpine climates, and it has been recommended for cultivation up to 2000 m altitude on sandy (10 cm deep) soils rich in organic compounds. The plant requires rainfall between 664.2 and 1485.7 mm. Its flowering months are between August and October from the third year of growth. The species grows on grassy Himalayan slopes between 3000 and 4200 m in height, with a maximum descent of 2200 m (Paramanick et al. 2017).

It belongs to Order: Ranunculales; Family: Ranunculaceae; and Genus: Aconitum where the stems are green in color; simple with branches, 15–20 cm in height. The leaves are Heteromorphous with dark green color, the lower parts are long petioles and the upper parts are amplexicaul (Paramanick et al. 2017).

This plant has been used in Ayurveda to treat patients with urinary infections, diarrhea, and inflammation. The plant shows pharmacological properties, like anti-flatulent, anti-atrabilious, anti-phlegmatic, anti-periodic, antioxidative, carminative, expectorant properties, and anti-inflammatory (Paramanick et al. 2017). There were 21 phytochemicals studied from the plant for its antiviral/immunity-boosting activity, Hetisinone was reported to show the highest binding affinity to the viral (Covid-19) surface protein, as a result, itis a possible inhibitory phytochemical that can help reduce the severity of SARS-CoV-2 infection by acting on both the host and viral targets (Singh et al. 2022).

1.3.2 Artemisia annua L.

Artemisia annua L. is an annual herb native to Asia and is predicted to be originated from China. Its common names are annual wormwood, sweet wormwood, or sweet annie. The flowering season starts from August to September, followed by the seed ripening season from September to October. The plant needs light (sandy) and medium (loamy) soils, grows abundant in well-drained soil (pH: mildly acid, neutral, and basic (mildly alkaline)), and is able to grow in nutritionally poor soil too (Feng et al. 2020). It belongs to Order: Asterales; Family: Asteraceae; and Genus: Artemisia L. The plant is single-stemmed with alternate branching with aromatic leaves dissected deeply. Leaves and flowers both have 10-celled biseriate trichomes and 5-cell filamentous (T) trichomes.

The traditional application of the plant was to treat respiratory infections, wound healing, longevity, fevers, and notably, "intermittent fevers" (Feng et al. 2020). Two major phytocompounds, namely artemisinin and artesunate, have been studied extensively as a treatment of malaria and rheumatoid arthritis and are a part of pharmaceutical research (Brown 2010). Majority of the secondary metabolites of the plant constitute of monoterpenoids, but the most studied secondary metabolite is Artemisinin (Sesquiterpene) which is reported to be a p21-activated kinase-1 inhibitor and is known to be inhibiting the covid-19 infections and thus boost the immune system. *A. annua* also shows promising apoptotic activity in human cancer cells along with antioxidant, antibacterial, antiworm, analgesic, and antispasmodic; and anticoccidial properties (Brown 2010).

1.3.3 Bergenia ciliate

The plant has pink flowers in a cyme and is perennial, evergreen, herbaceous, and drought-resistant. Fringed bergenia, hairy leaf bergenia, and winter begonia are all popular names for this plant. They spread through Central Asia, from Afghanistan to China in the Himalayan region with an altitude range between 1800 and 3000 m. Plants flourish in humid, temperate climatic conditions, with temperatures below 20 °C. The floe plant is from April to June while staying evergreen the rest of the year (Ahmad et al. 2018). Order: Saxifragales; Family: Saxifragaceae; and Genus: Bergenia. The morphology of the plant describes; less number of leaves, spreading; 4–11 × 3–10, Inflorescence is of raceme or corymbose type, often subtended by an ovate leafy bract, flowers are pink to purplish, pedicellate (Ahmad et al. 2018).

The Plant is heavily used in Unani and Ayurvedic medicine practice, the plant rhizomes and roots are utilized to cure kidney, heart diseases, lung, bladder diseases, and liver diseases, menorrhagia, ulcers, hydrophobia, spleen enlargement, cough, and fever. The most studied polyphenols of the plant constitute arbutin and "bergenin" which are currently a part of modern medicines (Koul et al. 2020). Bergenin alone has the potential to treat burn wounds, ulcers, fungal infections, diabetes and possess antilithiatic, anti-arrhythmic, anti-inflammatory, neuroprotective, anti-hepatotoxic, anti-nociceptive, anti-HIV, and immunomodulatory properties. The Plant shows pharmacological properties such as analgesic, anti-arrhythmic, immunomodulatory, and anti-inflammatory. There have been studies showing the ability of Bergenin to elevate T Helper 1 Responses and Anti-Mycobacterial immunity by stimulating the MAP Kinase Pathway of Macrophages. Berginin has also been used in combination with other antibiotics like isoniazid to treat tuberculosis-infected mice where Berginine was able to boost the immune cells CD4 and CD8 T cells in the spleen of the mice (Kumar et al. 2019).

1.3.4 Cinnamomum tamala

Cinnamomum spp is a short plant locally known as Indonesian cassia, Padang and Batavia cassia. The plant is distributed in Southeast Asia (Himalayan region) and is cultivated in Indonesia and Philippines. The plant grows abundantly in temperatures between 20 and 30 °C and 1250–2500 mm rainfall. Well-drained soil abundant in humus content is most preferred for proper cultivation of the plant. Sandy loam soils with sufficient quantity of organic manures are also suitable (Parham et al. 2020). It features oblong-elliptical leaves that are 4–14 cm long and oppositely oriented, as well as an ovoid 1 cm long fruit. The most important yield of the plant is its bark which is one of the popular spices used worldwide not only as an ingredient in cooking but also in traditional and modern medicines (Parham et al. 2020).

Traditional medicinal practices with the plant include: Cinnamon improves colon health hence reducing the chances of colon cancer. It is a good coagulant and is able to prevent bleeding. It is able to increase blood circulation in the uterus and contributes to tissue regeneration. Pharmaceutical properties of the plant include anti-inflammatory, antitermitic, nematicidal, mosquito larvicidal, insecticidal, antimycotic, and anticancer agent. Studies on the plant show that cinnamon extract is able to inhibit 157 wild-type SAR-CoV in vitro having IC50 value of 43 μM. The mechanism of action was also proposed to be due to endocytosis by blocking cell entry (Arora et al. 2021). The antiviral treatment called Gene-Eden159 VIR/Novirin is patented which contains a herbal broad-spectrum formulation containing Cinnamon as a major component. There are many studies showing the immunomodulatory effect of Cinnamon extract but the exact molecule is not known (Arora et al. 2021).

1.3.5 Tinospora Cordifolia

Tinospora cordifolia is commonly known as “Amrita” or” Guduchi” of “Giloy” a climbing shrub found throughout the Himalayas. The species is native to India and may be found at altitudes of 600 m across the tropical and subtropical zones. The plant is spread throughout the tropical region of India up to 1200 m above sea level. It flourishes in deciduous and dry forests of height up to 1000 ft. Many secondary metabolites having curative properties are present in the roots and stems of the plants (Khan and Rathi 2020). The leaves of the plant are simple, exstipulate, alternate, long petioles up to 15 cm long, pulvinate, and roundish, both at the base and apex. The pharmacological properties of the plant include antispasmodic, antipyretic, anti-allergic, anti-inflammatory, and anti-leprotic (Khan and Rathi 2020). The plants grow well in light medium sandy loam soil abundant in organic compounds and with sufficient drainage are best suited for cultivation. Order: Ranunculales; Family: Menispermaceae; and Genus: *Tinospora.*

Aqueous extract from the plant stem showed immunological activity due to the presence of arabinogalactan. Important immunomodulatory compounds like

Nformylannonain, magnoflorine, hydroxymustakone, cordifolioside A, N-methyl-2-pyrrolidone, and tinocordiside have been reported to be medicinally useful from this plant (Khan and Rathi 2020). The aqueous extracts of the plant have been studied for immune effect and cell stimulation; cytokine production and mitogenicity. In a preclinical study, in vivo, the extracts showed upregulation of IL-6 cytokine that resulted in B cell differentiation. *Tinospora cordifolia* exhibited immunomodulatory effect in patients with HIV disease as well (Khan and Rathi 2020).

1.3.6 Juglans regia

Its native range is from the Eastern Balkans to the Himalayas and Southwestern China, and it is commonly referred to as the walnut tree. Summer and winter temperatures are too hot and cold for the tree to thrive (Mao et al. 2020). Walnut trees are monoecious plants that grow to a height of 10–25 m. The male and female flowers are separated from one another on the pinnately complex leaves. The walnut tree can stand temperatures upto −11 °C during the winter dormancy period. In ethnomedicine, walnut roots are used to treat diabetes; walnut leaves are used to treat rheumatic pains, fever, diabetes, and skin disorders; and walnut blooms are used to treat malaria and rheumatic pain (Mao et al. 2020). Order: Juglandales; Family: Juglandaceae; and Genus: *Juglans.*

Flavonoids present in walnut plant exhibit potent antioxidant activity and have been useful in regulating immune function and improving anticancer activity. Pharmacological properties exhibited by the plant include antioxidant, anti-histaminic, bronchodilator, anti-fertility, analgesic, immunomodulatory, anti-ulcer, antidiabetic, hepatoprotective, antimicrobial, anti-inflammatory, lipolytic anti-hypertensive, neuroprotective, insecticidal, anticancer, wound healing, etc. (Mao et al. 2020). Walnut oligopeptides were isolated and their effect on the acceleration of innate and adaptive immune responses in mice is a result of multiple physiological mechanisms. These mechanisms include but are not limited to boosting phagocytic capacity of macrophages, activating NK cells, and improving cell-mediated and humoral immunities (Mao et al. 2020).

1.3.7 Matricaria chamomilla Linn

Chamomile (*Matricaria chamomilla L.*) is a well-known medicinal plant species from the Asteraceae family that has been dubbed “the star of medicinal plants.” It arrived in India during the Mughal era and is today cultivated in Punjab, Uttar Pradesh, Maharashtra, and Jammu and Kashmir (Singh et al. 2011). North Africa, Asia, North and South America, Australia, and New Zealand are all home to these plant species. It can be found in a variety of traditional, Unani, and homeopathic medicines. It is used to treat flatulence, colic, hysteria, and intermittent fever as a

medication. Chamomile is known by several names, including Baboonig, Babuna, Babuna camomile, and Babunj. It is generally utilized as an anti-inflammatory, antimicrobial, antispasmodic, and mildly sudorific agent (Laskova and Uteshev 1992). Chamomile has a strong tolerance for alkalinity in the soil. As the season progresses, crop development (increased height, branching, and bud production) becomes more active, and stray blooms may appear in the crop. In March, there is a lot of bud creation, the plants are growing all around, and the early-created buds bloom into flowers.

Antioxidant, antimicrobial, anti-inflammatory, anticancer, analgesic, anti-hypoglycemic, anti-stress, and hepatoprotective. A group of heteropolysaccharides are reported to exhibit immunomodulatory effect by start of immunostimulating qualities in heavy erythrocytes (macrocytes), activation of peripheral blood immunoregulation cells, and enhanced effector cell sensitivity to helper signals (Miraj and Alesaeidi 2016).

1.3.8 Nasturtium officinale

Nasturtium officinale L., also known as watercress, is a Brassicaceae family aquatic perennial plant. It is a water-loving leafy vegetable that thrives in and around water. The leaves have a strong pepper flavor, which helps to explain why they are so popular in salads. Western Asia, India, Europe, and Africa are the native areas of this plant. In cold, softly flowing, and low-running streams, it develops in clusters. It grows naturally in brooks, ditches, and pond borders. The plant grows in slow-moving water bodies such as lakes, ponds, rivers, canals, and streams (Zeb 2015). It is an important component of aquatic ecosystems. Watercress is a nutritious diet that preserves the body's immunity and health due to the presence of many phytochemicals and nutritional advantages. Carotenoids have medicinal properties that include cancer inhibition, cardiovascular difficulties, immune system enhancement, and protection against aging-related eye disorders and free radical responses (Chaudhary et al. 2018). Watercress has been used for so long to cure a number of ailments with claims that it can improve blood circulation to cure renal colic, liver disease as a mild stimulant, a diuretic, an expectorant, and a digestive aid. It is mainly used by rural healers as a nutritive, anti-inflammatory, and antioxidant agent. It is prized for its high nutritional density, which comes from a low-calorie count and a high concentration of vitamins (B1, B2, B3, B6, C, E), minerals (calcium, iron), and phytochemicals (B1, B2, B3, B6, C, E) (polyphenols, terpenes), anti-inflammatory, antidiabetic, anti-allergic, antibacterial, hypolipidemic, cardioprotective, and anticancer properties. After stimulation with concanavalin A (Con A), phenethyl isothiocyanate (PEITC) increased T cell proliferation. PEITC boosted phagocytosis by PBMC and peritoneal cavity monocytes and macrophages, as well as NK cell cytotoxic activity. PEITC's biological characteristics can stimulate immunological responses in normal mice in vivo (Schulze et al. 2021).

1.3.9 Viscum album L.

Mistletoe, or Santalaceae, is a hemiparasitic plant that has traditionally been used in cancer therapy. Mistletoe is a tiny shrub with leathery leaves that are linear and lanceolar and last for several seasons. In late fall and early winter, its yellowish-green blossoms turn transparent and pale berries. VA does not grow on the ground but it is transmitted to tree trunks by birds, whose droppings contain seeds (Jerant et al. 2000). VA, unlike other plants, has a 12-month vegetative phase, never touches the ground, and blooms in the winter. Viscotoxins have been shown in previous research to enhance the number of circulating natural killer (NK) cells and, as a result, strengthen the anti-tumor immune response. Similarly, viscotoxin has an efficient immunomodulatory impact on human and animal granulocytes. Lectins are carbohydrate-binding proteins with anti-tumor and immunomodulatory properties, among other things. Through the binding of Toll-like receptors, lectins can activate a variety of immune cells (TLRs) (Holandino et al. 2020). By connecting to TLR2, they can also activate macrophages and dendritic cells, causing them to secrete cytokines (IL-10 and IL-12). MLI (115 kDa) lectin for galactose, MLII (60 kDa) lectin for galactose and Nacetyl-D-galactosamine, and MLIII (60 kDa) lectin for N-acetyl-D-galactosamine were discovered in VA (Holandino et al. 2020).

1.3.10 Withania somnifera

Withania somnifera L. is a multifunctional medicinal plant belonging to the Solanaceae family that is widely grown in subtropical areas across the world. The common names of the plant are ashwagandha, suranjan, winter cherry, and Indian ginseng. Fever, cancer, asthma, diabetes, ulcer, hepatitis, eyesores, arthritis, heart issues, and hemorrhoids are among the ailments that local *vaidyas* treat with the herb (Saleem et al. 2020). Anticancer activity, low back pain therapy, and muscular strengthening are all documented benefits of the plant, which may be related to an approach that integrates alkaloids. The plant is a xerophytic plant that grows abundantly in Africa, the Mediterranean, Sri Lanka, Pakistan, and India. The pharmacological properties of the plant include *Neurotic regeneration activity, Immunomodulatory, hypolipidemic, and antidiabetic activities,* a*daptogenic activity,* etc. W. somnifera has a repertoire of withanolides, among which, based on drug-likeness score, modulated proteins, and docking score. Withanolide D, Withanolide G, Withanolide M, and Withanolide Q were projected to be the lead hits for boosting the immune system and inhibiting COVID-19 infection, and could predominantly act against COVID-19 (Khanal et al. 2020).

1.3.11 Andrographis paniculata

For millennia in Asian nations, *Andrographis paniculata* Wall (family Acanthaceae) has been used to cure a number of disorders which mainly include diabetes, high blood pressure, cancer, ulcer, leprosy, bronchitis, skin infections, flatulence, colic, influenza, dysentery, dyspepsia, and malaria. It is said to have originated in peninsular India and Sri Lanka, and is currently found in Southeast Asia, China, America, the West Indies, and Christmas Island (Hossain et al. 2014). It is in high demand and frequently grown because of its well-known medicinal usefulness, and it grows well in a variety of soil kinds, making it widely available. The plant is commonly known as King of Bitters (English), Mahatikta (Sanskrit), Kiryato (Gujarati), Mahatita (Hindi), Kalmegh (Bengali), or Fah Talai Jone (Thai). A. paniculata includes a variety of chemicals, including labdane diterpenoid lactones, flavonoids, and other substances, according to phytochemical research. The pharmacological effects of its various extracts and compounds include antimicrobial, cytotoxicity, anti-protozoan, anti-inflammatory, antioxidant, immunostimulant, antidiabetic, anti-infective, anti-angiogenic, hepato-renal protective (Hossain et al. 2014).

In mice, an ethanol extract of the fresh plant, as well as the purified diterpenes andrographolide and neoandrographolide, caused considerable ($P < 0.001$) antibody stimulation and a delayed hypersensitive response to sheep red blood cells. The plant extracts also induced a nonspecific immunological response in the mice, as indicated by macrophage movement index, phagocytosis of 14C-leucine-tagged E. coli, and splenic lymphocyte proliferation (Banerjee et al. 2021).

1.3.12 *Aloe vera*

The medicinal properties of this plant are well-known to all age groups of populations. Aloe vera gel contains small-molecular-weight immunomodulators such as G1C2F1, which is known to reduce UVB-induced immunological suppression in the skin, according to one study. This is done by repairing and building UVB-induced damage on epidermal Langerhans cells (Rajeswari et al. 2012). The major carbohydrate fraction obtained from the gel of the Aloe vera leaf is called Acemannan. This group of molecules has been claimed to have several important therapeutic properties including acceleration of wound healing, immune stimulation, anticancer, and antiviral effects. Acemannan could stimulate macrophage cytokine production, nitric oxide release, surface molecule expression, and cell morphologic changes. There are studies which state that the production of cytokines IL-6 and TNF-a was dependent on the dose of acemannan provided (Zhang and Tizard 1996). Pyrogallol-induced inhibition of humoral and cell-mediated immune responses was considerably reduced when patients were given a daily dose of Aloe vera saline extract. The immunostimulant action of Aloe vera might be related to the alkaloids content based on the findings and phytochemical research (Zhang and Tizard 1996).

1.3.13 Camellia sinensis

Camellia sinensis is commonly known as Tea. It is one of the most widely consumed beverages in the world after water. Camellia leaves, buds, and fragile stems are used to make the mixture. Tea consumption is high in Europe, particularly in the United Kingdom (~540 mL per day). According to reports, 3 billion kg of tea is produced and consumed annually throughout the world. Polyphenols, caffeine, minerals, and trace quantities of vitamins, amino acids, and carbs make up the majority of tea (Prasanth et al. 2019).

In traditional Asian folk medicine, tea is indeed a useful remedy for treating a variety of illnesses and problems. It is well-known for its high antioxidant content. Tea aids in the proper functioning of the cardiovascular system, weight loss, and even the prevention of cancer and neurological illnesses. The major component of green tea, epigallocatechin gallate (EGCG), has been proven in studies to have beneficial benefits on the immune system (Pastorino et al. 2018). The most prominent catechin in green tea is epigallocatechin gallate (EGCG), which accounts for roughly 50–80% of the entire 200–300 mg in a brewed cup of green tea. EGCG is a biochemically active molecule that has been shown to have anti-inflammatory, anticarcinogenic, and anti-free radical characteristics (Pastorino et al. 2018).

1.3.14 Glycyrrhiza glabra L.

Glycyrrhiza glabra is commonly known as licorice. Asia and areas of southern Europe are home to the medicinal plant. Licorice is thought to have originated in Iraq. Many local names include jaishbomodhu (Bengali), mulaithi (Hindi), licorice (English), and aslussiesa (Spanish) (Arab). Bangladesh, India, China, Spain, Russia, Iran, and Italy are the plant's native habitats which have fertile sandy soil and abundant water supply in the form of rivers, ponds, and dams (Pastorino et al. 2018). The plant has pinnate leaves that are 7–15 cm long and have 9–17 leaflets individually. Flowers have a narrow form, are born in an axillary spike, and the calyx is small and campanulated. The flowers are about a centimeter long and come in a variety of colors ranging from purple to pale white and blue. Cough, hoarseness, sore throat, bronchitis, asthma, and tonsillitis may all be treated with the roots peeled or unpeeled, and rhizomes are beneficial in treating most respiratory tract problems.

N-Acetylmuramyl, an analog of glycyrrhizin, a plant secondary metabolite, has the capacity to inhibit influenza and H1N1 virus replication. Glycyrrhiza polysaccharide extracts stimulate macrophages and boost immunological activation (Hasan et al. 2021). Neutrophils' phagocytic ability is increased by a Glycyrrhiza alcoholic extract. In another in vitro investigation, licorice at a dose of 100 g/mL had an immunostimulatory impact on human granulocytes, perhaps increasing the generation of TCD69 cells and macrophages (Table 1.1) (Hasan et al. 2021).

Patent information:

Table 1.1 Some medicinal plants known for their role in increasing the immunity of the host system

S. No	Botanical Name	Common Name	Family	Parts used	Solvent/Condition	pharmacological property	Other Medicinal uses/traditional uses	Reference
1	*Acacia nilotica* (L.) Willd. ex. Delile.	Gum arabic tree, babul, thorn mimosa	Fabaceae	Whole plant	Ethanolic extract	Immunomodulation; anti-inflammation	Gynecological disorders, for example, regulation of menstruation, abortion, prolapsed uterus, pain in female genital tract	Mishra et al. (2014)
2	*Allium cepa* Linn.	Bulb onion or common onion	Amaryllidaceae	Bulb	Methanolic extracts	Quercetin (immunomodulator) and COVID-19 protease inhibitor, antioxidant, anti-melanogenesis	Obesity, such as hyperlipidaemia, diabetes, hypertension, cardiovascular diseases, and inflammatory state	Marrelli et al. (2018)
3	*Allium sativum* Linn.	Garlic	Amaryllidaceae	Clove (bulbil of bulb)	Aqueous, chloroform, methanolic, and ethanolic extracts	Immunomodulatory, antibacterial, antifungal, antiviral, antiprotozoal, antioxidant, anti-inflammatory	infection in urinary system, indigestion, respiratory and heart disorders and it showed diuretic, carminative, antipyretic, sedative, and aphrodisiac	Batiha et al. (2020)

(continued)

Table 1.1 (continued)

S. No	Botanical Name	Common Name	Family	Parts used	Solvent/Condition	pharmacological property	Other Medicinal uses/traditional uses	Reference
4	*Andrographis paniculata* (Burm.f.) Wall. ex Nees.	Green Chireta, Creat, Nilavembu, Kirayat, Chuan Xin Lian, king of bitters	Acanthaceae	Whole plant	Methanol (MeOH), ethanol (EtOH), hexane, acetone, acetone-water, chloroform (CHCl3), and dichloromethane	Immunostimulatory, anticancer, antidiarrheal, antihepatitis, anti-HIV, cardiovascular , cytotoxic hepatoprotective, and sexual dysfunctions	Diabetes, dysentery, enteritis, helminthiasis, herpes, peptic ulcer, skin infections (topical use), snake bites (topical use)	Hossain et al. (2014)
5	*Asparagus racemosus* Willd.	Satawar, Satamuli, Satavari	Liliaceae	Root	Methanolic, ethanolic, aqueous extracts	Immunostimulatory, antioxidant, anaphylactic, adaptogenic, anti-stress, anti-ulcer	Dysentery, tumor, inflammation, biliousness, diseases of the blood and eyes, rheumatism and diseases of the nervous system	Singh and Geetanjali. (2016)
6	*Bauhinia variegata* Linn.	Orchid tree	Fabaceae	Stem bark	Petroleum ether, chloroform, methanol brown, aqueous	Immunomodulatory antiophidian, antidiabetic, antimalarial, and antioxidant potential	Fever, diarrhea, dysentery, hemorrhoids, piles, edema, skin diseases, wound healing, obesity, stomatitis, dyspepsia	Modh et al. (2011), Ghaisas et al. (2009)

7	*Boerhavia diffusa* Linn.	Red spiderling	Nyctaginaceae	Whole plant	Aqueous, methanolic, and ethanolic extracts	Immunomodulatory, antibacterial, antioxidant, antidiabetic	Inflammation, jaundice, asthma, rheumatism, nephrological disorders, ascites, anemia, and gynecological disorders	Mishra et al. (2014), Srikanth et al. (2012)
8	*Coriandrum sativum* Linn.	Coriander	Apiaceae	Whole plant	Ethanol, methanol, and aqueous	Immunomudolatory, antioxidant, antidiabetic, antimutagenic, antilipidemic, antispasmodic	Hiccough, suppuration, piles, inflammation, toothache, jaundice, scabies, and gland tuberculosis	Ahmed et al. (2018), Ahmed et al. (2020)
9	*Cassia occidentalis* Linn.	Septicweed, coffee senna, coffeeweed	Fabaceae	Whole plant	Methanol, aqueous, benzene, petroleum ether, and chloroform extract	Immunomodulatory, anti-Inflammatory, anti-diabetic	Malarial, filaria, constipation, fever	Hamid et al. (2020)
10	*Citrus limon* (L.) Burm.	Lemon	Rutaceae	Fruit	n-hexane, ethyl acetate, ethanol, and water	Immunomodulatory, anti-bacterial, anti-fungal, anti-inflammatory, and anticancer	Scurvey, high blood pressure, the common cold, and irregular menstruation	Klimek-Szczykutowicz et al. (2020), Diab (2016)
11	*Cucurbita pepo* L.	Pumpkin; summer squash	Cucurbitaceae	Seed	Methanol, aqueous extract	Immunomodulating, antioxidant, anticancer, antimicrobial, anti-inflamatory, antidiabetic, and anti-ulcer	Vermifuge, treat problems of the urinary system, hypertension, prevents the formation of kidney stones	Gutierrez and Martha (2016)

(continued)

Table 1.1 (continued)

S. No	Botanical Name	Common Name	Family	Parts used	Solvent/Condition	pharmacological property	Other Medicinal uses/traditional uses	Reference
12	*Curcuma longa* L.	Turmeric	Zingiberaceae	Rhizome	Aqueous extract, acetone extract, ethanolic extract, chloroform extract, and methanolic extract	Immunomodulatory, anticancer, antidiabetic, antioxidant, hypolipidemic, anti-inflammatory, antimicrobial, anti-fertility, anti-venom, hepatoprotective, nephroprotective, anticoagulant	Biliary and hepatic disorder, diabetic wounds, rheumatism, inflammation, sinusitis, anorexia, coryza, and cough.	Chanda and Ramachandra (2019), Yuandani et al. (2021)
13	*Emblica officinalis* Gaertn.	Indian gooseberry, amla	Euphorbiaceae	Fruit	Aqueous and acetate extract	Antioxidant, immunomodulatory, anticancer, cytoprotective, analgesic, antimicrobial	Dyspepsia, gastritis, hyperacidity, constipation, colitis, hemorrhoids, hematuria, menorrhagia, treat anemia,	Baliga and Dsouza (2011), Madhuri et al. (2011)
14	*Euphorbia granulate* Forssk.	Bottle gourd	Euphorbiaceae	Leaves	Aqueous methanolic extract, methanoic extract, dichromomethane extract	Immunomodulatory antioxidant, antibacterial, anti-fungal, diuretic, anti-ulcerative colitis	skin infections, worms of intestine, raised blood glucose, warts, gonorrhea, snake bite, and scorpion Sting	Ahmad et al. (2019)
15	*Linumus itatissimum* L.	Flax	Linaceae	Seeds	Aqueous, ethanol, and chloroform extracts	Immunomodulatory, anti-cancer, antioxidant, anti-microbial, anti-inflammatory, anti-obesity, antidiabetic, antidiarrheal	Controlling levels of cholesterol and blood sugar	Ansari et al. (2019)

S. No.	Patent No.	Title	Plants Used	Medical use claimed	Disorder/disease	Inventor	Current Assignee	Date of Filing	Priority Country
1	WO2005077393A1	Herbal formulation comprising extracts of adhatoda, hedychium and curcuma as cough syrup	Adhatoda, hedychium and curcuma	Cough syrup to improve immunity	Common cold and cough	Rahul Singh Aravind Padiyar Anil Kanaujia Navin Kumar Sharma	Ranbaxy laboratories limited	1/19/2004	India
2	WO2006008761A2	Anti-retroviral herbal formulation	Gycyrrihza glabra, bulbs of Allium sativum, seed kernels of Azadirachta indica	Herbal formulation for therapeutic immunity boosting	HIV	Raja A.K.	---	7/23/2004	India
3	CN1056286C	Traditional Chinese medicine ointment for AIDS	Himalayan teasel root	A antiviral formulation enhancing immunity and symptomatic treatment for AIDS	AIDS	Li Yongkang	---	7/26/1995	China
4	WO2013042131A1	A process for preparation of synergistic herbal preparation of extracts picrorhiza kurroa and glycyrrhiza glabra having medicinal value	Picrorhiza Kurroa Royle, Picrorhiza Scrophulariflora Pennel, and Neopicrorhiza Scrophulariiflora, and Glycyrrhiza Glabra	Antiviral and immune boosting activity against DNA virus-bacteriophage pi AT	Viral infection	Ramana Rao	---	9/23/2011	India

(continued)

Table 1.1 (continued)

S. No.	Patent No.	Title	Plants Used	Medical use claimed	Disorder/disease	Inventor	Current Assignee	Date of Filing	Priority Country
5	EP1032409A1	Herbal extract composition and method with immune-boosting capability	ARUM, extract of POMEGRANATE, extract of TEA and extract of HIBISCUS	An herbal extract composition for stimulating cell-mediated immunity	HIV	Ahmad Abdallah Shehadeh	---	6/23/1998	USA
6	CN101269198A	Pure Chinese medicine preparation for preventing and treating enteron disease and improving self phylactic power, and preparation method thereof	Himalayas red jessamine	Improve the immunity of the patients	Atrophic gastritis, ulcer, edema, and acute and chronic gastroenteritis, hepatocholangeitis, and malignancy	Solang bazu	XIZANG JIALI TIBETAN MEDICINE HOSPITAL	2/29/2008	China
7	EP2393503B1	Medicinal plants extract using processing of herbal medicine and composition of skin external application comprising the same	Commiphora mukul, Terminalia arjuna, Inula racemosa and Hippophae rhamnoides	Improve the immunity of the patients	Coronary heart disease	G.P. DubeyG. V. Rajamanickman	Sharma Anurag	2/4/2009	India

8	US20190224193A1	Compositions of natural extracts and use thereof in methods for preventing or treating diseases	Cannabis	Treating conditions related to immune dysfunction	Metabolic diseases and disorders, cancer, psoriasis and to improve health and Well-being in general	Christopher Brian ReidQue T. Collins	----	3/14/2013	USA
9	US7048953B2	Methods and apparatus to prevent, treat, and cure infections of the human respiratory system by pathogens causing severe acute respiratory syndrome (SARS)	Eucalyptus globulus, Melaleuca alternifolia, wucalyptus citriodora, and eucalyptus radiata.	Improve the immunity of the patients	Severe acute respiratory syndrome (SARS)	William banning Vail, IIIMarilyn L. Vail	Inhalation Inc	4/3/2000	USA
10	US6030622A	Herbal extract composition and method with immune-boosting capability	ARUM, extract of POMEGRANATE, extract of TEA, and extract of HIBISCUS	Stimulating cell-mediated immunity	Immunosuppressive and autoimmune diseases, disorders, infections, or conditions	Ahmad Abdallah Shehadeh	---	6/23/1998	USA
11	CN103519293A	Fresh extract capable of improving child immunity and preparing method thereof	Dandelion	Improving child immunity	Keep the intestine, spleen, and the entire body healthy	Fu Hongjie Li Zunchang Niu Xiaogui	---	11/4/2013	China

(continued)

Table 1.1 (continued)

S. No.	Patent No.	Title	Plants Used	Medical use claimed	Disorder/disease	Inventor	Current Assignee	Date of Filing	Priority Country
12	US20090175964A1	Composition for enhancing immunity	Shatavari	Enhance immunity	Cold or influenza	Gautam Banerjee Jyoti BhatVilas Pandurang SinkarAsha TelkarSmitha Ashok Upadhyaya	Conopco Inc	12/14/2007	India
13	CA2864816A1	A novel herbal formulation for the modulation of immune system of HIV-infected patients and a process of preparation thereof	Withania somnifera, Ocimum sanctum	Enhance cell-mediated immunity as well as humoral immunity among HIV patients	HIV	Sunil Kumar Swamy, Rajan Govind Prasad, Dubey Aruna, Agarwa lNirupama, Dubey Shipra, Dubey Vedant, ARUN ARUN Kumar Swamy Rajan	---	9/20/2011	India
14	US6476203B1	Safe pharmaceutical composition for treating and preventing infertility and increasing immune function	Withania somnifera, Ocimum sanctum	Preventing infertility and increasing immune function	Treating and preventing infertility	Xinxian Zhao	---	3/14/2002	USA

15	US6440448B1	Food supplement/herbal composition for health enhancement	Arctium Lappa, Curcuma longa, Echinacea Purpurea.	Increasing natural immunity	General health	Joseph Intelisano	---	3/16/1998	USA
16	CN105285977A	Healthcare product having efficacies of resisting aging and improving immunity	Dandelion, ginseng	Resisting aging and improving immunity	Nurse health of heart, liver, spleen, lung, and kidney five big systems, entirety safeguards organism balance	Li Ji	Henan Jiahe Kang biological food technology co. ltd.	9/23/2015	China
17	DE4102054A1	Euphorbia hirta L. to increase immunity, e.g., against influenza, winter colds, and AIDS and as antifungal agent to treat open wounds	Euphorbia hirta L	Stimulate the immune system	Flu, winter colds, and AIDS	Geb Szenasi Tamas	---	1/24/1991	Denmark
18	US9775870B2	Medicinal plants extract using processing of herbal medicine and composition of skin external application comprising the same	Chrysanthemum indicum	Immunity activating effect	General health	Jun Seong ParkHye Yoon ParkDong Hyun KimEun Jeong MoonJi Hye ChungJae Kyoung LeeDuck Hee KimHan Kon Kim	Amorepacific Corp	5/2/2008	Korea

(continued)

Table 1.1 (continued)

S. No.	Patent No.	Title	Plants Used	Medical use claimed	Disorder/disease	Inventor	Current Assignee	Date of Filing	Priority Country
19	CN101637258A	Health food used for improving immunity	Ginseng	Immunity activating effect	General health	Zhang Hongcha Shenghua	BEIJING TONGRENTANG HEALTH PHARMACEUTICAL co ltd	8/17/2009	China
20	CN105495620A	Plant extract composition simultaneously has immunological enhancement and blood fat reducing functions	Hawthorn, dendrobium	Enhancing immunity and reducing blood fat	General health	Wang Hongxin Jia qi Haima Chaoyang Lü Wenping Lou Zaixiang Zhang Yi Zhang Yu	GUIZHOU PROVINCE CHISHUI CITY COUNTRY GIFT DENDROBIUM NOBILE DEVELOPMENT co ltd	1/8/2016	China

1.4 Formulations

As stated earlier, medicinal plants are potent therapeutic reservoirs for a large number of diseases and ailments. These medically important plants have a distinct place in the pharmaceutical industry as they are a major resource of novel compounds that form the active pharmaceutical ingredient (API) in their medicine (Bhatt 2016).

The coronavirus pandemic crisis has shed light on the use of different herbs to boost the immune system against the virus. The prior art is filled with literature on various methods/approaches to utilize natural as well as synthetic therapeutic agents to fight against the virus, majority of it being the Himalayan medicinal plants. Along with the giant pharmaceutical companies, small-scale local medicine producers have started producing a plethora of different immunity-boosting formulations to combat coronavirus by diving deep into the prior knowledge of the ancient medicinal plants that originated from the Himalayas. Following is a summary of the different formulations of the abovementioned plants which are currently available in the market.

1. *Aconitum*: The alcohol extract of this group of plants is formulated as Aconitum napellus 3× which can be orally administered and used for improving immunity against diseases like epilepsy, hepatitis, jaundice, and pneumonia.
2. *Artemisia annua*: The active metabolite of the plant-Dihydroartemisinin is isolated and formulated into oral Artemisinin Capsule and drops which is known to treat fever and boost the immune response during malaria.
3. *Acorus calamus L:* The plant roots are used for formulating powder and capsule for oral consumption which contain their respective active metabolites for treating diarrhea associated with intestinal worms. There is also a concentrated extract of the plant available as *Acorus calamus* Dilution 30 CH which is used to get relief from fever, nausea, vomiting, and abdominal pain.
4. *Cinnamomum tamala:* The bark of the plant is formulated into powder and capsules to be administered orally to improve appetite and fight bacteria and fungi.
5. *Holarrhena:* The group of plants from this genus is used to formulate powder and capsules to treat Diarrhoea and to boost health in men.
6. *Hypericum perforatum*: The plant is used for formulating capsules and pellets for oral consumption and is used to treat mental health illness.
7. *Matricaria chamomilla Linn.*: The leaves and flowers of the plant are used for making organic herbal tea which is an excellent way to boost immunity during winter seasons.
8. *Onopordum acanthium:* The plant is used to formulate syrup that helps to improve the condition after cirrhosis and hepatitis, such as alcohol abuse, autoimmune disease, or viruses.
9. *Viscum album Linn:* The Tincture (powder dissolved in alcohol) is made of ripe berries leaves or the whole plant is used to make the medicine. The medicine is applied for the overall health of the body.

10. *Withania somnifera*: The leaves and petals are used to make organic tea and capsules, which are used to boost immunity and preserve overall system health throughout the hard winter months.
11. Aloe vera (L.) Burm.f.: The leaves of the plant are extensively used to produce gels and juice that is highly recommended to boost immunity of the body throughout the year. The compounds Aloenin and Aloesin are known as herbal remedies that inhibit COVID-19 protease.
12. *Andrographis paniculata*: The leaves of the plant are used to make capsules and extracts for improving immunity and seasonal wellness of the body.
13. *Camellia sinensis*: *Camellia* tea is formulated into regular tea and green tea to boost immunity and keep the body of the host healthy. *Camellia sinensis* oil is also available for application on skin and hair for better nourishment purposes.
14. *Curcuma longa L.:* This plant is heavily used in formulations having combinations of extracts (pepper, ginger). *Curcuma longa* is a famous plant which is widely available in majority of the local markets. This plant is used in the formulation for its immunoboosting property as well as other important pharmacological activities like antimicrobial and antioxidant. Capsules of turmeric are also available in combination with plants like Boswellia for the treatment of the joints.
15. *Emblica officinalis Gaertn*. This plant is again heavily exploited for making formulations to boost immunity with the help of Vitamin C present in it. The formulations are in the form of powders, capsules/tablets, and even semi-solid "*Chavanprash."*
16. *Glycyrrhiza glabra L.:* The plant root extract is formulated into powder, liquid extract, serum, capsule, and concentrated extract available in different concentrations. This plant extract is beneficial for the body, skin, and hair.
17. *Justicia adhatoda L.*: This plant leaves are formulated into powder, tablets, and concentrated extract available in different concentrations. The plant extract supports healthy respiratory functions, joint health and promotes healthy joint function, and promotes healthy digestion.
18. *Linumus itatissimum L.*: The plant seeds are getting attention as super-food for its antioxidant and immunostimulatory activities. This plant is formulated into seed oils, raw seeds, and concentrated seed extract.
19. *Mangifera indica L.*: Healthvit Mango powder: Rich in dietary fiber, vitamins, minerals, and polyphenolic flavonoid antioxidant molecules are all good sources of antioxidants. Aids in the regulation of heart rate and blood pressure. Mangifera indica extracts are also available in different concentrations.
20. *Ocimum sanctum Linn.*: *Ocimum sanctum* plant extract is available in different concentrations and Ocimum sanctum powder is commonly available in all the local markets.

1.5 Conservation/Sustainability

Sustainability and conservation go hand in hand when plants or any renewable source are the topic of concern. Looking at climate change and the overall behavior of human population there is a pressing priority to develop programs and initiatives that take appropriate measures to seek the desired quality and yield of these plants in their natural habitat. Examples of such initiatives have already been conducted which will be explained below (Phondani et al. 2011).

1. A series of workshops was conducted in traditional communities of the Uttarakhand state, where 150 *vaidyas* participated in making their system of healthcare more effective and practical thus connecting the *vaidyas* strongly among themselves. The revitalization of these healthcare systems will directly benefit in conserving age-old traditionally important medicinal plants which are the working wheels of these *vedic* practices. This approach gives dual benefits where the traditional knowledge is revised on one hand and the plants involved participating in it are conserved.
2. MAPs—Medicinal and aromatic plants are used for medical and household food consumption in Swat District, Pakistan. The MAPs are used to generate revenue in urban markets. In order to ensure the long-term usage of MAPs, research was done with the purpose of expanding capacity through improved awareness, training, exposure, and the establishment of market ties. The initiative included a series of meetings with the local Forest Department for consultation and coordination, focus group discussions with MAP dealers and collectors in each village, and ethnobotanical field surveys led by community members (Sher et al. 2017). People of all races used 20 medicinal and aromatic plant species of very intense market value in native medical systems, according to the study. Formal and informal trading networks, including cross-border smuggling between Pakistan and Afghanistan, were used to trade these species. Organizing interventions such as local awareness campaigns, capacity-building training, community mobilization for threatened species conservation, and exposure visits to connect local communities with processors and buyers in order to maximize net income in order to promote sustainable use and improved livelihoods. Capacity-building was a crucial intervention used to achieve the project's main goals. The initiative is planned to act as a strategic investment for rural people in Pakistan's Swat District, which would generate money through sustainable harvesting and marketing of MAPs (Sher et al. 2017).
3. In today's crowded world, conservation of mountain ecosystems' distinctive biodiversity necessitates cross-disciplinary approaches. Geographers, conservationists, ecologists, and social scientists used to operate in silos, even though they all had the same conservation goals. The necessity of integrating various conservation criteria and approaches is emphasized in this chapter. New biological and social data-based criteria for selecting species and habitats for conservation in controlled ecosystems have been presented (Brown and Shogren 1998). Mountain environments are hotspots for plant conservation efforts because populations

replace one another along altitudinal and climatic gradients. They have a lot of endemic species and a lot of overall plant diversity. This review contributes an enhanced understanding of.

(a) Plant variety in mountain ecosystems, with a focus on the western Himalayas;
(b) Mountain vegetation's ethno botanical and ecosystem service benefits in the context of anthropogenic effects; and
(c) Local and regional plant conservation methods and goals (Brown and Shogren 1998).

1.6 Conclusion

The field of medicinal plants from the western Himalayas is enormous, and research and development in this sector will keep on refreshing as newer plants are discovered for their immunomodulatory effect. This is a small contribution to documenting plants of medicinal importance grown in the valleys and mountains of the western Himalayas. There is still a high amount of traditional medicinal knowledge hidden deep in the folk cultures that are still being used and should be explored to fullest content. The patent literature available in the public domain is an example of the vast applications that can be attempted freely by inventors and researchers that have a deep interest in this area. Abovementioned patent analysis is a small example of the kind of formulations being developed around the world. With proper use of keywords, search engines, and databases, we can obtain the exact number of patents present on this given topic at a given date which will assist in better formulations. The formulations listed above are available locally and are an example of the types of different formulations present in the market. These local formulations are a way of livelihood for the local people from that area. Many local families depend on such small and medium-scale industries which run on the availability of the raw product, i.e., yield of the medicinal plant. Hence, it is our duty as humans to help in restoring the climate, habitat, and the overall conservation of these plants. Central and Local Governments should also set up strict rules and regulations to maintain the sanity of the environment as they have a huge role in maintaining these plants for the coming future generations.

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Chapter 2
Achillea millefolium L., Common Yarrow

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2.1 Introduction

Humanity has utilized plants to alleviate pain and cure diseases since ancient times. Even today, herbal medicine is the primary component of practically all folk remedies worldwide. The herbaceous perennial plant yarrow (*Achillea millefolium L.*) pertains to the *Asteraceae* family (Fig. 2.1), does have an ancient legacy of usage, dating back to prehistoric times when it was employed to treat wounds. Northern America, European countries, East Asian regions, and Northern Africa are all home to this plant (Si et al. 2006). To beautify the garden, yarrow, a significant therapeutic plant, is usually employed (Benedek et al. 2007a; Dempewolf et al. 2008). This has long been a well-known medicinal herb that has been around for more than 3000 years (Mitich 1990). It is cultivated commercially as a raw ingredient for the phytomedicine and tea sectors. *A. millefolium* has several pharmacological qualities, including anti-inflammatory, antidiabetic, analgesic, spasmolytic, hemostatic, anti-tumor, antioxidant, antifungal, disinfectant, and hepatoprotective capabilities; also, flavonoids and phenolic acids are abundant in this plant (Afshari et al. 2018; Ali et al. 2017; Fierascu et al. 2015). Essential oil and flavonoid derivatives such as apigenin,

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Fig. 2.1 *Achillea millefolium*

rutin, lutein, and campherol are the primary phytochemical substances extracted from *A. millefolium* (Bimbiraite et al. 2008). Partially biological implications of *A. millefolium* extract, including antidiabetic, gastroprotective, and antibacterial properties, seem to be attributable to the antioxidant ability of polyphenols, notably flavonoids (Chavez-Silva et al. 2018). Decoctions have traditionally been utilized to alleviate inflammatory conditions, including hemorrhoids and headaches. The blooming tops are the most medicinally active component of the plant. They were also used as a snuff due to their modest stimulating properties. Yarrow is a popular folk remedy in Europe because it possesses flavonoids (plant-based compounds) that enhance digestion by increasing saliva and stomach acid. Yarrow may help ease stomach and menstrual cramps by relaxing the smooth muscle in the colon and uterus. The flowers are used to cure hay fever and other allergic mucous disorders. Since the leaves promote clotting, they could be administered fresh to treat nose-bleeds (Crellin et al. 1990). The plant's aerial portions are being utilized to treat phlegm and promote bile flow; they are given as bitter digestive tonic and as a diuretic. Yarrow enhances the therapeutic effects of various herbs by adding yarrow (Henrichs 1994) and eliminating body toxins.

2.2 Taxonomy

The *Achillea* genus comprises roughly 110–140 species, is primarily limited throughout Eurasia (Ehrendorfer and Guo 2006), including some species native across northern Africa and North America. Subsp. *millefolium* (tiny white blooms), subsp. *alpestris* (pink flowered), and subsp. *ceretanum* Sennen (big white blooms) are all endemic to Europe and Spain and southern France are the only countries that have it (Applequist and Moerman 2011). The following table (Table 2.1) describes the classification of the plant.

The epithet *millefolium* alludes to the plant's "many leaves" or "thousand leaves," which Achilles used to cure the injuries of his companion soldiers in the Trojan War. Hence, the name *Achillea* (Mitich 1990). The common name "yarrow" has no definite origin; however, it is believed to be derived from "garawa" and existed in the past from around 725 A.D. (Mitich 1990).

2.3 Common Names of *Achillea millefolium*

Achillea millefolium is known by its various names. Common names are given below in Table 2.2.

Table 2.1 Classification of *Achillea millefolium*

Eukaryota	Domain
Plantae	Kingdom
Spermatophyta	Phylum
Angiospermae	Subphylum
Dicotyledonae	Class
Asterales	Order
Asteraceae	Family
Achillea	Genus
Achillea millefolium	Species

Table 2.2 Names of *Achillea millefolium*

Green arrow	Yarrow
Thousand-leaf	Soldier's woundwort
Sanguinary	Noble yarrow
Milfoil	Nosebleed plant

2.4 Flowering/Fruiting Season

From May through June, the plant blooms, and it is a popular addition to butterfly gardens. Grasslands and open woodlands have a lot of common yarrow, which grows on slightly disrupted soil. In the spring, active growth takes place. There are native and imported genotypes in North America, and plants are diploid and polyploid. Yarrow (*Achillea millefolium*) prefers a bright, warm environment and may be spotted in meadows, alongside the road, and on dry, sunny hillsides. During June and September, yarrow blooms. The blooms are usually white; however, in mountainous places, pink or pastel purple blossoms are prevalent. The flower's petals appear closely packed in the form of compressed bunches, as well as the leaves resemble feathers. The plant proliferates and spreads quickly. Due to the plant's drought endurance, yarrows can counteract soil erosion. Drought-tolerant common yarrow (*Achillea millefolium*) comes in various beautiful variants. Because seeds need sunshine to germinate, they should be placed no lower than ¼ inches. Seeds germinate best at temperatures ranging around 18 and 24 °C (64 and 75 °F). The optimum soil for common yarrow is weakly established and adequately drained. The plant has a limited lifespan; it may be extended by splitting it every year and then putting it 12–18 inches apart. The weedy common yarrow has the potential of becoming invasive. If not planted in well-drained soil, it may succumb to mildew.

2.5 Distribution

Plants of the Asteraceae family may be found worldwide although they are ubiquitous in the subtropical and lower temperate zones' dry and semi-arid environments. Approximately 130 blooming and perennial species of *Achillea* may be recognized in Europe, Asia, and North America. Turkey has 46 taxa, 25 indigenous, whereas Iran has 19 species, 7 of which are native (Mozaffarian 1996). Europe and Western Asia are home to *A. millefolium* although it can be found in temperate areas such as North America. There are roughly 85 species of *A. millefolium*, the majority of which are located in European regions, Asian countries, and Northern America. *A. millefolium* may well be spotted in meadows and open woodlands at an elevation above 3500 m above sea level. Flowers are most prevalent between May and June, and there is much growth in the spring.

Owing to its intricate taxonomy and management of all individuals across an "*A. millefolium* complex," *A. millefolium* is extensively dispersed (PROTA 2015). The yarrow plant is native to Eurasia and Northern American regions, where it develops mainly throughout Northern Hemisphere's temperate and boreal provinces and in the Southern Hemisphere, to a lesser amount (PROTA 2015). North America, Central America, Asia, and Europe are among its natural habitats.

2.6 Morphology

Asteraceae is the most prominent family of vascular plants, and *A. millefolium* is its member. As shown in Fig. 2.2 *Achillea millefolium* is a long-blooming perennial with a lovely fragrant perfume and a peculiar morphology. It is a 50 cm-tall herbaceous perennial having a cropping rootstock that is slender that throws multiple roots and stolons at every node, each with a succulent, blunt scale. The length of the leaves is about 5–20 cm (Fig. 2.2). They are bipinnate or tripinnate, nearly feathery, and grouped spirally at the center and bottom of the stem with variable levels of hairiness (pubescence). Flowers are generally white (Fig. 2.3), yet blooms are pink or pale purple, with corymbose, ovoid, flat tips at the stems and branches' extremities, along with compactly crammed petals in flat bunches. The fruits seem to be lustrous, oblong achenes with broad edges and without pappus, about 2 mm long (Akram 2013).

The stem of this plant is bare, upright, and fluffy, and it may grow to be 1–3 ft tall. Fibrous roots with a rhizome system hold the stalk. On alternate sides of the stalk, fern-like leaves with a feathery appearance surround it. The length of these leaves ranges from 1 to 6 inches. These hardy stalks and leaves typically make it throughout the winter months. Yarrow flowers blossom from June to September and are generally white but can also be pale pink or purple. The flowers are arranged with five ray flowers in a distinctive flat-topped dome group design. These blooms are tiny, measuring almost a quarter inch in diameter, and bloom for a prolonged time. Flowers develop seeds and perish. Yarrow may reach elevations of 3500 m above sea level.

Fig. 2.2 Leaves of Yarrow

Fig. 2.3 Flowers of *Achillea millefolium*

2.7 Traditional Uses

Inflammatory and spasmodic gastrointestinal disorders have long been treated with yarrow (*Achillea millefolium L. s.l.*), along with hepatobiliary symptoms and infection (Benedek and Kopp 2007). It is also used to boost hunger owing to its bitter flavor, mend wounds, and treat inflammation of the skin. Aqueous or alcoholic extracts of the plant's aerial portions are commonly used. *A. millefolium*, also known as Biranjasif in the Unani (Greco-Arab) medicinal system (Applequist and Moerman 2011), is being utilized in the conventional approach of medicine for centuries, both internal use in the form of herbal teas for headaches, hepatobiliary disorders, abdominal discomfort, and as a hunger stimulant, and extrinsically as moisturizers and creams for irritated skin including lesions, incisions, and many more (Cavalcanti et al. 2006; Benedek et al. 2007a, 2008; Nadim et al. 2011).

A Homo neanderthalensis burial at Shanidar was discovered with yarrow pollen, dating from 65,000 B.C., rendering it the earliest plant known to have been utilized by humankind (sensu lato) (Leroi-Gourhan 1975). Many cultures believe yarrow to be a remedy for a wide range of diseases; thus, it has become a constant fixture. The wound-healing effect is among the most common goals of the traditional yarrow species use. The wound-healing benefits of yarrow medicines are due to their hemostatic, anti-inflammatory, including antibacterial characteristics (Nemeth and Bernath 2008). *Achillea millefolium* is among the most used *Achillea* species for repair of wounds. Furthermore, the Indian Ayurvedic Pharmacopeia lists *Achillea millefolium* as a remedy for high temperatures and healing of wounds (Sayed and Bano 2018; Sharma et al. 2004). *A. millefolium* has recently been explored in clinical

studies. Hajhashemi and colleagues stated that an aqueous extract of *A. millefolium* airy sections was tested on 140 women in a clinical trial. The procedure for recovery was measured using five characterizations on the 7th, 10th, and 14th days: discharge, ecchymosis, wound dehiscence, redness, and edema. According to the findings, the ecchymosis, edema, redness, and discomfort levels were all decreased by using yarrow extract ointment (Hajhashemi et al. 2018).

Loss of appetite, abdominal convulsions, flatulence, gastritis, intestinal inflammations, internal and external bleeding (coughing up bloodstained fluid, bleeding of nose, hemorrhoidal and menses, bloodstained urine), bruises, lesions, skin infection, and dog and snake bites have all been treated with infusions, decoctions, or fresh juices, according to Ali et al. (2017). It seems to have oral and topical use, orally as a tea and as a moisturizer, salve, or bandage topically as reviewed by Ali et al. (2017) (Chandler et al. 1982).

A widespread species among many other *Achillea* species, *Achillea millefolium*, its aerial portions, are frequently administered as traditional medicine for treating gastrointestinal problems, hepatobiliary ailments, wound healing, and skin infections in Europe and Asia (Jaradat 2005; De Albuquerque et al. 2007; Ugulu et al. 2009). It was known as *Herba militaris* by the ancient Europeans, and an ointment prepared from it was applied as a vulnerable medicine for war injuries. The United States Pharmacopeia previously featured the flowers *A. millefolium*. In European traditional medicine, *A. millefolium* is employed as a diaphoretic for abdominal issues, a reduction in appetite, menstrual difficulties, skin infections, lesions, and external hemorrhaging (Wichtl 2002, 2004). The review by Ali et al. (2017) reveal that, in Iranian traditional medicine, *A. millefolium* is often utilized to cure various ailments, notably inflammation, discomfort, and gastrointestinal problems. For treating hemorrhoids, dyspepsia, dysmenorrhea, and gastritis, the dried flower extract seems to work excellent (Miraldi et al. 2001).

Yarrow has been employed in Italy to cure a wide range of ailments, including menstruation issues, diuretic, urinary infections, toothaches, and drowsiness, however largely for abdominal problems (Passalacqua et al. 2007). This plant has been renowned in Hungary as *cickafark* (kitten tail) and is administered to treat inner illnesses, burn, and injuries (Applequist and Moerman 2011). According to Bussmann et al. (2007), in Peru, *A. millefolium* has been claimed to treat gastroenteritis, diabetes, high cholesterol levels, and most dermatological disorders, diarrhea, and other abdominal problems with the following names Milenrama and Chonchón. Mil-folhas and erva de Cordura are two Brazilian names for it (Baggio et al. 2008; Pires et al. 2009) even though extract of plant or decoction of the airy sections of the plant is a shred of evidence for tranquility. Even this shred of evidence can also be found in Mexico (Molina-Hernandez et al. 2004). Injuries, nosebleeds, uterine bleeding, high blood pressure, breathing problems, high temperatures, and rheumatic symptoms accounted for one-third of the 125 reports of traditional usage of *A. millefolium* in Britain and Ireland as reviewed by Ali et al. (2017). Yarrow is well-known in China. Hemorrhaging, snakebite, lesions, hemorrhoids, varicose veins, dysmenorrhea, and tuberculosis have all been treated with *A. millefolium* (Applequist and Moerman 2011). In the Parvati valley of the Himalayan area of

India, the leaves and blossoms are administered to cure stomach disorders and high temperatures (Sharma et al. 2004).

2.8 Phytochemistry

Since the nineteenth century, the biochemical components of *A. millefolium* have been researched, and various other components have been discovered to date. Many components have been discovered in the Achillea genus (Si et al. 2006). The components are as follows:

- essential oils
- phenolic acids
- sugars
- fatty acids
- sesquiterpene lactones, primarily guaianolides and germacranolides
- coumarins
- alkanes
- sterols
- saponins
- polyacetylenes
- flavonoids
- alkaloids
- amino acids

Polyphenols: Polyphenols, a chemical category of natural substances, are vast and ubiquitous. Because of their regular occurrence and appealing biological features, this group of functional items is of tremendous relevance. According to their chemical structures, polyphenols from the Achillea genus may be classified into two categories: flavonoids and phenolic acids.

Flavonoids: Flavonoids are the most abundant polyphenolic substances found in *Achillea spp.*, and they are similar to those found in other Asteraceae families. In general, there is a propensity for methylation at the C6 and C4 sites in these flavonoids. Additionally, their structures' percentage of hydroxylation, substitution, and conjugation affects their biological activity. In 1961, the first isolation of *A. millefolium's* flavonoids, cynaroside and cosmoside, revealed two flavonoids has antispasmodic action.

Sesquiterpenes Lactones (SLs): Asteraceae is a family of plants that has a broad spectrum of structurally modified and substantially replaced SL skeletons. *Achillea* spp. SLs are an intriguing phytochemical class in the genus because of their structural variety and wide range of biological activity. Though other SLs have been described, the most typical classes include germacranolides, guaianolides, and secoguaianolides. *A. millefolium* from Mexico yielded achillin and leucodin (Arias-Durán et al. 2020). Achilles A was discovered in *A. millefolium* as a monomer (Li et al. 2012a, b). A second investigation of this plant's blooms found ten

1, 10-secoguaianolides containing two dimers. Millifolide A and B are dimeric compounds, whereas millifolide C, a unique guaianolide reorganized carbon skeleton compound, too was identified alongside 5-epi-seco-tanapartholide A, seco-tanapartholide B, seco-tanapartholide A, isoseco-tanapartholide, arteludovicinolide A, 3-methoxy-tanapartholide, and 3-acetyl-iso-seco-tanapartholide.

Other compounds: Several phytochemical analyses have indicated the existence of additional chemicals in addition to the genus' main components, other than sesquiterpene lactones and flavonoids. Alkamides (Althaus et al. 2014), fatty acids (Agar et al. 2015; Dias et al. 2013), and sterols (Ezzat and Salama 2014) have all been isolated in various investigations. These findings suggest that the taxa's phytochemical profile may vary; yet, *Achillea* species' bioactivity may be explained by their biodiversity.

2.9 Nutritional Composition

GC and GC-MS were performed to study the essential oils isolated from the leaves and blossoms of *Achillea millefolium L.* Both oils mainly were monoterpenes throughout the plant's blooming stage (about 80%). In flowers' oil (29%) and the leaves' oil (25%), 1, 8-cineole was the most prominent constituent, after that sabinene in the blossoms (15%) and the oil produced from the leaves (10%) contains trans-sabinene hydrate. Germacrene-D (0.7% in flower oil and 7% in leaf oil) is the primary sesquiterpene component in both cases. The monoterpene proportion of the essential oil secluded from vegetative leaf tissue was negligible (3%), but sesquiterpenes accounted for 92%, with germacrene-D accounting for the majority of the oil, i.e., 65% (Figueiredo et al. 1992).

2.10 Clinical Studies

There have been zero human investigations of single-component yarrow formulations for conventional applications. The study's goal conducted by Farnaz Hashemian and co-workers was to see how effective *Achillea millefolium* ointment was at treating recurring idiopathic epistaxis. Fifty individuals with idiopathic recurrent epistaxis underwent this arbitrary, double-blind, placebo-controlled experiment. Before research, complete initial evaluations and coagulation tests were conducted. One of two groups was randomly allocated to the participants: case or control, and they were given for ten days the "Vitamin A and *Achillea* Ointment" or "Vitamin A and Placebo Ointment" reciprocally (Vitamin A ointment on day one and *Achillea* or Placebo ointment on day 2). Nasal checks and coagulation tests were done on day 10, and patients were seen after the first and third months after starting therapy. The bleeding volume was measured before and after the intervention using

the Epistaxis Severity Score (ESS) (tenth Day, first and third Month). Regarding bleeding frequency and duration on day 10, between the two groups, there was no statistically significant difference ($p = 0.105$). Nevertheless, in the first and third months of investigations, the case group substantially reduced the incidence and extent of bleeding ($p < 0.001$). During the case group's treatment, no abnormalities were recorded. In this study, in those with recurrent idiopathic epistaxis, *Achillea* ointment 1% effectively lowered ESS and bleeding duration, with no drastic side effects (Farnaz et al. 2021).

The current study, conducted by Ufuk Okkaya et al., (Okkay et al. 2021), sought to investigate how *Achillea millefolium* affects cisplatin (a very efficient anti-cancer agent) biochemically, molecularly, and histopathologically, induced oxidative and inflammatory eye damage in rats. A total of 24 adult male mice were assigned to one of four groups at random ($n = 6$): (a) control, (b) cisplatin (7 mg/kg, intraperitoneally), (c) Cisplatin + *Achillea millefolium* (200 mg/kg, orally for 14 days), and (d) Cisplatin + *Achillea millefolium* (400 mg/kg, orally for 14 days). In ocular tissue, total antioxidant capacity and total oxidant status and SOD, MDA, IL-1β, and IL-10 were assessed. TNF-α, nuclear factor kappa B, and Caspase-3 mRNA expressions were measured. Ocular segments have also been investigated histopathologically. Overall, the findings show that *Achillea millefolium* has been shown to protect from cisplatin-induced eye damage and may be a feasible supplementary treatment for cisplatin-related ocular toxicity prevention.

The study conducted by MM Hashemi and co-workers investigated the cytotoxic and anti-cancer properties of *Achillea millefolium L.* (Yarrow) in vitro. Upon taxonomically identifying *Achillea millefolium L.*, its hydroalcoholic extract was obtained, and different extract dosages were given to the AGS gastric cancer and L-929 regular fibroblastic cell lines across three time periods (24, 48, and 72 h). The cytotoxic effects were assessed using the MTT assay. The cell viability was not affected by the 24-h treatment, but the IC_{50} values of 64 and 16 μg/mL were obtained after 48 and 72 h of incubation, respectively. The 72-h incubation period with 16 μg/mL proved to be the most effective on cancerous cell lines while remaining safe for regular cell lines. Long-term therapy of the AGS cancer cell line with low quantities of yarrow extract may benefit cytotoxicity in these cancerous cells (Hashemi et al. 2021).

2.11 Toxicology

Long-term health risks linked with *A. millefolium* extracts are not widely documented. Granted that the plant has been categorized as non-poisonous according to the Food and Drug Administration and authorized to be permitted for usages in alcoholic beverages, following its usage by people and in animal tests, inevitable hazardous consequences have been documented (Guédon et al. 1993). Attributed to the prevalence of stimulating substances like guaianolides (a classification of sesquiterpenoid) and notably alphaperoxyachifolid, certain

people may get allergic contact dermatitis after coming into contact with *A. millefolium*, which is existent at varying levels up to 0.6% in flower and 0.05% in leaf (Hausbn et al. 1991; Rücker et al. 1991, 1992). Chemical degradation might cause the percentage in processed or dried materials to decline as suggested by Rucker et al. (Rücker et al. 1992).

In Wistar rats, Dalsenter et al. (2004) assessed the toxicity of an aqueous extract from *A. millefolium* leaves affecting reproductive parameters (reproductive organ weights, sperm and spermatid counts, and sperm morphology). Yarrow extract was fed to adult male rats (0.3, 0.6, and 1.2 g/kg/day) orally for 90 days. The most critical dosage of *A. millefolium* extracts led to a significant rise in the proportion of aberrant sperm, without any additional alterations in the additional reproductive factors in male rats. Additionally, following a 3-day therapy of juvenile female rats with no uterotrophic effects, the extract's potential estrogenic/antiestrogenic activity was tested. The findings convincingly demonstrated that the dosages of *A. millefolium* frequently ingested by humans provide no long-term reproductive toxicological harm.

Ali et al. (2017) reviewed that Cavalcanti et al. (2006) used Aqueous *A. millefolium* extract, up to 10 g/kg oral and up to 3 g/kg intraperitoneally to evaluate biochemical, histological investigations in Wistar rats; it showed no symptoms of mortality. Longer term trials found no evidence of significant toxicity at dosages of up to 1.2 g/kg/day administered by tube feeding for up to 90 days. Substantial increases in the liver's mass, concentration of cholesterol in the blood, and glucose concentrations, on the other hand, were not associated with dosage or exposure duration and were not indicative of any toxicity.

2.12 Pharmacology

Numerous active compounds from *A. millefolium* have been demonstrated to display choleretic, antimalarial, antioxidant, antihypertensive, antibacterial, antispasmodic, hepatoprotective, gastroprotective, and other therapeutic properties utilizing various in vitro and in vivo techniques. Figure 2.4 describes various pharmacological activities of yarrow.

Antimicrobial: The antimicrobial effectiveness of an ethanol extract of *A. millefolium* airy components was examined towards *B. cereus*, *E. coli*, *Candida albicans*, *P. aeruginosa*, and *S. enteritidis*. *B. cereus* and *S. enteritidis* had the highest MIC values, both of which were 62.50 mg/mL. Nevertheless, none of the other three strains tested showed any action (Kokoska et al. 2002). These findings support *A. millefolium's* ethnopharmacological usage and position it among the most promising indigenous drugs for treating microbial infections.

Antibacterial activity: *Mycobacterium smegmatis*, *Acinetobacter lwoffii*, *Streptococcus pneumonia*, and *Clostridium perfringens* are all said to be resistant to yarrow essential oil (Candan et al. 2003). *A. millefolium* has been employed to treat gastrointestinal problems; Yarrow's methanol extract has been shown to be

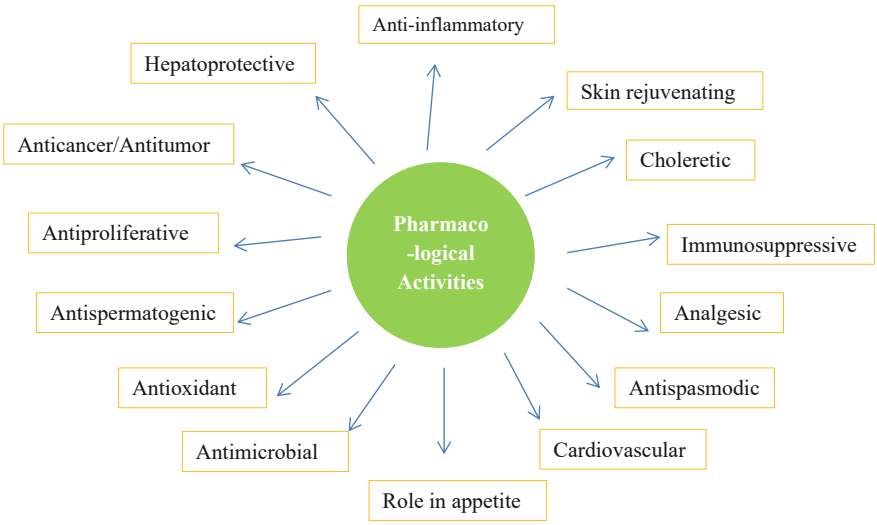

Fig. 2.4 Different pharmacological activities of *Achillea*

beneficial towards Helicobacter pylori. These bacteria end up causing stomach ulcers and gastritis, at least an inhibitory dosage of 50 g/mL, which could conveniently be accessed in the stomach upon oral administration (Mahady et al. 2005). *A. millefolium* apical parts essential oil was studied for antibacterial action towards *Klebsiella pneumonia, Enterococcus faecalis, Staphylococcus epidermidis Shigella dysenteriae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium, Escherichia coli*, and *Bacillus cereus*. *S. epidermidis* and *S. aureus* had the greatest antibacterial activity (33.6 0.5 mm; MIC 12.6 µg/mL and 31.4 0.8 mm; MIC 15.4 µg/mL, respectively).

Antifungal: Stojanović et al. (2005) found that *Achillea millefolium* and three additional kinds' extracts in hexane-ether-methanol, restricted *Aspergillus niger* and *Candida albicans* and that yarrow essential oil (the two *A. millefolium* subsp. *millefolium* and "*A. setacea*") prohibits species of *Candida* (Ünlü et al. 2002). Holetz et al. found no evidence of anticandidal activity (Holetz et al. 2002). Variations in grade of the raw materials, kind of extract, or process might cause contradictory findings. Holetz et al. (2002) examined 13 plants conventionally employed to treat contagious illnesses against eight pathogens; merely 11 of the 104 plant and disease-causing organisms combinations had a minimum prohibitive dosage of less than 100 g/mL, which was considered "good," while the majority of tests indicated no activity. This looks to be a modest success rate. *Aspergillus niger*, *Candida albicans*, *Aspergillus niger*, *Epidermophyton flavus*, *Microsporum gypseum*, *Microsporum canis*, *Candida tropicalis*, *Trichophyton verrucosum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Cryptococcus neoformans*, *Candida parapsilosis*, *Candida krusei*, and *Candida guilliermondii* were tested to check the *A. millefolium* essential oil's antifungal activity derived from aerial

portions. The oil had the best results towards the strains examined, for MIC values varying from 0.32 to 1.25 μL mL^{-1} (Falconieri et al. 2011).

Anti-inflammatory: Inflammation-associated proteases were suppressed by methanolic and aqueous extracts of *A. millefolium* airy constituents such as human neutrophil elastase (HNE) and matrix metalloproteinases (MMP-2 and -9), as shown by anti-inflammatory characteristics of the same in vitro. In addition to extracts, researchers looked at two fractions that were higher in flavonoids and DCQAs to see whether they may help the plant's antiphlogistic impact. At an IC_{50} of 20 g/mL, the extract, as well as the flavonoid-fortified component, suppressed HNE, whereas HNE was suppressed by a DCQA-fortified component with an IC_{50} of 72 g/mL. The dicaffeoylquinic acid component was more efficient than the flavonoid component, and the extract suppressed MMP-2 and MMP-9, with IC_{50} readings ranging from 600 to 800 g/mL (Benedek et al. 2007b). A gel comprising 6% yarrow extract was shown to be equally effective as a diclofenac sodium gel in decreasing carrageenan-induced paw edema in albino rats (with approximately 50%) (Maswadeh et al. 2006). The bulk of veterinary research has relied on separated yarrow chemicals, which may be helpful in pharmaceutical research yet do not thoroughly assess conventional usage. The mouse paw edema test was used to see whether various elements retrieved from an aqueous extract of *A. millefolium* dried flower heads have anti-inflammatory properties. At a 40 mg/kg dosage, the highest functional subset extracted (XII) decreased inflammation by 35% (Goldberg et al. 1969).

Anticancer: The growth inhibitory efficacy of ten 1, 10-secoguaianolides derived by *A. millefolium* bloom extract in methanol was investigated in vitro over the human tumor (MCF7WT) and human prostate cancer cell line (PC3). The 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) test was employed for cell viability. According to the findings, the inhibitory impact of seco-tanapartholide A on cell proliferation is substantial in a human cancer cell line MCF7WT (IC50 = 5:51 m) (Li et al. 2012a). Lin and co-workers (2002) investigated the effects of 15 botanicals on five human liver cancer cell lines. In three negative cell lines for hepatitis B virus, an extract of yarrow in boiling water at a 2000 g/mL concentration induced an average of 55.3% inhibition. At that dosage, the IC_{50} values for the two cell lines inhibited over 50% were 1422 g/mL and 430.4 g/mL, respectively; while the third, 40.7% inhibition at 2000 g/mL was the highest of any plant evaluated. Despite being ineffective towards two hepatitis B-positive cell lines, yarrow extract was one of only two plants evaluated to diminish one of them (Hep3B) by not appearing to be cytotoxic.

Hemmati et al. (2011) examined the *A. millefolium* hydroalcoholic extract blooms in Sprague Dawley rat with bleomycin-induced lung fibrosis (7.5 IU/kg) for anticancer effects. For 2 weeks, they were given varied extract dosages of *A. millefolium* (400, 800, 1600 mg/kg/day P.O.). The alveolar thickening was linked with fibroblasts, myofibroblasts proliferation, and collagen synthesis in interstitial tissue in bleomycin-treated mice as revealed via histopathological investigation, resulting in pulmonary fibrosis.

The anticancer ability of *A. millefolium* aerial component ethanol extract was tested on HFFF (normal fibroblast cell line) and six cancer cell lines, namely PLC/PRF/5 (human liver hepatoma), A375 (human melanoma cancer), SKLC6 (human lung carcinoma), AGS (human Caucasian gastric adenocarcinoma), SW742 (human colorectal adenocarcinoma), and MCF7 (human breast ductal carcinoma). 10 mg evaporated ethanol extract mixed in DMSO and ethanol (50 percent) were used to treat cell lines. PLC/PRF/5 was found to have the greatest IC_{50} value of 66.000 g/mL, along with MCF7 (64.058 μg/mL), A537 (49.438 μg/mL), SW742 (40.279 μg/mL), HFFF (34.431 μg/mL), SKLC6 (24.106 μg/mL), and AGS cell lines (22.051 μg/mL). The data might indicate that the extract has a selective impact in causing cytotoxicity in cancerous cell types (Ghavami et al. 2010).

Antispasmodic: In separated rat jejunum, crude yarrow extract has an antispasmodic action (Yaeesh et al. 2006). In isolated guinea pig ileum, a flavonoid-rich subset of yarrow had an antispasmodic action (Lemmens-Gruber et al. 2006). In the same test, many flavonoid aglycones, notably quercetin, luteolin, and apigenin, demonstrated substantial antispasmodic action, and the amounts of all these flavonoids in tea prepared from yarrow are powerful sufficiently to have such impact in vivo (Lemmens-Gruber et al. 2006). Calcium-channel obstruction has been blamed for a fraction of the activity's progression, while the other has been attributed to mediator-antagonistic activities.

Babaei and co-workers (Babaei et al. 2007) investigated the contractile reflexes of the detached guinea-pig ileum using a hydroalcoholic extract of *A. millefolium*. The contractile response was shown to be dose-dependently suppressed by the extract (EC50 = 1.5 mg/mL). The observations validated the *A. millefolium* extract's in vitro activity suppressing electrically generated ileal contractions in guinea pigs.

Hemostyptic: In an in vitro study employing human blood plasma, Sellerberg and Glasl (2000) examined different hot-water yarrow extracts to see how they affected blood coagulation. The recalcification period was significantly decreased by yarrow, suggesting that the herb may have hemostyptic action in vivo. The highly effective component was flowering material; the effectiveness of a complete herb extract was significantly higher than that of leaves or bloom extracts at a 5% concentration but not significantly more effective than stem extracts, indicating that the stem contained the majority of the activity. Despite no statistically significant difference in a wide variety of concentrations' activities, whole-herb concentrations of 1.0–5.0% looked to be better, cutting recalcification time to lower than half that of the control. The most notable concentrations employed for certain sections (12.5% for the total herb, 5% for the leaf) were wholly ineffective or almost so, presumably indicating the existence of chemicals in the leaf that has opposing actions that appear prominent at significant amounts.

Antiparasitic: Antiplasmodial efficacy of extract of methanol and chemicals derived from *A. millefolium* was tested in *Plasmodium falciparum* CQ-sensitive (D10) and CQ-resistant (W2) strains. In the D10 strain, the methanol extracts failed to produce 50% mortality, yet it had a detectable effect on the CQ-resistant W2 strain, with an IC_{50} of 44.6 ± 8.8 μg/mL. Nevertheless, apigenin 7-O-glucoside (IC_{50} 10.1 ± 1.3 in D10 and 6.1 ± 3.8 μg/mL in W2) and luteolin 7-O-glucoside (IC_{50}

26.2 ± 13.5 in D10 and 26.8 ± 3.6 μg/mL in W2) had been the highest power towards both *P. falciparum* strains amongt derived compounds (Vitalini et al. 2011). Murnigsih and co-workers (Murnigsih et al. 2005) tested antimalarial and antibabesial activities of 24 aqueous extracts of plants historically employed to treat malaria in Java (*Babesia gibsoni* is a tick-transmitted tick canine protozoan parasite that demolishes erythrocytes). In all experiments, yarrow was one of six species that showed substantial inhibitory efficacy (above 80% inhibition at 1 mg/mL concentration). However, clove oil is significantly more potent towards *Trypanosoma cruzi*; large doses of yarrow essential oil may have some action (Santoro et al. 2007). The fumigant toxicity of *A. millefolium* essential oil towards *Plodia interpunctella* adults was measured by Ebadollahi and Ashouri (2011). At 50, 65, and 80 μL concentrations, a 100% death rate was attained. After 24 h of fumigation, the LC_{50} value was 34.80 μl/L, and it declined as the exposure period increased. The effectiveness of *A. millefolium*, propolis hydroalcoholic extract, and systemic glucantime from *Cutaneous leishmaniasis* in Balb/c mice was studied by Nilforoushzadeh et al. (2008). The average ulcer size decrease seen in glucantime was 22.57%, *A. millefolium* (43.29%), and propolis groups (43.77%). The antileishmanial action of separated essential oils off the leaves and floral portions of *A. millefolium* was investigated in vitro towards *Leishmania amazonensis* as well as murine macrophages (J774G8 cell line). The IC_{50} value of *L. amazonensis* promastigotes was 7.8 μg/mL, but treatment with the oil at 6.5 g/mL reduced the lifespan of *L. amazonensis* amastigotes inside peritoneal murine macrophages by half. When evaluated against adherent (uninfected) J774G8 macrophages, the oil had a cytotoxic value of 72.0 μg/mL (i.e., 9.2 and 11.0 times greater than the IC_{50} towards the promastigotes and intracellular amastigotes). The oil-induced morphological abnormalities in the treated parasites fluctuate in size or shape, as per scanning electron microscopy. Promastigotes exposed to the oil (at an IC_{50} of 7.8 ug/mL) displayed ultrastructural changes in transmission electron microscopy as found by Santos et al. (2010). The efficiency of 11 flavonoids in inhibiting the development of the intraerythrocytic malarial parasite, chloroquine-sensitive (3D7) and chloroquine-resistant (7G8) strains, was investigated in the research. All flavonoids were active against the 7G8 strain, for the 3D7 strain, just eight were functional, with luteolin being the most effective in inhibiting parasite multiplication (Lehane and Saliba 2008).

Hepatoprotective: Yaeesh and co-workers (2006) used d-galactosamine and lipopolysaccharide to cause the mice hepatitis, using levels that eliminated all control mice. Mice pretreated with 300 mg/kg raw yarrow extract had a 40% reduction in mortality rates. Yarrow likewise increased the enzyme concentrations in the liver while decreasing histopathology. The experimental sample was a 70% aqueous-methanol extract that was separated and strained twice, then reduced to paste and redissolved in an aqueous mix. In isolated, perfused rat livers, According to Benedek et al. (2006) the choleretic action of yarrow extract is twice to three times that of cynarin (1, 3-dicaffeoylquinic acid, a recognized choleretic). Their research began with a 20% methanolic extract, subsequently supplemented with 48.8% dicaffeoylquinic acids and 3.4 per cent luteolin-7-O-beta-D-glucuronide via solid-

phase extraction. Nonetheless, the authors pointed out that because such chemicals are polar, they are easily removed through water and water-ethanol mixtures, which are often employed.

Serum transaminases, such as Glutamyl Pyruvate Transaminase (GPT) and Glutamyl Oxaloacetate Transaminase (GOT), alkaline phosphatase, and total bilirubin, were analyzed to evaluate the antihepatotoxic action on fasted Wistar rats of 5-hydroxy 3, 40, 6, 7-tetra methoxy flavone extracted via the airy portion's aqueous extract of *A. millefolium.* Paracetamol (PCL) and Carbon tetrachloride (CCl_4) were used to cause hepatotoxicity. When compared to CCl_4 and PCL, respectively, pre-treatment using extracted compound 5-hydroxy 3, 40, 6, 7-tetra methoxy flavone at 20 mg/kg b.w.i.p. drastically decreased GPT amounts (101.28 and 141.98%), whereas GOT values were brought down to 99.8 and 110.12%, alkaline phosphatase (39.2 and 17.97%), and overall bilirubin (55.28 and 43.64%). The findings backed up the therapeutic benefits of *A. millefolium*, which has long been employed in India's traditional medical systems (Gadgoli and Mishra 2007).

Gastroprotective: Yarrow extract in aqueous solution efficiently cures them in rats with persistent lesions caused from acetic acid (Cavalcanti et al. 2006). Mucosal erosion was decreased after 7 days of treatment by 75% at a dosage of 100 mg/kg/day, and at a 300 mg/kg/day dose, mucosal damage was reduced by 90%; these findings were more remarkable to the favorable pharmacological control of ranitidine, administered at 60 mg/kg. The yarrow's ED_{50} was determined to be 32 mg/kg, which is a safe amount for humans. Acute treatment with yarrow extract in conjunction with ulcer-inducing therapy with 70% ethanol or indomethacin considerably decreased mucosal harm inflicted by such chemicals (Cavalcanti et al. 2006). The researchers employed dosages of 125, 1500, and 2000 mg/kg, with the increased levels being extra helpful, particularly in avoiding ethanol harm.

Hatsuko Baggio and colleagues ended up finding that in rats, yarrow extract in hot water prevented the synthesis of ethanol and indomethacin-induced ulcers, despite the fact that the measured ID_{50} of 900 mg/kg was relatively high than the other two plant extracts used in medicine historically utilized in Brazil for digestive issues. The scientists discovered moderate antioxidant activity in vivo as well as minor, glutathione synthesis was shown to have non-significant organ-specific effects, despite the fact that it was thought to possess cytoprotective qualities. The same yarrow extract had no impact on gastrointestinal motility in rat research conducted at the same time. The impact of an *A. millefolium* hydroalcoholic extract on ileum locomotor activity was investigated. Wister rats had their ileum contractions triggered by 60 mM KCl (18.83 ± 4.91%) or 1-μM acetylcholine (18.31 ± 11.12%). Furthermore, adding 1% of the extract to the ileum reduced the contraction caused by KCl (59.96 ± 11.8%) or acetylcholine (54.16 ± 12.06%) ($p > 0.05$), which might be attributed to flavonoids like quercetin and apigenin (Sedighi et al. 2013).

The hydroalcoholic extract of *A. millefolium* aerial portion (35, 100, and 300 mg/kg) reduced ethanol-induced stomach bruises by 35, 56, and 81%, respectively, when given orally. Oral therapy with 1 and 10 mg/kg decreased chronic stomach ulcers caused by acetic acid exposure by 43 and 65%, respectively, and encouraged

considerable gastric mucosa restoration after ulcer formation, indicating enhanced cell growth. After acetic acid-induced chronic gastric lesions, *A. millefolium* therapy (10 mg/kg p.o.) reduced glutathione (GSH) and superoxide dismutase (SOD) activity by 53 and 37%, respectively. The findings showed that the hydroalcoholic extract's antioxidant capabilities might contribute to the benefits of its gastroprotective efficacy (Potrich et al. 2010). Rats were protected from stomach ulcers caused by ethanol and cold confinement by an aqueous hot-water extract of *A. millefolium*, yet not ulcers caused by indomethacin, according to Baggio et al. (2008). When examined for stomach ulcers caused by ethanol or indomethacin, the *A. millefolium's* aqueous extract had an inhibitory dosage of 50% (ID_{50}) of 900 mg/kg p.o. *A. millefolium's* antiulcer action might be attributable to a decrease in gastric secretions or an elevation in protective elements (including blood flow) in the stomach mucosa.

Antiproliferative: The antiproliferative efficacy of methanolic extract of *A. millefolium* aerial components (MEA) was investigated using the MTT assay on human prostate cancer (DU-145) and also on human non-malignant fibroblast cell lines (HFFF2), either alone or in combination with bleomycin. MEA was used to treat both cell lines at different amounts (20, 100, 500, 1000, and 2000 μg/mL). The extract significantly decreased bleomycin-stimulated cytotoxicity, with 60 and 49% viability rates at 1000 and 2000 g/mL, respectively. Bleomycin-treated cells had an 85% survival rate. MEA has no cytotoxicity when tested on HFFF2 cells. Although the pathways are unclear, cytotoxic flavonoids like casticin and sesquiterpenoids may be responsible for the enhanced bleomycin caused cytotoxicity in prostate cancer cells while causing no harm in normal cells (Shahani et al. 2015).

Five human lung cancer cell lines were tested in vitro using Achillinin A, a chemical derived from the floral portions of *A. millefolium* including adenocarcinomic human alveolar basal epithelial A549, human lung adenocarcinoma RERF-LC-kj, human lung carcinoma QG-90, QG-56, PC-3, and comparison was made with cisplatin. The researchers discovered that Achillinin A displayed antiproliferative effect towards adenocarcinomic human alveolar basal epithelial A549, human lung adenocarcinoma RERFLC- kj, and human lung carcinoma QG-90 cells, with 50% inhibitory concentrations (IC_{50}) of 5.8, 10, and 0.31 μM, respectively, and was more robust to that of cisplatin.

The antiproliferative properties of five flavonoids (apigenin, luteolin, centaureidin, casticin, and artemetin) and five sesquiterpenoids (paulitin, isopaulitin, psilostachyin C, desacetylmatricarin, and sintenin) extracted and discovered from chloroform extract of aerial portions of *A. millefolium* were examined using MTT test on three human tumor cell lines that include Centaureidin was perhaps the most efficient ingredient, inhibiting cell proliferation in HeLa (IC_{50} value of 0.0819 μM) and MCF-7 (IC_{50} value of 0.1250 μM) cells. Casticin and paulitin were likewise tremendously active towards all three tumor cell lines ($IC_{50} = 1.286–4.76$ μM), but apigenin, luteolin, and isopaulitin were reasonably active ($IC_{50} = 6.95–32.88$ μM). These cell lines were not affected by artemetin, psilostachyin C, desacetylmatricarin, or sintenin (Csupor-Löffler et al. 2009).

Antioxidant: The antioxidant capacity of different constituents recovered from the essential oil of *A. millefolium's* airy section was tested using the DPPH assay. Thymol had the greatest free radical scavenging activity towards DPPH (IC_{50} 12.0 ± 0.1 μg/mL), subsequently carvacrol (IC_{50} 13.43 ± 0.0 μg/mL), while bornyl acetate had the lowest activity (IC_{50} 25 ± 0.1 μg/mL). Nevertheless, α-pinene (20 ± 0.1 μg/mL), limonene (20 ± 0.3 μg/mL), and camphene (20.01 ± 0.3 μg/mL) showed similar action. The findings revealed that thymol and carvacrol serve a significant influence in essential oils' antioxidant characteristics (Kazemi 2015).

Georgieva et al. (2015) probed the antioxidant capacity of *A. millefolium* (leaves and stems) (DPPH, ABTS, FRAP, and CUPRAC tests). CUPRAC (55.08 ± 0.85–148.99 ± 1.94 μMTE/g dw) had the greatest free radical scavenging activity, after that FRAP (38.16 ± 0.47–132.71 ± 1.86 μMTE/g dw), DPPH (24.15 ± 0.15–116.74 ± 0.21 μMTE/g dw), and ABTS (18.59 ± 0.22–125.75 ± 2.24 μMTE/g dw). Furthermore, the action of the decoction extract was two to three times that of the remaining extracts examined. In vitro antioxidant ability of borneol, camphor, eucalyptol, α-pinene, and β-terpineol extracted from *A. millefolium* aerial parts essential oil significantly decreased the diphenylpicrylhydrazyl radical (DPPH) (IC_{50} = 1.56 μg/mL) and displayed hydroxyl radical scavenging action in the Fe^{3+}EDTA–H_2O_2 deoxyribose system (IC_{50} = 2.7 μg/mL). Non-enzymatic lipid peroxidation of rat liver homogenate was similarly reduced (IC_{50} = 13.5 μg/mL). The antioxidative effect of *A. millefolium* essential oil was proven by observations (Candan et al. 2003). According to the findings, *A. millefolium* might be employed as a readily available potent antioxidant and a dietary supplement or in the pharmaceutical business.

Didier et al. (2011) looked at how five different caffeoyl derivatives affected the antioxidant ability of *A. millefolium* as measured by the DPPH test. *A. millefolium* airy portions have an antioxidant capability of 8.29%, with chlorogenic acid (10.01%), 3, 5-DCQA (33.17%), 1, 5-DCQA (13.63%), and 4, 5-DCQA (13.63%) accounting for 61.80% of the total. In *A. millefolium* aerial components, the primary caffeoyl derivatives among polyphenols can be recognized as the chief antioxidant components.

In male rats, fertility characteristics were lowered by oral treatment of the alcohol extract of *A. millefolium* blossoms including fertility indices, body and reproductive organ mass. There was no substantial difference in body weight, sperm motility, or sperm viability after 50 days of treatment with 200 and 400 mg/kg/day. However, at 200 mg/kg of body weight, there was a substantial reduction in epididymis weight; epididymal sperm reserve (ESR), daily sperm production (DSP), and testosterone concentration. According to the findings, an alcoholic extract of *A. millefolium* blooms was found to have an antifertility effect; however, the process is unknown; it might be characterized by the accumulation of *A. millefolium* chemical makeup (Parandin and Ghorbani 2010).

Antispermatogenic effect: Montanari et al. (1998) investigated the spermatogenesis of Swiss mice using an ethanolic extract (200 mg/kg/day, intraperitoneally, over 20 days) also a hydroalcoholic extract (300 mg/kg/day, orally, for 30 days) of *A. millefolium* flowers. When *A. millefolium* was fed at dosages of 200 mg/kg/day

for 20 days or 300 mg/kg/day for 30 days, there was no discernible change in weight of the body or testis and seminal vesicle weight. Nonetheless, the organs of sexual reproduction in animals given 200 mg/kg/day intraperitoneally for 20 days showed macroscopic alterations, and the vacuolized seminiferous tubules of animals given 300 mg/kg/day exhibited remarkable multinuclear cells. The findings strongly indicated that *A. millefolium* is investigated further as an antifertility agent.

Immunosuppressive: The existence of glycosylated derivatives of caffeic acid extracted from the functional segment of *A. millefolium* might explain its immunological characteristics. The impacts of essential oil of *A. millefolium* on humoral immunological reactions in BALB/c mice were examined by Saeidnia et al. (2004). The anti-SRBC antibody titer in mice was lowered by the essential oil, which explains the plant's many immunological actions. The primary ingredient identified from *A. millefolium* essential oil was a sesquiterpene bisabolol. Using a microhemagglutination test, Yassa et al. (2007) investigated the immunosuppressive effect of methanolic extract and specific components of *A. millefolium* airy sections on humoral immunity in BALB/c mice. The anti-SRBC titer of mice was significantly reduced in just two components, at 125 and 61.5 mg/kg.

Analgesic effect: In a rat formalin test, Noureddini and Rasta (2008) investigated the analgesic efficacy of *A. millefolium* aqueous floral extract. Thirty minutes prior to formalin injection, an *A. millefolium* aqueous extract (5, 27, 40, 80, 160, and 320 mg/kg, p.o.) was administered. Following formalin injection, antinociception was measured from 0 to 5 min (first phase) and 15–60 min (second phase). The most substantial antinociceptive impact was seen at 160 mg/kg, whereas higher doses (320 mg/kg) had zero response in the formalin test. The findings of this investigation supported the traditional usage of *A. millefolium* for pain relief.

Choleretic activity: The choleretic effectiveness of a 20% methanolic extract component that has been enhanced in 3, 4-DCCA, 3, 5-DCCA, and 4, 5-DCCA, as well as luteolin-7-O-D-glucuronide from *A. millefolium* airy sections, was studied in perfused rat liver that had been detached. When compared to the internal standard cynarin, which demonstrates increases of 5.1% (±2.0), 15.9% (±3.6), and 21.6% (±8.9) at the identical levels (10, 20, and 40 mg/L), the fraction induced a dose-reliant rise in bile flow of 23.1% (±6.7), 44.1% (±17.2), and 47% (±12.2). DCCAs and luteolin-7-O-D glucuronides worked together to promote bile flow more efficiently than cynarin alone. The polar nature of these chemicals let them often be distilled to the teas and tinctures. They are vital choleretic factors in *A. millefolium's* conventional usage.

Rejuvenating action for the skin: Pain et al. (2011) used quantitative image analysis to assess the influence of extract of *A. millefolium* on the transcriptional profile of a variety of epidermal differentiation markers in routine human skin biopsies, as well as its potential to renew the look of the skin layer in vivo. In cultured skin biopsies, *A. millefolium* extracts raised epidermal thickness and enhanced the pattern of expression of multiple epidermal differentiation markers (cytokeratin 10, transglutaminase-1, and filaggrin). Compared to a placebo, in vivo, administrating 2% *A. millefolium* extract for 2 month marked a significant decrease in the impression of pores and wrinkles.

Cardiovascular: The inotropic and chronotropic impacts of an *A. millefolium* aqueous–ethanol extract on the detached heart of 24 Wistar rats were studied by Niazmand and Saberi (2010). For 30 s, the extract was administered into the heart at three doses (0.01, 0.0125, and 0.02 mg/mL). Throughout the infusion, the extract had adverse inotropic and chronotropic cardiac impacts.

Nevertheless, *A. millefolium's* negative chronotropic impact outweighed its negative inotropic impact. Thus endorses a few of *A. millefolium's* conventional applications while also suggesting new possible cardiovascular activities.

Applying the MTS test, Dall'Acqua et al. (2011) studied the effectiveness of an *A. millefolium* airy segment extract in methanol on the vascular smooth muscle (VSMC) cells proliferation on the primary rate at varied cell seeding density (2000 or 8000 SMC/well) and incubation period (24 or 48 h). At concentrations below 60 g/mL, *A. millefolium* extract promoted primary rat VSMC by partially functioning via estrogen receptors and inhibiting NF-κB signaling in human umbilical vein endothelial cells by roughly 30–40%.

Estrogenic: Innocenti et al. (2007) used recombinant MCF-7 cells to test the in vitro estrogenic potential of *A. millefolium* airy portions. The active compounds extracted from the aerial portion of *A. millefolium* (dihydrodehydrodiconiferyl alcohol 9-O-beta D-glucopyranoside, apigenin, and luteolin) are estrogenic agents. Around a level of 1.50×10^{-2} g/L, apigenin activated the estrogen receptor (ERβ), but luteolin did not. On the other hand, apigenin and luteolin turned on ER at minimal doses of 3.70×10^{-3} g/L and 2.20×10^{-3} g/L, respectively.

In a test employing recombinant MCF-7 cells, yarrow demonstrated mild estrogenic efficacy as demonstrated by Innocenti et al. (2007). Even though the identified most negligible content that exhibited any action was 8.75×10^{-5} g/L for the raw extract vis a vis 2.8×10^{-4} g/L for the methanol/water portion, the researchers suggested that after fractionation, estrogenic activity was observed in the methanolic/aqueous portion, implying that substances absent in that fraction play a part synergistically to activity.

Part in appetite: By monitoring plasma ghrelin levels, on 30 male Wistar rats, Nematy et al. (2017) explored the appetite stimulating (orexigenic) impacts of an *A. millefolium* hydroalcoholic extract. Rats were given a dosage of 50, 100, or 150 mg/kg of *A. millefolium* extract through gavage for seven days. This extract caused a considerable rise in food consumption in rats over 24 h at dosages of 50 and 100 mg/kg, whereas there was zero impact at 150 mg/kg. The explanation for the reduction in appetite upon receiving 150 mg/kg of the extract is unknown. It might, nevertheless, be attributable to a few of *A. millefolium's* adverse effects at high doses.

2.13 Cultivation, Harvesting, and Processing

Yarrow is a perennial composite family member. Although it is endemic to European regions and Western Asian countries, it is also native to Northern America. Flattened white flower heads and sharply split leaves characterize this plant. It grows well in

various climates and soil types and is simple to cultivate. Yarrow is utilized in dried flower decorations, herbal tea mixes, and medical purposes. The cosmetics sector uses essential oils steam distilled from the inflorescence in herbal shampoos and lotions. The occurrence of azulene, which generates during steam distillation, gives this oil its blue color (Guenther 1948). In bright light, yarrow thrives. Direct sowing in the field, transplants, and root division are all options for establishing yarrow. In the fall, direct-sown crops can be planted. Rhizomes propagate the plants' self-seed and older plants will lose strength, but they can be revived by cultivating strips across the crop. When 80% of the flowers are open during the bloom, the yarrow plant is picked. The second year after blossoming, we should expect an entire harvest. The stems are separated in herbal teas bypassing the dried material through a 2.5-mesh (8 mm opening) screen. Yarrow should be dried below 35 °C (95 °F) and away from direct sunshine. Dry herb yields range from 1.0 to 4.0 tonnes per hectare (0.45–1.8 tonnes per acre). The flowers of the yarrow contain the majority of the essential oil. The flower contains 0.2–0.5% oil, whereas the leaves and stems have 0.02–0.07%, according to Hornok.

2.14 Formulations and Market Product

Syakri et al. (2021) undertook a study to see how efficient anti-aging peel-off gel masks created from an ethanolic extract of yarrow (*Achillea millefolium*) gets affected by various concentrations of PVA (Polyvinyl alcohol: 7–10%) and HPMC (Hydroxypropyl methylcellulose: 2–4%) as film-forming and gelling agents. Different quality characteristics were analyzed, including the mask's organoleptic properties, uniformity, pH, syneresis, skin discomfort, fluidity, spreadability, and drying duration, which were all factors to consider. The various proportions of PVA and HPMC were used in the experiment to increase dispersal and drying rate yet did not influence viscosity value reduction. PVA and HPMC levels significantly impacted the effectiveness of the Yarrow ethanol extract peel-off gel mask ($p < 0.05$).

Andleeb et al. (2021) undertook research to produce a durable gel preparation for topical medicine delivery based on nanoethosomes abundant in *Achillea millefolium L.* (AM) extract. Phytochemical screening, antioxidant properties, total phenolics and flavonoid concentration, and FT-IR evaluation were initially performed on the AM extract. After being manufactured and evaluated by dimensions, surface charge, including shape, nanoethosomes carrying AM extract had their Entrapment Efficiency (EE) evaluated. Following that, the nanoethosomes were employed to make a topical gel composition tested for permeability, pH, viscosity, and organoleptic assessment on the skin for a maximum of 3 months. The AM ethanolic extract had phenolic concentration around 123 mg GAE/g and flavonoid contents around 42 mg QE/g and had 88% free radical scavenging activity. A surface charge of −31.1 mV was observed in nanoethosomes encapsulated with AM extract (240 nm) and was spherical (240 nm) and had a high entrapment efficiency (90%). Additionally, the

topical gel that was chosen stayed steady throughout the research. The ethosomal gel ex vivo permeation testing yielded the most remarkable release rate of about 79.8%. According to the findings, a topical gel incorporating nanoethosomes carrying AM extract for topical medication delivery is a promising method.

Till now, products out of *Achillea millefolium* are not prevalent commercially. Still, the usage of the products or the parts of the plants is restricted to the native households or used indigenously. Nevertheless, medicinal herb like *Achillea millefolium* has the great potential to be marketed for use by ordinary people as it possesses high medicinal value, which can be used for treating problems like stomach-ache, skin issues, and many more than applying chemically synthesized medicine.

2.15 Tissue Culture

In this study conducted by Chalabian et al. (2008) several hormones such as IAA, BA, NAA, Kin, and 2,4-D were used to evaluate in vitro culture of *Achillea millefolium L.* Explants included various sections of wild plants and seedlings grown on hormone-free media. A medium enriched with 1 mg L^{-1} of NAA and 2 mg^{-1} of Kin was the optimum media for callogenesis. After developing callus, the apical meristem of a seedling in media enriched with 1 mg L^{-1} of IAA and 2 mg L^{-1} of BA developed shoots. Development of calli and then shoots took place in medium supplied with 2 mg L^{-1} of IAA along with 1 mg L^{-1} of BA. When seedlings were placed in media supplied with 1 mg L^{-1} of BA, the apical meristem developed shoots that produced roots when carried to a medium complemented with 2 mg L^{-1} of IBA. As a result, this medium provided the optimum opportunity for propagation and regeneration compared to the other media.

2.16 Conservation

Knowledge and motivation are required for plant diversification conservation. In the Himalayas, religion has served an important part in conventional conservation, and there are presently many holy places and sites. Traditional doctors' outstanding expertise and social position offer a more secure platform for medicinal plant conservation in the Himalayas than anything else. Herbal physicians, often known as Himalayan botanists, have long been interested in collecting therapeutic herbs in environmentally friendly methods. Four of the world's main medicinal lineages converge in the Himalayas: Ayurveda, Chinese, Tibetan, and Unani—are practiced, the majority of people rely primarily on herbal therapy. There are a slew of other local medicinal traditions, including those related to the Bai, Dai, and other ethnic groups.

The creation of adequate management methods at the local level will be required to achieve sustainability in the wild medicinal plant commerce. The herbal industry may aid in a variety of ways. The most fundamental necessity is establishing partnerships between industry and producers (collector or farmer cooperatives) that stipulate assured and fair rates for high-quality, sustainably manufactured materials. To enhance ethno medicinal plants conservation in the Himalayas, Governments may play an important role by establishing the legislative foundation. There are numerous components, including community rights connected to nature reserves, shared forest management about forest reserves, acknowledgment of traditional medicine, and herbal industry standards. Community-based field initiatives assisted by non-governmental organizations (NGOs) and botanical institutions would be highly beneficial in creating and evaluating ideas and procedures for medicinal plant conservation in the Himalayas and identifying best practices. Suggestions based on on-the-ground expertise should be beneficial in constructing government policy. With their various policy settings and conservation histories, regional collaboration across Himalayan nations can provide many benefits. Some challenges will necessitate face-to-face collaboration (e.g., unsustainable cross-border trade).

2.17 Conclusion and Future Perspectives

Since the beginning of humankind, aromatic herbs have long been exploited in the cure of illnesses, and numerous studies in the field of herbal medications are being conducted to discover newer and safer options to treat a variety of disorders. Yarrow (*Achillea millefolium*) historically is being employed to alleviate respiratory infections, digestive problems, injuries, and inflammation. The pharmacological qualities of *A. millefolium* suggest that it might be used as a natural medication to treat various illnesses and pathologies, notably inflammatory diseases, cancer, dyspepsia, bacterial, viral, parasite, and helminth infections, among others.

Nonetheless, additional studies are essential to learning more about the mechanics behind some of their pharmacological actions, such as antifertility, gastric motility promotion, and stomach ulcer treatment, cytotoxic as well as genotoxic impacts, coronary heart disorders, and toxicology of fumigants for insect control in storage. Yarrow is harmless and well accepted in individuals who are not allergic to it, according to animal research and vast human experience. Yarrow may be readily grown on a residential or commercial scale, allowing for the long-term availability of inexpensive dietary supplements. As a result, it has much potential to help the general people by being used in alternative medicines and self-care for mild diseases. Furthermore, many of the plant's pharmacological properties have yet to be scientifically verified or ascribed to any of its ingredients. The outcomes of in vitro and pre-symptomatic studies must be rigorously assessed and incorporated within *A. millefolium's* practical uses. Offered the wide range of chemical constituents and quality of obtainable yarrow products, all yarrow studies must include details

on the origin of test specimens and their chemical makeup, such as chemical fingerprinting or measurement of certain bioactive marker molecules among a wide range of compounds. The moment has arrived for this traditional medication to join the contemporary pharmacopeia, and science must step up to the plate.

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Chapter 3
Arisaema Jacquemontii Blume, Jacquemont's Cobra Lily

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3.1 Introduction

Diseases have been associated with humans since the beginning of their existence and challenged our survival by affecting us in various ways. The current example is Spanish influenza (H1N1 virus) which caused the death of approximately 5% of the total population of the world. Such diseases undergo different mutations which lead to their evolution which in turn creates more resistant strains or species that affect humans by causing new problems such as high fever, respiratory tract infection, cough, cold to pneumonia, and sometime leads to death (Alschuler et al. 2020). Such diseases have created a terrible pandemic situation in the past. However, with the development of vaccination against these diseases, it helps to develop immunity that has lead to the survival of humans with these diseases (Pooladanda et al. 2020; Vellingiri et al. 2020). In recent years, Coronavirus outbreak happened around the world. This virus belongs to the Coronaviridae family and named due to spikes that resembles crown. Its rate of transmission is high due to genetic recombination in the S protein of the RBD region. Initially, various antibiotics having a broad spectrum

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effect and different anti-viral drugs were used to decrease the viral load. However, only remdesivir was found to show a positive effect in response to the viral effect (Shereen et al. 2020). Since ancient times, medicinal herbs are being used in various therapeutic practices not only as primary effectors but also in combination with other medications. The use of these herbs has proven to be one of the effective actions against the disease with lesser chances of any kind of side effects (Zhang et al. 2020). Plants such as *Verbascum Thapsus*, *Justicia adhatoda, Hyoscyamus niger*, *Ocimum basilicum, Plantago major,* and various other plants are well known for their effective response as antiviral. Various secondary metabolites such as alkaloids, flavonoids, and terpene are known for their antiviral effect (Amber et al. 2017).

The Himalayan region is one of the important reservoirs of the medicinal plant that is effective in antiviral, antimicrobial, and antifungal activity. *Arisaema jacquemontii* is one such medicinal plant which is found in the Kashmir region of India, and traditionally used to heal different disorders and diseases. The plant has morphological similarities to a snake therefore it is commonly known as a cobra lily. The plant comes under Araceae family and is used as food, as an anthelmintic, and in the curing of pulmonary infections, skin swelling, or irritation, and as an antagonist for snakebites (Zhang et al. 2020). *Arisaema jacquemontii* shows anti-insect and anti-proliferative effects due to the presence of lectin (Mukhtar et al. 2008). In addition, antioxidant, anticonvulsant, and immunomodulating properties were shown by the plant (Lubbe and Verpoorte 2011; Chen and Nakamura 2004). Although the plant is extensively used in traditional medicinal practices, few research has been performed to understand its pharmacological properties. This review elaborates the distribution, morphology, traditional uses, nutritional composition, pharmacology, and other aspect related to the species.

3.2 Taxonomy and Common Names

The plant *Arisaema Jacquemontii* (Fig. 3.1) is mostly known as Green, Jacquemont's Cobra lily, Cobra lily, Jack in the Pulpit, Snake lily, Cobra plant, Snake lily in English (Quattrocchi 2012; Wani et al. 2006; Roshan et al. 2017; Pandey 2006). However, in Bhutan, Nepal, China, and Tibet it is called Dav-ba, Timju, Zang nan lü nan xing, and Dahpa (Wangchuk et al. 2016) In India it is known by many names to local people, i.e., Basair, Saperi mausi, Haput, Gogej, Jinjok, Khaprya, Ki kukri, and Kirala, (Quattrocchi 2012; Roshan et al. 2017). Happat Makai (Khan et al. 2004), Meen (Singh and Rawat 2011), Kirala, Sarap (Khan et al. 2009), Bankh (Kala 2015), Khaprya, Saperi mausi (Bhatt and Negi 2006), Khyan bank, Sarpabheda (Ratha et al. 2015), and Surp, Hapat-Brand (Lone and Pandit 2007).

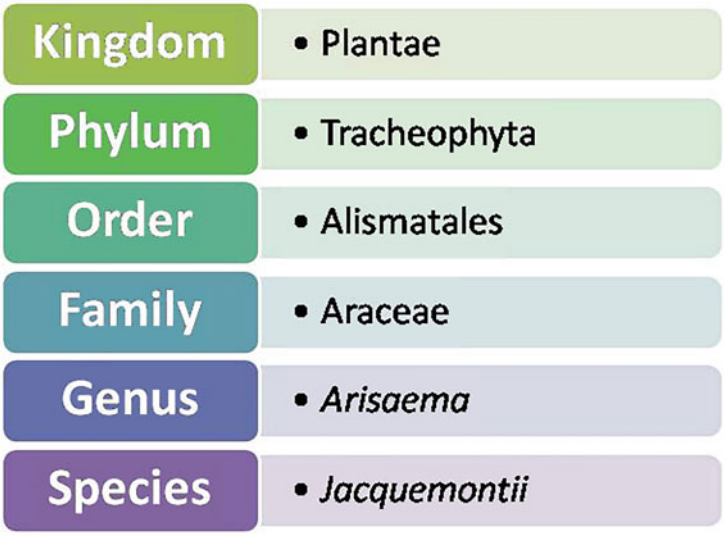

Fig. 3.1 Taxonomical structure of *Arisaema jacquemontii*

3.3 Distribution

The plant is distributed in different countries of Asia, i.e., Afghanistan, Bangladesh, China South-Central, East Himalaya, India, Myanmar, Nepal, Pakistan, Tibet, and West Himalaya. In India it is found in Shimla, Kashmir, hills of khasi (nilgiri), the north-east region, and the southern region of India. In Pakistan, its vegetation can be viewed at a range of 2400–4000 m in the upper forest as well as in the alpine zone in the drier areas of Himalayas, Lakary mountains, Shamshaki, District Karak, hilly zone of kalam Khyber pakhtunkhwa Pakistan (Ali and Yaqoob 2021; Roshan et al. 2017; Royal Botanical Gardens 2021).

3.4 Morphology

Arisaema jacquemontii Blume is native from Afghanistan to Tibet and grows to 70 cm. Tuber subglobose, 1.2–3 cm in diameter (Fig. 3.2). Cataphylls 1 or 2, membranous, apex acute or obtuse in shape, and are whitish in color and occasionally appear brown. Pseudostem, petiole, and peduncle are pale green in color and are unmarked. It has two sets of leaves, with numerous leaflets in an umbrella-like pattern. Petioles are 20–52 cm long, proximal 4/5 sheathing and forming pseudostem; ovate, oblong, or lanceolate in shape, base is attenuate, apex is acuminate; central leaflet are 7–18 × 2.5–7 cm; lateral leaflets are 3–7 × 0.8–2.5 m. Peduncle is as long as petioles. *Arisaema jacquemontii* Blume has a deep, narrow

Fig. 3.2 *Arisaema jacquemontii*

bright green spathe with white strips which is as long as the foliage or longer than that, the tube is cylindrical in shape and 3.7–5.5 cm long, the limb is curved and shape varies between oblong to oblong-ovate and it is slightly shorter than the tube. It has a hood that rises up instead of curving down like its other species and ends in a long whip. The tail is 4–8 cm long. It has a spadix with a long and purple-black or green whip. Spathe typically surrounds the spadix, bears appendage, curved, truncate (in male), and cuneate (in female). In male, spandex is 3–4.5 cm long and in female it is 5.8–6.2 cm long at post-anthesis. Spandix appears darker at the tips. Male flowers have stipitate stamens, subglobose-shaped anthers. Female flowers have subglobose ovary which is 1.5–2 mm long, subsessile style, disciform stigma, and globose berries which are 4–4.5 mm broad and turn red at maturity. Seeds are 3–8 in number and reddish brown in color. *Arisaema jacquemontii* has the ability to change sex during their lifecycle. During the flowering season, the flower which is shaped like the hood of a reptile complete with a forked-tongue-like part sticking out, starts blooming in, and at the end flowers starts disappearing giving its way to fruits with

red and green kernels resembling corn (Xing 1835; www.indiabiodiversity.org India Biodiversity Portal 2021; GBIF 2021; Plant of the World Online (2021).

3.5 Flowering and Fruiting Season

The flowering period of *Arisaema jacquemontii* is from June to July and with fruits usually looking like red berries when mature. Each plant can be a female or a male (dioecious), but not both. To get a seed, both genders need to be cultivated separately because plant is not self-fertile and thus require flies to pollinate (Roshan et al. 2017; Verma et al. 2012). The plant is approximately 2 ft. tall (0.6 m) and 1 ft. wide (0.3 m) and has 1 or 2 leaves with 5–7 leaflets. Arisaema jacquemontii generally have perennial behavior developed from tubers with flowering stem up to 50 cm tall. The fruiting season *Arisaema jacquemontii* began in late summer or early fall with fruits that look like green or red kernels resembling corn. The bright red-colored fruits have a distinctive purple or green whip-like projection that extends upright from the hood up to a foot in length.

The plant is approximately 2 ft. tall (0.6 m) and 1 ft. wide (0.3 m). Each individual member of the species additionally produces several hollow leaves, with no stalks. As these develop, they typically grow to the same approximate length as the single flower. *Arisaema jacquemontii* generally have perennial behavior developed from tubers with flowering stem up to 50 cm tall. The tubercles and older tubers help in the development of new plants in the subsequent years (Ali and Yaqoob 2021). The fruiting season *Arisaema jacquemontii* began in late summer or early fall with fruits that look like green or red kernels resembling corn. The bright red-colored fruits have a distinctive purple or green whip-like projection that extends upright from the hood up to a foot in length. The plant can grow in full shade (deep woodland), semi-shade (light woodland), or no shade and prefers moist soil.

3.6 Cultivation Practices

Soil: For the cultivation of plants of *Arisaema,* the soil needs to be humus-rich exceedingly aerated, pH should range from neutral or slightly acidic appropriate for its cultivation. Draining is crucial to avoid the rotting of tubers. The soil must be wet, leaky, and draining. Addition of various manures such as leaf mold to the soil will enhance the soil structure and quality (International Aroid Society 2021a; Adventurous Plants 2021).

Land Preparation: There is no report related to the land preparation for the cultivation of *Arisaema jacquemontii*. However, after the selection of the site, the area should be cleaned, ploughed, and leveled to create a good soil structure for the growth and development of planting materials in the production field. Generally, the purpose of land preparation is to provide suitable soil conditions necessary for the

successful establishment of the young offshoots produced in the nursery or directly planted in the production field. It helps the young plants to use growth factors including nutrients, water, sunlight, and aeration properly (Megersa 2017).

Planting material: Propagation could be raised from the seeds as well as from the tuber (International Aroid Society 2021a; himalayanwildfoodplants.com; International Aroid Society 2021b; Useful Temperate Plants 2021). It ought to be verified that the uniformity of seed/vegetative parts has to be specific to the true-to-type plant. All required details such as collecting area, grade, uniformity, and product performance must be provided for the germplasm of the raw material. Material which is tolerant to diseases should be preferred over the varieties which are less tolerant (Prajapati 2018).

By seed: To improve the quality of seed germination there is a need to treat the seeds before sowing it, stratification of seed coat, and provide a controlled environment that help the seeds to germinate to their full potential (Li et al. 2000). However, the seeds remain viable for a year in storage and they can be used in spring season under the greenhouse with a requirement of a period of cold stratification. The germination of seeds required a period of 1 month to half a year at 288.15 K (Useful Temperate Plants 2021). Its major species are dioecious due to which only female produces the seeds. Therefore, it requires two different plants having opposite gametes to make fertile seeds. The plant contains natural germination inhibitor in the berry part that is produced by plant containing an inhibitor which halts germination but it exhausts naturally in winter, that allows the seeds to sprout out in spring (International Aroid Society 2021b; Useful Temperate Plants 2021).

By Tuber: The tubers which are small in size can be stored in a refrigerator condition at 274.8–277.6 K for a quarter month, keeping them slightly moist in any of several ways. However, there is a conflict relating to the period for refrigeration. Moisture could have a bad effect on the tuber whereas the small tubers cannot survive in dry environment. To have a second crop of the year they are replanted which starts developing in a few weeks. However, it is also reported to have a third crop benefit if the month of winter is found to be sufficient (International Aroid Society 2021b; Useful Temperate Plants 2021).

Planting distance: A prime focus should be given to the sowing rate, depth, plating time, depth, and method. These must be auctioned accurately and in a timely manner for good growth of the plant (Prajapati 2018). Spacing between each plant and the time period of planting has a great impact on plant growth, yield, and bioactive constituents. Distance between each plant and two rows must be kept at 23.6–27.6 in. × 11.8–13.8 in. Tuber which is smaller in size should be planted at a small depth (2.6–2.9 in.). For the planting of tuber, the best season is from mid to late autumn to spring. However, they are mostly ready to be marketed by the late winter (International Aroid Society 2021b).

Irrigation: After planting light water should be poured to settle the soil around the plant. Sprinkles are a good technique for irrigation. In addition to irrigation, proper drainage is important in the field. Without proper drainage, plants will go under dormancy in winter (Easy to Grow Bulbs 2021). In addition to that, if soil parameters and the cultivating conditions are up to mark, the soil's moisture should be retained

and must not dry out extensively. However, the water requirement for the plant is not too high. As such, Arisaema should not require too much extra watering, beside the conditions such as peak of summer or in extremely hot weather intervals of a year. Plants that are water-stressed tend to make it pretty obvious: wilting leaves, crispy edges to the foliage, or premature autumn color are all signs that the plants may need a drink. The best period for watering in the day is evening using a sprinkler setting (Adventurous Plants 2021).

Manure and fertilizer application: In general practice, use of fertilizer is dependent on the location. For the initial preparation of the field 200–250 q/ha of decomposed organic manure must be added. Fertilizers need to be applied as needed by the crop (Prajapati 2018). Arisaema is a kind of plant that shows a positive effect on extra nourishment, specifically at the initial period of development when the leaves are unfurling. A prolonged development season in combination with the extensive availability of nutrition will lead the tuber to gain its volume and mass faster, which outcomes in definitive flowering. Sprinkling of chicken pellets in dried form around the plant leads to the good development of the plant. Further, a solution of seaweed extract and water could be watered to the soil. The fertilization of plant should be strictly avoided when the plant is dormant (Adventurous plants 2021).

3.7 Conservation

Many researchers have yet not assessed the conservation status of *Arisaema* species, but this plant is most likely at the risk of extinction (Prabhukumar et al. 2017). Currently, the IUCN (International union for conservation of nature) does not consider the Cobra Lily to be threatened at present. The flora is placed as "Least Concern" in the Red List of Threatened Species (Red List 2021). With a large range in remote high-elevation areas *Arisaema jacquemontii* requires no attention for practicing its conservation. Its habitat is mostly secured and does not emerge as safe at present. However, different species of *Arisaema* face challenges due to the increase in global warming worldwide (Our Breathing Planet 2021) (www.ourbreathingplanet.com).

3.8 Phytochemistry

Phytochemicals are naturally occurring bioactive components of plants, and their abundance and secretions differ from plant to plant. There are many significant classes of phytonutrients, including flavonoids, alkaloids, campesterol, glycosides, terpenes, oxalates, phenols, n-alkanes, tannins, saponins, n-alkanols sitosterols, stigmasterols, coumarins, lectins, triterpenoids, etc. They significantly contribute to human health in a variety of ways, including the effect on cell differentiation, maintenance of DNA repair, antioxidant properties, increased activity of detoxifying

enzymes, increase in cancer cell apoptosis, effect on DNA metabolism, and decreased cell proliferation. (Thakur et al. 2020). There have been different choices of solvents for the extraction of phytochemicals from plants. For example, various solvents such as methanol, hexane, and ethyl alcohol are used to extract antioxidants from plant parts (leaves, stem, and seeds). For the extraction of phenolic compounds from plants, various solvents of different polarities are used. Therefore, the solvents used to extract the bioactive component from plants are chosen based on the polarity of the solute of interest. Eloff (1998) discussed that the factors affecting the choice of solvent are the quantity of components to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, the toxicity of the solvent in the extract, potential health hazard of the extractants, etc. Although plants have various biological and pharmacological activities, there is very little literature showing the nature and class of compounds found in the *Arisaema jacquemontii.*

Sudan et al. (2014) conducted a phytochemical investigation of *Arisaema jacquemontii* and discovered that the plant contains terpenoids in five distinct fractions (Hexane, Acetone, Chloroform, Methanol, and Ethyl acetate). Additionally, coumarins, quinones, and glycosides were detected in all fractions. Further, hexane, chloroform, acetone, and methanol all included alkaloids and anthraquinones. The methanol extract contained flavonoids, whereas the acetone, ethyl acetate, and methanol fractions contained phenols in moderate amounts. Tanveer et al. (2013) and Jeelani et al. (2010) have isolated a triterpenoid 2-hydroxydiplopterol (1) from chloroform extract and 30-nor-lanost-5-ene-3β-ol (2) and 30-norlanost-5-ene-3-one (3) from methanol extract of the root of the plant. The earlier studies carried out by Iqbal et al. (2018) revealed the presence of oxalic acid (4) and calcium oxalate crystals (5), carbohydrates, tannins, protein, flavonoids, oils, sterols, alkaloids, and saponins. Besides that, several research demonstrated the existence of glycosides, terpenoids, flavonoids, quinones, phenols, coumarins, phenolics, alkaloids, and anthraquinones (Sudan et al. 2014; Baba and Malik 2015). Apart from that, *Arisaema jacquemontii* contains Arginine (6), Aspartic Acid (7), Leucine (8), Serine (9), Threonine (10), Tyrosine (11), Arisaeminone (12), valine (13), 13-phenyltridecanoic acid (14), Alanine (15) (Hiller 2001; Khan et al. 2007; Jeelani et al. 2010; Banyal et al. 2014).

Arisaema franchetianum is well known and is of medicinal value due to the presence of biologically active compounds. The chemical constituents like pyrrolidine alkaloid (2R*, 3S*,5S*)-N,2-dime thyl-3-hydroxy-5-(10-phenyldecyl) pyrrolidine (16) (Su et al. 2013), and many different compounds like bergenin (17; Fig. 3.3) (Miglani et al. 1978), nobiletin (18) (Jung et al. 1996), caffeic acid (19) (Zhao et al. 2010), emodin (20) (Ducki et al. 1996), perlolyrine (21) (Wang et al. 2007), qingyangshen genin (22) (Lamkadem et al. 2005), methylconiferin (23) (Nunomura et al. 2009), gagaminine (24) (Tao et al. 2011), indolo [2,3-a] carbazole (25) (Sun et al. 2009), 1-methoxycarbonyl-b-carboline (26) (Ma et al. 2007), and 4-hydroxycinnamic acid methyl ester (27) (Lee et al. 2000) have been extracted from *Arisaema franchetianum* tubers. The extracts obtained from *Arisaema amurense* revealed the presence of the compound 2,3-dihydroxypropyl,

9Z,12Z octadecadienoate (28) (Chung et al. 1995), Cytotoxic diacylglycerylgalactosides and antihepatotoxic cerebrosides (Jung et al. 1996). *Arisaema dicipiens* is one of the perennial herbaceous plants; three known tetranortriterpenoids and a novel piperidine alkaloid were obtained from rhizomes of this plant, and their chemical structures were identified as (−)-(2R*, 3S*, 6S*)-N,2dimethyl-3-hydroxy-6-(9-phenylnonyl) piperidine (29), 6-deacetylnimbin (30), 28-deoxonimbolide (31), and nimbin (32) Zhao et al. (2010). *Arisaema flavum* is a

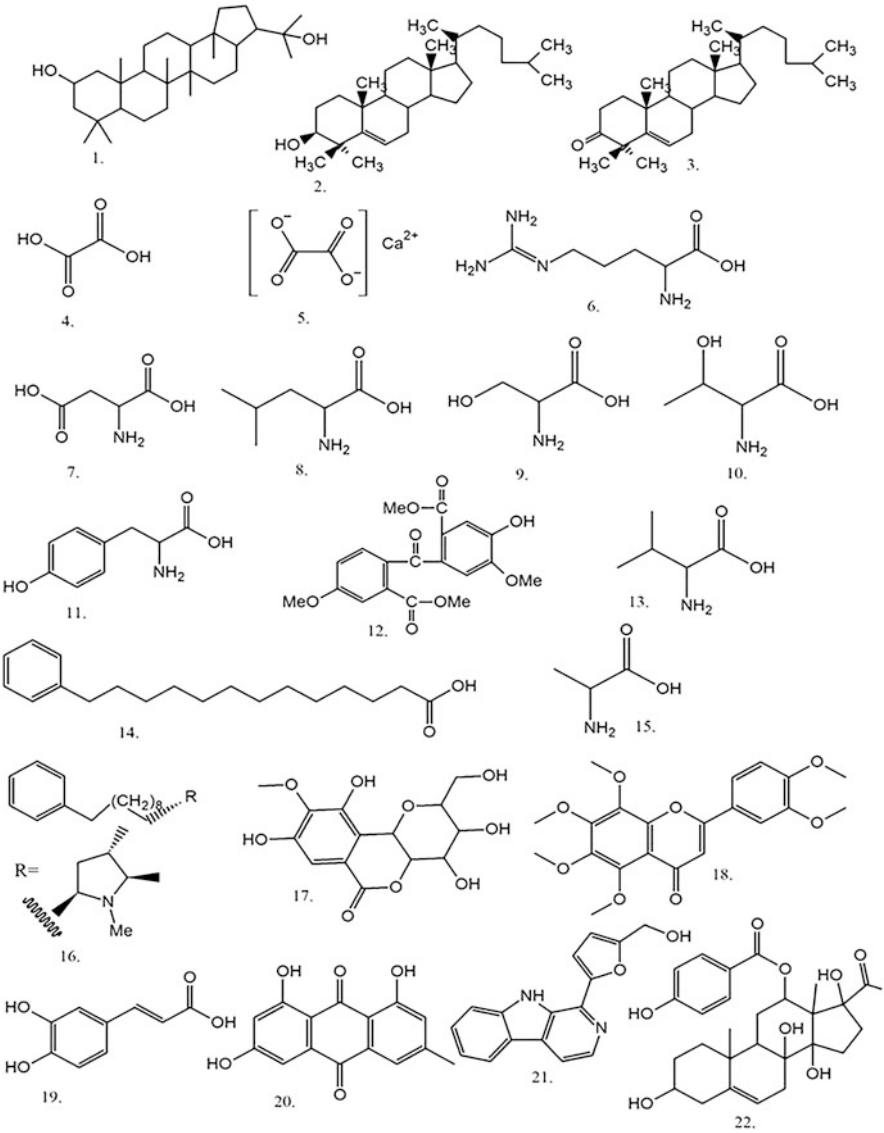

Fig. 3.3 Compounds identified in *Arisaema jacquemontii*

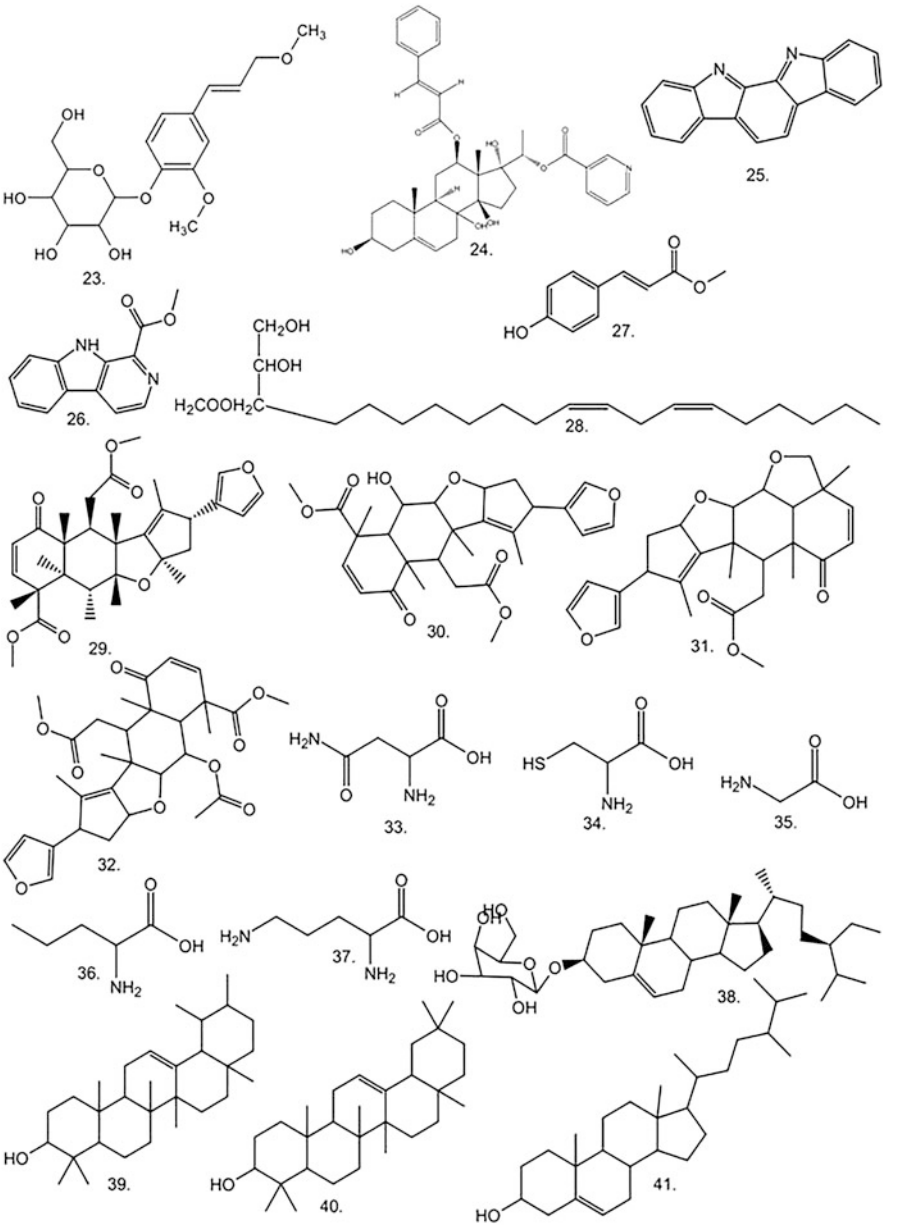

Fig. 3.3 (continued)

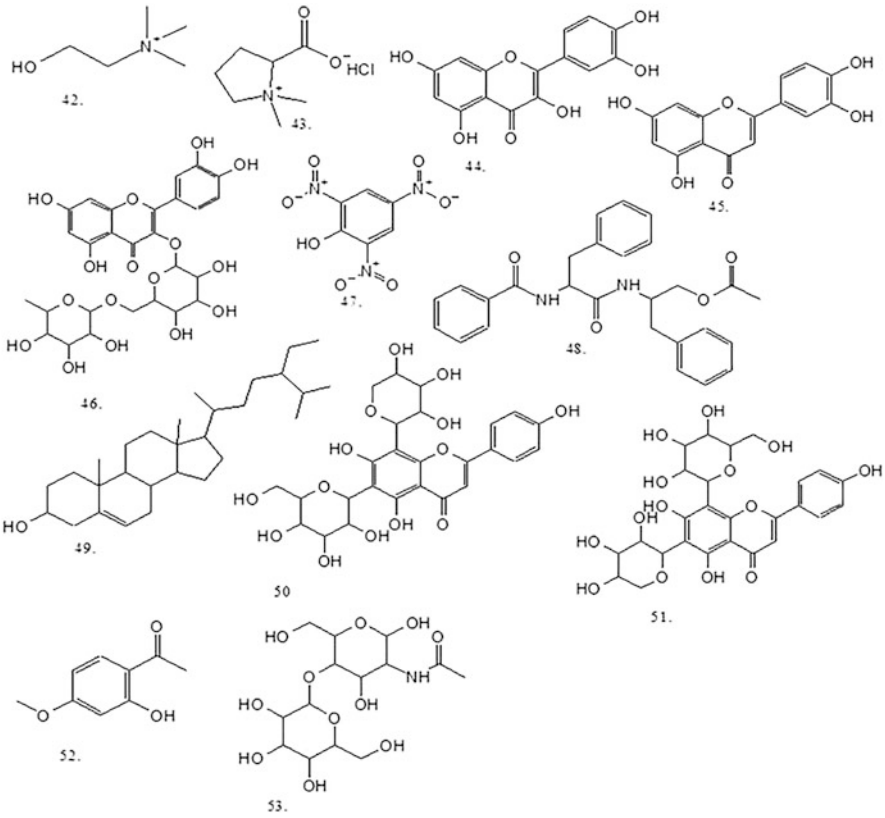

Fig. 3.3 (continued)

flowering plant known for that contains lectins, 13-phenyltridecanoic acid (14), Asparagine (33), Cysteine (34), Glycine (35), Norvaline (36), Ornithine (37), ß-setosteryl Galactoside (38), α and β amyrin (39 and 40) (Rastogi and Mehrotra 1979; Singh et al. 2004). *Arisaema tortuosum* tubers contain n-alkanes, n-alkanols, stigmasterols, sitosterols, campesterol (41), cholesterol, choline chloride (42), stachydrine hydrochloride (43), quercetin (44), Luteolin (45) (Dhuna et al. 2005); however, this plant contains flavonoids, alkaloids, saponins, and triterpenoids (Kamble et al. 2010). A phytochemical characterization study confirmed the presence of quercetin, rutin (46), luteolin, and lectin in *Arisaema tortuosum* (Nile and Park 2014). Picric acid (47), tannin, protein, sugar, anthraquinone, polyphenols, and other phytochemicals were present in the aqueous extract of *Arisaema murrayi*. In contrast, its alcoholic extract was found to contain glycosides, alkaloids, and flavonoids (Sagar et al. 2014). Paeonol and a crystalline substance designated as aurantiamide acetate (48) were extracted from dried *Arisaema erubescens* in methanol (Ducki et al. 1996). Several studies have demonstrated that the rhizome of *Arisaema* is abundant in beta-sitosterol (49), triterpenoids, dipeptides, alkaloids, saponins, and lectins (Shangary et al. 1995; Wang et al. 1997; Jeelani et al. 2010). The ethanolic extract of tubers of *Arisaema erubescens* (Wall.) yielded two

nematocidal lavone-C-glycosides, namely Schaftoside (50) and isoschaftoside (51) (Du et al. 2011).

Various chemicals substances, including paeonol (52), calcium oxalate (5), aurantiamide acetate (48), alkaloids, fatty acids, monoterpenoids, and flavonoids, have been obtained from *Arisaema erubescens* (Ducki et al. 1995). Phenolic compounds, triterpenoids, saponins, steroids, amino acids, alkaloids, anthraquinones, tannins, reducing sugars, catechins, and flavonoids were detected in ethanolic extracts of *Arisaema leschenaultii* Blume (Vinay et al. 2010; Selvakumari 2015). It is rich in triterpenoids, steroids, polyphenols, saponins, and terpenes but moderate in glycosides, tannins, anthraquinone, phylobatannins, carbohydrates, and flavonoids (Kumari and Narasimhan 2003; Gunde et al. 2009; Vinay et al. 2010, 2015; Vinay and Panigrahi 2012, 2010; Suruse et al. 2011; Suresh et al. 2017). N-acetyl-D-lactosamine (53) was detected in the methanolic extract of *Arisaema utile* (Mubashir and Shah 2012). The lectin from *Arisaema curvatum* Kunth was purified utilizing affinity chromatography with asialofetuin-linked amino-activated porous silica beads. Two novel lectins were isolated from the tubers of *Arisaema intermedium* and *Arisaema wallichianum* using affinity chromatography on asialofetuin-linked amino-activated silica beads (Kaur et al. 2005). Similarly, the lectin from the tubers of *Arisaema curvatum* was purified using affinity chromatography (Singh et al. 2008). Certain alkaloids, such as 2-alkylpyrrolidine and alkyl piperidine, have been isolated from *Arisarum vulgare*. Oxalates were identified in *Arisaema airorubens, Arisaema stewardsonii,* and *Arisaema Triphyllum* plants (Pandita et al. 2014). From the *Arisaema anurans*, oxygenated phenylpropanoids and terpenes were extracted (Jia et al. 2018). Lectin was also identified in *Arisaema helleborifolium* plant (Kaur et al. 2006). A plant lectin was extracted from the tubers of *Arisaema utile*, it is a homotetrameric protein with a molecular mass of 54 kDa and a subunit molecular mass of 13.5 kDa (Dhuna et al. 2010). The *Arisaema yunnanense* plant has been found to adulterate the tubers of another plant, *Pinellia ternate* (Liu and Guo 2010). Occasionally, the tubers of the plant develop small tubercles that separate from the tubers and might serve as the origin of a new plant in preceding years. A basic lectin was isolated from the bulbs of *Arisaema ringens*. This lectin has two carbohydrate binding sites and has been discovered as mono oligosaccharides and terminal N acetyllactosamine (Yagi et al. 2008).

3.9 Traditional Uses

The natural world is filled with indigenous methods, knowledge, and beliefs which are applied traditionally using medicinal plants singularly or in combination to provide healthcare and has developed over generations and proved the significance of the use of medicinal plants in traditional medical treatment. *Arisaema jacquemontii* is a very important plant from a medicinal point of view. It has been adopted in various countries to treat comorbidity. This plant is reported beneficial against various skin problems, antidote for snake bites, and relieves malevolent growth of tissues and bones, obstruction, impotence, and diseases related to female

reproductive system (Wangchuk et al. 2016). The tuber lectin, an important constituent of this plant is being used for medicinal purposes due to its potent anti-insect and anti-proliferative properties (Jeelani et al. 2010; Kaur et al. 2006). The plant *Arisaema jacquemontii* can be highly poisonous if they are eaten raw causing an extremely obnoxious sensation. The leaves of *Arisaema jacquemontii* are used to break down the toxic ingredients. They are also counterpoised by rigorously drying the plants or by macerating in water (Verma et al. 2012; Tanveer et al. 2013). The dried powder of the roots of these plants is used to treat boils by mixing the dried powder with ghee or oil and applied externally. Fruits, Roots, and rhizomes of the plants are also used to treat worm infestations, fever, stomach problems, toothache, scabies, lung infections, and menstrual illness (Pandey 2006; Ratha et al. 2015; Malik et al. 2011). It has also been used as food in various countries. The tubers of this plant are boiled and eaten like potatoes. In Nepal, this plant is used as food and in the preparation of alcoholic beverages (Verma et al. 2012; Ratha et al. 2015). In India, they are crushed and used for the preparation of juice to treat ringworm and dermatosis (Frohne and Pfainder 2005). The tuber and rhizome of this plant are also used as an antidote to snake bites and poisonous mushrooms in North-Western Himalayas region of India (Kala 2015; Bhat et al. 2013). Tubers are also used to treat chronic boils, ruptured wounds, pimples, blisters, cough, respiratory tract infection in cows and buffaloes, and other skin and kidney diseases of humans (Khan et al. 2007; Kaul 1997; Singh and Rawat 2011). Rhizome is dried and ground to make a fine powder which is used on infected sites of snake bites, pimples, and blisters. Fruits and powdered rhizomes are also used for psychic and nervous disorders due to their sedative properties and usually given in small quantity during meal. It is also used for relieving body pain. Rhizome paste made with oil proved beneficial to recover muscle-bound strength and dermatosis through massage therapy (Khan et al. 2004; Sheikh et al. 2016; Mir 2014; Singh et al. 2014). Skin eruptions and skin infections such as cold urticaria which result due to cold temperatures can be treated by water extracts of *A. jacquemontii* bulbs (Wani et al. 2006).

3.10 Pharmacology

Different extraction methods extracted different amounts of bioactive molecules from *Arisaema jacquemontii* Blume. Experimentally, it has been found that methanolic and ethanolic extracts have higher amounts of bioactive molecules that have great potency against different ailments like oxidative stress, cancer, apoptosis, etc. In some studies, it has been reported that the extract of this plant has higher free radical scavenging activity. Research work also reported that in in vitro studies, extracts of this plant inhibit the growth of prostate cancer cell lines, lukemic cancer cell lines, and psychic disorders (Jeelani et al. 2010; Ali and Yaqoob 2021). Root extracts and tuber extracts of this plant are highly enriched with terpenoids and lectins. These molecules are potent agents against inset and inflammation. They help in erythrocyte coagulation after binding with the carbohydrates that are present on the surface of the erythrocyte cell membrane (Kaur et al. 2005, 2006; Dhuna et al.

2005). The immune system is most effective after treatment with this plant extract (Singh et al. 2004). The extract of this plant is most effective as an antimicrobial, antifungal, and antibacterial agent (Baba and Malik 2015; Cushnie and Lamb 2005). This has a higher concentration of flavonoids, which are most effective against bacterial infection (Baba and Malik 2015; Cushnie and Lamb 2005). In the case of other plant species' extracts of this *Arisaema* species are also highly enriched with lectin, pyrrolidine alkaloid, piperdine alkaloid, N-acetyl-D lactosamine, etc., and they are being used as powerful bioactive molecules against snake venom, mitogenic activity, anticancerous (cell line of blood, breast, colon, liver, neuroblastoma, prostate, etc.) activity, anti-inflammatory, anti-helminthic (Ito et al. 1996; Dhuna et al. 2010; Ali and Yaqoob 2021; Su et al. 2013; Mir et al. 2020; Singh et al. 2004).

3.11 Conclusion

There are various medicinal plants which are distributed among the different regions of the world and have been utilized on the basis of their traditional uses and pharmacological studies. However, the investigations related to *Arisaema jacquemontii* and their pharmacological studies are still in scares. Therefore, there is a higher need to further studies to be conducted on the phytochemical and its medicinal uses. Among the present studies, it is visible that there are species in *Arisaema* genus which will have numerous medicinal uses that can be explored on the basis of previous studies and present-day uses. Various classes of secondary metabolites like tannins, anthraquinones, steroids, triterpenoids, reducing sugars, alkaloids, phenolic compounds, flavonoids, saponins, amino acids, and catechins have been isolated from the *Arisaema jacquemontii* and other species of *Arisaema* which have the potential to use against various disease and ailments. Some of the species have been studied for insecticides, fungal pathogens, and antimicrobial activity. These properties of species could be a major contributor to the industrial use of these plants for therapeutic and other beneficial uses. The medicinal values expressed by the genus have out laid that it would play an extensive role in the modern industry of medicinal drugs as well as in therapeutics.

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Chapter 4
Species of Arnebia Genus Found in the Western Himalayas: *Arnebia euchroma (Royle ex Benth.), Arnebia benthamii (Wall. Ex G Don) Johnston, Arnebia guttata Bunge*

Kanika Dulta, Gurpreet Kaur, Keshav Kumar, Mohd Mazhar, Garima Bhardwaj, and Ajay Sharma

4.1 Introduction

Plants, animals, microbes, and the oceans are all sources of natural goods. For many decades, plant components that have been discovered and isolated have been employed as a starting point for a variety of treatments. Approximately 40% of pharmaceutical medications used today are primarily derived from natural sources. Natural products are important for the development of new therapeutic agents due to their widespread abundance in nature. This leads to the spotting of bioactive compounds, which allows for the development of new pharmacological medicines as well as a tool for clarifying complicated molecular and cellular mechanisms of

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action involved in a pathological and biological processes. Due to the rising interest in the use of pharmaceuticals in recent years, natural substances have become the principal source of alternative or complimentary medicines, which are later used in the treatment of a wide range of problems (Calixto et al. 1998). The Arnebia genus was initially described in 1775 by Pher Forsskal and is primarily found in Asia. *Among the major genera in the Boraginaceae family are Pulmonaria, Pentaglottis, Echium, Symphytum, Lycopsis, Anchusa, Arnebia, Borago, and Onosma, which include 1600–1700 species in around 90 genera* (Abbaspour et al. 2009). *Arnebia* is a genus with 25 species that can be found all over the world (Kashiwada et al. 1995) from Africa to Asia. *Arnebia* chemical components are prominently found in the root's outer layer and have a variety of pharmacological functions (Ashkani-Esfahani et al. 2012a, b). *Arnebia* species can be found from north to south in Iran. In India, the genus Arnebia is represented by ten taxa, comprising eight species and two varieties, *A. benthamii* (Wall. ex G. Don) I.M. Johnst., *A. euchroma* (Royle) I.M. Johnst., *A. guttata* Bunge, *A. hispidissima* (Sieber ex Lehm.) A.DC., *A. linearifolia* A.DC., *A. griffithii* Boiss., and *A. nandadeviensis* are all species of A. DC. K. Chandra Sek. & R.S. Rawal, *A. euchroma* var. grandis (Bornm.) Kazmi, and *A. guttata* var. thomsonii (C.B. Clarke) Kazmi, a genus that can be found mostly in Jammu and Kashmir, Himachal Pradesh, and Uttarakhand in the North-West Himalayas, as well as in Uttar Pradesh, Punjab, and Rajasthan in other parts of India (Ambrish and Srivastava 2014). The species was also designated as endangered (E) in Himachal Pradesh in 2010 and critically endangered (CR) in Uttarakhand and Jammu and Kashmir in 2003 (Rather et al. 2018). *Arnebia euchroma* (Royle ex Benth.) is a valuable medicinal plant species commonly also known as Ratanjot that grows in the western Himalayas (Aswal and Mehrotra 1994). Because the plant has a variety of medical qualities, merciless harvesting is continuing, leading to a population reduction and a severely endangered status for this taxon. In addition to its other uses, powder made with root is used to ease coughs and lung-related problems. Shikonin derivatives, which were isolated from the roots of *A. euchroma*, have been shown to have antibacterial, anti-inflammatory, and anti-tumour properties, making them significant substances with potential medical applications (Houghton et al. 2005). *Arnebia benthamii* (Wall. ex G. Don) I. M. Johnston as Critically Endangered in the Northwestern Himalayas. It is known locally as *Gul-e-Kahzaban* or *Gaozaban* and has a wide range of medical properties. Plants include a variety of key phytochemicals that are effective against a variety of illnesses. "Himalayan Arnebia" is another name for this shrub. Overgrazing, overexploitation for local usage, and landslides are the most common risks. Efforts are currently being made to conserve this medical plant from extinction. Antiseptic, antifungal, antibacterial, anti-inflammatory, and wound-healing capabilities are all present in the plant (Harborne and Baxter 2001; Manjkhola et al. 2003; Sastri 1962). *A. guttata Bunge* is frequently used to treat a variety of illnesses in China and other nations, including viral infection, inflammation, arthritis, and cancer (Wang et al. 2019). Major active chemical component of Zicao is Shikonin (SKN),(+)-5,8-dihydroxy-2-(1-hydroxy-4-methyl-3-pentenyl)-1,4-naphthoquinone (Andújar et al. 2013). The roots include the secondary metabolites Arnebin 1 and Arnebin 3, which are reported to have

anticancer potential (Kashiwada et al. 1995). This research aimed knowledge on both modern and traditional usage of the Arnebia species as a medicinal herb.

4.2 Taxonomical Classification, Distribution, and Morphology

Kingdom: Plantae
Division: Tracheophyta
Class: Magnoliopsida
Superorder: Asteranae
Order: Boraginales
Family: Boraginaceae
Tribe: Lithospermeae
Genus: *Arnebia*
Species: *A. benthamii, guttata, euchroma*

Common Names: "Gul-e-Kahzaban" or "Gaozaban" is a local name for *A. benthamii* and its English name is Himalayan Arnebia. Ratanjot for *A. guttata* bunge.

Distribution and Morphology: Boraginaceae is a plant family found worldwide, particularly in tropical, subtropical, and temperate areas. Most variety in the northern temperate zone can be found in Irano-Turanian and Mediterranean regions. It is a tropical plant family that is found in Asia, Central America, North and South America. Boraginaceae has a wide variety of morphological features. It mainly consists of herbaceous species, but includes lianas, shrubs, and trees as well.

4.2.1 Arnebia benthamii *(Wall. Ex G. Don)*

Hairy perennial herb with a robust rootstock that grows 40–80 cm tall. Stems are fistular, hispid, simple, and tightly packed with white trichomes with tuberculated bases. They are also leafy. Basal leaves are narrowly lanceolate or oblong-lanceolate, 12.5–20.5 1.5–2.5 cm, attenuate at base, acute or obtuse at apex, entire on margins, whitish-hairy on both surfaces with spreading trichomes issuing from tuberculate roots, 3–5 nerved, cauline leaves are smaller.

Distribution: A plant that grows in alpine altitudes between 3000 and 3900 m. It grows at altitudes of 3000–4300 m above sea level in the alpine and subalpine Himalayas of the Hindu Kush Himalayan range in India, it grows report in Himachal Pradesh, Jammu and Kashmir and Uttaranchal.
Flowering and fruiting: August–September

4.2.2 Arnebia euchroma *(Royle ex Benth)*

Perennial, erect herb, 30–40 cm tall, with thick rootstock of purple dye. Many stems emerge from the root base and the leaf axils, densely covered in thick trichomes with white tuberculate roots. Petiole lanceolate basal leaves, 2.8–14.5 cm long, 0.5–1.2 cm wide, ciliate acute at apex, whole at margins, densely coated with thick trichomes of white tuberculate bases on both sides, Cauline leaves are sessile, ovate-lanceolate, 2.5–8.5 × 1–2.1 cm, upper ones much shorter and broader than lower ones, subcordate at base, acute at apex, glandular-hairy on both surfaces, entire on margins.

Distribution: This plant grows through western Himalayas at heights ranging from 3200 to 4500 m above sea level. Five of the three Arnebia species can be found in Spiti, a frigid desert that is a component of the trans-Himalaya and Temperate Zone that includes Himachal Pradesh, Jammu and Kashmir, Uttarakhand (India), Pakistan, and Nepal. It grows in the mountainous regions of Iran, especially in the north.

Flowering and fruiting: July–August

4.2.3 Arnebia guttata *Bunge*

Perennial hispid plant up to 30 cm tall, erect to suberect or procumbent, with a thin purple dye-stained tap root. Stems with stiff, white trichomes on tuberculate bases, grooved, simple, or briefly ascending branched. Leaves sessile, basal lanceolate to oblanceolate, margins coriaceous, coated in white trichomes on oblong-ovate, shorter and narrower; oblong-ovate, shorter and narrowed cauline leaves terminal, globular cymes, 1–2 cm across, bracts as long as calyx, inflorescence 1–3.5 0.5 1.2 cm, acute-acuminate at apex, whole on both surfaces, 1–2 cm. Flowers are orange or yellow, with a woolly stalk and a diameter of around 1 cm. They have a variety of styles. White trichomes cover the calyx, which is upright and has linear lobes.

Distribution*:* It has been observed in Himachal Pradesh, Jammu and Kashmir (India), Pakistan, and Tibet.

Flowering and fruiting: June–August (Fig. 4.1)

4.3 Ethnobotany and Traditional Uses of *Arnebia* Spp.

Arnebia is a plant that is being traditionally used in Unani, Ayurvedic, and Chinese medicines for a very long time. Previously, local experts mixed the liquid generated by *A. euchroma* with beeswax to make “Ghuriti”, which was used for hair remedies,

Fig. 4.1 (**a**) *Arnebia benthamii* (Wall. ex G. Don) (**b**) *Arnebia euchroma* (Royle ex Benth.) (**c**) *Arnebia guttata* Bunge

as well as for chronic illnesses, cough and cold, and other ailments by local experts (Hosseini et al. 2018; Jain et al. 2000). *Arnebia* plant dried root extract is useful in treating menorrhagia, as well as cough and lung problems. The root fibre turns red when combined with apricot or mustard oil, and it can be used to treat hair loss and dandruff. Burns and other skin diseases can also be treated using the roots (Pirbalouti et al. 2011). Further this plant can also be used to colour cups, dye textiles, and prepare a variety of dishes. To increase fertility, the entire plant can be combined with dirt (Uniyal et al. 2002). The root extract is used by experts in the western Himalayas to manufacture native remedies to purify blood and coughing (Shen et al. 2002). Spiti is a chilly desert region in India's Himalayan region where this plant's root colour is blended with mustard oil for hair strengthening, colouring Chog, Chatni, pickle, and other foods (Sharma et al. 2018). In the Spiti Valley, the root powder is used to treat coughs and lung ailments (Chawla et al. 2021). However, in addition to being used as a hair tonic by the inhabitants of Himachal Pradesh's Spiti region, it is also used to cure toothache, earache, eye sickness, wound healing, burns, and as a remedy for wounds (Chauhan 2011). Furthermore, the herb is utilised to cure backaches, colds, and blood vomiting (Singh et al. 2009). The roots of plant are mixed with butter for the purpose of treating wounds (Kosger et al. 2009). In Chinese traditional medicine, *A. euchroma* decoction, is used to treat cutaneous, heart disease, post-herpetic neuralgia, and dermatitis (Liang et al. 2013; Ma et al. 2014; Tse 2003). The genus *Arnebia* has four species in Turkish flora, in which, *Arnebia densiflora* is common in the Sivas area which is locally called as "egnik" and is used as a red dye for fitted carpet and mats. *A. euchroma* is one of the top 10 herbs for treating psoriasis (Tse 2003). *A. euchroma*, a traditional medicinal plant from India's freezing desert Ladakh, is used to treat a wide range of renal and urinary issues, as well as to calm, control urine discharge, and reduce inflammation and bleeding in the kidneys (Ballabh et al. 2008). Because of the severe side effects of synthetic medications, the study of this herb's phytochemistry is gaining importance in medical science (Gaury and Devi 2017). Previous study has shown that naphthoquinone can be used to treat HIV/AIDS and cancer (Kashiwada et al. 1995). Shikonin is a major phytochemical of this plant and is used to treat conditions like hair loss, toothaches, hearing impairment, eyesight problems, coughs, and colds

(Buck 1987). *A. euchroma* was previously studied for its pain-relieving capabilities by Aliasl et al. (2015)). It is also beneficial in the treatment of kidney-related problems and urinary diseases. *A. euchroma* extracts were proven to suppress adipocyte signalling and glucose sensitivity and could thus be used to cure diabetes in a clinical experiment (Lee et al. 2010; Ou et al. 2017).

A. euchroma crude extracts and bioactive components have been linked to a variety of biological functions and have potential health advantages.

4.3.1 Arnebia *in Persian Medicine*

According to Persian medicinal literature, the nature of four components forms the nature of all beings. Every ingredient has its own distinct characteristics. Some characteristics of objects will emerge as a result of the activity and interaction of these four elements, which are referred to as nature or temperament. Some of the properties of an item are controlled by these four factors. They have solidity and shape because of the soil, flexibility, and formability because of the water, lightness, and porosity because of the air, and motion because of the fire. All beings have a different ratio of these four pillars, which explains why they have diverse temperaments. Medicines are classified into four degrees based on their qualities. The first degree is linked to a small amount of medicine that has no dominating quality in the body, but higher and repeated dosages will cause minor changes in the body's quality. The second degree is a modest dose of medicine that produces a dominant quality in the body, and higher and repeated doses are not dangerous. The third degree is associated with a trace amount of medication that develops a dominant quality in the body, and higher and repeated doses are toxic but not lethal, but the fourth degree is lethal (Mirzaee et al. 2017; Tse 2003).

4.4 Phytochemistry

The activities of the plant are due to several secondary metabolites which are produced in the plant in response to many intrinsic and extrinsic stimuli. Many phytoconstituents like alkaloids, terpenoids, phenols, and quinones have been identified in *Arnebia spp.* (Kumar et al. 2021).

4.4.1 A. benthamii

Methalonic and ethanolic extract of *A. benthamii* showed the presence of alkaloids, phenols, flavonoids, saponins, glycosides, tannins, terpenoids, steroids, and carbohydrates (Fayaz et al. 2017). High-performance thin-layer chromatography

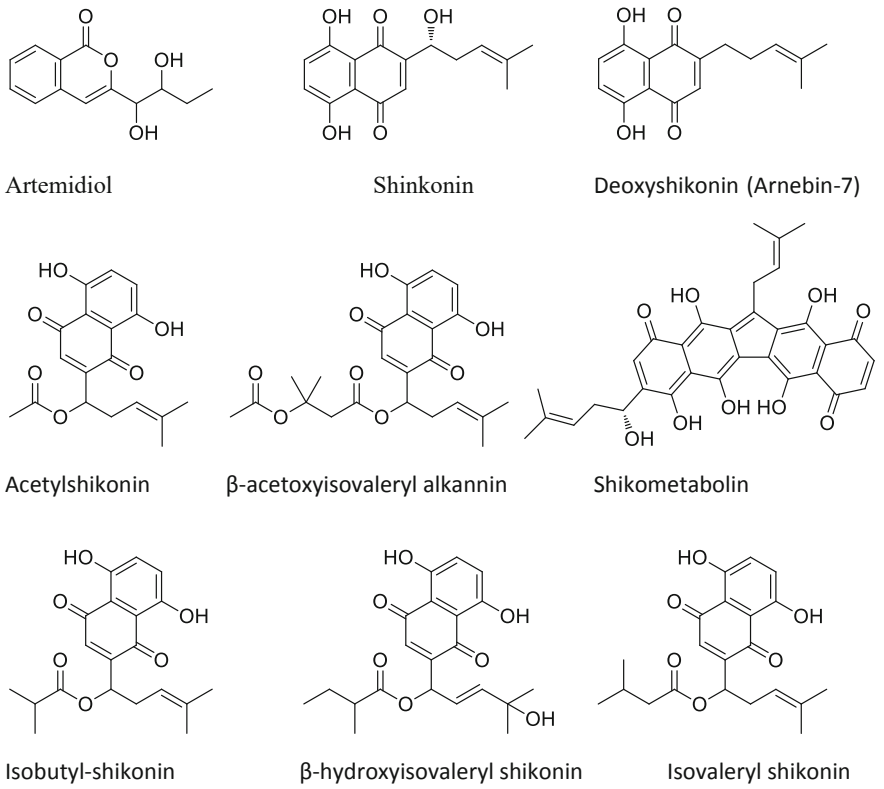
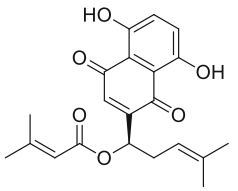
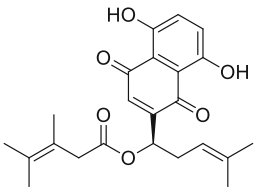


Fig. 4.2 Structure of key secondary metabolites obtained from different species of *Arnebia*

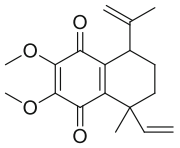
(HPTLC) analysis revealed the major active components shikonin (Rf, 0.37) and β,β-dimethylacryl shikonin (Rf, 0.58) in *A. benthamii* (Fig. 4.2) (Katoch et al. 2016). Similarly, (Sharma et al. 2009) showed the presence of naphthoquinone shikonin, acetylshikonin and beta-acetoxyisovaleryl shikonin in the *Arnebia* spp. Moreover, column chromatography of chloroform extract of *A. benthamii* displayed kaempferol, aromadendrin, sitosterol, and kaempferol-7-o-methyl ether (Rather et al. 2018). In another study, the major component shikonin has been isolated from the roots of *A. benthamii* (Parray et al. 2015). In addition to it, pyrrolizidine hepatotoxic alkaloids, heliotrine, echimidine, and lycopsamine, have been reported from *A. benthamii* from Neelum Valley of Azad Kashmir (Ahmad et al. 2018) (Table 4.1).



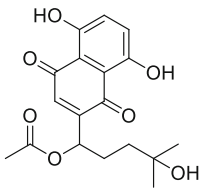
Arnebin-I (β,β-dimethylacryl shikonin)



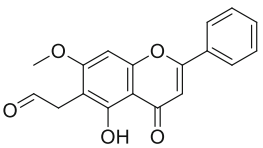
Teracrylshikonin



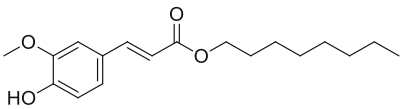
Arnebinone



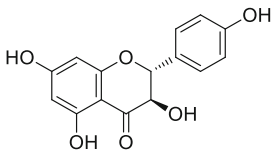
Arnebin-6



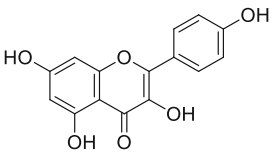
Hoslundal



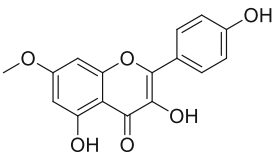
Octyl-ferulate



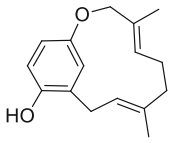
Aromadendrin



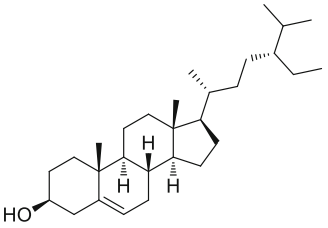
Kaempferol



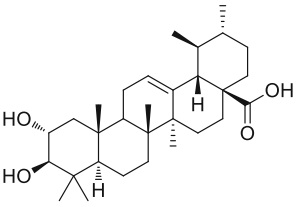
Kaempferol-7-o-methyl ether



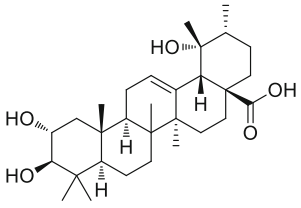
Arnebinol



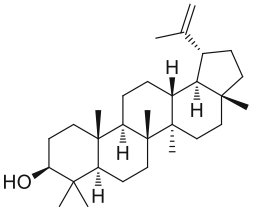
Sitosterol



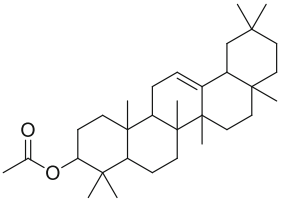
2 α-hydroxyursolic acid



Tormentic acid



Lupeol



Amyrin acetate

Fig. 4.2 (continued)

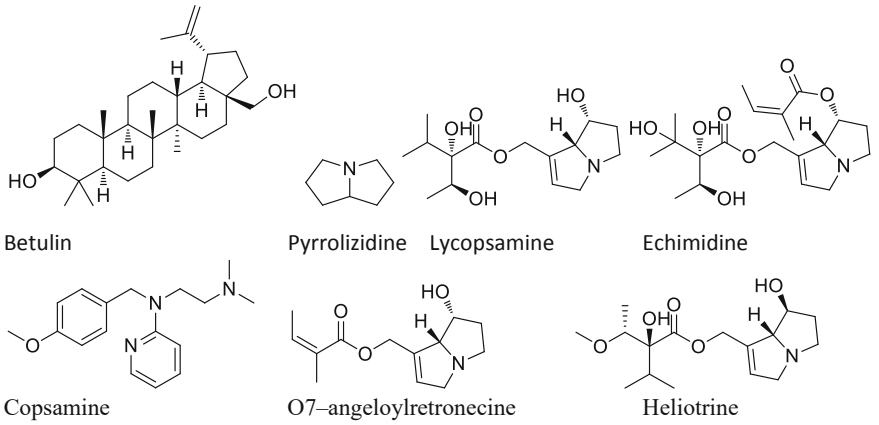


Fig. 4.2 (continued)

4.4.2 A. guttata

HPTLC studies was conducted for the screening of active phytoconstituents in *A. guttata*. It exhibited the existence of acetylshikonin, β-acetoxy isovaleryl alkannin, and dimethylacryl alkannin in the plant extract at the R_F value of 0.31, 0.46, and 0.64, respectively (Yan et al. 2021). Similarly, hydroquinone derivatives, like guttaquinol A and B were found in the roots of *A. guttata* (Gao et al. 2021). Petroleum ether extract of the *A. guttata* showed active components acetylshikonin, deoxyshikonin, shikonin, and β-hydroxy isovaleryl shikonin through HPLC and NMR studies (Fu-sun et al. 1983).

4.4.3 A. euchroma

Major phytoconstituents of *A. euchroma* are the coumarins, shikonin, isovaleryl-shikonin, isobutyl-shikonin, dimethylacryl-shikonin, deoxy-shikonin, acetyl-shikonin, β-hydroxy-isovaleryl-shikonin, arnebin, isobutyl-shikonin, arnebinone, and stigmasterol, which has been known for their pharmacological potential (Jain et al. 2000; Kashiwada et al. 1995; Zhu et al. 2019). Moreover, Jain et al. (2000)) described that the amount of shikonin in *A. euchroma* was found to be low in contrast to the other species. Roots of *A. euchroma* consisted of major terpenoid arnebinol A-D (Madarshahi et al. 2022). Various phytochemical studies demonstrated that the *A. euchroma* contained shikonin-angelate, benzoquinone, arnebin, deoxy-shikonin, acetyl-shikonin, isovaleryl-alkannin, and naphthoquinone which are known for their anti-HIV, antibacterial, anti-inflammatory, and anticancer activities (Xu et al. 2021; Yang et al. 1992). Moreover, another derivative of shikonin, i.e. β-acetoxyisovaleryl shikonin, *β*-dimethylacryl shikonin, β-hydroxy isovaleryl shikonin, and acetyl-shikonin, has also been purified from the *A. euchroma* roots by

Table 4.1 Traditional uses of *Arnebia spp.*

S. No	Disease	Part used	Mixture	References
1	Antimicrobial and antibacterial	Roots/ leaves	Ointment made from powdered roots	Gupta et al. (2013), Hosseini et al. (2018), Samant et al. (2007), Sharma et al. (2006)
2	Ear-ache problems	Roots/ leaves	Powdered roots are combined with olive oil, and herbs in a combination	Chauhan (2011), Gupta et al. (2013), Sharma et al. (2006)
3	Eye disease	Roots/ leaves	Powdered roots in combination with other plants	Chauhan (2011), Sharma et al. (2006)
4	Hair tonic	Roots/ leaves	Extracting the roots and combining them with mustard oil	Bibi (2019), Hosseini et al. (2018), Kala (2005)
5	Blood vomiting	Roots/ leaves	A decoction of *Arnebia euchroma*	Kumar et al. (2009), Rinchen and Pant (2014), Sharma et al. (2011), Singh et al. (2017), Singh et al. (2009)
6	Constipation	Roots/ leaves	Squashed root decoctions	Hosseini et al. (2018)
7	Anti-tumour/ ulcer	Roots/ leaves	Root powder, olive oil, and flower oil ointment	Chauhan (2011), Hosseini et al. (2018)
8	Eczema	Roots	Decoction produced from Arnebia and plant flowers, as well as mustard oil	Ma et al. (2014)
9	Cardiovascular	Roots	A decoction made from *Arnebia euchroma*	Ma et al. (2014)
10	Jaundice	Roots	Water and honey were used to squish the root	Ahmad et al. (2018), Singh (2012)
11	Abortifacient	Roots	*A. euchroma* root and flowers were combined to make a decoction	Hosseini et al. (2018), Sharma et al. (2011)
12	Tuberculosis	Roots	Roots powder decoctions	Singh et al. (2012)
13	Cough and cold	Roots	Root powder combined with other plant parts, floral decoctions	Jain et al. (2000), Singh et al. (2017)
14	Toothache, gum-bleeding infection	Roots	Root paste combined with other plant parts	Chauhan (2011), Sharma et al. (2006), Singh et al. (2012)
15	Bleeding and menstruation problem	Roots	*A. euchroma* root decoction made with flowers	Singh et al. (2012)
16	Religious (colouring of cloths and food items)	Roots	*A. euchroma* root powder combined with beeswax	Chauhan (2011), Hosseini et al. (2018), Samant et al. (2007)

(continued)

Table 4.1 (continued)

S. No	Disease	Part used	Mixture	References
17	Burns and skin disease	Roots	Powdered *Arnebia euchroma* roots + glycerine + eucerin + liquid paraffin	Lal and Singh (2008), Nasiri et al. (2014), Verma and Tewari (2016)
18	Lung problem	Roots	Squashed root decoctions	Kumar et al. (2009), Rinchen and Pant (2014)
19	Cuts (wound healing), antiseptic	Roots	Combine the root powder, rose, mustard oil, and butter in a mixing bowl	Gupta et al. (2013), Samant et al. (2007), Sharma et al. (2006)
20	Backache	Roots	Combine the root powder, mustard oil, and butter	Kumar et al. (2009), Rinchen and Pant (2014), Sharma et al. (2011), Singh et al. (2017), Singh et al. 2009
21	Pulmonary problem	Roots	Squashed root decoctions	Kumar et al. (2009), Rinchen and Pant (2014)
22	Neuralgia	Roots	Combine the rose, mustard oil, and butter with the root powder	Ma et al. (2014)
23	Anti-inflammatory	Roots	Ointment made from root powder	Ballabh et al. (2008), Hosseini et al. (2018), Sharma et al. (2006)
24	Psoriasis	Roots	Combination of various plant extracts	Tse (2003)
25	Kidney and urinary disorder	Roots	*A. euchroma* roots combined with other herbs	Ballabh et al. (2008)
26	Edible (pickle, chatni, chog, ghuriti)	Roots	Roots mixed with mustard oil	Chauhan et al. (2011), Sharma et al. (2018)
27	Ichthyosis	Roots	Grinded root macerated in vinegar	Hosseini et al. (2018)
28	Malignant ulcer	Roots	Ghiruti* is a root powder and olive oil-based drink.	
29	Diaphoresis	Roots	Root macerated in oil after being ground	
30	Scabies	Roots	Vinegar-macerated ground root	
31	Otalgia	Roots	Root decoction in rose or olive oil	
32	Sciatica	Roots	Plaster the root with lard or goat fat	
33	Anal fissure	Roots	Ghiruti* made from root powder	
34	Hard swelling of uterus	Roots	Decoction of root	
35	Induced abortion	Roots	Decoction of root and flower	

(continued)

Table 4.1 (continued)

S. No	Disease	Part used	Mixture	References
36	Snake bite	Roots	Root powder in wine	
37	Scrofula	Roots	Root ground in vinegar or root coated in lard or goat fat	
38	Erysipelas	Roots	Plaster of root with barley flour	
39	Dysuria	Roots	Decoction of squashed root	
40	Worms	Roots	*Tamarix gallica* and *Lagoeciacuminoides*	

*Also known as Ghrta and Ghrita, is an incredibly important and revered ingredient in the Ayurvedic system of medicine.

high pressure liquid chromatography (He et al. 2016; Pu et al. 2021; Shukla et al. 2011; Sykłowska-Baranek et al. 2014).

The root extract of *A. euchroma* revealed the main organic constituents isohexenyl-naphthazarin, arnebiabinone, octyl-ferulate, naphthoquinone, ethyl-9-(2, 5-dihydroxyphenyl) nonanoate, and butyryl-alkanninare (Ashkani-Esfahani et al. 2012a, 2012b; Liu et al. 2010). *A. euchroma* contained major phenols O^9–angeloylretronecine, tormentic acid, O^7–angeloylretronecine, and pyrrolizidine alkaloids (Sharma et al. 2009; Smyrska-Wieleba et al. 2017). In another study, lupeol, amyrin, O^7-angeloyl retronecine pyrrolizidine, betulin, and O^9-angeloyl-retronecine has been reported in the extract of *A. euchroma* (Singh 2001). Naphthoquinone derivatives di-methoxy-alkannin, tetra-benzoyl-alkannin, di-carboxy-alkannin, Shikometabolin H, β-methyl-anthracene, tetra-bromo-alkannin, epoxyarnebinol, and 2, 3-secodiplopterol dioic acid were observed in the roots of *A. euchroma* (Sharma et al. 2009; Smyrska-Wieleba et al. 2017). Thin-layer chromatography showed the presence of acetyl-shikonin in *A. euchroma* (Sharma et al. 2009). In another investigation, (Shukla et al. (1969)) purified Arnebin from the roots of *A. euchroma* and *A. nobilis.* (Liao et al. 2020a) and (He et al. 2016) also reported the active components isovaleryl shikonin, arnebin-7-methoxy-acetyl-shikonin, isobutyryl shikonin, tetra-acryl-shikonin, propionyl shikonin, aphthoquinones, isovaleryl alkannin, and β-acetoxy isovaleryl alkannin in the roots of *A. euchroma* (Song et al. 2022; Yan et al. 2022) (Table 4.2).

4.5 Pharmacological Activities

4.5.1 Antioxidant Activity

The antioxidant activity of *A. benthamii* EA root extract implies that it could be utilised as a dietary supplement or traditional medicine to prevent and/or cure

Table 4.2 Major phytoconstituents in different species of *Arnebia*

Phytoconstituents	Species of Plant	References
Acetyl shikonin	*A. guttata* *A. euchroma*	Chawla et al. (2021), Shen et al. (2002) Yan et al. (2021)
β-Acetoxy isovaleryl alkannin	*A. guttata* *A. euchroma*	Liao et al. (2020a), Shen et al. (2002), Yan et al. (2021)
Dimethylacryl alkannin	*A. guttata* *A. euchroma*	Chawla et al. (2021), Ganie et al. (2012), Yan et al. (2021)
Arnebinol	*A. euchroma*	Cao et al. (2020)
β-Methyl-anthracene	*A. euchroma*	Ashkani-Esfahani et al. (2012a, b)
Guttaquinol	*A. guttata*	Gao et al. (2021)
Deoxy-shikonin	*A. guttata* *A. euchroma*	Chawla et al. (2021), Fu-sun et al. (1983), Ganie et al. (2012)
Shikonin	*A. guttata* *A. benthamii* *A. euchroma*	Fu-sun et al. (1983), Katoch et al. (2016), Parray et al. (2015), Shen et al. (2002)
Isobutyl-shikonin	*A. euchroma*	Andújar et al. (2013), Parray et al. (2015)
2, 3-secodiplopterol dioic acid	*A. euchroma*	Cao et al. (2020)
β-Hydroxy isovaleryl shikonin	*A. guttata* *A. euchroma*	Fu-sun et al. (1983), Shen et al. (2002)
Isovaleryl shikonin	*A. euchroma*	Andújar et al. (2013), Liao et al. (2020b)
β,β-dimethylacryl shikonin	*A. benthamii* *A. euchroma*	Chawla et al. (2021), Katoch et al. (2016)
Kaempferol	*A. benthamii*	Rather et al. (2018)
Hoslundal	*A. benthamii*	Shameem et al. (2015)
Aromadendrin	*A. benthamii*	Rather et al. (2018)
Sitosterol	*A. benthamii*	Rather et al. (2018)
Kaempferol-7-o-methyl ether	*A. benthamii*	Rather et al. (2018)
Lycopsamine	*A. benthamii* *A. euchroma*	Ahmad et al. (2018), Singh (2001)
Echimidine	*A. benthamii* *A. euchroma*	Ahmad et al. (2018), Singh (2001)
Heliotrine	*A. benthamii*	Ahmad et al. (2018)
Octyl-ferulate	*A. euchroma*	Singh (2001)
Arnebin-3	*A. euchroma*	Ramawat et al. (2014)
Teracrylshikonin	*A. euchroma* *A. guttata*	Shen et al. (2002)
Arnebinone	*A. euchroma*	Yang et al. (1992)
Arnebin-7 Arnebin-1 Arnebin-6	*A. euchroma*	Sharma et al. (2009), Smyrska-Wieleba et al. (2017)
2 α-hydroxyursolic acid Pyrrolizidine	*A. euchroma*	Ashkani-Esfahani et al. (2012a, 2012b), Baran et al. (2020)

(continued)

Table 4.2 (continued)

Phytoconstituents	Species of Plant	References
Butyryl-alkanninare Iso-hexenyl-naphthazarin Tormentic acid	*A. euchroma*	Thomson (2012)
Lupeol Amyrin acetate Betulin Copsamine	*A. euchroma*	Singh (2001)
Di-methoxy-alkannin Di-carboxy-alkannin	*A. euchroma*	Ashkani-Esfahani et al. (2012a, 2012b)
Shikometabolin H	*A. euchroma*	Cao et al. (2020)
O^9–angeloylretronecine	*A. euchroma*	Smyrska-Wieleba et al. (2017)
O^7–angeloylretronecine	*A. euchroma*	Smyrska-Wieleba et al. (2017)
Naphthoquinone	*A. euchroma*	Xu et al. (2021)
Epoxyarnebinol	*A. euchroma*	Smyrska-Wieleba et al. (2017)
Tetra-bromo-alkannin	*A. euchroma*	Ashkani-Esfahani et al. (2012a, 2012b)
Arnebiabinone	*A. euchroma*	Thomson (2012)

diseases caused by oxidative damage, as well as to protect DNA from hydroxyl radical damage. For quantifying shikonin, the HPLC method provides excellent sensitivity. With an 85.9% recovery, a 50 g/g ethyl acetate extract yielded a shikonin concentration of 5.19 g/g (Parray et al. 2015). The methanol extract of *A. benthamii* was found to have 71.29% scavenging activity at 800 g/mL concentration. With a scavenging efficacy of 90.67% at 800 g/mL concentration, the scavenging effect was comparable to that of traditional Vitamin E. It has excellent antioxidant activity and could be exploited in the pharmaceutical industry as a source of lead compounds (Ganie et al. 2012, 2014).

4.5.2 Antidepressant Activity

A force swim test and a tail suspension test were used to assess the antidepressant activity of *A. benthamii* aqueous root extract in rats. These behavioural models caused rats to develop depressed symptoms such as immobility. In the force swim and tail suspension tests, aqueous root extract of *A. benthamii* significantly improves immobility time by shortening the immobility period. Higher doses of *A. benthamii* aqueous root extract (150 and 300 mg/kg) were more efficacious than lower values. A higher dose of *A. benthamii* aqueous root extract resulted in a substantial rise in SOD and brain glutathione levels when compared to the control group.

Malondialdehyde and nitrite levels were considerably reduced in the *A. benthamii* aqueous root extract treated group, especially at the higher dose. The aqueous root extract of *A. benthamii* has been demonstrated to be an efficient antidepressant. It has the ability to reduce symptoms of depression (Kumar et al. 2017).

4.5.3 Anticancer Effects

Acetylshikonin appears to cause tumour cell apoptosis via activating the pro-apoptotic bcl-2 family, releasing cytochrome c, and activating caspase-3. Acetylshikonin decreased the proliferation of A549, Bel-7402, MCF-7, and LLC cells in a dose-dependent manner, according to the findings. Acetylshikonin, which was isolated from *A. euchroma* (Royle) Johnst cell suspension cultures, has anticancer action both in vivo and in vitro (Xiong et al. 2009). Shikonin has additive to synergistic interactions when combined with existing chemotherapeutics, immunotherapeutic methods, radiation, and other treatment modalities, highlighting the potential of this phytochemical to be integrated into standard treatment regimens and pharmaceutical medicines used in cancer therapy (Boulos et al. 2019).

4.5.4 Anti-inflammatory Effects

CAEP not only reduced morphological injury, oedema, and lung permeability in ALI rats, but it also lowered oxidative stress in bronchoalveolar lavage fluid considerably (BALF). CAEP therapy reduced both the febrile reaction caused by LPS and the acute lung injury caused by LPS with ischaemia-reperfusion. CAEP may have an anti-inflammatory impact by lowering the inappropriate activation of the complement system (Ou et al. 2017). Shikonin reduces inflammation and chondrocyte death in a rat model of osteoarthritis through modulating the phosphoinositide 3-kinase/Akt signalling pathway. Shikonin at 10 mg/kg per day effectively suppressed the increase in IL-1 and TNF-expression levels in an osteoarthritic rat model, when compared to the osteoarthritic group (Fu et al. 2016). *A. euchroma* is employed in traditional treatments and the pharmaceutical sector because of to its antimicrobial and anti-inflammatory characteristics. Even today, herbal remedies derived from *A. euchroma* are commonly used to treat body pains as well as other microbiological infections (Ge et al. 2006; Kim et al. 2001; Shen et al. 2002; Terada 1990) and anti-HIV (Kashiwada et al. 1995). Shikonin significantly reduced the levels of TNF-, IL-6, and IL-1 in LPS-induced bronchoalveolar lavage fluid. Shikonin inhibits the NF-B signalling pathway, which regulates the expression of pro-inflammatory cytokines, and so has anti-inflammatory characteristics in LPS-induced ALI. Shikonin has the potential to be used to prevent ALI (Liang et al. 2013). *A. euchroma* (Royle) Johnst's hydroxynaphthoquinone combination has anti-inflammatory and analgesic properties (HM). HM provided prophylactically

and curatively shown significant anti-arthritic action by decreasing paw swelling and inflammation, lowering TNF- and IL-1 levels, and protecting cartilage and bone from injury (Fan et al. 2012).

4.5.5 Anti-genotoxic and Anti-Photogenotoxic Activities

The V79 cell line is particularly pathogenic and cytotoxic to *Arnebia* extract. The inclusion of *A. euchroma* callus extract (EXT) at all concentrations (5.6–720 mg/L) resulted in considerable cytotoxicity in the current investigation. It implies that the mammalian cell line is far more sensitive to *A. euchroma* callus extract than the bacteria strain. Shikonin and its derivatives' cytotoxicity has been well documented, albeit largely against malignancies lacking normal cell lines. Shikonin demonstrated no detectable anti-photogenotoxic activity although acetylshikonin did. EXT was extremely cytotoxic to the V79 cell line, inducing considerable alterations in cell morphology as well as cell death (Li et al. 1999; Skrzypczak et al. 2015). Shikonin is one of the active components recovered from the root of *A. euchroma* (Royle), and it has been tested in clinical trials and has been shown to have potent antibacterial, anti-inflammatory, and anticancer effects. The in vitro metabolism of shikonin and other naphthoquinone compounds with pharmacological activity in *A. euchroma* (Royle) Johnst will be valuable for studying the in vivo metabolism of shikonin and other naphthoquinone compounds with pharmacological activities in *A. euchroma* (Royle) Johnst.

4.5.6 Antidiabetic Activity

AE can also help to reduce blood glucose levels to some extent. When the primary insulin-secreting cells were harmed by STZ, they also reduced blood glucose levels when compared to diabetics who were not treated. Normal rats (C1) treated with AE at doses of 100 mg/kg (E1) and 300 mg/kg (E2) showed significant differences in pancreatic islet volume densities and total volume; volume densities and total numbers of beta cells in AE-treated rats also showed significant differences when compared to untreated diabetic rats (Noorafshan et al. 2017). *A. euchroma* could possibly be utilised to treat diabetic foot ulcers, which are a common cause of amputation and have huge social consequences (Sharif et al. 2019). The role of pro-/anti-inflammatory factor antagonism on the transforming growth factor-(TGF-) superfamily (activin and follistatin) in the re-epithelialisation of diabetic wound healing in vivo, as well as activin/follistatin protein expression regulation, phospho-Smad (pSmad2), and nuclear factor kappa B p50 (NF-B) p50 in diabetic wound healing (Kuai et al. 2018).

4.5.7 Wound Infection and Healing

Traditional medicine has utilised the roots of *Arnebia* (Boraginaceae) to treat wounds. The root barks and an olive oil extract of the roots were used to open wounds for rapid healing. *Arnebia densiflora* has shown remarkable wound-healing qualities (Akkol et al. 2009). Anti-inflammatory, antibacterial, and antioxidant activities of *A. euchroma* have the potential to promote wound healing. It was also stated that AEO at 5% and 10% may successfully prevent following burn problems such as oedema, erythema, secretion, or wound infection, which could be induced by the ointment's antibacterial properties (Nasiri et al. 2016). Several researchers have reported similar findings in which the rat was utilised as a model organism (Aliasl et al. 2014; Ashkani-Esfahani et al. 2012a, 2012b; Nasiri et al. 2015; Pirbalouti et al. 2011). Antibiotic tolerance improved when *Pseudomonas aeruginosa* and *Staphylococcus aureus* were cultivated together in planktonic cocultures, and it improved even more when they were grown together in the wound model. This demonstrates that *P. aeruginosa* and *S. aureus* may benefit from each other by co-infecting wounds, and that the host-derived matrix may play an equal role in shielding bacteria against antibiotics as the bacterium-derived matrix (Yang et al. 2020). AE has promising promise for mending various types of skin lesions, including burns and excisional wounds in rats (Mohsenikia et al. 2017). Topical *A. euchroma* had a beneficial effect on third-degree burn wounds, according to the wound-healing effects of AE and the outcomes of this investigation. However, before presenting AE as an alternative healing agent for wounds, more research into its efficacy, safety, and potential harmful effects on people is required (Ashkani-Esfahani et al. 2012a, 2012b).

4.5.8 Leukaemia Treatment

Increase in the number of white blood cells causes leukaemia, and the compounds derived from *A. euchroma* play an important part in illness management (Salik et al. 2020). (Wang et al. 2020) discovered that *A. euchroma* can trigger apoptosis in cells, hence preventing leukaemia. In that study, a compound-target network with 10 targeted and 14 components in a target gene was used to explain the pharmacological action mechanisms involved with leukaemia treatment.

4.5.9 Anti-obesity Properties

Obesity is a worldwide health problem that has been linked to a variety of metabolic disorders. Metabolic syndrome is a comorbidity that is linked to the size of the waist and the thickness of the abdominal fat. To reduce fat thickness around the belly,

liposuction and other treatments for removing fat in specific locations are readily available. External application of an ointment containing *A. euchroma* extracts was proven to reduce body weight (2.96 kg), abdominal fat thickness (2.3 cm), and abdominal circumference in obese women (11.3 cm) (Siavash et al. 2016).

4.5.10 Antimicrobial Activity

The plant extract of 250–500 g/mL concentration for antimicrobial activity was tested against *Pseudomonas aeruginosa* CD0023, *Escherichia coli* CD0006, *Shigella flexneri* CD0033, *Klebsiella pneumonia* CD0049, *Salmonella typhimurium* CD0003, *Staphylococcus aureus* CD0001, *Aspergillus versicolor* CDF0011 and *Candida albicans* CDF. The aerial component exhibits an efficient antibacterial activity against almost all of the pathogens tested, with *E. coli CD0006* and *P. aeruginosa* CD0023 having the biggest inhibition zone diameter (IZD). According to the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values obtained, the *P. aeruginosa* CD0023 was inhibited by the lowest concentration of 75 g/mL of the aerial part methanol extract. The plant has antibacterial properties across a wide range, making it an excellent alternative for sickness treatment (Shameem et al. 2015). The chloroform extract of *A. benthamii* leaves demonstrated antibacterial activity comparable to standards (Guna 2019).

4.5.11 Hepatotoxic Activity

The researchers were interested in seeing how an aqueous extract of *A. benthamii* affected dichromate-induced hepatotoxicity and nephrotoxicity in rats. As a hepatotoxicity and nephrotoxicity index, the researchers compared the levels of blood hepatic and renal markers in the treated and toxic models to those in the control group. The serum levels of alkaline phosphatase, alanine, and aspartate aminotransferases were found to be considerably higher in rats given dichromate (10 mg/kg b. w., i.p.), indicating liver injury. Similarly, renal function indices such as BUN and creatinine increased significantly in dichromate-treated rats. Pretreatment with *A. benthamii* aqueous extract increased serum levels of hepatotoxicity markers, showing that it has a hepatotoxic effect. However, no statistically significant difference in kidney function markers could be found between the treated and toxic groups (Ahmad et al. 2018; Hamid et al. 2014).

4.5.12 Cytotoxic Activity

Natural meroterpenoids (2, 5–9) and seven recognised meroterpenoids (1, 3, 4, 10–13) were extracted from *A. euchroma* root plant. Meroterpenoids 1–13 was investigated for cytotoxicity on liver cancer cell on human lines SMMC-7721, HepG2, QGY-7703, and HepG2/ADM. The meroterpenoids isolated from *A. euchroma* outperformed the positive medication, cisplatin. propionyl alkannin, a novel phytochemical compound isolated from the callus and cell suspension culture of *A. euchroma* (Royle) Johnst were tested for cytotoxicity in cancer cells (human leukaemia cell CCRF-CEM, breast cancer cell MDA-MB-231, human glioblastoma cell U251, and colon cancer cell HCT 116); it demonstrated potent cytotoxic activity with low IC_{50} values. The side chain is more significant for action than the naphthoquinone molecule, and it works as an activity modifier. *A. euchroma* had no substantial killing impact on Adenocarcinoma gastric cell line (AGS). Only at 0.02 mg/mL doses was the reduction significant three times, with a fatality rate of roughly 20%, which is insufficient to be termed cytotoxic (Parray et al. 2015; Wang et al. 2018).

4.6 Agrotechnology

Several agrotechniques have been established for reproducing plants at faster rate without the harm of active principles. In vitro seed germination, tissue culture, in vitro plant regenerations and metabolic engineering are the methods which can be useful for the preservation and protection of the various species of *Arnebia* (Guna 2019; Manjkhola et al. 2005).

4.6.1 Propagule Collection, Enhanced Rooting, and Seedling Survival

Various studies were conducted on the conservation and usage of *A. benthamii* in order to monitor the optimal time of propagule collection, enhance rooting of root cuttings, and find the best conditions for seedling survival. Individuals at propagative stage were determined to be adequate for propagule collection due to the presence of 3–5 buds at the terminal root point. These buds can be used efficiently for vegetative growth. Chilling of the root cuttings for 40 days caused the improvement in the rooting considerably. As a result, seedling survival and growth performance were substantially more in the high-altitude regions. This action will not only relieve strain on the natural population; however, also stimulate rural economic activity. The potential of generating new vegetation in the damaged natural habitats

and establishing nursery centres in low-altitude locations can also be an advantageous approach (Manjkhola and Dhar 2002).

4.6.2 In Vitro Seed Germination

Seeds consist oil as a reserve food material and they possess 98% viability. The water holding capacity of the seeds is good and the seed coat showed very less impact on the physical dormancy. Scarification was observed to be the most efficient technique to boost the rate of germination and decrease the mean germination time. Scarification increased seed germination to 97% and decreased the duration of germination to 4.03 days in contrast to the control, where seed germination was observed to be 32% and duration of germination was 9.2 days. After the treatment of scarified seeds with seed coat extract, the rate of germination was severely reduced to 28%, signifying that the seed coat consists of inhibitors that exhibit inhibitory impact on seed germination (Khursheed et al. 2011). In another investigation, ex situ experiments were conducted on the germinability and seedling survival of *A. benthamii*. It was observed that scarification of the seeds caused enhancement in the germination which was hampered due to the presence of inhibitors in the seed coat. Treatment with gibberellic acid at 25 and 50 ppm was found to be most beneficial, whereas higher doses (100 and 200 ppm) reduced seed germination (Ganai and Nawchoo 2002). In another investigation, Thiourea treatment enhanced the germination in the alpine population over the control; however, cooling in the subalpine population markedly improved germination in contrast to the control (Manjkhola et al. 2003).

4.6.3 Tissue Culture

A tissue culture method for *A. benthamii* was devised by employing various combinations and suitable media formulations, such as several adjuvants Murashige and Skoog (MS) medium, growth hormones, carbohydrates, and agar. The impact of several media combinations was calculated, and the MS, thiadiazuron, Indole 3-acetic acid combination favours a greater regeneration ability. The in vitro herbal extracts also revealed a notable effect of hydroxyl radical scavenging in terms of safety from DNA damage (Parray et al. 2018).

4.6.4 In Vitro Multiplication

An effective in vitro multiplication and propagation technique was devised for *A. benthamii*. Half-strength Murashige and Skoog (MS) media supplemented with

varied doses of 6-benzyladenine (BA) was utilised for the growth of shoots from shoot tip explants. Treatment of 5 μM benzyladenine induced the formation of multiple shoots. Moreover, it was observed that the half-strength MS medium supplemented with 4 μM BA and 1 μM IBA caused the induction of maximum number of multiple shoots. Different doses of IBA, indole-3-acetic acid (IAA), and naphthaleneacetic acid (NAA) were utilised for the development of roots from shoots. Half-strength MS medium mixed with 4 M IBA produced the finest roots, and 80% of plantlets moved to field conditions endured (Quadri et al. 2012).

4.6.5 Metabolic Engineering

Metabolic engineering is a common approach of improving regulatory and genetic systems within the cell in order to improve the synthesis of certain compounds. Geranyl pyrophosphate (GPP) and p-hydroxybenzoate (PHB) are fundamental components of the shikonin biosynthesis pathway in *A. euchroma*. GPP is generated by the cytosolic mevalonate (MVA) route, whereas PHB is created via the phenylpropanoid pathway. Mevinolin, a HMGR enzyme inhibitor inhibits the MVA pathway. GPP and PHB react to generate m-geranyl-p-hydroxybenzoate via a process catalysed by PHB. The obliteration of the mevinolin-expressing gene can raise the concentration of HMGR, which can upsurge the amount of shikonin, and overexpression of GHB and PHB can also cause the intensification of shikonin content. Moreover, addition of bacterial gene UbiA was found to be potent modulator of shikonin production (Boehm et al. 2000; Chawla et al. 2021). It catalysed the production of 3-geranyl-4-hydroxybenzoate (GBA) from GPP and induced 50-fold enhancement in GBA concentration when compared to the control. However, shikonin content increased very little (22%) in transformants compared to controls. This recommends that more expression of UbiA alone is inadequate to raise shikonin content, and that shikonin amount is augmented by a variety of additional genes. Shikonin production may also be boosted by irradiating cell suspension cultures with 2, 16, or 32 Gy of radiation. PHB, a crucial enzyme in shikonin production, can be activated by gamma irradiation. Shikonin content rose 400% after 16 Gy gamma irradiation, but it amplified only 240% and 180% after 2 and 32 Gy gamma irradiation, respectively (Chung et al. 2006).

Monitoring the synthesis of diverse plant metabolites by cell cultures is also alternative method for the enhancement of shikonin concentration. It reduces strain on the plant species' native habitats and also creates favourable circumstances for metabolite synthesis (Sood 2020). Many biotic or abiotic elicitors in cell cultures can boost the plant secondary metabolite synthesis. Addition of fungal elicitors (*Aspergillus niger* and *Rhizopus oryzae*) to the culture medium resulted in an immediate rise in shikonin concentration. On the sixth day of addition, highest concentration of 89.75 mg/L was obtained in contrast to the control group. Shikonin content was enhanced 2.24-fold by *R.oryzae* elicitors as compared to the control. Furthermore,

combination of fungal elicitation and in situ hybridisation boosted the quantity of shikonin to 6.2-fold than the control (Fu and De-Wei 1999).

4.7 Conservation

The over-utilisation of natural habitats, and overusage in the pharmaceutical sector due to traditional medicinal properties has hastened the demise of *A. euchroma* all over the world. Moreover, *A. benthamii* has also been encompassed in the Indian Red Data Book due to constant overexploitation. Anthropogenic actions like unintended economic development, road building, and other infrastructure-related projects have recently been exposed to contribute to the fast loss of this species and its habitat (Sofi et al. 2022). It is a huge challenge for humanity to protect this plant. Currently, the goal of the International Union for Conservation of Nature and National Mission on Himalayan Studies is the conservation of natural resources, improving environmental awareness policies, avert abuse, maintaining gene banks and preserve biosphere reserves (Hamilton 2004). Local residents and doctors should be made aware of the issues created by the overexploitation of plant roots (Singh 2012). Long-term monitoring is also necessary for warranting the persistence of decreasing plant populations in their early stages. Better understanding of the extrinsic and intrinsic reasons for reduction of the plant population is critical to attaining conservation goals (Lal et al. 2020).

4.8 Conclusion and Future Perspectives

The current study depicts the worth of *Arnebia* species as a vital environmental indicator in the Indian Himalayan region. *Arnebia* is highly utilised by the traditional remedial experts for the cure of several fatal infections. It is also used as colourant in food and textile industry. The phytoconstituents of *Arnebia* species like acetyl-shikonin and shikonin are responsible for the therapeutic as well as prophylactic medicinal applications. Due to the vast medicinal properties of *Arnebia*, its demand is constantly growing in the market worldwide. The overuse of *Arnebia species* in various industrial sectors has expedited its annihilation all over the world. Thus, it is imperative to conserve and propagate the *Arnebia* sp. with various agrotechniques in order to offer adequate raw materials for its commercial utilisation. Further research are needed for the exploration of new agrotechnological processes to restore the population of *Arnebia* sp. Moreover, more studies are required on the screening of plant for active components which might prove to be an important step in medication formulations. There is very less data published on the pharmacokinetics of the plant which further necessitates more research into the bioavailability of *Arnebia* and its allied species. The mechanistic studies of *Arnebia* for the cure of HIV and cancer is also required to be investigated in the future.

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Chapter 5
Species of the *Berberis* Genus Found in the Western Himalayas

Ramandeep Kaur, Urvashi, and Amit Kumar

5.1 Introduction

The Himalayas have long been recognized as a rich source of biodiversity including flora and fauna. Due to its climatic conditions, it is a house of several plant families which are used in the Ayurvedic systems where many of them are already in use to prepare several ayurvedic medicines and a large number are still unexplored. Berberis is among one of the major dicotyledonous genera which belong to the Berberidaceae family (Tiwari et al. 2012; Bruckner 2000). To date, more than 17 genera and 650 species belonging to the Berberidaceae family have been identified worldwide (Khan et al. 2014). The basic origin of this genus is the Himalayan regions of Asia and from here it gets migrated to different continents, and all this happens around 34 million years ago (Li 2010). The main distribution of this genus occurred in Asian countries like India, China, Pakistan, etc. while few of its species are also distributed to the different countries of Europe, America, and Africa. The plants were divided into three groups based on their habitat: the Rocky Mountain group, which comprises *B. aquifolium* Pursh, the Asian group, which comprises *B. aristata*, and the European group, which includes *B. vulgaris* (Bhardwaj and Kaushik 2012).

Out of the total 50–60 species reported in India, the bulk of berberis species are located in the temperate and subtropical parts of the Himalayan valley. The genus *Berberis* is distributed into 8 sections which are further divided into 14 subsections (Rao and Hajra 1993; Rao et al. 1998a, b). In India, mainly *B. aristata* DC, *B. lyceum* Royle, *B. angulosa*, *B. asiatica* Roxb. ex. DC, *B. coriaria* Royle ex Lindl, *B. Chitria* Lindl, *B. tinctoria* Lesch, *B. umbellata*, *B. virescens*, *B. coriaria* Royle, and *B. floribunda* are found and are being planted as hedges due to their straggling

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habit. Taxonomic characterization of Berberis is difficult due to common features in leaves, stems, flowers, and the size of the fruit. Even in some species of this genus, the texture of leaves and serrations showed variation concerning the season as well as age (Lucas et al. 2012).

Long back, *Berberis* is considered an indicator of environmental changes. Wild animals and birds were dependent on berries for their food (Champion and Seth 1968). The genus *Berberis* is a key medicinal plant that has both culinary and therapeutic values. The fruit/berries are sweet and sour in taste and are consumed either raw or cooked throughout the world. Rasaunt, a concentrated root/stem juice, is described in Ayurveda as essential medication for the treatment of eye-related ailments. The use of berberine (an alkaloid isolated from the plant) for curing oriental sore is documented in British Pharmacopoeia.

Almost every part of the plants of this genus is being utilized for treating various ailments. For example, the bark, stem, and root are used to cure a range of eye and ear infections, rheumatoid arthritis, liver diseases, diabetes, skin disease, malaria, and metabolic disorders (Ali et al. 2015). Even in classical Ayurvedic texts, its role in treating infected wounds has been very well described.

Overall, the roots of this plant are a substantial source of bioactive chemicals that are responsible for their therapeutic qualities in the treatment of fever, acting as an antipyretic, antiperiodic, and diaphoretic, and are thought to be as effective as quinine. The bark is also reported to show antiperiodic properties as well as it is utilized as tonic also. The plant of this species is also well proven to cure heart and liver diseases, as well as several microbial infections. In some latest reports, the study to explore the anticancer properties is also discussed (Pai et al. 2012). Hence, this chapter will provide a deep insight into the general characteristics of the genus Berberis, its distribution, phytoconstituents, pharmacological activities, patents, etc. Two main plants of this genus are *B. aristata* and *B. lycium* are much documented in the literature for their phytoconstituents and pharmacological properties. Hence, this chapter will provide a detailed account of phytoconstituents and pharmacological properties of these two species reported to date. The other plants of this genus are still unexplored and can be taken up to explore their vast potential in the field of medicine.

5.2 Taxonomy

Berberis genus includes more than 600 different species distributed all over Himalayan and other hilly areas. Out of these the most commonly studied for their pharmacological properties and phytoconstituents are *B. aristata* and *B. lycium.*

Kingdom	Plantae	Plantae
Phylum	Tracheophyta	Tracheophyta
Division	Magnoliophyta	Magnoliophyta

(continued)

Kingdom	Plantae	Plantae
Class	Magnoliopsida	Magnoliopsida
Order	Ranunculales	Ranunculales
Family	Berberidaceae	Berberidaceae
Genus	Berberis	Berberis
Species	*Berberis lycium*	*Berberis aristata*

5.3 Common Name

B. aristata and *B. lycium* have been extensively used in our traditional medicinal systems for curing several diseases. These plants bear different names in different languages. *B. aristata* has been most commonly called Daru haldi owing to its vast range of pharmacological properties similar to turmeric (haldi). The other common names for this plant are Indian Barberry, tree turmeric, chitra, etc. India is a country of multiple languages and this plant is quite popular in its traditional medicinal system so it is used all over for the preparation of several ayurvedic medicines. It bears different names in local languages; it is called as Daruharidra in Bengali, Daruhuladur in Gujrati, Sumalu in Punjabi, Katamkateri or Dirvi in Sanskrit, Gangeti or Varattu manjal in Tamil, Maradarishana in Kannada, Maramannal in Malayalam, Daruhala in Marathi, Manupsaupu in Telugu, Darhald in Urdu, etc. Likewise, *Berberis lycium* has also been called with different names in different languages such as Ishkeen (Urdu), Kashmal or kasmal (Hindi), Kwaray or ZiarLargay (Pastu), and Zirkash (Persian) (Sood et al. 2012).

5.4 Flowering/Fruiting Season

Berberis plants have a flowering and fruiting season that lasts from March to August. The plants start blooming with yellow-colored flowers in mid-March and this continues by the end of April month. The ripening of fruits starts by mid of May and continues up to the end of June. The ripened fruits (berries) are purple or bright reddish and can be enjoyed in the June–July month. The fruits can sustain as such for longer periods but fall as soon as the rainy season starts. Hence, the fruiting period varies according to the onset of the rainy season. The ripened fruits can be consumed raw whereas unripened fruits can be cooked or preserved. The fruit is a bit juicy, tangy, and acidic in nature.

5.5 Distribution

Berberis is found throughout Asia, Europe, North America, and Africa. Globally, *B. aristata* plant is widely distributed in Asia from Himalayan regions to Sri Lanka. The main areas of distribution are the Himalayan regions of India, Pakistan, Bhutan, Bangladesh and Nepal. Being the native of the hilly area, this plant was found growing at a height of 2000–3000 m covering the major area of Chamba and Kumaon of Himachal Pradesh. In northern regions, it is available in Jammu and Kashmir, Himachal Pradesh, Sikkim, and Uttarakhand. In the southern area of India, it is located in the Nilgiri hills of Tamil Nadu (Ray et al. 2011). *B. lycium* is inherent to South Asian countries viz. India, Pakistan, Afghanistan, Nepal, and Bangladesh. It may be found in the Himalayas at altitudes of 850–3500 m, from Kashmir to Uttaranchal, in temperate and semi-arid climates.

5.6 Morphology

Almost every plant in the genus Berberis is a spiny shrub with yellow flowers. *B. aristata* is a woody shrub with stiff spines that grows to a height of 6–9 ft. The wood is hardy and yellowish, and the bark is yellowish-brown on the exterior and dark yellow on the interior side, with three-branched spines (which may be readily removed as longitudinal strips by hand). The flowers are hermaphroditic, complete, cyclic, actinomorphic, perigynous, and stalked. A full-bloomed flower has an average diameter of 0.49 in. (12.5 mm). Flowers are grouped around the central stalk in a racemose inflorescence (11–15 flowers/raceme). Actinomorphic, polysepalous blooms with two sets of yellow-colored sepals (3 little and 3 big), caduceus 4–5 mm long. This plant has a polypetalous (six golden petals) and actinomorphic corolla. The androecium is adnate and polyandrous (6 stamens) (5–6 mm long). The solitary gynoecium is 4–5 mm long, with a narrow stigma and a short style. The violet-colored fruits that appear when the rainy season begins are globose to ovoid in shape and are mostly covered in blossom. These are 7 mm in length with 4 mm diameter. The fruit weighs approximately 227 mg. The plum and color of the juice are mostly purple. These yellow and pink fruits bear 2–5 seeds with an approximate weight of 25 mg.

B. lycium is an appealing, spiky, deciduous shrub that grows to a height of 6–12 ft. The stem and branches are pale white to grey are of alternately arranged spines throughout the stem. The leaves are usually lanceolate and have big spines with a leathery texture, and they are placed alternately along the stem. The leaves have a bright green upper surface and a bluish-green bottom side. The brilliant yellowish blooms, which are cupped in shape and have racemose corymbs inflorescence, are 11–16 in number. The plant is self-pollinating; however, pollination through insects can also occur. The fruits are oval-shaped, reddish, or purplish berries with 4 mm diameter, 7 mm long, and 227 mg weight. The plant contains

2–5 seeds/fruit. The color of the seed varies from yellowish to pinkish. The plant bears hard and broad roots (3–8 cm diameter) with thick bark (3 mm). The bark of the root is fissured outside and smooth inside whereas the wood is smooth and yellow in color (Sood et al. 2010; Ahmed et al. 2017; Parra et al. 2018).

5.7 Traditional Uses

B. aristata has been well documented for its traditional benefits, and it is basically having the properties similar to turmeric so it is also documented as Daruharidras. It has been known for its properties in treating hemorrhoids, wound healing, skin disorders, toxicity, excess fat, and purification of breast milk, excessive sweating, and pruritis itching. It is also used as a part of folklore medicines in India for the treatment of skin and eye problems as well as metabolic disorders where it is used as a laxative. It is also used as a part of medicine which constitutes 73 plants for treating skin disorders in Nepal and surrounding villages (Joshi and Joshi 2007). In some rural parts of India, it is one of the ingredients in a multi-herbal therapy for bleeding piles (Saraf et al. 2010). A research on ovariectomized rats verified the traditional use of this herb in the cure of joint pain, osteoporosis, and menopause. These rats were treated with methanolic aqueous extract and the results showed control in ailment associated with the same (Yogesh et al. 2011). The decoction of its leaves has long been used to cure various skin disorders, stomach, eye/ear infections, urinary tract infections, cholera, and jaundice as well. This Rasaut (decoction of leaves) is having antifungal, anti-inflammatory, antipyretic, and analgesic effects (Shahid et al. 2009; Sati and Joshi 2011; Meena et al. 2009). One of the other traditional uses includes utilization of its root decoction in the treatment of eye-related problem practiced in Bhotiya communities of the Himalayan region of India (Phondani et al. 2010). Further its fruits are consumed as antiscorbutic and laxative (Sharma et al. 2005). Apart from treating infectious diseases, *B. aristata* is also used in treating psychological-related diseases in children in Garhwal, Himalaya (Tiwari et al. 2010a, b). Almost every part of these plants is utilized to treat a variety of health issues. Its leaf and fruit juice are used to treat stomach disorders such as dysentery and diarrhea in Nepal, while its root decoction and bark is used to cure jaundice (Acharya and Rokaya 2005; Kunwar and Adhikari 2005). Along with the treatment of infectious ailments, some people from Sikkim and Darjeeling use the plant extract for the treatment of diabetes and liver disorders (Chhetri et al. 2005). This herb is used to cure leprosy in the Unani Medicine system.

B. lycium has been applied in Ayurvedic and Unani therapy for centuries to cure different ailments. In Kashmir, the roots are known as Kaw DachiMool, while the extract is called as Rasaunt (Raizada and Saxena 2000). *B. lycium* fruits (kashmal) have been used in Himalayan locations for centuries. Traditionally, the plant is widely used to cure a variety of human diseases, including leprosy, piles (Sood et al. 2010; Hamayun et al. 2006), diabetes (Waseem et al. 2006; Tiwari et al. 2010a, b), ophthalmia (Khan et al. 2010), backache (Zabihullah et al. 2006),

rheumatism (Kaur and Miani 2001), menorrhagia, gingivitis, jaundice, intermittent fever, sun blindness, remittent fever, broken bones, and wounds (Sood et al. 2013; Ahmed et al. 2017). It is helpful in intestinal colic and bacterial dysentery (Afzal et al., 2009). It is used in diuretic, diaphoretic, chronic ophthalmic, acute conjunctivitis, and throat inflammations (Hamayun et al. 2006; Kaur and Miani 2001). The sap of early vegetative shoots is effective for eye disorders. Diabetes, pustules, and scabies have all been treated with root bark water extract, while bone fractures have been treated with root powder paste (Ahmed et al. 2009a, b). Traditional practitioners treat jaundice with a rhizome decoction (Zabihullah et al. 2006).

5.8 Phytochemistry

The major compounds present in *B. aristata* are alkaloids, and there are few reports regarding the presence of flavonoids in flowers. Blasko et al. (Blasko et al. 1982a, b) and Atta-ur-Rahman et al. noticed the presence of karachine (a protoberberine alkaloid) and other alkaloids such as berbamine, berberine chloride, aromoline, oxyberberine, and oxyacanthine in the root bark of *B. aristata*. In another such study, pseudopalmatine chloride, pseudoberberine chloride, 1-O-methylpakistinine, and pakistanine were isolated from the bark of this plant (Bhakuni et al. 1968; Lect et al. 1983). Further pseudobenzylisoquinoline type alkaloid-taxilamine was isolated from this plant (Blasko et al. 1982a, b). In one study, nearly four alkaloids (palmatine chloride, tetrahydroberberine palmatine, terahydropalmatine, berberine) and their mixture were isolated from the alcoholic extract of powdered bark of this plant (Chakravarti et al. 1950; Chakarvrati 1950). The main constituents of the flowers of this plant are polyphenolic flavonoids like rutin, meratin, and quercetin. Ecaffeic acid and chlorogenic acid were the most abundant acids found in the flowers (Sivakumar and Nair 1991). In an ethanolic extract of *B. aristata* heartwood, the presence of n-docosane (an aliphatic hydrocarbon) was found (Katiyar et al. 2011). Overall, the most prevalent alkaloid in *B. aristata* is berberine, which can be recovered with an approximate yield of about 2.23%, while the second most abundant alkaloid is palmatine, according to the literature. Furthermore, the composition of berberine is affected by height, as well as soil potassium and moisture content (Andola et al. 2010). The roots and stems of plants growing at lower altitudes contain more berberine than those growing at high altitudes (Ray and Roy 1941). Moreover, with the advancement of the techniques, the quantification of berberine has also become simple, precise, and easy with the help of spectrophotometers and HPTLC fingerprinting (Chauhan et al. 2000). The quantification of berberine concentration in root and bark of *B. aristata* was determined with the aid of HPTLC fingerprinting which showed its abundance in the roots than bark (Andola et al. 2010).

Cardiac glycosides, saponins, hydrolyzable tannins, and alkaloids were identified in aqueous extracts of *B. lycium* (Ahmed et al. 2009a, b). Sood et al. (2010) reported that fruits of *B. lycium* possessed 8.9, 2.5, 343.0, 0.78, 6.01, 82.47, 85.65, and

7.94 mg/100 g percentage of tannins, phytic acid, β-carotene, phytate phosphorus, hemicellulose, anthocyanin, vitamin A, and cellulose, respectively. Malic acid, tartaric acid, and citric acid were present in fruits (Sharma 2003; Sood et al. 2010).

This plant is a major source of alkaloids (Khan et al. 2010) and alkaloids contributed to about 2.45% of the total chemical composition of roots (Srivastava et al. 2010). Berberine is chief alkaloid present in *B. lycium* along with berbamine, palmatine, and jatrorrhizine (Bhardwaj and Kaushik 2012). The other compound reported were baluchistanamine, jhelumine, karakoramine, gilgitine, punjabine, chinabine, sindamine, and umbellatine, acetic acid, ascorbic acid, and maleic acid (Baquar 1989; Manske 1998; Khare, 2004). Ikram et al. (1966) have isolated berbericine, berbenine, and berbericinine iodide alkaloids from *B. lycium*. Miana and co-workers (1970) have identified palmatine iodide, berberine chloride, and berbamine from the roots of *B. lycium*. Three artefact alkaloids, palmatine-$CHCl_3$, berberine-$CHCl_3$, and oxyberberine from roots were isolated and characterized by Miana (1973). The alkaloid baluchistanamine was detected in this plant by Krane and Shamma (1982). Other polar alkaloids such as karakoramine, jhelumine, chenabine, and berbamunine were also isolated from roots by a similar researcher team (Leet et al. 1983). Jhelumine, baluchistanamine, punjabine, karakoramine, gilgitine, sindamine, umbellatine, and chinabine as well as acetic acid, ascorbic acid, and maleic acid were also reported. Khare (2004), Baquar (1989), Manske (1998), Baquar (1989), and Ikram et al. (1966) identified three alkaloids from *B. lycium*: berbenine, berbericine, and berbericinine iodide. Berberine chloride, palmatine iodide, and berbamine were found in the roots of *B. lycium* by Miana and colleagues (1970). Leet et al. (1982) identified three seco-bisbenzylisoquinoline alkaloids from *B. lycium* roots: sindamine, punjabine, and gilgitine. Khan et al. (2010) have found berberine and palmatine from its three different solvent root extracts (butanol, ethyl acetate, and aqueous). Different researchers have reported different concentrations of berberine in different parts of a plant. Berberine contributed to about 80% dry weight of root of *B. lycium.* The amount of Berberine reported in root and fruit of *B. lycium* was 4.5 and 2.9%, respectively (Gulfraz et al. 2004). Another study also observed a similar berberine content in roots and fruits of *B. lycium* (Andola et al. 2010). Five new compounds (sitosterol, 4-methyl-7-hydroxy-coumarin, 4,4-dimethylhexadeca-3- ol, 3-(4^1-(6-methyl-butyl)-phenyl) propan-1-ol, and butyl-3-hydroxypropyl phthalate along with berberine were isolated from petroleum ether and methanol extracts of *B. lycium* (Sabir et al. 2013). Recently, HPLC examination revealed the existence of berberine, quercetin, rutin, chlorogenic acid, hydroxy benzoic acid, and mandelic acid in *B. lycium* roots (Nazir et al. 2021). Bukhari et al. (2021) reported total phenolic contents and total chlorophyll contents of 88.66 μg/g of gallic acid equivalent phenolic (GAE) and 3.75 μg/mL, respectively, in the roots of *B. lyceum,* Royle collected from Muzaffarabad and Neelum Districts of Azad Jammu and Kashmir. They have also reported 4.76% berberine in the roots. The structures of key secondary metabolites are presented in Fig. 5.1.

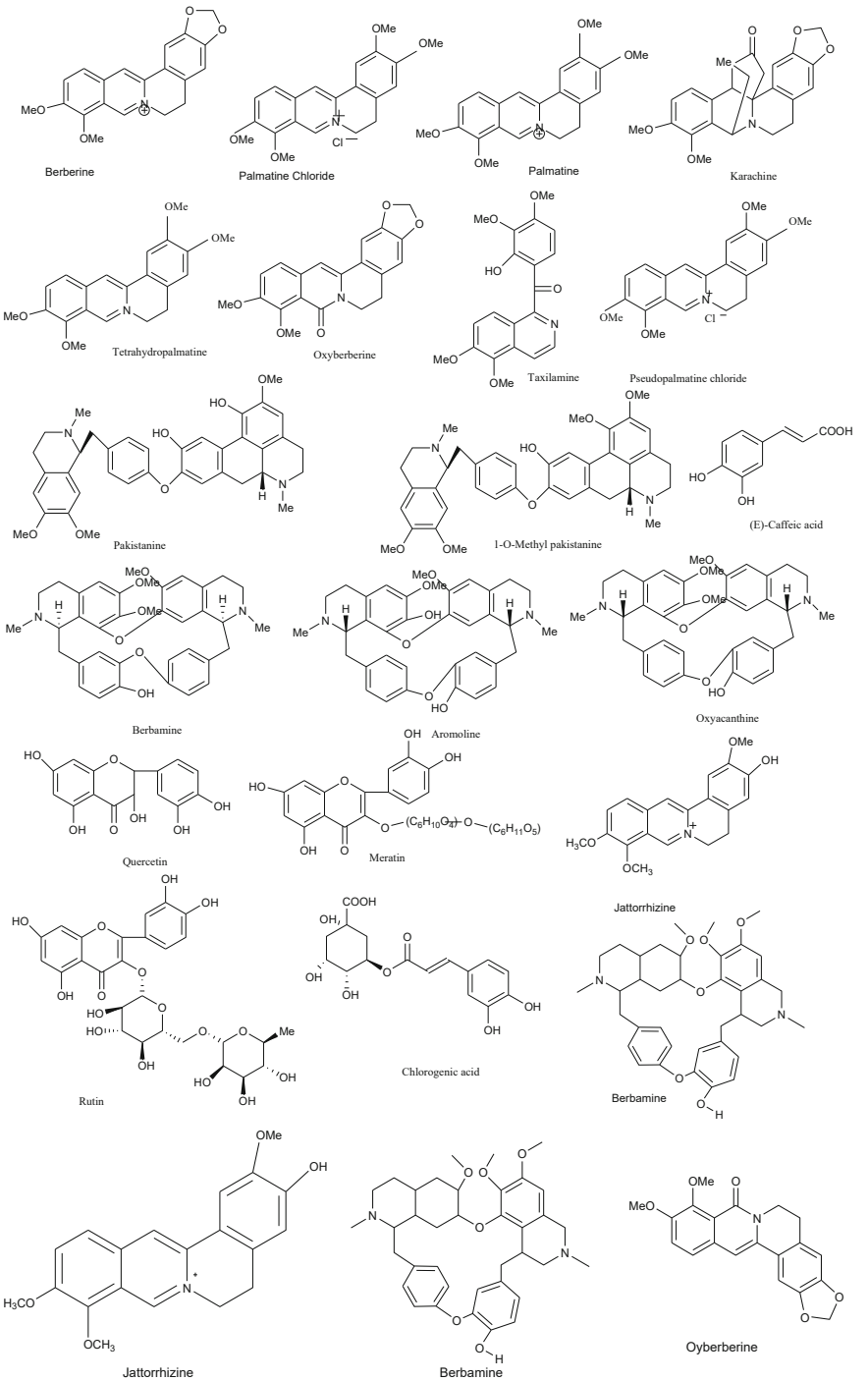


Fig. 5.1 Main compound isolated plants of *Berberis* species

5.9 Nutritional Composition

The berberis genus is explored for its medicinal properties. The proximate and mineral analysis of a few species has revealed it to be enriched in proteins, minerals, and fiber. Moreover, depending upon the plant part its composition varies. The roots of *B. lycium* are rich in copper, sodium, and potassium whereas its leaves constitute zinc, copper, iron, phosphorous, potassium, manganese, calcium, and sodium (Shah et al. 2003; Gulfraz et al. 2004; Srivastava et al. 2006). Zinc, sodium, and copper were found to be the highest in the root, whereas manganese, phosphorous, and calcium were maximum in the leaves, and potassium was most in the stem (Shah et al. 2003). It has been stated that the fruit of *B. lycium* contained a significant amount of iron (Rahimi-Madiseh et al. 2014). The proximate analysis of various parts of *B. aristata* revealed that roots showed the presence of 61.2, 20.5, 4.5, 2.6, 3.5, 2.5, and 0.34% of dry matter, moisture, protein, fat, sugar, fiber, and vitamin C, respectively, while the fruits composed of dry matter (62.5%), moisture (12.5%), sugar (4.5%), protein (2.5%), fat (1.8%), fiber (1.5%), and vitamin C (0.8%) in significant amount. Overall roots were found to be a major source of fat as well as fiber whereas leaves were found to be rich in proteins (Shah et al. 2003; Sood et al. 2010). Five Berberis species collected from different parts of the West Indian Himalayas were investigated for their nutritional composition. Different parts contain different compositions of nutrients. The fruits of this plant were an important source of fiber, protein, and fat in which pulp contained 7–8% fiber, 4.7–7.2% protein, and 2.6–4.0% fat, and seeds contained lesser fiber content (4.4–5.3%) in comparison to a pulp, whereas protein (5.9–8.5%) and fat (4.6–5.3%) content was higher as compared to a pulp. Overall carbohydrate content in the food is lower, but all these species were rich in mineral content (especially calcium and potassium) (Andola et al. 2011).

5.10 Pharmacology

B. aristata is a distinguished medicinal plant, and it is very well documented in Ayurvedic and Siddha Pharmacopoeia of India. In line with their traditional uses, several activities against various ailments had been conducted from time to time to find their scientific relevance. This plant and its parts are used during the preparation of various herbal compositions constituting various plants. Out of several species, the most studied ones for their medicinal potential are *B. aristata* and *B. lycium,* so under this subheading, we have compiled the different pharmacological activities of both these species reported to date.

5.10.1 Pharmacological Activities of B. aristata

5.10.1.1 Anticancer

The anticancer potential of the alcoholic extract and alkaloids found in *B. aristata* was tested against various cancer cell lines, and favorable findings were achieved. The effects of a methanolic extract of *B. aristata* stem on colon cancer cell lines were examined. After 72 h of inhibition, the extract inhibited the HT29 cells with IC50 values of 1.96 g/mL (Das et al. 2009). Berberine, the plant's main alkaloid, was found to be helpful in preventing 20-methylcholanthrene-induced carcinogenesis in mice and rats (Anis et al. 2001). Prostate cancer cells were shown to be resistant to the butanolic fraction, which is high in berberine. In PC-3 cells, this fraction was able to decrease NFB activity (both promoter and DNA binding) (Muralimanoharan et al. 2009). Berberine also showed its effectiveness against nasopharyngeal cancer. It was found to induce NM23-H1 expression which is responsible for inhibition of tumor implantation and cachexia thereby controlling nasopharyngeal carcinoma (Liu et al. 2008). This is also effective in controlling neuroblastoma cancer cells via induction of their apoptosis of SK-N-SH and SK-NMC cell growth (Choi et al. 2008). Moreover, this plant has also shown its potential in reducing the nephrotoxicity caused by cisplatin (one of the anticancer drugs) via a reduction in lipid peroxidation (Adikay et al. 2010). In mice with Ehrlich ascites carcinoma, the antineoplastic activity of *B. aristata* was tested in both aqueous and ethanolic forms. The ethanolic extract was shown to be more efficient (6.5 mg/kg i.p.) than the aqueous extract (100 mg/kg i.p.) in reversing the effects of tumor-induced changes; however, both extracts were less effective than the positive control cisplatin (3.5 mg/kg i.p.). Overall, the presence of alkaloids and flavonoids was assumed to be responsible for the ethanolic extract's action (Pai et al. 2012). The potential of Indian berry against human breast cancer cell lines (MCF-7) was investigated due to its traditional effects against skin-related illnesses, inflammations, ulcers, and other conditions. Further, methanolic extract was able to decrease colony formation at 500 ppm concentration as well as it also inhibited 50% of cell migration at 250 ppm concentration. This extract was also able to induce apoptosis up to 68% at 500 ppm as done by live/dead assay (Serasanambati et al. 2015).

The synergistic effects of *B. aristata* along with *Azadirachta indica* methanolic extracts, prepared via soxhlet extraction and sonication was tested for their potential to cure or inhibit bone cancer. These extracts alone or in combination were evaluated against human osteosarcoma cancer cells. The best results were shown by extracts prepared by the soxhlet extraction method in the case of *B. aristata* and sonication in the case of *A. indica*. These extracts were capable of inducing apoptosis in both drug-sensitive and drug-resistant cells. The combination of these extracts was also evaluated to see if the chemicals contained in both extracts had a synergistic impact. The combined extracts of *B. aristata* and *A. indica* prepared by both the methods soxhlet and sonication showed a synergistic effect against cancer cell lines. Even the combined extract obtained by the sonication method showed its efficacy against

cancer cells which have otherwise developed resistance against cisplatin drugs. Two major compounds (berberine and palmatine) were found to be present in *B. aristata* extract which was thought to be accountable for the activity of these extracts (Sengupta et al. 2017).

5.10.1.2 Antioxidant

B. aristata has long been used for the treatment of metabolic problems. Its aqueous-alcoholic root extract (1:1) showed strong antioxidant potential by lowering oxidative stress in diabetic rats. This extract was able to enhance the activity of catalase and superoxide dismutase by around 91 and 41% which was otherwise suppressed in diabetic rats (Singh and Kakkar 2009). An aqueous extract of bark of *B. aristata* was assessed for its antioxidant and antibacterial potential. The results indicated that the bark can also act as a potential drug (Sharmila et al. 2020). In the case of DPPH radical and NO scavenging activities, the methanolic extract of *B. aristata* stem demonstrated excellent antioxidant capacity with IC50 values of 0.90 mg/mL and 0.80 mg/mL, respectively. In the case of DPPH activity, methanolic extract was shown to be as efficient as gallic acid ($IC_{50} = 0.61$ mg/mL) (Dar et al. 2012). In a DPPH radical scavenging experiment, the aqueous: alcoholic stem bark extract of this plant had an IC_{50} value of 16 ± 0.5 g/mL, and in a nitric oxide radical scavenging assay, it had an IC_{50} value of 2500.5 g/mL. The presence of phytoconstituents such as quercetin (approx. 3.0 ± 0.02 g of quercetin/mg of dry extract) and phenolic compounds were implicated for the action (Thakur et al. 2016).

5.10.1.3 Antimicrobial Activity

B. aristata plant extracts have been around since the dawn of time. The isolated compounds from them have been used for the treatment of various diseases caused by variety of microorganisms. The extracts of different parts or the decoctions of this plant have been used to treat several diseases caused by different categories of microorganisms viz. bacteria, protozoans, fungi, viruses, nematodes, and chlamydia (Singha et al. 2007; Malik et al. 2014; Sharmila et al. 2020). The plant extract gel formulation of *B. aristata* was effective in treatment of skin-related bacterial infections caused by different bacterial strains (*Pseudomonas aeruginosa*, *Corynebacterium,* and *Staphylococcus aureus*) which had otherwise developed resistance against ointments available in the market (Deshmukh et al. 2009). This plant finds its applications against a wide range of bacterial strains. In one such study, it was found effective against *Helicobacter pyroli* (stomach ulcer-causing bacteria) thereby useful to treat gastric ulcers and other stomach-related disorders (Zaidi et al. 2009). Berberine is a principal alkaloid present in this plant. The salt of berberine, i.e., Berberine sulfate was tested against *Trichomonas vaginalis* under laboratory conditions, and the results showed its effectiveness at par with commercially available drug metronidazole. Hence, this can act as a replacement and safer alternative for this

synthetic drug (Soffar et al. 2001). This plant is not effective against human pathogens but is also found to be active against certain plant pathogens. In one such report, berberine extracted from the roots of this plant was found to inhibit spore germination in several phytopathogens. Berberine in combination with santonin or alone was able to inhibit spore germination thereby contributing towards the control of plant diseases (Singh et al. 2001). The extract of various parts of *B. aristata* was found to be effectual as an antibacterial agent against various bacterial strains. The methanolic stem extract of this plant reflected its effectiveness against *E.coli, B. subtilis,* and *S. aureus* bacterial strains. *B. subtilis* was the most inhibited bacterial strain with a maximum inhibition zone of 19 mm diameter (Dar et al. 2012). The hexane extract of roots of *B. aristata* showed antifungal activity against several clinical (Mathur et al. 2011) and phytopathogens (Sharma et al. 2008). The root bark extract of *B. aristata* was tested against 13 different bacterial strains and the extract showed its effect in the range of 12–25 mm. Furthermore, ethyl acetate was the best eluting solvent which extracted bioactive compounds accountable for the activity of the extract against bacterial strains. Out of different categories of the compounds, diterpenes were the most abundant. However, ethyl acetate extract was best (IC_{50} value 0.05–1 mg/mL), followed by diterpenes (IC_{50} value 0.05–5 mg/m) and flavonoids (IC_{50} value 0.05–10 mg/mL). Overall, the extracts were safe and also possessed antioxidant potential (Sood et al. 2019). *B. aristata* owing to its antimicrobial potential was effective to treat ear-related infections. The leave and root extract of this plant exhibited a wide range of action against six bacterial strains that cause ear infections. For both leaves and root extracts, the acetone extract was shown to be the most efficient against *S. aureus*. However, the highest MIC value against *S. aureus* was shown by acetone root extract and aqueous leave extract, i.e., 3.12 mg/mL in each case (Sharma et al. 2011). Even the stem bark extract of this plant showed an antimicrobial efficacy against a carbapenem-resistant strain of *E. coli*. The efficacy was assumed to be owing to the extract's synergistic action of many bioactive components (Thakur et al. 2016). Khan et al. (2020) assessed the antimicrobial potential of *B. aristata* stem bark extract against two bacterial and two fungal pathogens. The results showed it to be developed as a plant-based antimicrobial drug.

5.10.1.4 Hepatoprotective Activity

In recent years, the potential of *B. aristata* in the treatment of liver disorders or drug-induced damage has been investigated. Because of its cell membrane stabilizing properties, this plant was able to modify the harmful effects of bile salts in hepatic disorders (Upadhyay 2001). Moreover, its formulation was able to reduce the infection rate in golden hamsters caused due to amoebic-induced liver abscess as well as its effect was also studied in immunomodulation studies (Sohni and Bhatt 1996). The role of this plant in drug-induced hepatic disorder was also studied. In one such study, acetaminophen-induced hepatic disorder which resulted in raised glutamic pyruvic transaminase, serum alkaline phosphatase, and glutamic

oxaloacetic transaminase levels was treated with this plant (Gilani and Janbaz 1992). *B. aristata* is also a component of the polyherbal formulation known as Hepjaun syrup, which has been shown to have liver-protective properties. As a result, the formulation, either directly or in modified form, can be used to treat liver-related disorders (Patel et al. 2010). The water-methanol extract of the *B. aristata* fruits was able to show a hepatoprotective effect by inhibiting microsomal-drug metabolizing enzymes (Gilani and Janbaz 1995). The alcoholic extracts of this plant itself and its parts are used to treat various liver-related disorders. Besides from that, the chemicals in this plant have been shown to have hepatoprotective effects. Berberine is a key alkaloid present in this plant family's roots. In one such study, berberine was able to inhibit the calcium-potassium modulated signals thereby showing their role as hepatoprotective agents (Fang et al. 2004). The confirmation of the role of berberine was done by Tsai and Tsai (2004) which showed that Cyt P-gsp and P-450 played an important role in regulating liver metabolism and hepatobiliary excretion. Berberine is also found to be effective against Chinese medicines (Feng and Cheung 2009). Berberine also finds its application to inhibit the synthesis of triglycerides and cholesterol in liver cells—human hepatoma cells and primary hepatocytes (Brusq et al. 2006; Ge et al. 2011). Berberine is also known to regulate Hepatoma H4IIE cells via increased glucose consumption with an increase in the dose (Yin et al. 2008). *B. aristata* lowers total cholesterol, triglyceride, and low-density lipoprotein levels while raising high-density lipoprotein levels, boosting LDLR expression (Pirillo and Catapano 2015).

5.10.1.5 Cardioprotective Effect

Other medicinal uses linked to *B. aristata* has also been shown to help those with heart problems (Ahmed et al. 2009a, b). Various portions of this plant have been utilized to treat a variety of ailments. This plant's fruit extract exhibited inotropic potential, with distinct bioactive chemicals responsible for a selective inotropic effect including modification of actin-myosin cooperativity (Gilani et al. 1999). Triglycerides, serum cholesterol, and LDL cholesterol were all reduced by *B. aristata* extract (low-density lipoproteins). Similarly, it reduced thrombin and fibrinogen time, and the study was conducted regardless of the rabbits' gender (Razzaq et al. 2011). In diabetic mice, Suman et al. (2016) discovered that berberine had cardioprotective and myocardial saving properties.

5.10.1.6 Anti-inflammatory

An aqueous extract of *B. aristata* and *Curcuma longa* was tested for its anti-inflammatory effect in endotoxin (from the lipopolysaccharide of *E. coli*) induced uveitis in the rabbit. In rabbits with endotoxin-induced uveitis, topical administration of this extract had an anti-inflammatory effect (Gupta et al. 2008). One of the Unani eye drop formulations contains *B. aristata* stem extract which was able to treat the

inflammatory action induced in rabbit eyes by turpentine liniment. The same formulation was also able to alter the histamine effects caused during inflammation by blocking H1-receptors in guinea-pig ileum (Latif et al. 2010). With the advancement in anti-inflammatory medicines, new nanogels containing the plant extract were developed to be utilized to treat inflammation, etc. In one such study, nanovesicular gel containing *B. aristata* ethanolic root extract was developed to treat psoriasis induced by imiquimod and positive results showing anti-inflammatory and anti-psoriatic effects were observed in both in vitro and in vivo studies (Nimisha et al. 2017). *The aqueous-alcoholic extract of B. aristata was able to show anti-inflammatory and anti-granuloma effects in carrageenan-induced paw edema, complete Freund's adjuvant-induced stimulation of peritoneal macrophages, and cotton pellet induced granuloma formation in rats. There was a strong relation between dose and anti-inflammatory and anti-granuloma effects (Kumar et al.* 2016*). The anti-inflammatory efficacy of aqueous and ethanolic extracts of several portions of the B. aristata plant was examined, and heartwood extract showed the highest impact, with percent inhibition of 52 and 57% for ethanolic and water extracts, respectively, at a dose of 50 mg/kg* (Singh et al. 2014).

5.10.1.7 Anti-diarrheal Activity

The *B. aristata* extract has been traditionally known to relieve stomach-related ailments. The in vitro and in vivo studies of *B. aristata* extract confirmed its antidiarrheal properties (Joshi et al. 2011). An aqueous stem extract showed an antidiarrheal and antispasmodic effect in magnesium sulfate-induced diarrhea in mice. The main mechanism of action involved a decline in intestinal secretions via inhibition of intestinal motility (Shamkuwar et al. 2013). The crude extract was used to prepare a formulation that inhibited toxins released during cholera infection thereby decreasing the severity of diarrhea (Sabir et al. 1977). Even its major alkaloid Berberine was known to inhibit the symptoms caused by two bacteria, *Vibrio cholera,* and *E. coli*, and was thought to act as an antidiarrheal compound (Sack et al. 1982). Further in vivo studies also confirmed the effects of berberine as an antidiarrheal compound @ 10 mg/kg, the main effect was that it elongated the latent period and severity of diarrhea (Sabir et al. 1977).

5.10.1.8 Antidiabetic Activity

One of the traditional uses of *B. aristata* includes its potential to treat diabetes more specifically diabetes mellitus. The part of the plant used to treat diabetes is its stem (Upwar et al. 2010). However, the roots also possessed anti-hyperglycemic activity. In one such study, ethanolic root extract of the plant showed antidiabetic potential in alloxan-induced diabetic rats (Semwal et al. 2008). The major mode of action as antidiabetes includes regulation of glucose homeostasis by decreasing gluconeogenesis. This also added to its antioxidant potential by decreasing oxidative stress (Singh

and Kakkar 2009). The commercial formulation containing *B. aristata* (D-400) is effective against diabetes mellitus (Sundaram et al. 1996). Diabetes is mostly associated with higher cholesterol levels. The methanolic extract of *B. aristata* was found to alleviate the enzymatic activity which is responsible for bile acid synthesis and its excretion which thereby decreases cholesterol and triglycerides in serum. This study suggested the antidiabetic and hypolipidemic potential of this extract thereby showing its potential to prevent coronary heart diseases (Upwar et al. 2011). Moreover, the major alkaloid presents in this plant showed its efficacy to mimic insulin, activating 5′adenosine monophosphate-activated protein kinase which thereby improved the insulin action. It also reduced insulin resistance via several mechanisms including inhibition of DPP-1 V, promoting secretion of GLP-1, etc. Overall berberine also acts as an antidiabetic compound (Steriti 2010; Chakrabarti et al. 2011). The ethanolic extract was analyzed for its antidiabetic potential in vitro and further studies revealed that quercetin was the compound responsible for its potential. The in vivo test of ethanolic extract was performed on alloxan-induced rats was performed and similar results were found as shown by standard drug glibenclamide which showed that *B. aristata* and its phytoconstituents can act as a promising alternative for the development of medicine to treat diabetes mellitus (Soundarrajan 2017).

5.10.1.9 Ophthalmic

Different studies were conducted to date to test the potential of *B. aristata* extracts on ailments related to the eyes. In one such report, the formulation (Netrabindu and Madhudarvyadi) and containing *B. aristata* were tested for their potential to treat conjunctivitis and this was found effective (Sharma and Singh 2002). Another formulation containing *B. aristata* was able to treat symptoms related to cataract (Dhiman et al. 2010). *B. aristata* is found in an ayurvedic medicine called Elanir kujambu, which is used to diagnose a range of microbial eye diseases (Premnath and Yoganarashimhan 2009). The clinical trial was performed to test the effectiveness of *B. aristata* extract (Rasanjana) on the various eye ailments infective conjunctivitis. The extract was able to minimize the various symptoms of conjunctivitis such as redness and burning sensation. Overall, this extract was further recommended to be used as a treatment for eye ailments (Agarwal et al. 2014). Animal models were used to assess the anti-inflammatory and antihistamine properties from another Unani formulation containing *B. aristata*. The results were comparable to those obtained with the commonly used medication flurbiprofen sodium ophthalmic solution (Abdul et al. 2010).

5.10.2 Pharmacological Activities of B. lycium

5.10.2.1 Antidiabetic Property

In a study of aqueous, MeOH, $CHCl_3$ and n-hexane extracts of *B. lycium* in normal and diabetic rabbits, the aqueous extract had the highest hypoglycemic potential (6 h), whereas the other extracts had activity up to 4 h. The aqueous extract results were comparable to normal insulin (Dhar1983). When evaluated for an antidiabetic activity using glibenclamide as a standard, aqueous and ethanolic extracts of *B. lycium* roots displayed excellent results at a dose of 100 mg/kg (Gulfraz et al. 2004). Ethanolic root extract and berberine, an alkaloid derived from *B. lycium* roots, showed equal levels of effectiveness. *B. lycium* and its root preparations can help to lower blood glucose levels considerably (Li et al. 2019). The antidiabetic potential of *B. lycium* root extracts (ethanolic) and pure berberine in normal and alloxanized diabetic rats demonstrated that both extracts and berberine were ineffective in controlling glucose levels.

5.10.2.2 Antimicrobial Property

Bacteria and fungi were discovered to be sensitive to *B. lycium*. Berberisspare stem and root extracts are known to have antibacterial properties (Singh et al. 2007). The hydroalcoholic (50%) extracts of *B. lycium* were shown to be the most effective among the several berberis species. When compared to fungal strains, stem and berberine had higher efficacy against bacteria (Singh et al. 2007). *B. lycium* extracts depicted better immunity against infectious bronchitis, Newcastle disease, and infectious bursal disease. The response of coccidial oocysts/gram of feces to this plant was significantly reduced (Nidaullah et al. 2010).

5.10.2.3 Hepatoprotective Property

Hepatoprotective efficacy was discovered in methanolic extract and crude powder of *B. lycium* stem. Increased intensities of serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, and alkaline phosphatase enzymes in the experimental organism were dramatically lowered by the extracts (Ahmad et al. 2008). In mice, the stem powder of *B. lycium* was likewise beneficial in preventing carbon tetrachloride-induced hepatotoxicity (Khan et al. 2008). A herbal formulation containing *B. lycium* also showed hepatoprotective activity in paracetamol-induced hepatotoxic mice in a comparable investigation (Girish et al. 2009).

5.10.2.4 Antihyperlipidemic Property

Male albino rabbits were used to investigate the antihyperlipidemic capabilities of *B. lycium* roots. The oral treatment of crude powder (250 and 500 mg/kg) of roots for 4 weeks resulted in a substantial drop in triglyceride, total cholesterol, and low-density lipids (LDLs), with a rise in high-density lipids (HDLs) in rabbits. In diabetic rabbits, weight stabilization was also found (Ahmed et al. 2009a, b). In broilers, a comparable concentration-dependent decrease in triglycerides, LDL, and total cholesterol was seen (Chand et al. 2007). In broiler chickens, treatment of *B. lycium* at 2-day intervals resulted in a considerable improvement in liver function and serum lipid profile (Manan et al. 2012). Similar outcome was observed via the application of fruit extracts @ 600 mg kg^{-1} (Rahimi-Madiseh et al. 2014).

5.10.2.5 Pesticidal Property

At a dose of 10,000 ppm, petroleum ether extracts killed 26% of *Tetranychus urticae* Koch, 96% of *A. craccivora* Koch, and 28% of *H. armigera* Hub, and *Plutella xylostella* L, whereas aqueous methanolic extract killed 26% of *A. craccivora* Koch, 44% of *H. armigera* Hub, 41% of *P. xylostella* L, 43%. The fatality rates were comparable to those of the commonly used insecticide Dimethoate. (Tewary et al. 2005).

5.10.2.6 Wound Healing Property

The property of aqueous and methanolic extracts of *B. lycium* root to heal wound was studied in Swiss Wistar rats using excision, incision, and dead wound space as models. Both extracts improved hydroxyproline content, epithelialization area wound contraction, skin breaking strength, dry weight, tissue granulation, and hydroxyproline content. Collagen deposition with high fibroblasts and macrophages was seen in the aqueous extract, but collagen deposition with low fibroblasts and macrophages was observed in the methanol extract. Methanol extract was shown to be more efficient than aqueous extract. (Asif et al. 2007). Another similar study on animal models reported that root extracts have properties to enhance the wound healing property as these are rich in nutrients such as calcium and magnesium (Ahmad et al. 2011).

5.10.2.7 Antioxidant Property

The root extract and compounds isolated from *B. lycium* are identified for antioxidant properties and strong reduction potential (Gupta et al. 2009; Mashwani et al. 2013; Ahmed and Shakeel 2012). The petroleum ether extract *B. lycium* showed

maximum antioxidizing properties as compared to chloroform and methanol extracts (Sabir et al. 2013). The chemical analysis of the wine prepared from fruits of *B. lycium* revealed the presence of 186.7 mg/L of total antioxidant compounds (Rana and Singh 2013). A study reported that the phytochemicals such as steroids and alkaloids are responsible for the antioxidant potential of *B. lycium* roots extract (Sharma et al. 2019) while recent research correlated the high antioxidant activity to the amount of berberine and chlorogenic acid reported in the root of *B. lycium* (Nazir et al. (2021). Bukhari et al. (2021) has also reported the antioxidant activity of root bark of *B. lycium* Royle collected from different districts of Azad Jammu and Kashmir.

5.10.2.8 Antihyperglycemic Activity

In healthy rats, an oral administration of *B. lycium* ethanol extract caused a considerable reduction in blood glucose levels. Similar findings were reported in alloxan-induced diabetic rats via oral administration of ethanol extracts of *B. lycium* at doses of 50 and 100 mg kg^{-1} (Gulfraz et al. 2008). Another investigation on normal and diabetic rats verified the antihyperglycemic activity of the powdered root extracts of *B. lycium*, which found that root extract may be used as a supplement to insulin for the treatment of diabetes mellitus (Ahmad et al. 2009). A daily intake of 500 mg kg^{-1} of body weight of aqueous and methanolic extracts of *B. lycium* extracts induces a considerable drop (50%) in blood glucose levels and an increase in glucose tolerance. The rats' blood glucose levels were normalized after 150 min of glucose injection (Mustafaa et al. 2011). In type 2 diabetic male mice, a comparison of the antihyperglycemic property of the bark aqueous extract of *B. lycium* and glimepiride found that the extract was more efficient than glimepiride in regulating glucose levels (Aslam et al. 2015).

5.10.2.9 Anticancer and Antitumor Activity

The study of the anticancer property of the root extracts (ethyl acetate, butanol, and aqueous) of *B. lycium* and alkaloids (berberine and palmatine) revealed that butanol extract was the most active in inhibiting the cell cycle in HL-60 human leukemia cells. The antitumor effect of butanol extract is mainly attributed to the berberine content (Khan et al. 2010). The high berberine content causes breakage of DNA strand. The authors correlated the anticancer property of the herbs with cholesterol levels lowering (Issat et al. 2006). The crude extract of *B. lycium* Royle (IC50 = 47 g/mL) has a potential antiproliferative impact against the Human Hepatocarcinoma (HepG2) cancer cell line. HPLC analysis of the most active fraction revealed berberine, benzoic acid as the main components of the extract. The study concluded that *B. lyceum* Royle extracts can be utilized for treating liver cancer (Mustafa et al. 2020).

5.10.2.10 Antimicrobial Activity

Antimicrobial activity has been found in *B. lycium* root extract (Hussain et al. 2011). Among the hydroalcoholic extracts of different *B. spp* tested against 11 bacterial and 8 fungus strains, *B. lycium* and two other species root extracts exhibited potential antibacterial properties. Because of the high alkaloid berberine content, *B. lycium* has the lowest minimum inhibitory concentration (MIC) (Singh et al. 2007; Singh et al. 2009). In another study, *B. lycium* root methanolic extracts were shown to be more effective than water extracts against different bacteria and fungus species (Gulfraz et al. 2007). On the contrary, a research found that aqueous root extracts of *B. lycium* were ineffective against multidrug-resistant bacteria such as *E. coli*, *Staphylococcus aureus*, and *Klebsiella pneumonia* (Mansoor et al. 2013). Silver nanoparticles derived from *B. lycium* aqueous extracts exhibited remarkable antibacterial activity (Mehmood et al. 2014).

5.10.2.11 Anticoccidial Activity

A decrease in coccidial oocysts per gram of feces was observed in a study (Nidaullah et al. 2010). Using broiler chicken, the anticoccidial activity of various extracts of *B. lycium* and its main alkaloid berberine was investigated in vivo. Methanol extract demonstrated maximal efficacy at a concentration of 300 mg kg^{-1} body weight and no toxicity up to a concentration of 2000 mg kg^{-1} body weight. The anticoccidial potential is attributed only to berberine content (Malik et al. 2014).

5.10.2.12 Immunity Enhancer

B. lycium extracts have been known to boost immunity. It has been discovered that feeding broiler chicks 20 g kg-^{1} of *B. lycium* increased their immunity against Newcastle disease and infected bursal disease (Chand et al. 2011). In broiler chicks, infusions of *B. lycium*, either alone or in combination with other herbal plants, improved immunity and performance (Mushtaq et al. 2013; Raziq et al. 2012).

5.10.2.13 Antiurolithic Activity

In artificial urine, the infusion and decoction of *B. lycium* root bark prevented calcium oxalate crystallization by 80.2 and 92.4%, respectively (Shah et al. 2014).

5.10.2.14 Anthelmintic Activity

The anthelmintic action of *B. lycium* root bark decoction and infusion against earthworm (*Pheretima posthuma*) has been demonstrated (Shah et al. 2014). The anthelmintic activity of the bioassay was analyzed by measuring the model animal's paralysis, and death time.

5.10.2.15 Hepatoprotective Activity

Hepatoprotective activity of *B. lycium* was observed in carbon tetrachloride exposed rats (Khan et al. 2008; Sood et al. 2012). A mixture of *B. lycium*, *Galiuma aparine,* and *Pistacia integerrima* tend to improve liver functioning in broiler chicks (Chand et al. 2011). Another study also confirmed that methanol extracts of the root of *B. lycium* can reduce hepatotoxic effect due to carbon tetrachloride in rabbits, and the results of the study were comparable with the Silymarin, standard drug (Sherani et al. 2019).

5.10.2.16 Antiprotozoal Activity

Berberine crude extracts showed more effectiveness as compared to berberine salts (Kaneda et al. 1990). Berberine administration eased gastrointestinal symptoms and reduced Giardia positive stools significantly in a clinical trial, and it was efficacious at half the dose of the popular Giardiasis medicine, metronidazole (Choudhary et al. 1972).

5.10.2.17 Antibacterial Activity

The components present in *B. lycium* root extracts were found to have inhibitory activity (Irshad et al. 2013). It should not be taken with Glycyrrhiza species (Liquorice) since the berberine's benefits will be neglected.

5.10.2.18 Cardiac Diseases

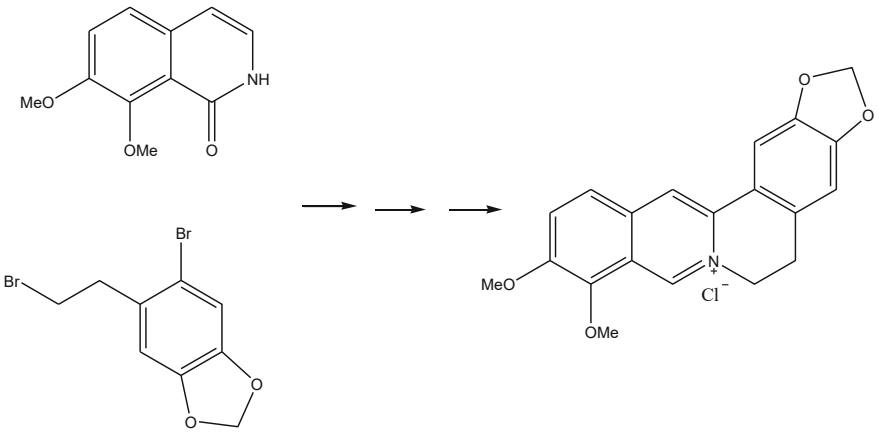
Berberis species have been demonstrated to reduce triglyceride levels in the blood. Berberine, the plant's main alkaloid, prevented the development of left ventricular hypertrophy by inhibiting ischemia-induced ventricular tachyarrhythmia, promoting cardiac contractility, lowering peripheral vascular resistance and blood pressure, and reducing peripheral vascular resistance and blood pressure (Chand et al. 2007).

5.10.2.19 Analgesic Activity

The crude methanol extracts of *B. lycium* showed promising ($p < 0.05$) analgesic effect against both the acetic acid produced pain and formalin tests. Crude methanolic extracts of *B. lycium* revealed substantial ($p < 0.05$) central nervous system-depressant activity in neuropharmacological activities, as well as anxiolytic effects in the forced swimming test (Samiullah et al. 2020).

5.11 Synthetic Strategies for Key Secondary Metabolites

Alkaloids are the most common secondary metabolites found in Berberis plants, and "Berberine" is the most common alkaloid found in practically all of the genus" plant species. Although berberine is very important as per medicinal point of view still very few reports are available on the synthesis of this compound. The reason for this may be attributed to its easy extraction and that too in bulk quantity from different genus apart from Berberis as well its difficult synthetic strategies with lower yields as compared to regular isolation from natural sources. So few reports are available in the literature on its synthesis. Epiberberine, a bioactive protoberberine alkaloid found in nature, was synthesized in four simple, low-cost steps that included cyclization, condensation, reduction, and ring-closing, yielding 26.1% overall (Lu et al. 2012). Tajiri et al. (2021) reported a 16% yield eight-step berberine synthesis using intramolecular Heck reaction of the N-alkylation product of isoquinoline and alkyl bromide. Tajiri and co-workers also carried out the derivatization of berberine.



5.12 Cultivation/Harvesting

The plant grows at an altitude of 2000–3000 m above sea level. The suitable climatic condition for the growth of plants of Berberis genus is a temperate climate. More precisely, the best conditions for the plant are moist and humid conditions as the plant is unable to grow under hot and dry conditions. The most favorable temperature range lies between 15 and 30 °C. This plant does not require excessive irrigation; normally, 60–70 cm rainfall is enough for the proper growth of this plant. This plant grows well in different types of soil ranging from sandy alluvial loamy soil to red laterite loam as well dark loam are also suitable for the proper growth of this plant. The seeds are propagated during the spring season, i.e., during a highly humid and moist atmosphere. The self-grown seeds can be propagated by stem cuttings. Like rice crops, seedlings can be raised in nursery beds and can be transplanted afterward. This crop is rainfed and hardly any irrigation is required, but during the dry season, this is irrigated at regular intervals to keep the soil moist and humid. Moreover, cultivated plants are required to be kept weed-free for proper growth of the plant. Hence regular weeding at the interval of 20 to 30 days is required to keep the crop weed-free. As this plant can resist pest attacks so pesticide spraying is not required. The overall total period for harvesting from sowing is 2 years. The bark of the roots is removed and the roots are sun-dried and cut into tiny pieces and kept in a dry place for marketing purposes and utilization.

5.13 Patent

Several patents have been filed and issued from time to time pertaining to the therapeutic characteristics of Berberis species as a result of extensive research into their efficacy in curing a variety of ailments. Most of the patents filed are on herbal formulations of Berberis species along with other medicinal plants. Out of which major number includes activities related to heart and metabolic disorders. Most of the patents filed are on herbal formulations in which apart from these herbal formulations related to skin problems which constitute Berberis species as their part are also filed. Hence, the patents which included Berberis species have been compiled in Table 5.1.

5.14 Formulations and Market Product

B. aristata is widely utilized in the Ayurvedic system for the treatment and prevention of a variety of health conditions. It is a great dietary supplement that is known for strengthening of immune, gastrointestinal, and cardiovascular systems. *Rasaut* (wound dressing oil), *Darvyaditaila* (oil), *Darvyadilepa* (ointment), *Darvyadikvatha*

Table 5.1 Patents on Berberis species

Sr. no	Title	Inventors	Issuing agency	National/ International	Patent no.	Year of publication
1	Composition for controlling and reducing blood cholesterol levels	Rosa Maria Raventós Colomer Ana Fortuny Solà Alberto Sala Llinares Carlos Fernández Navarro	European Patent Office	International	EP3590356A1	2020
2	Herb mixture, useful, e.g., to slow the aging process, comprises, e.g., *Crocus sativus, Elettaria cardamomum, Cinnamomum zeylanicum, Hedychium spicatum, Terminalia chebula, Trichosanthes dioica, Coleus forskolin,* and *Berberis aristata*	Brigitte Muenster De Luenemann	Germany	International	DE102007040798A1	2009
3	*Berberis aristata* plants extracts for treating osteoporosis and the extraction process thereof	Villoo Morawala Pattell	WIPO (PCT)	International	WO2008007215A2	2007
4	Composition containing lacquer tree used as feed additive for livestock	Park Mi-seong	Korean	International	KR2011051820	May 18, 2011
5	Combinations of botanical extracts for promoting cardiovascular health	Yann Wang, Jeffry Zidichouski	USA	International	US20100239603	Sept 23, 2010
6	Crude drug containing composition used for improving metabolic syndrome, obesity, and liver function comprises Garcinia, *Terminalia beleria, Commiphora mukul, Gymnemasyl vestre, Boswellia serrata,* and *Salacia reticulata*	Chizuko Saito	Japan	International	JP2010202634	Sept 16, 2010
7	Methods and compositions for the treatment of metabolic and cardiovascular disorders	Arnold Stan Lippa	WIPO (PCT)	International	WO2010104595	Sept 16, 2010

(continued)

Table 5.1 (continued)

Sr. no	Title	Inventors	Issuing agency	National/ International	Patent no.	Year of publication
8	Agent useful for whitening skin and preventing and suppressing pigmentation and liver spots by inhibiting Dopa oxidase activity, contains extract of plant, e.g., *Berberis vulgaris* and *Berberis aristata*	Kyoko Amano, Mitsuru Sugiyama	Japan	International	JP2010195731	Sept 09, 2010
9	Nasal irrigation solutions and methods of using the same	Rajmani Tigunait, James L. Miles	United States	International	US7771757B2	Aug 10, 2010
10	Herbal formulation for wound healing	Manish Saxena	United States	International	US20100178367	Jul 15, 2010
11	Intramammary teat sealant	Majid Razzak, Robert Holmes, Alan Johnson, Jitendra Goswami, Atul Awasthi	United States	International	US20100143510	Jun 10, 2010
12	Herbal formulation for prevention and treatment of diabetes and associated complications	G. Geetha Krishnan	WIPO (PCT)	International	WO2010032267A72	Mar 25, 2010
13	Bioactive composition for the treatment of the HIV/AIDS and method for manufacturing and using the same	Mukesh Harilal Shukla	WIPO (PCT)	International	WO2010029562A1	Mar 18, 2010
14	Synergistic antipyretic formulation	Palpu Pushpangadan, Ajay Kumar Singh Rawat, Chandana Venkateshwara Rao, Sharad Kumar Srivastava, Sayyada Khatton	United States	International	US7658954	Feb 09, 2010
15	Compositions containing berberine and/or analogs thereof or extracts containing it, for the prevention and treatment of alterations of the lipid and carbohydrate balance	Francesco Di Pierro	European Patent Office	International	EP2149377	Feb 03, 2010

16	Method and system for producing medicinal alcohol as a prophylactic or remedy for cancer, HIV, AIDS, and autoimmune diseases	B. Dr. Kapur, Rajesh Kapur	European Patent Office	International	EP2090315	Aug 19, 2009
17	Formulation for oral administration with beneficial effects on the cardiovascular system	Paolo Senin, Ivo Setnikar, Luigi Angelo Rovati	United States	International	US20090136469	May 28, 2009
18	Herb mixture, useful, e.g., to slow the aging process, comprises, e.g., *Crocus sativus, Elettaria cardamomum, Cinnamomum zeylanicum, Hedychium spicatum, Terminalia chebula, Trichosanthes dioica, Coleus forskolin,* and *Berberis aristata*	Rolf Dieter Buecker, Gopi Krishan	Germany	International	DE102007040798	Apr 02, 2009
19	An anticancer medicine including berberine	Hong Jin-tae, Choi Myung-sook	South Korea	International	KR20090032617	Apr 01, 2009
20	Antipyretic vasodilators	Oron Zachar	WIPO (PCT)	International	WO2008126088	Oct 23, 2008
21	Methods and compositions for the treatment of metabolic syndrome	Arnold Lippa, Jian-Dong Jiang, Jing WeiWei-Jia Kong, Li-Xun Zhao, Dan-Qing Song	United States	International	US20080081781	Apr 03, 2008
22	*Berberis aristata* plants extracts for treating osteoporosis and the extraction process thereof	Villoo Morawala Pattell	WIPO (PCT)	International	WO2008007215	Jan 17, 2008
23	Enteral compositions for the prevention and/or treatment of sepsis	Robert Johan Joseph Hageman, Gelske Speelmans, Adrianus Johannes Maria Vriesema	European Patent Office	International	EP1411951	Sept 26, 2007
24	Method and composition for controlling oral pathogens	Christine Wu, A. Kinghorn, Sara Roberts	United States	International	US20070098649	May 03, 2007
25	Compositions effective in altering the perception of malodor	Virginia Pinney	United States	International	US20070065394	Mar 22, 2007

(continued)

Table 5.1 (continued)

Sr. no	Title	Inventors	Issuing agency	National/ International	Patent no.	Year of publication
26	Compositions for veterinary and medical applications	Betty Jin, Wen-Yang Wu	United States	International	US20070027176	Feb 01, 2007
27	Methods and compositions for the treatment of hyperlipidemia	Jian-Dong Jiang, Jing Wei, Wei-Jia Kong, Li-Xun Zhao, Dan-Qing Song	United States	International	US20060223838	Oct 05, 2006
28	Herbal compositions for effective treatment of AIDS, preparation thereof and method for the treatment of AIDS patients	Shambabu Ayare	WIPO (PCT)	International	WO2005030232	Mar 02, 2006
29	Composition for treating and preventing diabetes comprises crude drug component of Guggul, Licorice, Balsam Pear, Gymnema sylvestre, Boerhavia diffusa, Guduchi, Amla, Turmeric and Neem, and mineral component	Chizuko Saito	Japan	International	JP2005325025	Nov 24, 2005
30	Herbal water treatment method for prosperity of biological	Kannan Devaraj	WIPO (PCT)	International	WO2004092078	Oct 28, 2004
31	Skin care preparation with high safety and anti-aging effect	Kazuhisa Osumi, Tsutomu Sakaida, Tomonori Katada, Satoru Nakada	Japan	International	JP2003095856	Mar 04, 2003
32	Herbal composition and method of manufacturing such composition for the management of gynecological disorders	Chandra Kant Katiyar, Ramesh Kumar Duggal, Bodapati Venkata Jagannadha Rao	United States	International	US6455077	Sept 24, 2002
33	Application of protoberberine alkaloid, berberine, an immunosuppressive agent	Shakti N. Upadhyay, Raman Prasad Yadav	United States	International	US5856487	Jan 05, 1999
34	Cell adhesion inhibitor/novel use of extract isolated from Berberidaceae plant as cell adhesion inhibitor	Tadashi Hase, Takatoshi Murase, Ichirou Tokimitsu	Japan	International	JP10158184	Jun 16, 1998

35	Herbal compositions	Eladevi Shah	United States	International	US5693327	Dec 02, 1997
36	Composition for a dietary supplement for the treatment of hemorrhoids	Som C. Pruthi	United States	International	US5591436	Jan 07, 1997
37	Natural antimicrobials, insecticides, acaricides, and nematocides for plant protection—are prepared. From Rosa, Allium, Brassica, Mahonia, Sambucus, Pelargonium, and/or Hypericum plants	Dagmar Dr Plumhoff, Clas Von Dr Ramm, Hubertus Dr Kleeberg	Germany	International	DE4327792	Apr 06, 1995

(for treatment of excessive vaginal discharge), *Rasanjana*, *Daruhaldi powder*, *Madhumehantak churna* (for treatment of diabetes), *Navkarshik churna*, *Dasangalepa* (ointment), and *Vati* are some important polyherbal Ayurvedic formulations having *B. aristata* as one of the constituents (Potdar et al. 2012; Amalraj and Gopi 2021). Livokin, a herbal medication used for the treatment of hepatic dysfunction, contains *B. lycium* along with other medicinal plants like *Carum copticum*, *Tephrosia purpurea*, *Eclipta alba*, *Cyperus rotundus*, *Terminalia chebula,* etc. (Girish et al. 2009). Other than these traditionally used Ayurvedic tonics, oils, and ointments, *B. aristata* is also an important constituent of many commercially available dietary supplement capsules, tablets, and powders (Rout et al. 2008) (Table 5.2). Prasad and Kaur (2019) also formulated an antiacne gel from ethanolic extract of *B. aristata* in polyethylene glycol-400 using triethanolamine as a neutralizer and methyl, propylparaben as preservatives (Prasad and Kaur 2019).

Table 5.2 Commercially available products containing *B. aristata*

Name of product	Brand name	Formulation	Health benefits
Leucoforte	Alopa Herbals	Tablets	Relieves leucorrhea
Piloguard	Maxima Proyurveda	Capsules	Reduces piles swelling
Berberine	Amazing formulas	Capsules	Dietary supplement-support immune system, glucose metabolism, cardiovascular and gastrointestinal function
Berberine	HMS Nutrition	Capsules	Dietary supplement-support cardiovascular, immune, and intestinal health
Berberine	Whitaker nutrition	Capsules	Dietary supplement
Barberry root powder	Bixa botanicals	Root powder	Food supplement-aids in the proper functioning of the liver and kidneys
Berberis aristata root powder	MB Herbals	Root powder	Aids in the proper functioning of the liver and kidneys
Berberis aristata	Nusa Pure	Capsules	Supports immune and cardiovascular system, supports healthy cholesterol levels
Berberine	Physician's Healthy Alternatives	Capsules	Supports optimal blood sugar and cholesterol
Berberine Plus	Doctor's recommended	Capsules	Dietary supplement
Berberine	Puritan's pride	Capsules	Supports heart health
Berberine	We like vitamins	Capsules	Support cardiovascular and immune system
Berberine Gluco Gold+	Whitaker nutrition	Tablets	Support healthy insulin sensitivity, blood sugar, and cardiovascular system

5.15 Conservation

The genus Berberis is one of the gems of Himalayan plant treasure which has been utilized for its vast therapeutic potential since time immemorial. Berberis species has plethora of medicinal and pharmacological potential which led to its huge demand all over the world. But this situation causes its indiscriminate use and uprooting of plant from its natural habitat resulted into mass destruction. Adding to this, the germination rate of its seed is also very low as well as seed collection during rainy season, especially at high altitude is also very difficult. Due to the huge demand of this plant, it was declared as endangered as per IUCN list in past its conservation is very important (Kala 2002). Although both the species *B. aristata* and *B. lycium* are categorized as LC (least concern) in the latest updated IUCN red list of threatened species (Plummer 2021), still their conservation is required to prevent these species from becoming endangered or extinct. Several strategies have been employed for its conservation in which leaf-derived callus was one of the most cost-effective and better alternatives to seed propagation method (Khan et al. 2016).

5.16 Conclusion

Berberis genus has a wide distribution among different parts of the world. It was originated from the Himalayas and constitutes more than 650 species on its list. Still, many more species are either unexplored or unidentified. Therefore, there is a huge scope to explore these unidentified species for their therapeutic potential. Among a large number of species, *B. aristata* and *B. lycium* are the most important species of this genus. These are thoroughly explored for their therapeutic and pharmaceutical potential. In general, these plants are found at an altitude of 2000–3000 m from sea level. The mention of these plant species in the Indian Ayurvedic system for the cure of a vast range of diseases from diabetes to the skin as well as metabolic problems have shown their huge therapeutic potential. Medicinally, almost every component of this genus's plants is useful. Due to its wide range of medicinal properties, it has been a part of traditional medicinal systems for the last many 100 years. The roots of this plant are the most important source of its major alkaloid "Berberine" which itself is a golden compound with a wide range of medicinal properties. Due to their huge therapeutic potential, the plants of this genus were overexploited in the past with less emphasis done on their conservation. So these species had come under the category of endangered and needed immediate and practical strategies for their conservation. Among all the species reported to date very few are assessed for their pharmacological potential under laboratory conditions, and many more are still required to be validated via clinical trials. Hence, there is a huge scope to explore the species of the genus for their proper utilization in the medical field.

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Chapter 6
Dactylorhiza Hatagirea (D.Don) Soo: Himalayan Marsh Orchid

Anu Shrivastava and Swati Jain

6.1 Introduction

The Himalayas are spread over 18% of the India subcontinent, contain 50% of India's forest cover and harbour 30% of endemic species. Out of the 8000 higher plant species found in the Indian Himalayan region, 1748 species are known for their medicinal use (Semwal et al. 2010). Medicinal plants are those plants in which one or more parts of the plant produce certain substances capable of being used for therapeutic purposes or function as a precursor for drug synthesis. Plants containing secondary metabolites such as glycosides, alkaloids, tannins, volatile oils and nutrients possess medicinal properties. Since ancient times, medicinal plants are being used by people in healthcare for treating a wide range of health problems. Several traditional systems of medicine use medicinal plants, such as Ayurveda and Unani. The medications formulated from the medicinal plants are known to boost the natural recovery power of the body and have greater cultural acceptability and compatibility with human body and exhibit fewer side effects (Ranpal 2009).

India is a treasure trove of well-documented and well-practised traditional knowledge related to herbal medicine which has a great economic potential if capitalized by promoting its use in the developed world with an ever-increasing interest in herbal medicines. About 2000 miraculous medicinal plant mentioned in Ayurveda are widely renowned for being both safe and effective in curing many bodily ailments. *D. hatagirea* is also an extensively used medicinal orchid of high value in Ayurveda, Siddha and Unani system of medicine (Giri and Tamta 2010; Thakur 2019).

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As per WHO estimates, traditional medicine is extensively used in developing countries by about 80% of the people in these countries. India's rich cultural heritage is also evident from the extensive knowledge of traditional herbal medicine which is found in the Vedic literature, especially the Rigveda, Charaka Samhita and Sushruta Samhita. Many of the remotely located areas of Indian Himalayas have rich traditional knowledge regarding the use of these medicinal plants which are yet to be documented. Lack of modern healthcare facilities make the people living in these regions highly dependent on these plants for their health problems (Semwal et al. 2010).

Orchids, known for their beautiful and fragrant flowers, are the largest family of flowering plants after Asteraceae, with around 25,000–35,000 species worldwide. These are grown and traded for numerous reasons such as medicinal products and ornamental plants. Chinese people are credited to be pioneers in cultivation and usage of orchids in healthcare system. In India, orchids have been used in medicinal practices since Vedic times. Orchids have been used in the treatment of nerve disorders, debility, bone fractures, fever, skin problems, etc. (De 2020; Magar et al. 2020; Wani et al. 2020; Kumar et al. 2021).

Dactylorhiza, derived from Greek words *dactylos* meaning finger and *rhiza* meaning root, was named by Necker ex Nevski in 1937 is a member of Orchidaceae family. Dactylorhiza consists of almost 75 species, which are found in Northern Temperate Zone. *D. hatagirea* (*D. Don*) *Soo* is a perennial orchid native to the Himalayan region (Warghat and Sood 2013; Choukarya et al. 2019). Endemic to the Hindu-Kush region of the Himalayas, this species has numerous vernacular names such as Hath panja, Hatajadi and Salam panja, and it is used in many traditional systems of medicine such as Ayurveda, Unani, Amchi and Siddha. It is a critically endangered species as per Conservation Assessment and Management plan (CAMP). A number of reasons have been speculated to be the cause of its decreasing population out of which the prime cause is anthropogenic disturbance caused by habitat degradation, over-extraction and climate change which have resulted in small or fragmented patches of the plant population (Dhiman et al. 2019). The plant is very significant for both the indigenous and the scientific community owing to the numerous bioactive phytochemicals present in it such as resveratrol, Dactylorhins A-E, Dactylose A and B, militarin, loroglossin, flavonoids, alkaloids, glycosides, carotenoids and phylloquinone. Presence of these compounds gives the plant many of its medicinal properties such as antioxidant, anti-microbial, aphrodisiac, anti-tumour, anti-diabetic, anti-inflammatory, antipyretic and immunomodulatory properties (Dutta and Karn 2007). Indigenous people have been using this plant for treating numerous other health conditions such as wound healing, nerve tonic, cough and cold which are yet to be studied (Wani et al. 2020). The plant is in urgent need of conservation using both in situ and ex situ techniques to prevent the species from further deterioration and replacement of the already endangered population.

6.2 Botanical Description and Taxonomical Classification

Dactylorhiza hatagirea is a beautiful, food-deceptive orchid with medicinal properties which is endemic to the Hindu-Kush Himalayan region. Dactylorhiza is made up of two Greek words, "dactylos" which means finger and "rhiza" which means roots and was coined by Necker ex Nevski in 1937 (Warghat and Sood 2013; Thakur et al. 2018; Kumar et al. 2021).

It is a temperate and perennial ground dwelling herb. It grows in abundance in moist meadow wetland, with porous soil having rich humus and sloppy mountains, especially in the moist and shady regions. The hardy tuberous geophyte remains erect even during heavy snowfall (Maikhuri et al. 1998; De 2020; Magar et al. 2020; Kumar et al. 2021). The height of the plant has been reported to be different in various studies conducted in different regions and is thought to be affected by a number of factors such as age of the plant, topography of the area, soil and climate. The height of the plant ranges from 20 to 60 cm (Dutta and Karn 2007; Chamoli and Sharan 2019). The Trans Himalayan Ladakh Region of India has even taller plants; the height of the plant ranging from 70 to 90 cm. The mean diameter of the plant is 0.77 cm when measured at a height of 5 cm above the ground (Ranpal 2009; Warghat and Sood 2013).

The tuberoids of this plant are fleshy and flattened, with palmately divided 2–5 lobes (Fig. 6.1). In order to survive the arid conditions, the underground stem is thickened in which water can be stored in large quantities. The moisture content of

Fig. 6.1 Tubers of *Dactylorhiza hatagirea*. (Source: https://commons.wikimedia.org/wiki/File:Paanch_aaule.jpg)

Fig. 6.2 *Dactylorhiza hatagirea.* (Source: https://commons.wikimedia.org/wiki/File:Dactylorhiza_hatagirea_(7832429742).jpg)

the tubers is 79%. The leaves are palmately lobed and shaped lanceolate with sheathing leaf base. The mean length of leaves is 15 cm, speckled and arranged through the length of the stem. The stem is erect, hollow and obtuse (Maikhuri et al. 1998; Warghat and Sood 2013; Warghat et al. 2013; Chamoli and Sharan 2019; Magar et al. 2020). The flowers are 1.7–1.9 cm in length and possess curved spur with green bracts. The structure of the flower consists of 3 sepals forming the outer whorl and 3 petals forming the inner whorl out of which 2 are similar and one is modified (rounded and lobed) called as "lip". The gynostemium is massive, located at the centre of the flower and consists of the female pistil to which the male stamens are attached. The colour of the flowers varies widely and can be lilac purple, rosy, purple, pink, red or white. When compared to the length of the plant, the inflorescence is short, with compact raceme which is 5–15 cm long, in which the axillary buds bear 25–50 flowers (Fig. 6.2) (Warghat and Sood 2013; Magar et al. 2020; Kumar et al. 2021). The phenological stage of initiation of growth begins in the spring season, i.e. May. Its vegetative phase is in the summer months of June–July during the time of melting of snow, the flowering period is from June to July while the fruiting period is from August to September. It is an early flowering species since its flowers emerge at the time when the other co-occurring species are still in their vegetative phase. During the winters, the plant survives below the ground as tubers (Dutta and Karn 2007; Thakur et al. 2018; Dhiman et al. 2019; Kumar et al. 2021). The seeds are monocotyledonous, minute (200–1700 μm) and dark-brown to black in colour. The seeds lack endosperm and therefore require symbiotic association with a fungi for germination in normal conditions (Aggarwal and Zettler 2010; Giri

and Tamta 2012; Warghat et al. 2013; Warghat et al. 2014). Pollination takes place mainly by insects. Dispersal of seeds by wind enhances gene flow among the populations (Chamoli and Sharan 2019). Its mode of propagation is through seeds and also root cutting. For higher yield there should be a gap of 5 years between cultivation and harvesting of the plant; however, it is harvested after 2–3 years sometimes (Maikhuri et al. 1998; Giri and Tamta 2010).

Synonyms: *Orchis latifolia var. indica, Orchis hatagirea D. Don* (Ranpal 2009; Chamoli and Sharan 2019).

Taxonomy:

Kingdom: Plantae
Division: Angiosperms
Class: Monocots
Order: Asparagales
Family: Orchidaceae
Subfamily: Orchidoideae
Tribe: Orchideae
Sub-tribe: Orchidinae
Genus: Dactylorhiza
Species: hatagirea (Ranpal 2009; Warghat and Sood 2013)

Vernacular names: *Dactylorhiza hatagirea* is known by different names in different regions and languages such as panja, Salam panja, Hath panja or Hatajadi in Uttarakhand; Salam panja in Kashmir, Wanglak or Angmo-lakpa in various parts of Ladakh and Hatajari (Uttaranchal), Panch aunle, Hatajadi (Nepali), Aralu, Salap (Sanskrit), Ongu lakpa (Sherpa), Lob (Gurung), Spotted Heart Orchid or marsh orchid in English, Buzidan and Salam Misri in Unani, Salam panja in Ayurveda, Hatajadi in Kumaon, Ambolakpa and Hath panja in Sowa Rigpa and Zhang Lie Lan in Chinese (Ranpal 2009; Giri and Tamta 2010; Pant and Rinchen 2012; Warghat and Sood 2013; Sirohi and Sagar 2019b; Kumar et al. 2021).

Parts used: Whole plant, rhizome, leaves, flowers.

6.3 Distribution: India and World

D. hatagirea occurs in the temperate zone and is nearly endemic to Hindu-Kush region, in the central and western Himalayas, the biodiversity hotspot (Badola and Aitken 2003; Bhatt et al. 2005; Pant and Rinchen 2012; Chamoli and Sharan 2019). The species is reported to occur in the sub-alpine and alpine regions of India (Jammu and Kashmir, Ladakh, Uttarakhand, Himachal Pradesh, Arunachal Pradesh, Sikkim), Nepal, Pakistan, Tibet, Bhutan and Afghanistan at an altitude of 2800–4200 m above sea level (Giri and Tamta 2010; Pant and Rinchen 2012; Choukarya et al. 2019; Magar et al. 2020; Wani et al. 2020).

The Conservation Assessment and Management Plan (CAMP) has identified and listed this plant as critically endangered, the Convention of International Trade in

Endangered Species (CITES) has listed it under Appendix II and is critically rare as per IUCN (Bhatt et al. 2005; Murkute et al. 2011; Chamoli and Sharan 2019; Wani et al. 2020).

6.3.1 Population Density

According to previous studies, the population density of the species seems to differ in different regions of the Himalayas and often have patchy distribution. Uniyal et al. (2002) reported the population density of the plant to be 4756.1/ha in the Upper Gori Valley, Kala (2000) reported it to be 161,900/ha in the Indian-Trans Himalayan Region, 42,000/ha in Nanda Devi Biosphere Reserve Area and 10,000/ha in Valley of Flowers (Maikhuri et al. 1998; Bhatt et al. 2005). The density was also reported to be higher in the protected areas when compared to the unprotected areas, i.e. 0.60–1.89 individuals/m^2 in unprotected areas and 0.70–2.19 individuals/m^2 in protected areas (Uniyal et al. 2002; Bhatt et al. 2005; Chapagain et al. 2021). In the Manaslu Conservation Area in central Nepal, the population density has been recorded to be 2.18 individuals/m^2 with relative frequency being 7.48% (Bhattarai et al. 2014). In the Western Himalayan region of India, the areas with high frequency of plant extraction have been reported to have plant density of about 1–6.6 individuals/m^2 while regions with low frequency and intensity of plant extraction have13.8–46.29 individuals/m^2 (Thakur et al. 2018).

Orchid populations worldwide have been adversely affected due to habitat destruction and changes in the land use patterns resulting to changes in the natural habitat. The decline in population has been more drastic in the unprotected areas while the population has remained almost stagnant in protected areas (Swarts and Dixon 2009; Gonzalez et al. 2011; Kusum 2014; Chapagain et al. 2021). A large number of factors have together led to its dwindling population from its wild habitats, of which anthropogenic factor is the prime cause. Some of these factors have been discussed below.

Over-exploitation: The plant is collected by local inhabitants for domestic uses and by local herbal healers for treating various health conditions (Semwal et al. 2010). Owing to its high medicinal potential, the species is in increasingly high demand both in domestic and international markets with the market value of its dry tubers being Rs. 2700–3200 per kg. It is estimated that its demand is nearly 5000 tons every year, and collection of raw materials from the wild species has been the most common way to meet this demand. In India, the annual consumption of its salep is estimated to be around 7.38 tonnes which is valued at USD 83,333 (or Rs. 50 Lakhs) (Olsen and Helles 1997; Badola and Pal 2002; Kala 2004; Murkute et al. 2011; Warghat and Sood 2013; Warghat et al. 2013; Warghat et al. 2014; Wani et al. 2020).

Illegal trading: There is a wide gap between the demand and the supply of this plant which leads to traders collecting and selling the plant illegally in both national and international markets. Almost 90–100 mature plants are harvested to obtain 1 kg

of dried roots which the local inhabitants could collect at a rate of Rs. 100–200 (Chaurasia et al. 2007; Warghat and Sood 2013).

Excessive weed proliferation: Most of the dominant associates of *D. hatagirea* are inedible such as *Anemone tetrasepala, Anaphalis triplinervis, Morina longifolia* and *Polygonum polystachyum* (an over-growing species) which increase the pressure on the plant, making it easier for the inedible species to proliferate (Bhatt et al. 2005).

Overgrazing: A study reported that the livestock were taken to higher regions of the valley by the locals, and the resultant trampling leads to disturbance in the plant life cycle due to the destruction of the parts of the plant above the ground and underground part of the plant getting exposed. Grazing also causes proliferation of weeds further suppressing the growth of the desired species (Ranpal 2009; Warghat and Sood 2013).

Low rate of propagation and poor seed germination: Vegetative propagation is very slow in the species, and it has only 0.2–0.3% seed germination rate. Due to the absence of metabolic machinery and endosperm, very few out of the millions of seeds present in the orchid capsule germinate (Vij 2002; Giri and Tamta 2012; Warghat et al. 2014). Also, the species has slow rate of growth, low capability of regeneration along with requirement of mycorrhizal association and high pollinator specificity. Since the seeds of this plant lack endosperm, they require fungal association to grow in their natural environment (Bhatt et al. 2005; Pant and Rinchen 2012; Bhattarai et al. 2014; Kumar et al. 2021).

Low genetic diversity: *D. hatagirea* has low genetic diversity which differs across different locations, with only 40% of the genetic diversity attributable to differences within populations and the rest 60% to among population (Ranpal 2009; Warghat et al. 2013). Inter Simple Sequence Repeats (ISSR) and random amplified polymorphic DNA markers when employed to study genetic diversity among populations of *D. hatagirea* revealed the occurrence of moderate genetic variations among populations (Warghat et al. 2012; Warghat et al. 2013). The causal factors for this are localized distribution of the species, fragmented habitats and rapidly deteriorating population of the species. This has led to reduction in the ability of the species to evolve in the absence of new allelic varieties immigrating into the population (Magar et al. 2020). The deleterious effects of genetic drift and inbreeding pose genetic risk to these species by affecting population fitness and genetic diversity (Thakur et al. 2018).

Other than these improper collection and cultivation practices mainly due to inadequate knowledge, poor conservation practices resulting from lack of awareness, destruction and degradation of forests, global climate change are some other crucial factors (Ranpal 2009; Pant and Rinchen 2012; Bhattarai et al. 2014; Warghat et al. 2014). There is a need to take serious efforts at the national level to prevent depletion, along with both ex situ and in situ conservation, protection and management strategies to reinsure rapid recovery of plant populations; otherwise, the species will likely vanish even before its complete medicinal importance has been established (Pradhan 1975; Kusum and Verma 2014; Thakur et al. 2018). One of the imperative ways of ex situ conservation is tissue culture. Protocorm development and mass multiplication of *D. hatagirea* under in vitro and in vivo conditions have

been undertaken by Warghat et al. (2014). Asymbiotic seed germination was tested in 10 media. Highest seed germination (37.12%) and maximum protocorm formation (23.40%) was seen in Lindemann (LD) medium followed by BM-1 and Murashige and Skoog (MS) medium. Protocorms were further cultured in MS media containing different concentrations and combinations of growth regulators Indole Butyric Acid (IBA) (0–3 mg/L) and Kinetin (Kin) (0–3 mg/L). Maximum growth and development were seen in MS medium supplemented with IBA (3 mg/L) and kin (1 mg/L). After 28–30 days, number of shoots and roots were recorded to be 18.12 and 8.25, respectively. While the mean length of shoot was 17.80 cm and root length was 8.02 cm. In a different study, plant growth hormone 6-Benzylanimopurine (BAP) was used along with IBA in the MS medium, and maximum growth and development were recorded at the concentration 4 mg/L IBA and 3 mg/L BAP. Number of shoots and roots were recorded to be 43.50 and 15.00, respectively. While the mean length of shoot was 31.06 cm and root length was 14.20 cm after 28–32 days. Fully growth plantlets were placed in different combinations of potting mixtures of which 100% plantlet survival was seen in the mixture containing cocopeat, vermiculite and perlite in the ration of 1:1:1, along with highest number of plantlets, i.e. 25, shoots (75) and roots (23), longest shoot length (18.8 cm) and root length (44.7 cm) after transplantation in the greenhouse for a month (Warghat et al. 2014; Popli et al. 2016).

Similar study was undertaken by Giri and Tamta (2012) with different basal media and several concentrations of plant growth regulators (PGRs) for in vitro propagation of green pod, shoot bud, tuber and leaf segment. Green pods culture resulted in poor, slow and difficult seed germination with 57% of treatments resulting in failure of germination. Good seed germination was seen in only 1 out of 28 treatments used which was in half strength MS medium supplemented with peptone (1 g/L), morphoinoethane sulphonic acid (1 g/L) and activated charcoal (0.1%). Average seed germination was also seen in only one, i.e. Knudson C (KC) medium with kinetin (1 mg/L) and activated charcoal (0.1%). Only four treatments resulted in the formation of protocorm-like bodies (PLBs); however, none of them multiplied in any culture. Only few plantlets were obtained of which none could survive when transferred to soil for hardening. In shoot bud culture, only MS medium supplemented with thidiazuron (TDZ) resulted in sprouting (11.10%). However, the shoots died in the shoot multiplication medium. Similarly, no rooting was observed in tuber and leaf segment culture. Vegetative propagation of the apical (consisting of dormant shoot bud), middle and basal tuber segments was also carried out with the help of PGRs, in which only the apical segments resulted in sprouting and rooting (Giri and Tamta 2012).

Use of robust molecular markers can be an efficient way of conservation and genetic improvement of the species. However, absence of genotypic linkage disequilibrium in populations and cross-specific amplification have been anticipated for use of efficient markers in conservation genetic studies. A study by Lin et al. (2014) for assessing intra- and inter- population genetic diversity of *D. hatagirea* in China resulted in identification of 14 simple sequence repeat (SSR) markers for different repeats. Study of variability on the basis of morphological, biochemical and isoenzyme patterns has indicated significant diversity among the populations (Chauhan

et al. 2014). In the recent years, use of "omics" approaches has increased immensely in plant research to gain better understanding of mechanisms behind various pathways and processes. Advanced next-generation sequencing (MGS) techniques have commissioned the role of "omics" and have enabled genome and transcriptome sequencing for characterization of chloroplast genome, phylogenomic relationship and ecological divergence among the species of the genus dactylorhiza. Studies have reported use of ngs platforms nova-seq and genome analyzer IIx (both from Illumina Inc.) for elucidation the biosynthetic mechanisms of secondary metabolites dactylorhin, resveratrol and stilbenes from tissues of *D. hatagirea*. This transcriptomic characterization has been used to identify molecular cues linked with environmental factors such as freezing stress (Dhiman et al. 2019; sood 2021)

6.4 Phytochemical Composition

The mature tubers of the plant contain glucosides, starch, mucilage, loroglossin, phosphate, chloride and volatile oils. Chemically, the major constituents of the plant are dactylorhins A–E, dactyloses A, B and lipids (Table 6.1) (Maikhuri et al. 1998; Dutta and Karn 2007; Ranpal 2009; Warghat and Sood 2013).

Table 6.1 Phytochemicals present in *Dactylorhiza hatagirea* (Kizu et al. 1999; Wani et al. 2020)

Common name	Scientific name	Description
Dactylose A	1-deoxy-1-(4 hydroxyphenyl)-L-sorbose	$C_{12}H_{16}O_6$; Mol wt.-256.254 g/mol
Dactylose B	1-deoxy-1-(4 hydroxyphenyl)-L-tagatose	$C_{12}H_{16}O_6$; Mol wt.-256.254 g/mol
Dactylorhin A	(2R)-2-β-D-glucopyranosyloxy-2(2-methylpropyl) butanedioic acid bis (4-β-D-glucopyranosyloxybenzyl) ester	$C_{40}H_{56}O_{22}$; Mol wt.-888.866 g/mol
Dactylorhin B	(2R-3S)-2- β-D-glucopyranosyloxy-3-hydroxy-2 (2-methylpropyl) butanedioic acid bis (4 β-D-glucopyranosyloxybenzyl) ester	$C_{40}H_{56}O_{23}$; Mol wt.-904.865 g/mol
Dactylorhin C	(2R)-2- β-D-glucopyranosyloxy-2(2-methylpropyl) butanedioic acid	$C_{14}H_{24}O_{10}$; Mol wt.-352.336 g/mol
Dactylorhin D	(2R-3S)-2- β-D-glucopyranosyloxy-3-hydroxy-2 (2-methylpropyl) butanedioic acid 1-(4 β-D-glucopyranosyloxybenzyl) ester	$C_{27}H_{40}O_{17}$; Mol wt.-636.6 g/mol
Dactylorhin E	(2R)-2- β-D-glucopyranosyloxy-2(2-methylpropyl) butanedioic acid 1-(4 β-D-glucopyranosyloxybenzyl) ester	$C_{27}H_{40}O_{16}$; Mol wt.-620.60 g/mol
Resveratrol	(E)-5-(4-hydroxystyryl)benzene-1,3-diol	$C_{14}H_{12}O_3$; Mol wt.-228.24 g/mol
Trans-stilbene	(E)-stilbene	$C_{14}H_{12}$; Mol wt.-180.24 g/mol

Synthesis of Dactylose A and B takes place from L-ascorbic acid and 4-hydroxybenzyl alcohol via 2-c-(4-hydroxybenzyl)-α-L-xylo-3-ketohexulofuranosono-1, 4-lactone. Enzymatic emulsion of Dactylorhin A and Dactylorhin E using almond emulsion gives Dactylorhin A. Loroglossin is formed on hydrolysis of the compound (-2-3-dihydroxy-2-2-methylpropyl) butanedioic acid which is formed from enzymatic emulsion of Dactylorhin B and Dactylorhin D using cellulose (Dutta and Karn 2007; Warghat and Sood 2013). Qualitative phytochemical analysis of the hydroalcoholic extracts of the crude powder of the roots of *D. hatagirea* as shown the presence of flavonoids, saponins and carbohydrates, with total flavonoid compounds (TFC) content being 0.866 mg/100 gm of quercetin equivalent of dry extract sample (Choukarya et al. 2019). Its resveratrol content has been found to be 3.21 μg/100 mg of fresh weight (f. wt.) and trans-stilbene is 2.49 μg/100 mg f. wt. (Dhiman et al. 2019).

The phytochemicals have great medicinal potential and are therefore highly valued in both local and international markets. Alkaloids act as defensive elements which protect against predators, particularly mammals owing to their general toxicity and the ability to deter. They also have analgesic, anti-inflammatory and adaptogenic activities which play a role in alleviating pain, along with developing resistance to disease and stress (Hartmann 1991; Gupta 1994; Ali and Prasad 2016). Flavonoids have a range of beneficial biological activities and are generally nontoxic and an important component of diet. Glycosides and terpenoids are necessary for disease prevention and therapeutic effects in traditional medicine (Nakatani 2000). Saponins from plant sources are also responsible for some pharmacological effects like anti-inflammatory, molluscidal, anti-microbial, antispasmodic, anti-diabetic and anticancer, hypocholesteromic, antioxidant, anticonvulsant, analgesic, and cytotoxic activities (Ali et al. 2011). Generally saponins are toxic, but their consumption by human beings has been shown to have numerous beneficial effects on human health (Price et al. 1987).

The production and composition of secondary metabolites in plants is dependent on numerous factors such as physiological variations which includes the developmental stage of the plant organ, diurnal and seasonal variations which may be monthly or annual, the activity cycle of the pollinators, part of the plant being used, the type and location of the secretory structures which often have heterogeneous distribution within the plant body, presence of mechanical or chemical injuries, environmental conditions including climate, presence of diseases and pests, edaphic factors, geographic variations, genetic factors, evolution, etc. (Figueiredo et al. 2008; Magar et al. 2020). Plants growing at higher altitude, in natural environments (or the wild varieties) have higher amounts of bioactive compounds as compared to the ones growing at lower altitudes or the cultivated varieties (Bahuguna et al. 2000; Badola and Aitken 2003).

Other than these, numerous other compounds have been found from different parts of the plant such as indole alkaloids (1H-Indole by strictosidine synthase), Naphthoquinone (Napthalane-1,4-dione), ascorbic acid ((R)-5-((S)-1,2-dihydroxyethyl)-3.4 dihydroxy furan-2(5H)-one), phylloquinone (2-methyl-3((7R, 11R,E),3,7,11,15-tetra methylhexadec 2-en-1-yl) naphthalene-1,4-dione),

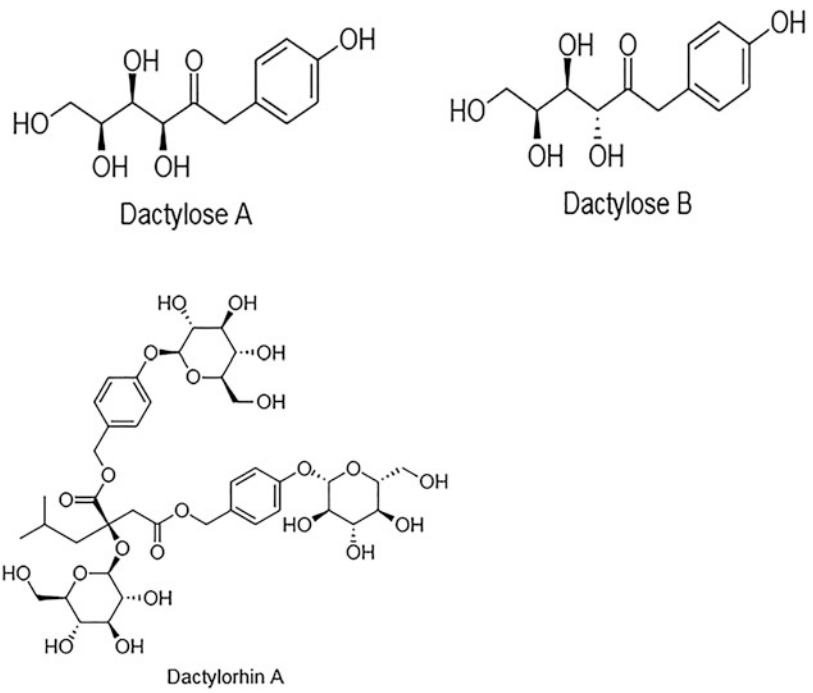
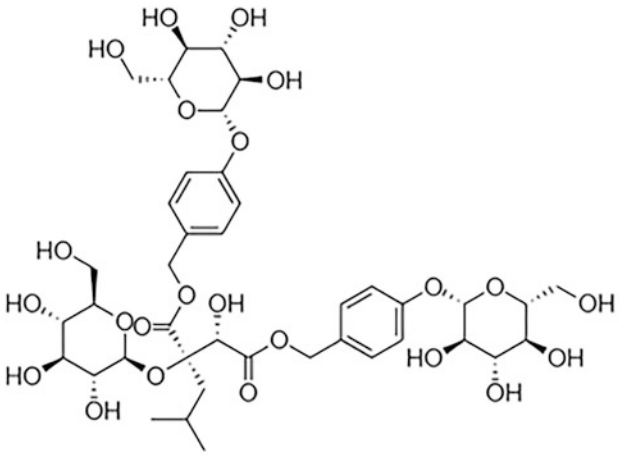


Fig. 6.3 Structures of secondary metabolites isolated from *Dactylorhiza hatagirea*

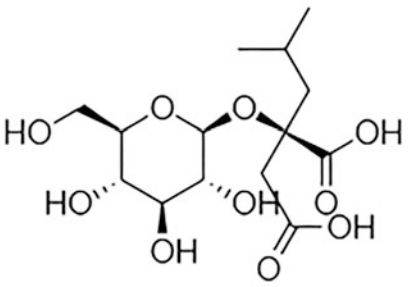
militarine, albumin, butanedioic acid, hydroquinone, loroglossin (bis (4-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)benzyl) (2R,3S)-2,3-dihydroxy-2-isobutylsuccinate; $C_{34}H_{46}O_{18}$), pyrocatechol, glucomannan, glycoside, saponin, tannin, carotenoids (synthesized from zeaxanthin-dioxygenase and prolycopene isomerase), terpenoids, steroids, phylloquinone (vitamin K) (synthesized from 2-carboxy-1,4-naphthoquinone phytyltransferase) (Kizu et al. 1999; Ali and Prasad 2016; Dhiman et al. 2019; Kawra et al. 2020; Wani et al. 2020). The metabolites dactylorhin A, B and E have also been isolated from the orchids *Gymnadenia conopsea* R. Br. and *Coeloglossum viride* (Li et al. 2009; Sood 2021). The structures of phytochemical present in *D. hatagirea* have been shown in Fig. 6.3.

6.5 Ethnomedicinal Uses

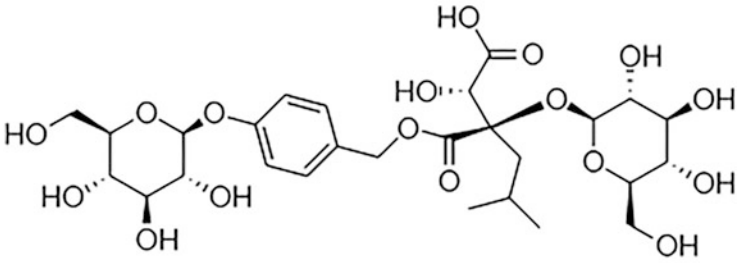
As per the estimates of WHO, almost 80% of the world's population in developing countries depends on traditional medicine for their healthcare needs. The Himalayas lying in the Northern region of India and covering around 18% subcontinent,



Dactylorhin B



Dactylorhin C



Dactylorhin D

Fig. 6.3 (continued)

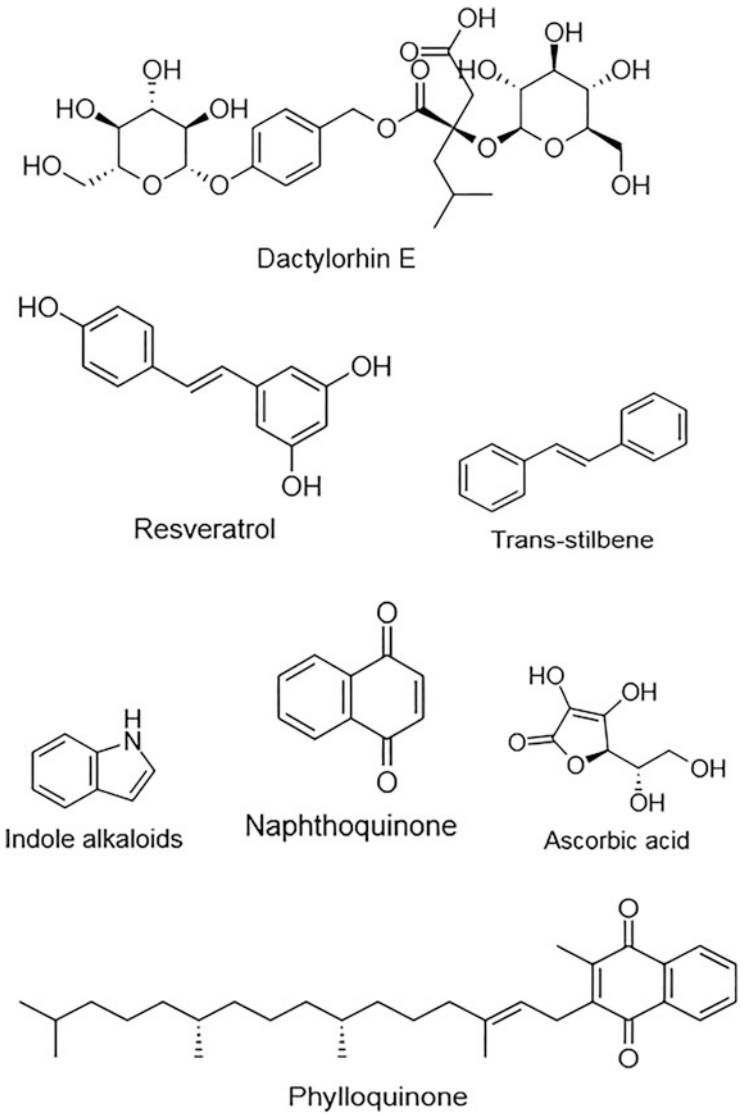
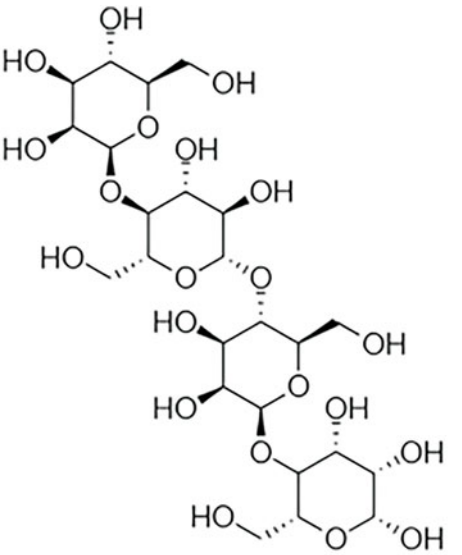
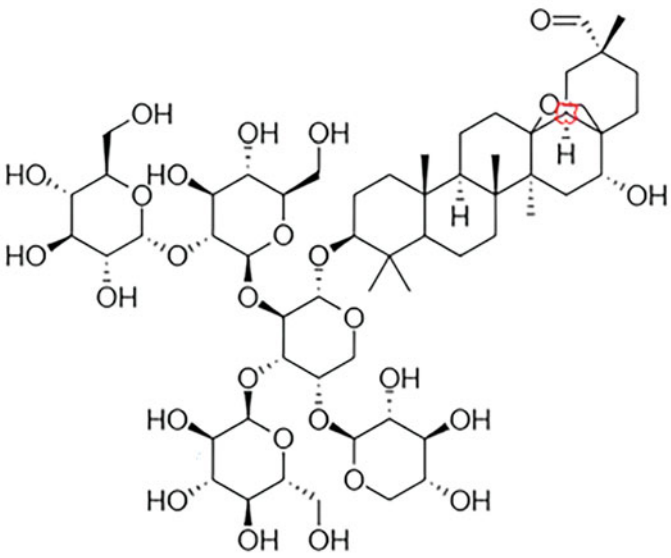


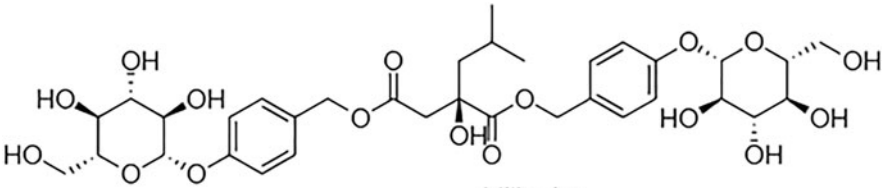
Fig. 6.3 (continued)



Glucomannan



Saponin



Militarine

Fig. 6.3 (continued)

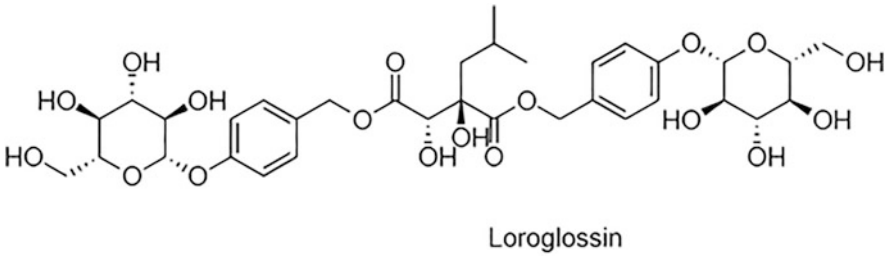


Fig. 6.3 (continued)

harbours about 30% of India's endemic species. It is a biodiversity hotspot of not just the wide variety of endemic plant species found in the region but also of the rich traditional knowledge of their medicinal uses, much of which remains largely undocumented (Semwal et al. 2010). Since the outreach of modern medicinal services is inadequate in our country, people in many remote areas are still dependent on locally available plants for medicinal properties. *D. hatagirea*, is one such plant which is extensively used in traditional Indian medicinal systems such as Ayurveda, Unani, and Amchi since time immemorial for treating myriad health conditions, and in numerous different ways, which tend to differ across regions (Pant and Rinchen 2012).

Orchids are widely used as "salep" which is a flour prepared by grinding dried tubers of *D. hatagirea,* with glucomannan being its major constituent it is known to have high nutritional value and is considered a nervine tonic and immunomodulator. Salep is also a high quality astringent which possesses aphrodisiac properties (Baral and Kurmi 2006; Warghat and Sood 2013; Verma 2014; Dhiman et al. 2019). It is widely used in many systems of medicine such as Ayurveda, Amchi, Unani and Siddha. It is estimated that the annual consumption of the salep is about 7.38 tonnes (Dhiman et al. 2019). In Ladakh, milk-containing salep is boiled and consumed as a rejuvenating tonic. According to local people, in adverse conditions, consumption of 25 gm powdered tubers is sufficient for a day's diet. Decoction prepared using salep, sugar and flavoured spices is used as a nutraceutical (Warghat and Sood 2013).

In the local households of Ghasa, Nepal, *D. hatagirea* is commonly used. Paste of the rhizome is prepared and applied on cuts, burns, skin problems and infectious wounds for swift recovery. After cleaning and drying, power of its rhizome is prepared, which is also used as a spice or consumed with milk for health benefits (Ranpal 2009).

The indigenous people in Rudraprayag, Uttarakhand, consider the plant to be useful for its therapeutic potential and use it along with other ingredients to cure numerous common health problems like diarrhoea, cough, dysentery, weakness, burns, etc. For treating normal weakness, roots of *D. hatagirea* along with other ingredients such as kunja (wild rose) pulp, Shilajeet are mashed together forming paste and consumed in the form of pills. Extracts of fresh tubers is consumed as a remedy for cough. Boiled tubers extract along with milk is known to be beneficial in

healing bone fracture. A mixture of refined tubers, misri and milk is consumed to control spermatorrhoea. Juice obtained from the fresh roots of the plant is taken for curing stomachache and for treating wounds due to burns, the tubers are rubbed on stones and the paste, thus obtained is applied on the affected part. Tuber paste is also consumed orally to cure diarrhoea. It is also used to cure bleeding and wounds (Pant and Rinchen 2012; Chamoli and Sharan 2019; Ojha et al. 2020).

The ethnomedicinal uses of orchids found in Kashmir Himalayas by the inhabitants of the region has been studied by Shapoo et al. (2013). The study results revealed that the plant *D. hatagirea* was being used in different forms and dosages along with certain dietary restrictions in order to cure numerous health conditions. Almost 100–200 mL of dried tubers in powdered form along with sugar mixed in water is consumed daily and food items containing excessive fat is avoided for stomachic. For treating headache, poultice is prepared by crushing fresh tubers and applied 1–2 times a day along with avoiding consumption of cold water. A paste prepared by adding crushed fresh tubers and turmeric powder is applied once daily during the bedtime and cold water is avoided for treating fracture. For treating cough and cold, decoction is prepared by boiling dried flowers and tubers of the plant in water for 5 min followed by adding honey and is consumed 2–3 times. A mixture of crushed tubers and milk along with honey/sugar is consumed once or twice daily as vermifuge and also for curing nervous system weakness. Decoction of dried flowers and tubers boiled in water for 5 min is consumed twice a day by the person suffering from diarrhoea. Poultice prepared from crushed leaves and tubers once or twice per day. Powdered and dried plant along with ghee is consumed twice or thrice daily for general weakness after delivery. A mixture of fresh tubers, milk, sugar and almonds is believed to have aphrodisiac activity. Cold water, sugary, fatty foods and pickles are avoided with most of these preparations (Shapoo et al. 2013).

Several ethnomedicinal plants are used in the postpartum period to reduce childbirth-related complications among the Marwari community in Jodhpur, Rajasthan, and one of them is *D. hatagirea*. The plant along with numerous other medicinal plants such as *Acacia Senegal, Coriandrum sativum*, and *Withania somnifera* are powdered and a ladoo is prepared out of it with the help of ghee and is consumed by pregnant women as both prophylactic measure and for management of postpartum complications. *D. hatagirea* is believed to function as a uterine tonic and diuretic (Goyal 2017). Himalayan marsh orchid is also in many other areas for curing debilitated condition after childbirth and for increasing regenerative fluids (Pant and Rinchen 2012).

Intake of dried and powdered tubers along with milk is believed to enhance vigour in Ladakh. Its tubers are considered to have emollient, astringent, demulcent properties (Kumar et al. 2021). Juice from tuber is given for curing pyorrhoea while poultice prepared from root paste is applied on cuts and wounds (Vishwakarma and Karole 2021). A study on the use of medicinal plants for treating cold, cough and fever, by the Amchis (herbal practitioners of Amchi system of medicine) in Ladakh region of Himalayas, documented use of *D. hatagirea* (called Ambolakpa) for treating fever. The tubers of the plant are collected in the month of October, dried in shade and powdered along with *Aconitum heterophyllum* roots, *Punica granatum*

seeds, fruits of *Emblica officinalis, Terminalia chebula*, leaves of *Ficus religiosa, Azadirachta indica* and mineral salt. It is consumed twice daily in the form of tablets (2–3) with warm water until recovery (Ballabh and Chaurasia 2007).

In the Kullu District of Himachal Pradesh investigation of the indigenous uses of the locally available medicinal plants on Parvati Valley found that the Himalayan Marsh Orchid was used as an expectorant, which enhances sputum secretion in the respiratory tract. It is also used for purifying blood, treating rheumatism, sexual disability, bone fractures, cuts, wounds and as an antibiotic (Sharma and Samant 2014).

The roots in the form of decoction are believed to be energy boosting and therefore, recommended for weak people especially by Balti and Brokpa ethnic groups in Ladakh (Haq et al. 2021). According to a study on ethnomedicinal plants in Devikund, Sunderdhunga Valley, Bageshwar District, Uttarakhand, the tuber extract of the plant is used in the treatment of whooping cough and fever (Sekar and Rawat 2011).

In the Sowa Rigpa system of medicine, the plant is known as *d.bang-lag* and is known to have spermatogenesis boosting, aphrodisiac and nourishing properties (Yeshi et al. 2017). A study in the Garam Chashma Valley, of the Chitral valley in Pakistan reported the use of *D. hatagirea* plant (indigenously called Juwari Joshu) for treating anaemia and as an aphrodisiac. The roots of the plant are dried and consumed orally in the form of powder while in the Hindu-Kush area it is mainly used as a nerve tonic and as sex stimulant. Ethnic groups residing in hilly districts of Nepal, such as Magar and Tangbeton, use *D. hatagirea* plant for treating head ache, stomachache, piles and typhoid (Miya et al. 2020; Birjees et al. 2021; Hassan et al. 2021).

*Immunomodulatory potential of Dactylorhiza hatagirea***:** Certain bioactive substances derived from plant sources have also been reported to enhance immunocompetence. Although several chemical and synthetic immunomodulators are available in the market they suffer from major drawbacks such as nephrotoxicity, hepatotoxicity, gastrointestinal toxicity, neurotoxicity, cardiovascular toxicity, metabolic toxicity, among many others. This has led to an upsurge of interest in usage of plant-based immunomodulators. In plants, several compounds like glycosides, alkaloid, volatile oils, tannins, polypeptides, etc. also influence various physiological processes along with essential nutrients. Secondary metabolites from plants such as flavonoids, isoflavonoids, alkaloids, polysaccharides, glucans, have immunomodulatory potential (Shukla et al. 2012).

There are many medicinal plants which have been proven to be immunomodulators (Agarwal and Singh 1999; Kumar et al. 2012; Shukla et al. 2012; Sharma et al. 2017). Salep prepared from the roots of the plant *D. hatagirea* has been traditionally used as an immunomodulator (Choukarya et al. 2019). Several compounds from plant have potential to modulate the immune response. They can do so by virtue of their antioxidant and anti-inflammatory potential.

In vitro and human cells studies have shown that polyphenols like resveratrol have pro-inflammatory cytokine (such as TNF-α and IL-6) inhibitory properties. Resveratrol has both innate and adaptive regulation potential. It has shown NADPH

oxidase inhibitory property in cell culture studies. Studies have also demonstrated that it inhibits spleen cell proliferation which is induced by concanavalin A, interleukin-2 or alloantigens. It also hinders IL-2, IFNγ, TNF-α and IL-12 production by lymphocytes and macrophages, respectively. It participates in T cell, natural killer cell and macrophage activation (Malaguarnera 2019; de Arruda et al. 2020).

6.6 Pharmacological Importance

6.6.1 Anti-microbial Activity

Indiscriminate use of commercial anti-bacterial drug use leads to multiple drug resistance. Hence, the anti-microbial potential of medicinal plants is being widely evaluated since these have fewer side effects and also prevent resistance build up against pathogens (Ranpal 2009).

Dactylorhiza hatagirea has been found to possess significant anti-microbial activity. In a study, *Staphylococcus aureus*, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Bacillus subtilis* were used to test anti-microbial potential of the rhizome and aerial extract of plant. The plant extracts were prepared using petroleum ether, chloroform, methanol and water as solvents. Results showed that the chloroform extract of the aerial part formed the most active Zone of Inhibition (ZOI), i.e. 14 mm, for the bacteria *E. coli*. The solution was found to inhibit bacterial growth at concentration above 125 mg/mL. While the aqueous extract of the rhizome formed most active ZOI, i.e. 13 mm for the bacteria *S. flexneri*. The solution was found to inhibit bacterial growth above the concentration of 250 mg/mL. Both aerial and rhizome extracts were found to form ZOI in the range of 7–11 mm for the bacteria *S. aureus* for most of the solvents. Comparing ZOI of the plant extract with the standard antibiotics, namely Azithromycin, Amikacin, Ciprofloxacin, Norfloxacin and Nitrofurantoin, it was found that the ZOI of *D. hatagirea* was larger than Ciprofloxacin and similar to Norfloxacin for *S. aureus*, thereby indicating similar efficacy in inhibiting bacterial growth. For *E. coli*, the effectiveness of the aerial part of *D. hatagirea* was close to that of Ciprofloxacin which is surprising since this bacteria is highly resistant to many of the synthetic drugs. The ZOI value of Ciprofloxacin found to be nearly the same as that of aerial part of *D. hatagirea*, and similar results were obtained in case of rhizome part of bacteria Sh. flexneri. The results also concluded that *D. hatagirea* rhizome extract showed resistance against all Gram-positive and Gram-negative bacteria, with better effectiveness (except in case of E. coli), and hence had better anti-bacterial properties than the aerial part which exhibited resistance only against some bacteria (Ranpal 2009).

6.6.2 Antioxidant Activity

Free radicals have been speculated to be a critical factor in the development of many diseases of the humans such as ischaemia, atherosclerosis, cancer and central nervous system-related problems. Antioxidants typically function by quenching ROS and/or forming chelates with catalytic metal ions. Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are considered quite unsafe and toxic. And so, sources of natural antioxidants which are both safe for human consumption and bioactive, capable of neutralizing free radicals, are constantly being researched (Ranpal 2009; Kumar et al. 2012). The antioxidant activity of *D. hatagirea* based on inhibitory concentration value (IC_{50}) using DPPH assay has been estimated to be 0.065–0.21 mg/mL (Sirohi et al. 2019; Kawra et al. 2020). The maximum antioxidant activity in *D. hatagirea* using DPPH assay has been found to be 40.13% and the maximum ferric reducing antioxidant power has been found to be 0.33% (Ali and Prasad 2016). The inhibitory concentration value using hydrogen peroxide and nitric oxide radicals has been found to be 53.01 µg/mL and 62.50 µg/mL for *D. hatagirea* as compared to 17.92 µg/mL and 24.17 µg/mL for ascorbic acid, respectively (Sirohi et al. 2019).

6.6.3 Aphrodisiac Activity

Dactylorhiza hatagirea is frequently used for its aphrodisiac properties by traditional medicine practitioners. A study on Wistar strain albino rats showed beneficial effects of the plant extract on the sexual organ and sexual behaviour. The study results showed that the resulting anabolic effect was similar to that of testosterone treatment. There was an increase in the weight of the sexual organs which indicates increased production of steroidal hormones. Rats which were treated with lyophilized extract experienced 2.5 times more attraction compared to the non-treated ones while the testosterone treated animals exhibited 2 times more attraction. Significant increase in copulation and number of bouts in the treated animals was observed. There was also an increase in number of ejaculating animals. Mount latency time decreased by 36% in animals treated with *D. hatagirea* extract as compared to 34% in testosterone-treated animals. Also there was 36% reduction in intromission as post-ejaculatory latency in the animals treated with *D. hatagirea*, and 17% reduction in group treated with testosterone as compared to the control group (Thakur and Dixit 2007a). Lyophilized aqueous extract of *D. hatagirea* exhibits aphrodisiac activity both in vitro and in vivo. An increase in the sperm count (141×10^6) was observed in Wistar strain albino rats administered with 100 mg/kg body weight *D. hatagirea* extract which was significantly higher than the sperm count of the rats in both control group ($110 \times .10^6$) as well as in testosterone-treated group (121×10^6). The Penile Erection Index (PEI) was higher in treated group (i.e. 49.8) as compared to the control group (24.6). Relative increase in inducible nitric oxide release was also

observed, which was 12.9 μM for *D. hatagirea* and 4.93 μM for the control group (Thakur et al. 2011). *D. hatagirea* also increases the pendiculatory activity in male rats which reflects an enhancement of sexual behaviour. Administration of 200 mg/kg body weight of extract increased mean number of yawning to 1.33 on 14th day from 0.31 (on 0 day) and stretching increased to 1.36 on 14th day from 0.44 (on 0 day) which was also higher than the control group, i.e. 0.33 and 0.44, respectively. Average number of sperms/chamber of WBC counting chamber was also higher, i.e. 86.4 as compared to 61.4 in case of control group after 30 min incubation (Thakur and Dixit 2007b).

6.6.4 Anti-cancerous Activity

The anti-cancerous activity of the *D. hatagirea* extract was evaluated using MCF-7 and MDA-MB-231 cell lines, for which HEK-293 was used for normal cell line. In this study, MCF-7 and HEK-293 were grown in Dulbecco's Modified Eagle Medium (DMEM) and MDA-MB-231 in Leibovitz (L-15). The results of the cytotoxicity study showed that the root and shoot extracts had difference in the percentage viability of the cells being tested, which was also dose dependent. The reduction in the number of cells was not significant, even with increasing concentration of plant extract. Percentage viability of root extract treated HEK-293 cells, increased to merely 94.44%, compared to 98.55% for the concentrations 1000 μg/mL and 250 μg/mL, respectively. While the percentage viability of shoot extract treated HEK-293 cells, decreased from 99.56 to 92.41% for concentrations 250 μg/mL and 1000 μg/mL, respectively. When MDA-MB-231 cell line was treated with root extract, there was significant reduction in viable cell population from 96.86% at 250 μg/mL to 82.38% at the concentration 1000 μg/mL. In case of shoot extract, the percentage viability of root extract decreased from 99.65% at 250 μg/mL concentration to 83.81% at the concentration 1000 μg/mL. The percentage viability of root extract treated MCF-7 cells declined from 99.17% at 250 μg/mL concentration to 84.24% at 1000 μg/mL concentration. Similarly, the percentage viability of shoot extract treated MCF-7 cells declined from 96.53% at 250 μg/mL concentration to 87.09% at 1000 μg/mL concentration, respectively. For HEK-293, the IC_{50} value was 9900 μg/mL for root extract and 6362.5 μg/mL for shoot extract. Hence based on the study, it can be concluded that the extracts of the plant *D. hatagirea* can be used to treat cancer which functions by killing the cancerous cells without causing significant harm to normal cells (Popli and Sood 2017).

6.6.5 Anti-diabetic Activity

Anti-diabetic potential of *D. hatagirea* has been studied by administering *D. hatagirea* root extract in diabetic rats. For in vitro analysis, hydroalcoholic root

extract of the plant was used and the percentage inhibition of α-amylase was calculated, with acarbose serving as positive control. α-amylase was found to be dose dependent on *D. hatagirea* extract, with IC_{50} value being 35.33 for acarbose and 224.45 μg/mL plant extract. For in vivo analysis, alloxan monohydrate was used to induce diabetes in Wistar rats, which functions by causing β-cell necrosis, ultimately resulting in insulin deficiency. This resulted in variation in the biochemical parameters such as an increase in blood glucose, increased cholesterol and triglyceride, decreased protein content and body weight. The effect of root extract was compared with reference drug "glibenclamide". Gradual significant reduction in blood glucose level was observed in all treatment group from 250 mg/dL to 125.20 mg/dL for *D. hatagirea* (100 mg/kg), 245 mg/dL to 117.8 mg/dL for 200 mg/kg plant extract and 240–112.7 mg/dL for glibenclamide treated group after 15 days. Similarly, significant reduction was also observed for other biochemical parameters such as total cholesterol which reduced to 109.6 mg/ dL for 100 mg/ kg plant extract and 105.1 mg/ dL for 200 mg/kg plant extract and 101.1 mg/ dL for glibenclamide treated group while for untreated diabetic group it was 180 mg/dL. For triglycerides, it was 95.5 mg/dL for plant extract (100 mg/kg), 92.5 mg/dL for plant extract (200 mg/kg) and 89.2 mg/dL for glibenclamide (600 μg/kg) treated. Total protein content also increased in the treated group, with its values being 7.90gm/dL for plant extract (100 mg/kg), 8.35 mg/dL for plant extract (200 mg/ kg) and 8.75 mg/dL for glibenclamide (600 μg/kg) treated compared to 5gm/dL in the untreated diabetic group. A significant increase in the body weight was also observed in the weight of treated groups. In the plant extract treated group, it increased by 30 g (for 100 mg/kg extract) and 35 g (for 200 mg/kg extract) while for glibenclamide treated group it increased by 40 g. In case of untreated diabetic group, a reduction of 10 g was noted. This study supports the use of *D. hatagirea* for treatment of diabetes (Choukarya et al. 2019; Magar et al. 2020). The methanolic leaf extract of *D. hatagirea* has been shown to possess anti-diabetic property without any cytotoxic effect on the cells. *D. hatagirea* leaf extract (500 μg/mL) can inhibit the activity of α-amylase to 75% as compared to 85% by standard drug acarbose, with IC_{50} values of 51 μg/mL and 210 μg/mL for acarbose and leaf extract, respectively. Similarly, it can inhibit α-glucosidase activity to 72% as compared to 94% using acarbose with IC_{50} values of 39 μg/mL and 200 μg/mL for acarbose and leaf extract, respectively. Bioactives in the leaf extract can also reduce post-prandial blood sugar levels by enhancing cellular uptake of glucose by inducing GLUT-4 translocation inhibition (Alsawalha et al. 2019).

6.6.6 Anti-inflammatory Activity

A number of phytochemical compounds found in *D. hatagirea* have potential anti-inflammatory activity. The hydroalcoholic extract exhibits dose-dependent anti-inflammatory response in Carrageenan-induced paw oedema with results similar to that of standard drug Diclofenac, with maximum effect being at fourth hour, i.e. 39%

and 56.34% inhibition for doses 100 mg/kg and 200 mg/kg, respectively, thereby supporting the traditional use of the plant in inflammation management. (Sharma et al. 2020).

6.6.7 Antipyretic Activity

Rise in body temperature results from increased prostaglandin E2 concentration in the brain. Antipyretic activity of *D. hatagirea* was assessed by administering increasing doses of *D. hatagirea* extract to Wistar rats with Brewer's yeast-induced pyrexia. A dose-dependent relationship was found to exist between the amount of extract and decrease in body temperature. There was a significant reduction in the rectal temperature which started 1 h after administration of its extract and continued till 4 h (Sirohi and Sagar 2019a).

6.6.8 Neuropharmacological Activity

Soporific drugs or hypnotic drugs, which are psychoactive drugs used to treat insomnia and are also used as surgical anaesthesia. Hydroalcoholic extract of *D. hatagirea* has been found to be safe and dose-dependent in prolonging duration of sleep in Swiss albino male mice which increased from 35.30 min for the group receiving 100 mg/kg, to 57 min for the group receiving 300 mg/kg. It is being speculated that the sedative effect of the plant extracts could be the result of facilitation of GABAergic transmission. (Sirohi and Sagar 2019b).

6.6.9 Other Applications

D. hatagirea tubers are used in silk industries as sizing material. Due to their beautiful flowers, they have been widely used for decoration and ornamental purposes and in perfume industries. The plant stem and leaves are also used as fodder for the livestock, vegetables and as insect repellent (Wani et al. 2020). Further studies are however required to elucidate the mechanism behind these effects and their active compounds responsible for the same.

6.7 Conclusion

Himalayas are the biodiversity hotspot which harbour many endemic species of high value medicinal plants such as *Dactylorhiza hatagirea*. Also known as Himalayan marsh orchid, *D. hatagirea* has several vernacular names such as Salam panja and

Hatajadi. It grows mainly in the moist and shady regions and belongs to the family "orchidaceae". It is a critically endangered with less population density and patchy distribution. A number of factors such as over-exploitation, illegal trading, excessive weed proliferation, overgrazing, low rates of propagation, poor seed germination, low genetic diversity have resulted in decline in their population. Its major chemical constituents are responsible for its medicinal properties are dactylorhins A–E, dactylose A and B, starch, mucilage, loroglossin, glucomannan, saponin, etc. *D. hatagirea* has been a part of many traditional systems of medicine such as Ayurveda, Unani, Amchi and Siddha. Locally, it has been used to treat a number of health conditions such as cough, cold, cuts, burns, skin problems, diarrhoea, fractures, general debility, head ache, sexual disability and weakened immunity. Several studies have been undertaken to establish pharmacological efficacy of the plant. In vitro and in vivo studies have shown that the plant has significant anti-microbial, antioxidant, aphrodisiac, anti-cancerous, anti-diabetic, anti-inflammatory neuropharmacological activity. However, its use in numerous other health conditions, and the effects on human consumption remains largely unverified. Other than improving health, the plant is also used in other industries as sizing material, decoration and ornamental purposes, insect repellent, etc. There is an urgent need to take suitable measures to prevent this plant population from further deterioration and studies need to be undertaken to elucidate the mechanisms behind their health promotive effects and role of their bioactive components.

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Chapter 7
Fritillaria Roylei Hook.f.: Himalayan Fritillary

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7.1 Background

The genus *Fritillaria* (Liliaceae) include about approximately 130 species which are generally used for medicine, starch source, and ornamental purpose (Ali-Zade 2014). One such potential species is *Fritillaria roylei* Hook., commonly known as Himalayan fritillary, kakoli, payasya, ksirakakol, and ksirasukla. It is among the 36 species of worldwide important remedial plants of Western Himalayas. According to the Conservation Assessment and Management Prioritization (CAMP), it has been stated that the worldwide status of this medicinal herb is under endangered category (Kuniyal et al. 2015). Additionally, the International Union for Conservation of Nature (IUCN) has allocated high risk of extinction status for North-West Himalayas and endangered status for Jammu and Kashmir state of India (Kumar et al. 2011). For Uttarakhand, the species assigned critically endangered status (Bisht et al. 2016). It is an important Himalayan curative herb (Chauhan et al. 2011a; Bisht et al. 2016; Kumar et al. 2021) and inhabitant of shrubberies, alpine slopes, and grows well in light sandy or medium loam well-drained acidic soils. The bulb of the plant is of highly medicinal value as its constituents are used in various ayurvedic products like Chyavanprash, Astavarga, Dhanwantharam Thailam, Jeevanthyadi Ghrutham, and Mahatriphala Ghritham (Kaul 2010). Currently, it is among the 18 species which has

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gained importance and is active in trade market (Chauhan et al. 2013) and due to the rise in demand and need in herbal medicinal market for this species, supply chain is gradually decreasing (Ved and Goraya 2008).

F. roylei is in increasing demand by pharmaceutical industries, but the supply chain is decreasing day by day as its harvesting is banned in Uttarakhand, India. Legal harvesting of bulbs from natural habitat is permitted from native place of Himachal Pradesh, India. Besides the illegal collection from some places in Uttarakhand is going unchecked, and this has resulted in depletion of plant species from wild (Badola and Butola 2004). The bulb of the plant species is required continuously by the pharma industries, with an international market value of USD 400 million and the local Indian valuation of about Rs 15,000 per kg/dry bulb (Luo et al. 2020; Kumar et al. 2020). The plant species is propagated conventionally through seeds and asexually via daughter bulbs, but the propagation is hindered by its prolonged life cycle, which approximately takes 80–90 days for aerial growth and 270–280 days below the ground due to geophytic nature. Low temperature causes the seeds to become dormant throughout the winter season and blooms during spring season (Carasso et al. 2011; Petric et al. 2012).

7.2 Botanic Classification and Distribution

Kingdom:	Plantae						
	Phylum:	Tracheophyta					
		Class:	Liliopsida				
			Order:	Liliales			
				Family:	Liliaceae		
					Genus:	*Fritillaria*	
						Species:	*roylei*

Distribution: The genus *Fritillaria* is dispersed in the northern temperate zone (Mabberley 1997). The species *Fritillaria roylei* has been reported to be present in highest number of taxa in India, China, Turkey, Greece, and California. In India, it is distributed in western Himalayan range of Jammu and Kashmir, Himachal Pradesh, and Uttarakhand. It covers the area between Kashmir to Uttarakhand within the altitudinal range of 2400–4000 m above sea level while in Uttarakhand, several authors reported different areas where *F. roylei* is found in the area ranging between 2800 and 4000 m above sea level (Singh and Rawat 2011), 2900–4200 m (Chauhan et al. 2011a) and 3250–6919 m above sea level (Kala 2005). The plant thrives vigorously in light sandy to medium loam well-drained acidic soil which are rich in organic components, generally found in habitat which is slight hilly and open area with proper sunlight (Bisht et al. 2016). *F. roylei* is mostly found in the region Minimarg and Gurez Valley of Jammu and Kashmir, in Uttarakhand it naturally flourishes well in various alpine meadows such as Tungnath, Kedarnath, Rudranath, Dronagiri, Valley of flowers and Dayara (Fig. 7.1).

Fig. 7.1 Geographical distribution of *F. roylei* in India

7.3 Morphology

F. roylei is an erect bulbous perennial herb which grows approximately 15–60 cm in height with mottled stem. The leaves are linear lanceolate and obtuse often pointed (5–10 cm long), arranged in whorls (3–6) or opposite. The vegetative phase of this plant species starts in the month of April and blooms during June–July, flowers are solitary or raceme (2–3), bell shaped, hanging down face and the floral buds are produced single or sometimes in cluster of two or more. The petals are ovate (4–5 cm long) and the color varies from yellowish green to brownish purple with checkered pattern in dull purple or yellowish green. Fruiting in this plant species occurs during July–August, fruits are oblong, obtusely angled, capsules are 6-winged. Bulbs are generally covered with membranous bulb scales (Goraya et al. 2013), these characters help them to survive under dormant phase of life cycle. *F. roylei* completes its vegetative and reproductive phase in very short time span (April to September) (Muraseva et al. 2015).

7.4 Phytochemistry

F. roylei is a bulbous plant species, the bulb consists of three major steroidal alkaloids which are pharmaceutically important viz. (1) Peiminine ($C_{27}H_{43}NO_3$) having 429.64 molecular weight and 135 °C melting point, also known as peiminine, raddeamine, fritillarine, imperialine, imperialin, kashmirine, zhebeinone, verticinone, or 3β,20-dihydroxy-5α-cevan-6-one. (Fig. 7.2) (2) Peimine ($C_{27}H_{45}NO_3$) having 431.66 molecular weight and 224 °C melting point.

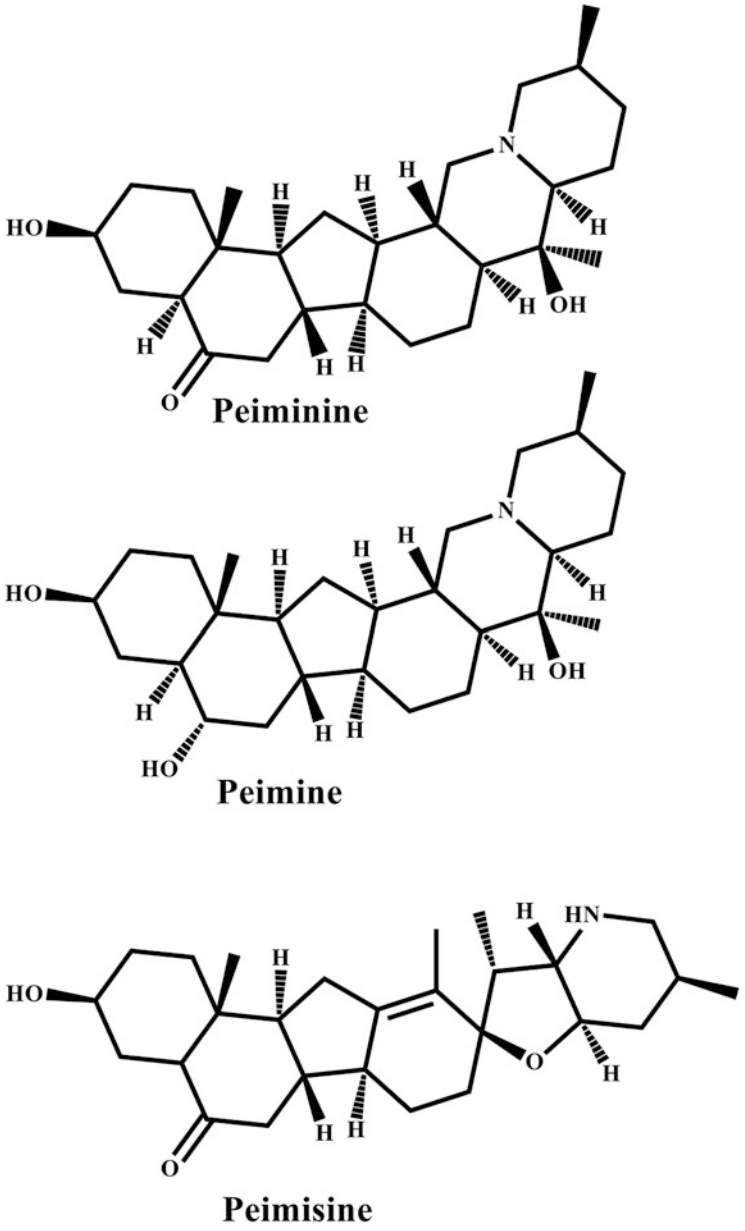

Fig. 7.2 Structures of main phytochemicals present in *Fritillari roylei* Hook.

Synonymously known as verticine, dihydroisoimperialine, wanpeinine A, zhebeinine, or Cevane-3,6,20-triol (3β,5α,6α,22β) (Fig. 7.2). (3) Peimisine ($C_{27}H_{41}NO_3$) having 427.62 molecular weight and 270 °C melting point. Synonymously known as Peimissine, Ebeiensine, Veratraman-6(5H)-one, 17,23-epoxy-3-hydroxy-(3β) (Fig. 7.2) (Chi et al. 1940; Wu 1944; Chou 1947; Jiang et al. 2001; Chatterjee et al. 1976; Goraya et al. 2013; Bisht et al. 2016; Kumar et al. 2020). Besides, these three major active constituents the bulb of *F. roylei* also consists of other alkaloids like peimidine ($C_{27}H_{45}NO_2$), peimiphine ($C_{27}H_{46}NO_3$), peimitidine ($C_{27}H_{44}NO_3$), fritillarin ($C_{28}H_{62}NO_3$), fritimine ($C_{38}H_{62}N_2O_3$), verticin ($C_{19}H_{32}NO_2$), and verticilline ($C_{19}H_{33}NO_2$). It also contains some neutral molecules such as propeimin ($C_{29}H_{48}NO_3$) and sterols ($C_{27}H_{46}O$) (Bisht et al. 2016).

7.5 Medicinal Properties

Bulb of the plant is medicinally important as it possesses properties to treat chronic respiratory disorders like asthma, bronchitis, and tuberculosis. In conventional medicine system, it is also utilized as medication for rheumatism, wounds, burns, aphrodisiac, tumor, ulcer, stomach problems, and hypertension, and it has properties like galactagogue, febrifuge, homeostatic, ophthalmic, oxytocic, anti-viral, and anti-microbial (Muto et al. 1994; Ping et al. 1995; Kang et al. 2002). In India, different regions of western Himalayan range have their own way of consuming the bulb of this plant species. In some regions of Jammu and Kashmir, the bulb of *F. roylei* is boiled with orange peel and given as a tonic to treat the patients having tuberculosis, asthma, and rheumatism (Srivastava et al. 1986; Shaheen et al. 2012). In Uttarakhand, most of the tribal people consume powder of the dried bulb with milk as a health tonic to increase stamina and for body weakness (Bisht et al. 2013). In Pakistan, the local or tribal people near Himalayan range use bulbous part to treat inflammation of bladder, soften and soothe the skin by consuming buttery paste of dried powder of bulb (Khan et al. 2013). In Chinese traditional medicine system, *F. roylei* is used as a strong source of cough suppressant, helps to treat various ailments like pneumonia and bronchial disorder. Its active constituent is also used commercially in Chinese drug named as Szechuan-Pei-Mu, which is used as lactogogue, expectorant, and anti-pyretic. Sometimes it helps to treat fever, hemorrhage, milk deficiency (Kaul 2010), chest injury, congenital pulmonary gout, dyspepsia, dysuria, stomatitis, boils, sinus, malaria, anemia, insanity, oligospermia (low sperm count), remedy for child skinniness, immunity promoter, and also work as a remedy for spider poisoning (Balkrishna et al. 2012). Additionally, *F. roylei* is exploited to cure heart diseases, decreased pulse rate, stimulation of the heart muscle, defective breathing, and nervous system (Erika and Rebecca 2005; Shah et al. 2014).

7.6 Pharmacological Activities

As per the available literature, it has been reported that the dried bulb of *F. roylei* is used as a potent source of drug to combat various diseases of respiratory system viz. asthma, anti-tussive, expectorant in conventional medicine system. Nowadays in vivo and in vitro studies on pulmonary ailments are focused by several researchers using dried bulb of *F. roylei*. Hence, increasing the advancement in pharmacological studies specifies that active constituents or herbal extracts from medicinal plants have potential antihypertensive, anti-inflammatory, antineoplastic, anti-tumor, and bacteriostasis effects.

Respiratory diseases: Respiratory disease is a serious health ailment which affects the physical well-being of patients with symptoms like difficulty in breathing, less oxygen supply to blood, cough, sneezing, chest pain which are leading causes of illness and death (Bai et al. 2018). The herbal materials extracted from *Fritillaria* have major pharmacological effect on respiratory system as it directly works on the immune system such as interferons, interleukins, and antibodies which ultimately lessen the negative effects on the body caused by asthma, cough, acute lung injury (ALI), or chronic obstructive pulmonary disease (COPD).

Anti-asthmatic effect: Asthma is a chronic disease of lungs; it causes the patients airways to get inflamed and narrow which causes difficulty in breathing. Anti-asthmatic drugs mechanism of action comprises the bronchial smooth muscles relaxation, bronchus spasm, relief of trachea, and helps to improve ventilation status. It has been reported by several scientific researchers that effect of *Fritillaria* on anti-asthmatic activity is linked to the antagonism of the tracheal M receptor (Zhou et al. 2006). Aqueous extract of the dried bulb possesses repressive effects on trachea/airway inflammation by various pathways including suppression of helper T cell-2 cytokines and immunoglobulin-E, increase in interferon-γ (IFN-γ) production, reduction of eosinophilic accumulation, and histamine production (Yeum et al. 2007).

Anti-tussive effect: The anti-tussive efficiency is a reliable and stable pharmacological action between the traditional and modern clinical utilization in routine life. A comparative study between 11 commercial *Fritillaria* species for anti-tussive effect was conducted, and it has been observed that the total alkaloid showed highly significant effect on cough induced by ammonia in mice. Additional studies reported that steroidal alkaloids are the major constituents played important role and having effectiveness in anti-tussive activity (Chen et al. 2004; Huang et al. 2013), the results showed that steroidal alkaloids decreases the coughing time and extended the latent period (Jiang et al. 2005). The study demonstrated that the potential mechanisms of action of these steroidal alkaloids which state that steroidal alkaloids were potential inhibitors of Ca^{2+} influx and act against muscarinic pathway (Chen et al. 2011).

Anti-inflammatory effect: The dried bulb of *F. roylei* possesses certain relaxing and healing effect on COPD and ALI; this process is related to reduce the inflammation or swelling. The ethanolic extract of the bulb of *Fritillaria species* inhibited the development of ear edema of an anti-inflammatory evaluation on a

concentration-dependent manner (Wu et al. 2018). Some isosteroidal alkaloids showed same anti-inflammatory effect (Li et al. 2006; Wei et al. 2020).

Expectorant effect: Dried bulb powder of *F. roylei* is consumed along with the mixture of fresh pear as a natural expectorant, older people use this remedy for ages for therapeutic effects. The presence of saponins, steroids, and total alkaloids are the main chemical constituent which contributes in the expectorant effect. Alkaloids from different species of *Fritillaria* are used to impart expectorant effect, as alkaloids from *F. cirrhosa* and *F. wabuensis* has improved the phenol red output from a mouse tracheal (Wang et al. 2011; Wang et al. 2012), it helps in relaxing smooth muscles. The amount of mucus was amplified without a vagus nerve after the drug was fed in rats; hence, it confirms that the bulb of *Fritillaria* does not have any adverse effect and can be used as an expectorant (Wang et al. 1993).

Other respiratory diseases: As we have discussed about the effectiveness of the steroidal alkaloids extracted from dried bulb powder of *Fritillaria* against respiratory problems, its efficiency is known to combat various respiratory diseases such as COPD and ALI. In a set of experimental analysis, the effect of an alkaloid-imperialine was tested on inflammation and pulmonary function in a COPD-like rat model, for this the rat was exposed to smoke of cigarette and intratracheal administration using lipopolysaccharide for the induction of symptoms (Wang et al. 2016). The observations revealed that imperialine reduced respiratory damage and inhibited the inflammatory response by intervening the expression of related cytokines in lung tissues. Peiminine has healing effect, it can decrease the wet-to-dry ratio and also lessen the activity of myeloperoxidase on LPS-induced ALI. IL6 was unable to act after peiminine treatment and in human lung adenocarcinoma cells (A549) the production of LPS-induced IL-8 was also inhibited by peiminine. However, in western blot analysis it has been observed that peiminine significantly inhibited the mechanism of action of the NF-κB pathway. Although peiminine interrupted lipid raft formation by reducing the cholesterol content (Du et al. 2020).

Anti-cancer or antineoplastic effect: The chemical or active constituents derived from plants bulb has a positive role to cure ailments like cancer, the therapeutic properties of *Fritillaria* has been revealed in cancer or cancer like cells, for example, A549 cells (Matsuo et al. 2013), endometrial cancer cell lines and ovarian (Kavandi et al. 2015; Bokhari and Syed 2015), non-small cell lung cancer (NSCLC) (Lin et al. 2020), oral keratinocytes (Yun et al. 2008), human promyelocytic leukemia cells (HL-60) (Pae et al. 2002). HeLa cells (Abu et al. 2016), human colorectal carcinoma cells (Zheng et al. 2016), glioblastoma (GBM) cells (Zhao et al. 2018), and prostate cancer (Tan et al. 2020). Recently, some research has been conducted to study and explore the mechanism involved in anti-cancer activity by using herbal extract of *Fritillaria* bulb, the process involved in anti-cancer are stimulation of apoptosis without affecting the activity of caspase-3 (Matsuo et al. 2013) and arrest of G_0G_1 cell cycle via caspase pathway (Wang et al. 2015; Wang et al. 2016), TGF-β/SMAD signaling pathways are downregulated (Bokhari and Syed 2015). The steroidal alkaloid peimine exhibited the quality of in vitro reversal of multidrug resistance (MDR) as it facilitates the reverse the MDR activity of tumor cells. However, the reversal mechanism of MDR can be understood that of the presence peimine

increased amount in drug-resistant cells and the suppression of P-glycoprotein protein in drug-resistant cells (Hu et al. 1999).

Antihypertensive effect: Traditional ethanopharmacological studies showed that *Fritillaria* species also possesses antihypertensive effect. It has been reported that aqueous extract of the bulb can check the rise in systolic blood pressure in induced hypertension by N^G-nitro-l-arginine methylester and inferred as the improved generation of vascular NO and amelioration of renal functions (Kang et al. 2004). Aqueous extract of *F. ussuriensis* has been injected intravenously to anesthetized rats, as a result it helps to decrease the mean arterial pressure. Ethylacetate (292 mg mL^{-1}) and butanol (320 mg mL^{-1}) inhibits the activities of angiotensin-converting enzyme (ACE). However, the aqueous extract possesses hypotensive effect by using its ACE inhibition properties, and it also helps the vascular tissue of rats to release NO/cyclic guanosine 3′,5′-monophosphate (Kang et al. 2002).

Anti-microbial activity: In traditional medicine system, the active constituents present in herbal extract are known to possess negative effect on bacterial, fungi, or virus; in some cases, the plant extract is also helpful in combating the problem of antibiotic resistance (Ferrazzano et al. 2011). Li et al. (2005) reported that aqueous and ethanolic extract of *F. thunbergii* showed inhibition against *Helicobacter pylori* strains (the minimum inhibitory dose should be close to 60.0 μg mL^{-1}). In a study Kim et al. (2003) reported that *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus* activities were inhibited or checked by β-Sitosterol-3-*O*-glucopyranoside. In addition, against influenza H1N1 virus the aqueous extract of *Fritillaria* exerts anti-viral without inducing toxicity in vitro or in vivo (Kim et al. 2020).

Antioxidant activity: Most of the plants extract shows antioxidant activity, and it varies from species to species on the basis of type and quantity, similarly in *Fritillaria* the antioxidant is fraction and species dependent. The bulb of *Fritillaria* is rich in steroidal alkaloids viz. peimisine, peimine, and peiminine (acidic water-soluble heteropolysaccharides); additionally, the acidic fraction of steroidal alkaloid possesses strong antioxidant effect against free radicals present in body. In some species, the antioxidant activity declines with various polarity (crude flavonoid extract > crude saponin extract > ethanolic extract) (Li et al. 2010).

7.7 Conservation Strategies

Growth and dispersal of specific plant species depends on environmental condition and adaptability to survive. Loss of habitat, unscientific and illegal exploitation, anthropogenic activities and change in climate are the main reason behind the loss of population of *F. roylei*. Various other factors like biotic and abiotic factors also affect the habitat resulting into decline of the species up to 58–77% over the past few years in Western Himalayas (Chauhan et al. 2011b). The main reason behind the rate of decline is of early snowfall which causes hindrance in reproductive phase of plant species which prevents seed maturation and regeneration (Nautiyal et al. 2001).

Due to high medicinal value and large demand of the bulb of *F. roylei* plant in many pharmaceutical products, it is continuously being over-harvested from wild ultimately leading to its depletion and becoming an endangered species. The illegal harvesting is banned in the region of Uttarakhand and Jammu and Kashmir; therefore, it is necessary to conserve the plant species via conventional and unconventional method. Many efforts have been made for producing sustainable conservation protocol of *F. roylei*. Traditionally, it is propagated via conventional method, the frequency of asexual propagation via bulbous part is very low as the mother plant produces only 2–3 daughter bulb depending upon environmental condition and cultivation procedures (Uluğ et al. 2010). However, it is also propagated through seeds also, but it is even more tedious and difficult process than propagation via plant bulb, seeds take approximately 5–6 years to grow into a mature plant (Kumar et al. 2020).

Therefore, to overcome the limitation of conventional propagation numerous efforts have been attempted for the mass production of plant via morphogenesis or in vitro regeneration system. Plant cell and organ culture has provided an alternative opportunity to develop a protocol for *F. roylei* plant regeneration. The bulbous part of the herb is used as explants, i.e., whole bulb, bulb scale, or bulb segment. Callus culture, somatic embryogenesis, and bulblets have been developed and regenerated by culturing explants on suitable regeneration medium augmented with various concentration and combination of phytohormones growing under controlled environmental condition (Petric et al. 2012).

Joshi et al. (2007) has reported in vitro bulblet regeneration of *F. roylei*, using two different parts (basal or distal) of bulb scale as explant. Among the various growth regulators tested MS medium improved by adding kinetin (5.0 μM) and α-naphthalene acetic acid (2.0 μM) exhibited maximum regeneration rate (95.8%) and number of bulblet produced per explant (10.1 ± 0.63 bulblets) after 8 weeks of culture. In vitro inventions are useful for conservation and propagation of many valuable medicinal (Khanam and Anis 2018).

7.8 Analytical Techniques Used for Chemical Evaluation

There are number of chromatographic and spectrometric techniques which can be utilized for extraction, estimation, quantification, and identification of active molecules present in the dried bulb of *F. roylei*. Gas chromatography (GC) along with mass spectrometry (MS) techniques provides a powerful fusion tool for analysis of phytochemicals because they provide maximum separation efficacy and short-time analysis. The chemical constituents present in the bulb of isosteroidal *Fritillaria* were identified by GC analysis and GC-MS with pre-column derivatization. Although derivatization of column is required because of the polarity of isosteroidal alkaloid as it is unable to elute from regular GC columns (Kiani et al. 2015).

High-performance liquid chromatography (HPLC) is a useful standard procedure to identify isosteroidal compound in the shade/sun dried bulb of *Fritillaria*,

furthermore, in conjugation with HPLC an evaporative light scattering detection (ELSD) and charged aerosol detector (Long et al. 2016) used for the detection of chemicals which are usually not detected by traditional techniques. The derivatives of isosteroidal compounds are appropriate for ultraviolet and fluorescent determination, thus displaying efficient detection sensitivity and facilitating to meet the demands of analysis. Consequently, the formation of derivatization reagents is an essential step to the quality assurance of isosteroidal alkaloids of *Fritillaria*.

7.9 Conclusion and Future Prospects

Geographical distribution in the Himalayan range of *F. roylei* has shown that this valuable medicinal herb is at risk of extinction because of various environmental stress and anthropogenic activities. The high demand of dried bulb in the market cannot meet requirement; therefore, it is required that the complete biological research and considering ecological and physiological aspect must focus on artificial breeding, it will protect the natural resources from unnecessary uprooting. To overcome the cultivation obstacle modern molecular-assisted breeding practices proves beneficial and useful as it avoids the long growth period. The phytochemicals have become the evaluation index for the substitution and alternate production of endangered herbal medicine. Moreover, these herbal medicines are the mixture of varying complex chemical compounds depending upon species or plant part. The herbal drug based of *F. roylei* possesses various pharmacological activities, and it is important to consider that it may facilitate the degradation of few pounds and intermingled reactions occur during the process of heating and metabolism in the body and hence, potential plant for investigation of new therapeutic activities in the future for clinical studies. The mechanism of action of pharmacological activities is still ambiguous, consequently modern scientific approaches should be used to decipher the pathways involved in the multi-target and multi-channel mechanism. It is necessary for future that pharmacological investigation must concentrate on possible metabolism of the separated components, which will later help to conduct detailed research on animals and will also help in understanding the association between their pharmacokinetics and pharmacodynamics. The research should also be focused on new dosage forms, nano-preparations, inhalation preparations, and conservation strategies. Illegal or unscientific collection from natural habitat of *F. roylei* should be banned and strategies should be developed for conservation and sustainable utilization.

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Chapter 8
Gentiana kurroo Royle: Himalayan Gentian

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8.1 Introduction

Plant-based secondary metabolites (SMs) are significant and incipient elements of global pharmacopeia. The knowledge of their therapeutic applications is rising progressively because of advanced investigations and research, which may make these elements more and more safe or appropriate substitutes to modern allopathic medicine. Presently, there is augmented curiosity in SMs due to their rising demand for new and more medicine from plants. This revolution of inquisitiveness in plant-based medications is basically accredited to the topical widespread trust that "green medicines" are eco-benign, cost-efficient, and may provide harmless medicine in comparison to synthetic ones, which are more expensive and have many antagonistic effects (Sharma et al. 2018, 2021; Bhardwaj et al. 2021). Hence, the curiosity is growing in plant-based SMs with potential therapeutic activity. Also, several scholars around the world are presently being coddled to explore the relationship among the plant-based SMs and its botanical, pharmacological, and therapeutic properties (Sharma et al. 2018, 2021; Bhardwaj et al. 2021).

Gentianaceae is a flowering plant family comprises of 1700 species grouped into 91 genera, with a diverse variety of habitats, appearance, and behavior (Mukherjee

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2017). Around 16 genera and 145 species of this family are found in India. With over 400 species, *Gentiana* is the biggest genus in the Gentianaceae family which is mainly distributed in Europe and Asia. Gentianaceae species, particularly those in the Gentiana genus, have ornamental importance and are also of pharmacological interest due to their fascinating SMs composition. The name Gentiana is derived from a monarch named Gentius (a king of Illyria, Europe), who was the first to recognize the herb and be cured by it. According to Dioscorides (the Greek physician), King Gentius understood the virtues of this medicinal plant and used its roots to treat the Plague in 167 BC. The name Gentiana refers to the plant's genus and comprises its complete species, according to traditional medicine sources (Shabir et al. 2017a).

Traditionally, the species of the genus *Gentiana* have been frequently used to treat liver jaundice, dispel rheumatism and ease pain since ancient times (Mirzaee et al. 2017). Further, the SMs and extracts isolated from the different species of this genus have been known to show several pharmacological properties such as cardiovascular protection, bone protection, joint protection, hepatic protection, pulmonary protection, immunomodulation, reproductive protection, and gastrointestinal protection. Also, the SMs and extracts obtained from species of this genus show no substantial animal genotoxicity, cytotoxicity, and other toxicity (Jiang et al. 2021).

Gentiana kurroo Royle is a medicinal plant native to the northwestern Himalayas. It is known as Indian Gentian in the United States but known as Nilkanth in Kashmir. *Gentiana kurroo* is a rosette-forming perennial herb that grows at a height of 1500–3000 m in the sub-alpine region of the northwestern Himalayas. The alkaloid gentianin, iridoid glycosides gentiopicrine, and gentiamarin are the key SMs found in the roots of plants. Usually, the plant's roots are used as bitter tonics, expectorants, astringents, stomachic, anthelmintics, antipsychotics, and sedatives, as well as antibacterial. The herb can also be used to treat leucoderma, bronchial asthma, and urinary infections. Owing to its over-exploitation, nowadays it is a critically endangered therapeutic herb of the northwestern and western Himalayan (Sajjad et al. 2019).

Owing to its high demand in the global market and inadequate supply from natural habitat, *G. kurroo* has been known to be substituted or adulterated with roots of other *Himalayan* species such as *Gentiana decumbens, Gentiana tenella*, *Exacum bicolor,* and *Picrorrhiza kurroa*. But, the adulteration in the crude drugs can be evaluated by anatomical, microscopic, and macroscopic examination along with ash and chemical analysis (Shabir et al. 2017b and references therein).

From ancient times, variety of plants and their products have been in use for the cure of various ailments. Though, a handful of these has been authenticated scientifically by in vivo animal studies, in vitro bioassay, and clinical trials. Further, the maximum of the existing scientific data related to the biological activity of conventionally used plant products lacks an organized analysis of their mode of action, stability, usefulness, safety, and toxicity. Therefore, awareness about plant-derived therapeutic formulations and medications is incessantly increasing globally. Henceforth, the recognition and authentication of plant-based traditional medications and formulations by comprehensive scientific examinations is the demand of time. Till

date, very little work has been done related to the compilation of phytochemical composition, biological applications, and toxicity study data of *G. kurroo Royle*. Thus, in this chapter the experimental data explaining the promising phytochemical composition, folkloric uses, biological potential, toxicity, and agrotechnology of *G. kurroo Royle* have been presented.

8.2 Traditional Uses

Gentiana kurroo's therapeutic properties have been well-recognized since humans first learned about herbal treatments for various disorders using natural resources. The plant is used to treat ulcers, stomachaches, urinary infections, liver complaints, migraine, respiratory disease, cough, skin disorders, leprosy, indigestion, colic flatulence, blood purifier, indigestion, gastro infections, malnutrition, and high fevers in the Chinese folk medicinal system (Skinder et al. 2017). The SMs Amaroswerin is a gastroprotective agent, whereas Gentianine has anti-inflammatory, antipsychotic, sedative, hypotensive, diuretic, analgesic, anticonvulsant, antimalarial, anti-amoebic, and antibacterial activities. The plant's medication is useful in reducing all types of weakness and tiredness in the body caused by long-term disease, as well as in the manufacturing of stomachic tonics (Behera and Raina 2012a, b). In Ayurveda *G. kurroo* has been extensively used for the cure of skin diseases, fever, wound, dysmenorrhea, and liver disorders. Several ayurveda scriptures and periodicals suggest that the *G. kurroo* have been used for the treatment of a number of ailments such as Jvara (useful in fever), Dahahara (burning sensation), Pachana (helps in digestion), Hridaya (treats heart problems), Pandu (treats skin disorders), Trutahara (relieves excessive thirst), Anulomana (improves breathing), Mehahara (treats urinary tract disorders), Deepana (enhances stomach fire), Yakrit Vikara (prevents liver infections), and Prameha (manages diabetes). The Roots of the plant have been used as tonics, expectorants, astringents, stomachics, anti-inflammatories, antidepressants, sedatives, anthelmintics, and antibacterials. The powdered leaves are used to treat ulcers and fungal infections. Pain, inflammation, fever, and liver disease are all treated with flowering tops. The plant is also effective against leukoderma, helminthiasis, and bronchial asthma (Behera et al. 2011). Following are the other Ayurvedic benefits of *G. kurroo* (Behera et al. 2011; Skinder et al. 2017; Behera and Raina 2012a, b; Prashanth 2022):

- Fever is treated with a decoction of Trayamana root (*G. kurroo)*, which is also a blood cleanser and carminative.
- *G. kurroo* root decoction is being used to cure syphilis and leucoderma.
- The root powder of the plant with honey is given in 3–5 g doses to cure cases of poisoning.
- The root powder paste is applied on the affected area with alopecia as part of treatment.
- The root's cold infusion is given to cure the piles and indigestion.

- The powdered root is substituted with Katuki root (*Picrorhiza kurroa*) and utilized for purgation.
- The medicated ghee (prepared from the juice of *G. kurroo)* along with honey is given in cases of hemorrhage from small vessels.
- The cold infusion prepared from the root of *G. kurroo* is given to treat difficulty in dysmenorrhea and micturition.

8.3 Botanical Classification

Gentiana kurroo Royle (karu, kutki) is an important critically endangered medicinal plant of genus *Gentiana* and family Gentianaceae. The botanical classification of *G. kurroo* is given in Table 8.1 whereas the various vernacular names of *G. kurroo* are given in Table 8.2.

Table 8.1 Botanical classification of *Gentiana kurroo royle*

Kingdom	Plantae
Sub-kingdom	Viridiplantae
Infra-kingdom	Streptophyta
Super-division	Embryophyta
Division	Tracheophyta
Sub-division	Spermatophyta
Class	Magnoliopsida
Super-order	Asteranae
Order	Gentianales
Family	Gentianaceae
Genus	*Gentiana*
Species	*kurroo*

Table 8.2 Vernacular names of *Gentiana kurroo royle*

Hindi	Kutki, kadu, kore, karu, chireta
Kannada	Kiriyatu, karadihanni
Manipuri	Kirayet
Sanskrit	Trayamana, girija, anuja, trayanthi
Malayalam	Trayamana, Kiriyat
Telugu	Nelavemu, buroni
Tamil	Nilavimbu, kampantirai
Bengali	Trayaman, Karu, chireta, koutki
Urdu	Neel-kanthi
English	Himalayan gentian, Indian gentian
Marathi	Pakanbhed
Gujarathi	Pakanbas
Kashmiri	Kadu

8.4 Geographical Distribution

The Himalayan region of India, Nepal, and Pakistan is home to various *Gentiana* species. *G. kurroo* is a native to the northwestern Himalayas, where it may be found growing at elevations of 1500–3000 m, particularly in Kashmir and Himachal Pradesh. It is mostly growing on south-facing steeper slopes in Kashmir, along sloppy and dry rocky grasslands and sparsely shrubby scrubs, although due to its overexploitation it is now rare in Himachal Pradesh's sub-alpine to alpine meadows. India accounted for more than 80% of the species population reduction due to over uprooting of plant from wild habitats in the last 10-year span (Shabir et al. 2017b). *G. kurroo* was first discovered in Kashmir in the Pahalgam area, then in the Kangan and Wangat areas, at an elevation of 1850–2000 m (Raina et al. 2003; Shabir et al. 2017b).

8.5 Morphology and Lifespan of *Gentiana Kurroo*

G. kurroo can be propagated by a variety of techniques like micro proliferation of shoot nodal segments, seeds, rhizome cuttings, and somatic embryogenesis. The shoot system is made up entirely of blooming branches with culine leaves. The root system comprises of adventitious root and rhizome, while the stem is a modified rhizome. Flowering begins in the mid of August and lasts until the first week of November. The peak is between September 15 and October 20. A plant produces 20 blooms on average, and the best time to collect seeds is in the first 2 weeks of November (Kaushal et al. 2015; Lal et al. 2019). Morphological characteristic features of different parts of *G. kurroo* are presented in Table 8.3.

8.6 Phytochemistry

G. kurroo is taxonomically valuable for its diverse classes of phytochemicals and numerous pharmacological activities. Iridoids, C-glucoxanthone mangiferin, flavonoids, xanthones, and C-glucoflavones are among the prime phytochemicals isolated from the species (Yang et al. 2010; Skinder et al. 2017). These SMs are mainly isolated from the root and rhizome, which are normally taken from their natural habitat. Qualitative phytochemical analysis of rhizome and roots showed the presence of flavonoids, phenolics, tannins, alkaloids, glycosides (Gentiopicrine, Gentianine), terpenes, saponins, genianic acid, carbohydrates, and pectin; Flowering tops revealed the presence of alkaloids, flavonoids, free phenols, glycosides, terpenes, and sterols, while the phytochemical analysis of leaves revealed the occurrence of iridoid glycoside and volatile aroma compounds such as 20-(2,3-dihydroxybezoyloxy)-7-ketologanin, 1,3-propanediol, 2-ethylfuran,

Table 8.3 Morphological characteristic features of different parts of *G. kurroo* (Latif et al. 2006; Kaushal et al. 2015; Lal et al. 2019)

Part of plant	Characteristics
Habitat	Mainly thrive in temperate and subtropical climates
Rootstock	It consists of a thick perennial rhizome and sporadic roots
Stem	Underground rhizome The aerial section is a decumbent stem
Leaves	In this species, there are two types of leaves: Radical and cauline *Radicle:* Simple, sessile, lanceolate, and complete lamina with acute apex. Leaves last the whole life cycle of the plant, finally being replaced by new leaves. The majority of new leaves appear in the rainy season. These leaves are connected in pairs at the base to create a shared sheath *Cauline:* Contracted, linear, conflicting, the bases forming a sheath around the flowering shoot. On blooming shoots, the cauline leaves are short, linear, and borne in pairs at the base, creating a tube around the flowering stalk. When compared to the radical leaves, they are narrower and smaller, and they shed with the blooming stalk. Leaves have a pinnate or unicostate reticulate venation pattern. The veins get more noticeable and thicker as you go closer to the base of the lamina
Inflorescence	Monochasial cymose/solitary
Flower	Complete, bracteate, hermaphrodite, pedicellate, hypogynous, pentamerous, and actinomorphic are all terms used to describe flowers that are bracteate, pedicellate, complete, hermaphrodite, actinomorphic, hypogynous, and pentaentomophile (love of insects), different types of dichogamy protandrous, odorless, and necterless. Flowering season is September through October, with flowering beginning at 7:00 a.m. *Calyx:* Sepals are five, gamosepalous, tabular, persistent, lobes are linear, spiky, and green in color. The odd sepal is posterior to the mother axis. *Corolla:* Petals are five, infundibuliform, gamopetalous, with five-lobed limbs. The odd petal is the one that is closest to the mother axis. Plicae are blue, the neck is dotted with white dots, and the base is white from the inside. *Androrcium:* The stamens are five, haplostamenous, epipetalous, alternating with the petal lobes, and introrse. The anthers are bicelled, entities or individuals fixed, obovate, and white in color. Pollens are round in shape. The period of dehiscence is 7:30–10:00 a.m. *Gynoecium*: Unilocular bicarpillary syncarpous ovary. The ovule is anatropous, the placentation is parietal, and the style is identical
Fruits	Fruit is a capsule that is 5.47 cm in length on average. After fertilization, it takes 18–20 days for the capsule to develop. The capsule dehisces lengthwise, commencing at the tip, revealing countless seeds that are carried away by the wind. A single freshly plucked fruit weighs around 2.03 mg
Seeds	Tiny and slightly curved, with one end pulled out. The average seed yield/fruit was 8.95 mg, with a total seed set of 167.5 mg per plant. The 1000 seeds have a weight of around 0.1275 g

dimethyl sulfide, alpha-terpinyl acetate, hexanal, methandriol, pentanol, 1,8-Cincole, 2-methyl sulfide, 3-methyl butanol, and 7-oxabicylo (4,1,0)-heptanes (Yuan et al. 2017; Skinder et al. 2017). Further, the LCMS and LC-ESI-MSMS analysis of the methanol extract of *G. kurroo* showed the presence of swertiamarin, swertisin, sweroside, swertianolin, norswertianolin, loganic acid, gentiopicroside,

gentisin, gentioside, isogentisin, lupeol, and 4-O-β-d-glucosyl-6′-O-(4-O-β-d-glucosylcaffeoyl)-linearoside (Ghazanfar et al. 2017; Wani et al. 2013). Furthermore, the thin layer chromatographic analysis of ethanol extract of flowering tops of *G. kurroo* revealed the presence of robinetin-O, luteolin, apigenin, kaempferol, kaempferide (Latif et al. 2006). The structures of various SMs obtained from different parts of *G. kurroo* are presented in Fig. 8.1.

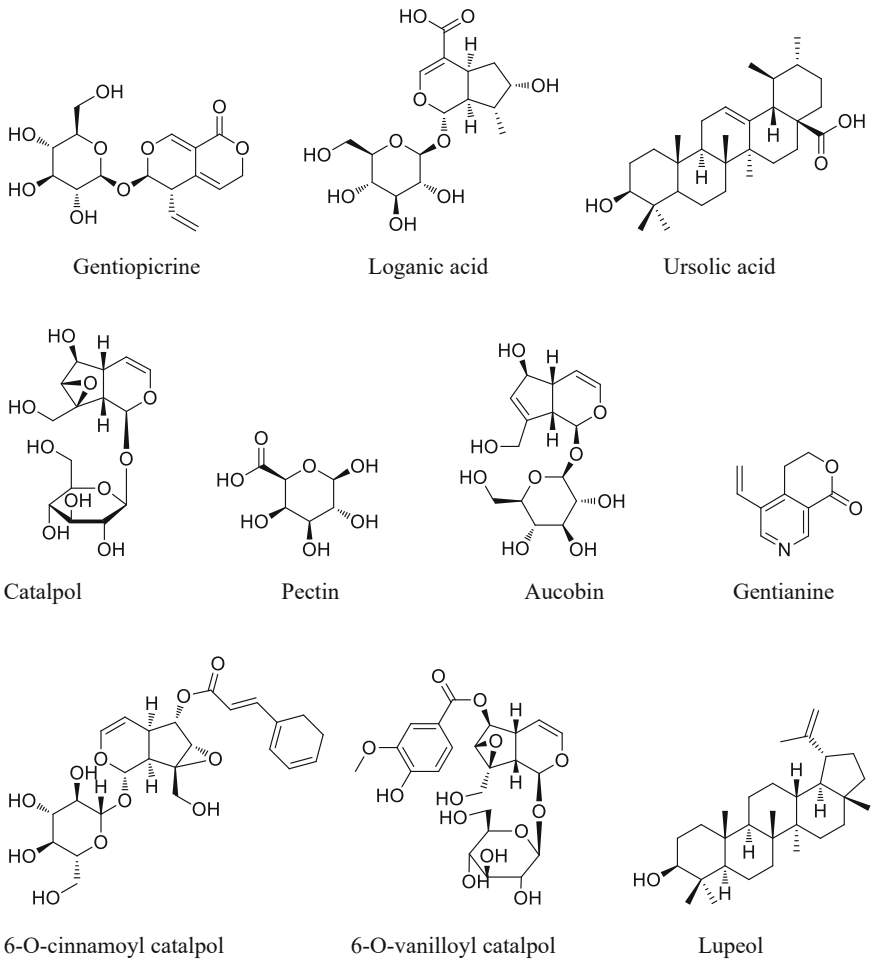
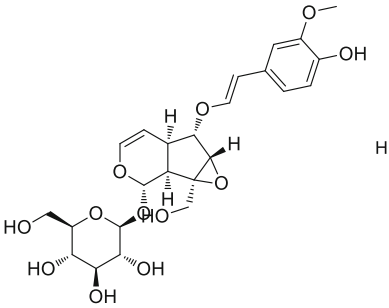
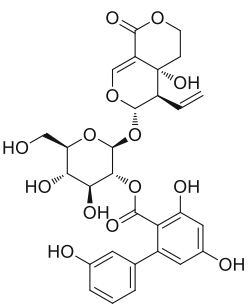


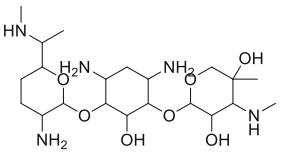
Fig. 8.1 Structure of Key SMs (volatile and nonvolatile) present in different parts of *G. kurroo*



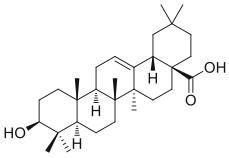
6-O-feruloyl catalpol



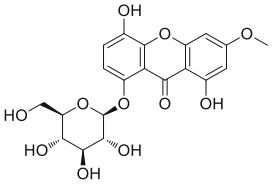
Amaroswerin



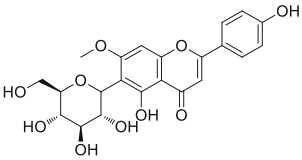
Gentamicin



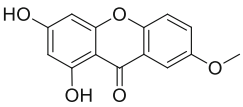
Oleanolic acid



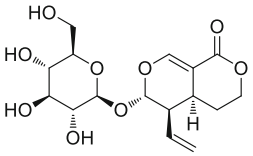
Swertianolin



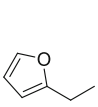
Swertisin



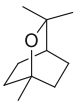
Isogentisin



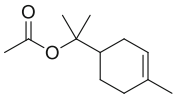
Sweroside



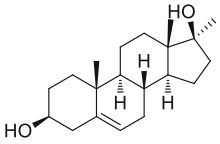
2-ethylfuran



Eucalyptol



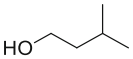
Alpha terpinyl acetate



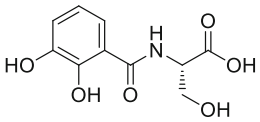
Methandriol

Fig. 8.1 (continued)

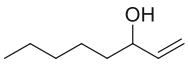
1, 3-propanediol



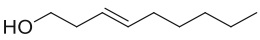
3-methyl butanol



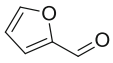
2,3-dihydroxybenzoylserine



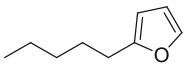
Oct-1-en-3-ol



Non-3-en-1-ol

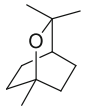


Furan-2-carbaldehyde

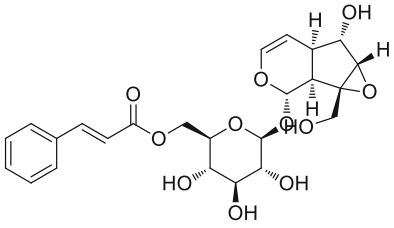


2-Pentylfuran

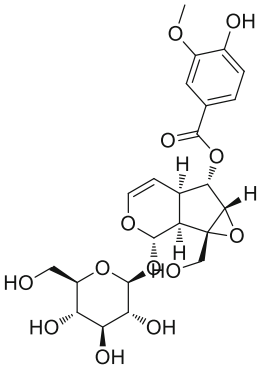
7-Oxabicyclo[4.1.0]heptanes



1,8-Cineole

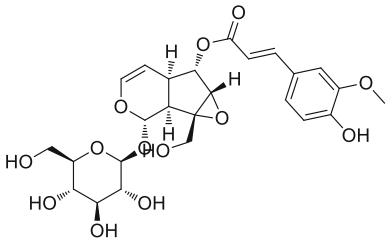


Picroside 1

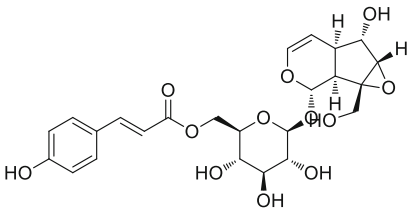


Picroside 2

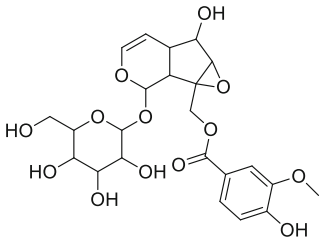
Fig. 8.1 (continued)



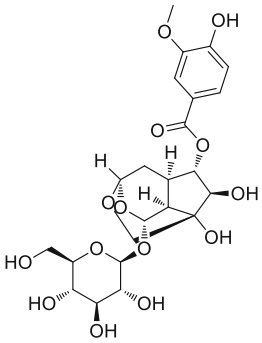
Picroside 3



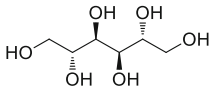
Picroside 4



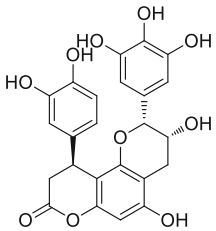
Kutkoside



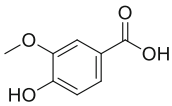
Pikuroside



d-mannitol



Apocynin



Vanillic acid

Fig. 8.1 (continued)

8.7 Pharmacology

8.7.1 Antibacterial Activity

In a recent study, the extracts of *G. kurroo* were discovered to exhibit antibacterial activity. These extracts inhibit the growth of both gram-positive and gram-negative bacteria. *G. kurroo* root and leaf extracts were shown to have stronger antibacterial

action against gram-positive bacteria than gram-negative bacteria. The high level of flavonoids, which hinder nucleic acid production and metabolic activities, might be the cause of antibacterial action (Baba and Malik 2014).

8.7.2 Antioxidant Activity

G. kurroo leaf and root extracts contained a high content of flavonoids and other phenolic compounds. Phenolic compounds are crucial plant phytoconstituents responsible for their antioxidant potential which is mainly attributed to their capacity to scavenge free radicals and other reactive species. Thus, the total phenolic and flavonoid content could be used as a rapid source for antioxidant activity screening (Mubashir et al. 2014b). Most of the oxidizing compounds present in the cell responsible for the number of disorders are scavenged by flavonoids and other polyphenols. These flavonoids and polyphenols reduce reactive oxygen generation, scavenge reactive species, chelate free-radical-producing trace elements, and boost the antioxidant defenses (Baba and Malik 2014). The total flavonoid content for the leaf extract was found to be 30 ± 1.3 rutin equivalent/g dry weight while that of the root extract was 51 ± 2.2 rutin equivalent/g dry weight. Further, the total phenolic content for the leaf extract was found to be 43 ± 1.5 gallic acid equivalent/g dry weight while that of the root extract was 86 ± 2.4 gallic acid equivalent/g dry weight. The methanolic extract of root (DPPH assay–72%; NBT assay–63%) showed better antioxidant potential as compared to the methanolic extract of leaves (DPPH assay–53%; NBT assay–51%) which may be attributed to higher polyphenolic content of roots as compared to leaves (Skinder et al. 2017 and references therein).

8.7.3 Analgesic Activity

Two procedures (Eddy's hot plate test and acetic acid-induced writhing test) were used to assess the *G. kurroo's* analgesic activity. The hot plate test is the most sensitive to centrally active analgesics, while the acetic acid-induced writhing test detects both central and peripheral analgesia. The methanol extract obtained from the root of *G. kurroo* was administrated at doses of 250 and 500 mg/kg body weight reduced the number of writhing (63.38% and 73.70% inhibition) in a dose-dependent manner in the acetic acid-induced writhing test, and the results were equivalent to the diclofenac sodium standard drug (71.61% inhibition) (Mubashir et al. 2014a). The analgesic effect of the extracts was most likely due to a reduction in prostaglandin production or activity. In Eddy's hot plate test however the extract showed a dose-dependent increase in reaction time (pain threshold potential elevation) in response to the heat stimulus at various periods of observation (0–120 min) as compared to the control. *G. kurroo* has a high analgesic action against a range of stimuli as a result of the studies. The probable mechanism was revealed to be the

retardation of both centrally and peripherally mediated nociceptive receptors (Latif et al. 2006).

8.7.4 Immunomodulation Potential

G. kurroo triterpenoids showed immunomodulatory action similar to cichoric and boswellic acids, which might be used as immunomodulatory drug-like compounds. The methanolic fraction of *G. kurroo* has been proven to have the best ability to inhibit both humoral (57.57% and 54.05%) and cell-mediated immunity (65.27% and 75%) (Mubashir et al. 2014b).

8.7.5 Anti-Diabetic Activity

There is no scientific evidence to back up *G. kurroo* anti-diabetic activity; nevertheless, a recent study conducted in 2017 demonsrated *G. kurroo's* anti-diabetic potential. *G. kurroo* extracts have been shown to improve glycemic control in oral glucose tolerance tests and in normal rats. The plant extracts swiftly discharge the glucose load, according to an analysis conducted on the rats (Wani et al. 2011). The primary symptoms of diabetes, polyphagia, polydipsia, and polyuria were shown to be alleviated by methanolic and hydroethanolic extracts of plant. In rats, it has a regulating effect on hyperglycemia and may effectively combat other metabolic abnormalities caused by diabetes. The anti-diabetic effect of *G. kurroo* might be attributed to the presence of bioactive components such as Swertiamarin, swertisin, and lupeol (Shabir et al. 2017a).

8.7.6 Antileishmanial Activity

Leishmaniasis is common in developing countries and nowadays it has become a global health issue. Due to the lack of viable vaccination and safe chemotherapeutic treatments, controlling the disease becomes more challenging. Various drugs like pentavalent antimonials, pentamidine, and liposomal versions of amphotericin B, miltifosine are being used to treat leishmaniasis, but each has one or more limitations that limit their usage. (Sidana et al. 2018) Worldwide, research is being conducted in the hunt for a novel leishmaniasis control agent, with a focus on natural bioactive secondary metabolites rather than synthetic chemicals. Novel therapies against diverse kinds of leishmaniasis can be found in biological compounds derived from plant and marine natural resources. The methanolic extract obtained from the roots of *G kurroo* showed antileishmanial potential against *L. donovani*. The *in silico* docking studies exposed that both norswertianolin and gentioside may have a role in

the in vitro antileishmanial potential of methanolic extract isolated from *G. kurroo* roots (Kaushal and Group 2015).

8.7.7 Anti-Inflammatory Activity

Various extracts obtained from *G. kurroo* were examined for their anti-inflammatory potential at a dose of 250 mg/kg body weight using the edema model test. The methanolic extract exhibit the highest potential for conquering the inflammatory response. The noted inhibitory potential in the paw edema assay was 47.62%. This was found to be noteworthy in comparison to the control group (55.24%). Further, maximum activity (67.27%) was noted at a higher dose of 750 mg/kg body weight which was even better as compared to the standard drug (56.36%). Anti-inflammatory potential could be attributed to its natural products like flavonoids (6-methoxytricin) and terpenoids (camphene, b-pinene, borneol).

8.7.8 Antiproliferative Activity

The antiproliferative potential of different extract obtained from *G. kurroo* root was evaluated by SRB (sulforhodamine B) assay against different cancer cell lines. The result revealed that the root extract inhibited the growth of cancer cells by inducing cell cycle arrest in Miapaca-2 cells (at G0/G1 phase of the cell cycle) and also by inducing a notable diminution in mitochondrial membrane in the studied cell line (Wani et al. 2013).

8.8 Green Synthesis of Nanoparticle

Scientists are currently very interested in nanotechnology because of its power to deal with the day-to-day challenges of environmental issues and the development of sound scientific principles. The creation of nanoparticles with homogeneous shape is a crucial notion in nanotechnology research. Due to the huge amount of organic and inorganic pollutants in the environment, scientists have recently attempted to focus on an eco-friendlier technique for the synthesis of a variety of metal nanoparticles. The green synthesis of metallic NPs using plant extracts is regarded as a substitute for a variety of chemical synthesis. The greener synthesis is competent, time-saving, non-hazardous, and environmentally friendly in nature. Silver nanoparticles were synthesized from its precursor silver nitrate salt using a *G. kurroo* rhizomes extract. The UV-visible spectra indicated a peak at 430 nm, showing that Ag-NPs were successfully produced and stabilized (Mukhtar et al. 2020). Further, the *G. kurroo* extracts are rich in flavonoids and other phenolic compounds owing to this these

extracts can be efficiently used for the synthesis of a variety of other metal nanoparticles.

8.9 Toxicology

Mice were used to test the effects of the extracts on their general behavior and protection. Methanol extract of *G. kurroo* (GKME) was given to mice at single dosages of about 1500–2000 mg/kg orally. After dosage, the mice were kept under observation for 4 h to see if they showed any hazardous signs. The number of animals that survived was counted after 24 h and the animals were kept under surveillance for another 13 days (Sajjad et al. 2019). There were no noticeable changes reported in the common activities, heaviness, mortality, or other physiological functions. In an acute toxicity experiment, no adverse effects or mortality were recorded after a single dose of GKME of 2000 mg/kg body weight (Wani et al. 2011). Further, the methanol extract obtained from *G. kurroo* was noted to be safe at a dose of 2000 mg/kg both in Wistar rats and Balb/C mice (Ghazanfar et al. 2017).

8.10 Agrotechnology

Seeds, rhizome cuttings, somatic embryogenesis, and microproliferation are the key options for the propagation of *G. kurroo*. It needs a cold, temperate environment and well-drained soil to thrive. When developing, plenty of sunlight and water is required (Behera and Raina 2012a, b).

Propagation through Seeds: Flowering takes place from September to October, and capsule maturation takes 18–20 days following fertilization. The first 2 weeks of November are good for seed harvesting. To avoid a large drop in germination percentage, seeds are needed to be stored at a very low temperature after harvesting. Seeds that have been sitting for more than a year have lost their viability and will not germinate. June, when 70–75% of seeds germinate, is the preferred time period to plant seeds. Germination begins on the 6th day after seeding and continues for the next 28 days. Despite the fact that the plants produce a lot of seed and the seed germinates quickly, seedling establishment is slow. Without injuring the root system, as soon as the first two leaves appear the seedling should be potted. The plant flourishes well on humus-rich, well-drained rocky stoney topsoil (Behera and Raina 2012a, b and references therein).

Propagation through rhizome cuttings: *G. kurroo* may be propagated using macro proliferation by rhizome cuttings. Rhizomes with a diameter of 5–8 cm are divided lengthwise into two halves, each containing the mother rhizome's above-ground segment with budding buds. Before the cuttings are put in a raised bed, they are given rooting hormones. Indole-butyric acid is the most widely used rooting

hormone and delivers the greatest outcomes. (Tomar et al. 2011; Behera and Raina 2012a, b and references therein).

Somatic embryogenesis: *G. kurroo* seedlings, isolated protoplasts, and callus tissue cell suspension have a great capacity for forming somatic embryos and regenerating shoots. Protoplasts can come from any type of plant cell, but the optimum source is embryogenic suspension with a high morphogenic potential. The efficiency and efficacy of *G. kurroo* regeneration by protoplast culture are influenced by the enzyme treatment, source of cell suspension culture, aggregate fraction size, and culture medium composition. When the culture media is added with 0.2–0.4% sucrose, the photosynthetic activity of the somatic embryo-derived germlings is at its peak (Raina et al. 2003; Behera and Raina 2012a, b and references therein).

Clonal propagation through shoot proliferation: *G. kurroo* propagation is made simple by rapid clonal multiplication via auxiliary branching. Nodal segments and shoot tips of mature plants are washed with detergent for about 15 min before being cleaned with tap water (2 h). These were then surface cleaned for a few minutes with 0.1% mercuric chloride and washed with distilled water before being implanted vertically on nutrition media. The clonal plantlets generated are hereditarily constant, allowing commercial in vitro multiplication of this species indefinitely without concern of genetic instability (Behera and Raina 2012a, b and references therein).

Substitute: Picrorhiza kurrooa Royle ex Benth, found mainly in the Himalayan area, has traits that are comparable to *G. kurroo*. Both *G. kurroo* and *P. kurrooa* are referred to as Kutki as their common name. The rhizome of *P. kurrooa* is being contaminated with or replaced for those of *G. kurroo*, taking advantage of the trade name kutki and also utilized as a replacement and adulterants of the drug (Behera et al. 2011; Behera and Raina 2012a, b).

8.11 Formulation and Ayurvedic Medicines

G. kurroo has been employed in several ancient Ayurvedic remedies due to its acute bitterness and excellent therapeutic powers (Prashanth 2022, https://www.easyayurveda.com/2016/12/21/trayamana-gentiana-kurroo-indian-gentian/ accessed 15 Aug 2022).

1. Ardraka Ghritam is an Ayurvedic medicine that comes in the form of herbal ghee. It is used as a Panchakarma preparation technique as well as a remedy for dyspepsia, persistent diarrhea, anorexia, and other ailments.
2. Maha Tiktaka Lepa of Kottakkal: It is a patented drug used to treat a variety of skin illnesses as well as nonhealing ulcers.
3. An Ayurvedic medication Patoladi Choornam is used in the form of herbal powder to treat digestive, heart, and liver problems.

8.12 Conservation

G. kurroo is a Himalayan medicinal plant that is critically threatened due to its overexploitation owing to its medicinal values. Because of its widespread extraction, the species is on the government of India's negative list of species (Shabir et al. 2017b; Skinder et al. 2017). The species is being harvested for its rootstock (as these are the rich bitter principles which are mainly responsible for its traditional uses), along with plant diversity depletion which also results in deforestation. Many investigations revealed that the key bitter constituents for which the plant is known are also present in both its rootstock as well as the leaves. So, there is need to educate the people not to uproot the plant from rootstock for bitter constituent, the same purpose can also be solved by the leaves. This will help to protect the species population in its natural habitat. Further, to prevent these plants from becoming severely endangered, traditional medicinal plant uprooting on a large scale should be banned or restricted. This might be achieved by looking into new ecological niches for renewable, ecologically friendly, and conveniently available natural bioactive chemicals from other plants which are not endangered. Bio-prospecting of endophytes from medicinal plants is one method of obtaining bioactive phytochemicals for the creation of new drugs. Bio-prospecting of medicinal plant endophytes will be a revolutionary drug discovery technique with little environmental impact and the potential to aid in the conservation of critically endangered medicinal plants (Raina et al. 2011; Skinder et al. 2017). Presently, a variety of strategies have been needed with an aim of conservation and sustainable use of medicinal plants. Even though ex situ and in situ conservation approaches shall remain to be the prevalent choices, the achievement of long-term objectives of conservation and sustainable uses is only possible through cultivation, avoiding destructive harvesting and increased productivity. Furthermore, It has been noted that the composition of active metabolites and essential oil constituents differ with geographical and altitudinal changes in most of the aromatic and medicinal plants. Therefore, species-specific actions are desirable to ensure both simultaneous conservation as well as sustainability in the substantial production of raw materials.

8.13 Conclusion

The aim of the present study was to scrutinize and highlight the possible phytochemicals and therapeutic properties of the medicinal plant *Gentiana kurroo Royle*, which is native to the western and northwestern Himalayas. The medicinal plant's root and rhizome are frequently used in different traditional medicinal system for the cure of a variety of ailments. This species had become critically endangered pertaining to its high rate of extraction from its natural environment and endemic nature. The phytochemical analysis revealed the presence of several important phytoconstituents (iridoids, C-glucoflavones, C-glucoxanthone mangiferin, and

xanthones) that are well-known for their medicinal benefit for a variety of acute and chronic disorders. Several researchers have conducted experiments to validate the medicinal plant's folkloric use for a variety of diseases, including antioxidant, anti-arthritic, antibacterial, analgesic, anti-inflammatory, and anti-diabetic properties. The data relating to many aspects of the species like mechanisms of action, pharmacokinetics, metabolism, potential drug interactions and toxicology is still inadequate which calls for further investigations mainly in animal models and humans. Therefore, to authenticate the various medicinal uses, to confirm the safety, to fix the effective dose, and to evaluate the mechanisms of action further in vivo and in vitro studies are still required. Owing to the advancement of computer-aided drug design, molecular biology, modern pharmacology, experimental and theoretical technologies, the bioactive secondary metabolites from *Gentiana kurroo Royle* will shine in the coming future, particularly in the field of medicine and pharmacology.

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Chapter 9
Habenaria Intermedia D. Don and *Habenaria Edgeworthii Hook., f. ex Collett.*: The Western Himalayan Medicinal Plants

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9.1 Introduction

One of the oldest botanical diversities exists in the southern region of the Himalayas, i.e., at the world's highest elevation (more than 8000 m). According to an estimation, over 10,000 species of plants are present in Himalayan's hotspot. The largest family of the flowering plants in the hotspot is the Orchidaceae with around 750–800 genera (Dressler 1993; Singh 2001; Hossain 2009; Hossain 2011).

The Orchidaceae family of flowering plants is varied and extensive, with colorful and fragrant blossoms. Orchids have been around for about 120 years (Verma 2014). Orchids are a stunning and appealing set of natural wonders that represent a highly developed group of blooming plants. These are known for their healing benefits as well as their decorative appeal (Pant 2013). These have been used in traditional medicines to treat various disorders and diseases including tuberculosis, paralysis, stomach disorder, arthritis, chest pain, syphilis, cholera, jaundice, eczema, acidity, inflammations, tumor, boils, piles, leucoderma, muscular pain, menstrual disorder, diarrhea, etc. since millennia (Dobriyal 2002; Hossain 2011). Even the medicinal importance of orchids has been discussed in Atharvaveda (one of the four ancient

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Vedas, which is the oldest Sanskrit scripture). The orchids having medicinal importance mainly originate from Anoectochilus, Bletilla, Coelogyne, Calanthe, Cypripedium, Dendrobium, Eria, Ephemerantha, Gastrodia, Galeola, Habenaria, Luisa, Ludisia, Nevilia, and Thunia genera (Szlachetko 2001; Singh and Duggal 2009; Pant 2013).

It has also been stated that there is a dramatic increase in the demand for plant-based and herbal medications throughout the world in recent years, resulting in significant exploitation of medicinal plants. Seventy percent of all plant-based herbal medication comes from a single source, the Himalayas (Bhat et al. 2013). Therefore, the conservative and sustainable use of medicinally relevant plants is vital for maintaining a balance between mankind and mother nature. The assortment of medicinal plants in the western Himalayas is manifested by the presence of native and threatened species. Because of their versatile usage, these orchids have been exploited a lot over a great period of time, and this over-exploitation accounts for a major threat to these valuable plant species (Shrestha 2000). The illegal and absurd harvesting before the maturation of seeds, heavy grazing, and poor regeneration has created a burden on these wild herbs (Pant et al. 2002).

One of such versatile and important genera of the orchid is Habenaria. Habenaria is also known as bog orchid and rein orchid and about 800 species of *Habenaria* have been formally described. Plants in this genus are mainly terrestrial with tall, thin, or sometimes fleshy stems. This was firstly described by Carl Ludwig Willdenow in *Species Plantarum*. The word *habena* means "thong," "strap," or "rein."

Out of all the species of Habenaria, two famous species *H. intermedia* and *H. edgeworthii* are very important and versatile species. *H. intermedia* and *H. edgeworthii* are two very important ingredients of Ashtavarga, which means eight (Ashta) groups (varga) (Chauhan et al. 2007; Dhyani et al. 2010; Balkrishna et al. 2012; Sahu et al. 2013). Ashtavarga constitutes some of the very important classical ayurvedic formulations such as Chyawanprash (Singh and Duggal 2009; Hossain 2011; Kaushik 2013). The eight groups present in Ashtavarga are namely, Jeevaka, Rishbhaka, Meda, Mahameda, Kakoli, Kshira-kakoli, Riddhi (*H. edgeworthii*), and Vriddhi (*H. intermedia*). Whereas Chyawanprash constituted with four main species of orchids named *Malaxis muscifera*, *Malaxis acuminata*, *H. intermedia*, and *H. edgeworthii* (Singh and Duggal 2009). Other names of *H. intermedia* are *H. arietina*, Rishisrista, Saravajanpriya, Vasu, Yuga, Siddhi, and Sukha. Except *H. arientina*, *H. edgeworthii* is also called by all similar names by locals of Himalayas as *H. intermedia*.

H. edgeworthii originates in temperate Himalayas, Kashmir, Himachal Pradesh, Sikkim, and Uttarakhand at the heights of about 800–2500 m, whereas *H. intermedia* is majorly located in Uttarakhand, Himachal Pradesh to North-West Himalayas at the height of 800–2800 m (Dhyani et al. 2010). Both *H. intermedia* and *H. edgeworthii aka* Riddhi and Vriddhi, respectively, possess important medicinal properties majorly because of secondary metabolite production including phenolic compounds. *H. intermedia* exhibits a high content of total phenol, thiamine, calcium,

and tannins whereas *H. edgeworthii is* considered a good source of sodium (Sedai 2015). From the literature survey, it is clear that both species have a huge potency as antioxidant (Rawat et al. 2014; Rawat et al. 2016), antimicrobial (Bharal et al. 2014; Rawat et al. 2016), antibacterial, and antianxiety effects against acute and chronic physiological stress (Habbu et al. 2012) and also for the treatment of the nervous disorder, skin disorder, and asthma (Kumar et al. 2017a).

Besides all such important uses hardly any effort is reported to cultivate them in commercial ways which is a need of an hour to restore the natural heritage. Therefore, in the present chapter, we have discussed their taxonomy, distribution, morphology, phytochemistry, pharmacology, cultivation, harvesting, and conservation techniques to increase awareness about these biologically important herbs.

9.2 Taxonomy

Genus *Habenaria* (habena, a Latin word meaning strap or rein), belonging to the tribe Orchidaceae, is a widely distributed genus of orchids and is commonly referred to as "Bog orchids" or "Rein orchids." Carl Ludwig Willdenow, first formally described this genus in 1805 in Species Plantarum, and to date over 800 of its species have been described, growing in both tropical and sub-tropical zones and are native to all continents except Antarctica (Willdenow 1805). *H. intermedia* and *H. edgeworthii* are among the biologically important species of this genera, found in the Himalayan region. The taxonomic classification of *H. intermedia* (Singh and Duggal 2009).

	H. intermedia	*H. edgeworthii*
Kingdom	Plantae	Plantae
Phylum	Tracheophyta	Tracheophyta
Class	Liliopsida	Liliopsida
Order	Asparagales	Asparagales
Family	Orchidaceae	Orchidaceae
Genus	Habenaria Willd.	Platanthera
Species	*Habenaria Intermedia* D.Don	*Habenaria edgeworthii* D.Don
Synonym	*Kryptostoma intermedium* (D.Don) Olszewski and Szlach *Ochyrorchis intermedia* (D.Don) Szlach *Habenaria arietina* H.f.	*Platanthera edgeworthii* (Hook.f. ex Collett) *Habenaria acuminata*

Common names: *H. intermedia* and *H. edgeworthii* were used extensively in traditional medicinal systems owing to their wide range of therapeutic and pharmaceutical properties. These are known with different names in distinct languages and regions. Mangala, Riddhi, Rathanga, Lakshmi, Rishisrista, Siddhi, Saravajanpriya, Vasu, Sukha, Yuga, Talgranthisamakand, Vamavartal, Vasu, Vrisya, Yuga, etc. are Ayurvedic names of *H. intermedia* (Dhyani et al. 2010). In English, it is known as a

Wild orchid (Singh and Duggal 2009). Common names for *H. edgeworthii* are Vriddhi, Dakshinavarta, Himadrija, Siddhi, Lakshmi, Vasu, Mangala, Sukha, Rathanga, Yuga, Rishisrista, and Saravajanpriya (Dhyani et al. 2010).

9.3 Flowering/Fruiting Season

Both *H. intermedia* and *H. edgeworthii* grow in terrestrial habitats at an altitude of 2000–3000 m above sea level in the Himalayan region. Loose and sandy loamy soil with high organic content is suitable for their proliferation. Tubers serve as the propagation material with mean annual rainfall of 100–150 cm and mean annual temperature of 10–15 °C is optimum for their growth (Kant et al. 2012).

Flowering months: July to August

Fruiting months: September–October (Balkrishna et al. 2012).

9.4 Distribution

India has the higher percentage of medicinal plants acknowledged for their medicinal and therapeutic importance than land in the world. Geographically, India is an affluent and largest habitat for numerous medicinal plant species in the world (Kala et al. 2006). *H. intermedia* is a monopodial terrestrial orchid with tuberous roots and is well distributed at altitudes of 1500–2800 m above sea level in the Himalayan region. It is distributed in India (Kashmir, Himachal Pradesh, Sikkim, Uttarakhand, Meghalaya), Pakistan, Tibet, and Nepal. Associated vegetation comprises grasses, ferns, and *Rhododendron arboreum* (Kant et al. 2012; Kumar et al. 2017b). The occurrence of *H. edgeworthii* is also reported in the Himalayan region of India (Jammu and Kashmir, Uttarakhand, Himachal Pradesh, Pakistan, Nepal). In the Indian state of Himachal Pradesh, it is found in different regions viz. Chamba, Jangtoo, Kasauli, Kothi, Manali, Narkanda, Nauradhar, Rahla Forest, Rampur, Sarain, Srahan, Shimla, Summer Hills, and Taradevi. It is found as an individual plant or in groups of three to five plants in dry grassy slopes at an altitude of 1500–3000 m above mean sea level. Associated vegetation includes ferns, grasses, and liverworts (Kant et al. 2012).

9.5 Morphology

H. intermedia have 25–50 cm long terete stem bearing robust, alternate, fleshy, spreading long, ovate-oblong, acuminate leaves with cordate base, generally five or many in numbers. Inflorescence comprises one to six flowers, large green-white in

color and distant. Acuminate and Lanceolate bract leaves are either equal or more than ovary. Petals are white, nerved, crescent-shaped, recurved, and adherent to dorsal sepal with pale yellow-green colored lip except for longer white claw (three-lobed and longer than sepals); sepals are long green, reflexed spreading tips, dorsal is smaller but wider than lateral sepals, acute, ovate-lanceolate, forms hood with petals, acuminate; 5–6 cm stout green, more or less curved spur and longer than ovary; deeply fringed side lobes; short column without foot, pollinia, bipartite, and elongated (Kant et al. 2012).

Tubers: oblong or oval tuber with a broad base and tapering towards the apical region, tubers are buff-colored, fleshy, mucilaginous internally, smooth surface covered with hairs and few grooves like pits (Kumar et al. 2018).

H. edgeworthii plant grows up to 30–60 cm in height, leafy, erect, and stout. Its leaves are 4–10 cm alternate, ovate, oblong-lanceolate, acuminate thick, gradually smaller upper leaves, five to seven nerves, and base sheathing. Its spike is long (7–25 cm) bearing numerous flowers of yellow-green color and deflexed in the bud. Flowers have lanceolate acute bracts (lower—short and upper—long), green ovary sepals with slightly fringed margins, thick yellow erect and obliquely triangular (broad base) petals, yellow lip longer than the sepals with thick and strap-shaped distal part, spur almost double the ovary length, short column, and yellow-green curving upwards with the tip curved down, pollinia, bipartite, pyriform, and granular (Singh and Duggal 2009; Kant et al. 2012).

Tubers: fleshy, mucilaginous internally, oblong, oval, fusiform or sub-globose in shape, buff-colored, smooth surface with numerous hairs (small white) with few groove-like pits (Kumar et al. 2018).

9.6 Traditional Uses

H. edgeworthii and *H. intermedia* are important constituents of traditional rejuvenating medicine "Astavarga" ("Asta" meaning eight and "Varga" meaning group) which is a formulated mixture of eight different medicinal plants used for treating cough, fever, asthma, diabetes, blood disorders, skin diseases, fractures, acid reflux, and burning sensations (Balkrishna et al. 2012). Tubers and leaves of *H. edgeworthii* have been used in combinations of other Ayurvedic medicines namely—Mahamayura ghrta, Ashtavarga churna, Madhura Varga, and Chyawanprash for curing facial paralysis, semen-related diseases, arthritis, gout, leprosy, and insanity (Manmohan et al. 2012; Sedai 2015). Tonic prepared from *H. edgeworthii* tubers has cooling effects, and it has been used to relieve burning sensations. It acts as a blood purifier, brain tonic, and appetizer in the traditional system of medicines (Sedai 2015). It is known for providing strength and immunity to the body and curing rakta dosha (blood disorders), pitta dosha (digestion and metabolism-related problems) (Kumari et al. 2012). *H. intermedia* provide physical and reproductive strength, its tubers and soft leaves can be cooked and used in the form of a meal which is beneficial for curing muscular pains, sprains, facial paralysis, anorexia, hyperdipsia,

insanity, vaginal diseases, cold, fever, and asthma. (Singh and Duggal 2009; Rawat et al. 2014; Sedai 2015). These herbs can be consumed in powder form (dosage 2–3 g) or can be applied on the skin in the form of oil or clarified butter. Chyawanprash, an important herbal medicine, is a nutritive paste, prepared using different herbs including *H. edgeworthii* and *H. intermedia*, which has been very popular in the Indian traditional system of medicine as an immunity booster (Sharma et al. 2019). This herbal jam rejuvenates tissues in the body, promotes muscle mass, enhances strength, supports healthy functioning of the heart and reproductive system, strengthens the respiratory system, reduces digestive disorders, and supports urinary health (Rawat and Roushan 2018).

9.7 Phytochemistry

Phytochemistry is the branch of science dealing with the study of all the chemicals that are procured from plants. This branch is used to know the structure of secondary metabolites, their functions in plants and human biology, and their biosynthesis. Though many types of compounds are found in flora species, yet the four major classes can be categorized as alkaloids, terpenoids, phenylpropanoids, and polyketides. Due to the variable climate and environmental conditions, the phytochemical composition of plants collected from different geographical regions vary. Various scientists have studied the phytochemistry of the tuber part of *H. intermedia* D. Don. and *H. edgeworthii*, and many biologically important constituents have been found. Virk et al. (2020) carried out the phytochemical analysis of toluene and methanolic extracts of *H. intermedia D. Don.* and found the presence of many biological constituents-alkaloids, flavonoids, terpenoids, tannins, steroids, phenolics, and carbohydrates along with the isolation of sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) (1). Giri et al. (2012b) performed phytochemical screening of seed-derived callus suspension culture of *H. edgeworthii* and harnessed its total phenol and phenolic compounds. In addition to this, they also studied the concentration effect of benzyl adenine and methyl jasmonate on the growth of callus suspension and found that with 3 μM concentration of benzyl adenine, total phenolic content was maximum (14.30 ± 0.03 mg/g DW) which kept on declining with a decrease in its concentration. The opposite trend was obtained with methyl jasmonate, which showed maximum total phenolic content (14.70 ± 0.72 mg/g DW) at the lowest concentration (10 μM). Among the testing of five phenolic compounds, only three compounds were found to be present, i.e., gallic acid (2), catechin (3), and hydroxybenzoic acid (4). Comparison of total phenols and phenolic compounds of in vitro callus culture and wild tuber was also carried out where in vitro callus culture was found to exhibit maximum content of phenolic compounds with a gallic acid content of 113.69 ± 3.69 mg per 100 g in methyl jasmonate culture followed by hydroxybenzoic acid (4) (5.66 ± 0.79 mg/100 g) and catechin (3) (2.44 ± 0.06 mg/100 g). Giri et al. (2017) also conducted similar study on phytochemical screening of tuber part of *H. intermedia* and *H. edgeworthii* and

observed more total phenolic content (6.42 ± 0.01 mg GAE/g DW) in *H. edgeworthii* than *H. intermedia* (4.83 ± 0.01 mg GAE/g DW) in addition to tannins, flavonoids, and flavonols. Quantification of individual phenolics was also done and out of 15 tested phenolic compounds, 13 were detected, i.e., gallic acid (2), catechin (3), 3-hydroxy benzoic acid (5), 4-hydroxybenzoic acid (6), caffeic acid (7), chlorogenic acid (8), 3-hydroxy cinnamic acid (9), ellagic acid (10), rutin (11), p-coumaric acid (12), vanillic acid (13), and ferulic acid (14) with their variable contents in *H. edgeworthii* and *H. intermedia*. It was found that gallic acid content (2) was more in *H. intermedia* (69.96 ± 1.52 mg/100 g) than *H. edgeworthii* (126.96 ± 4.24 mg/100 g), which was observed in accordance with previous results (Giri et al. 2012a). The assessment of total phenolic content of *H. edgeworthii* was done by Giri et al. (2012b) by taking in vitro raised and wild tuber parts of respective species and concluded that in vitro tuber part yielded more total phenolic content (10.05 ± 0.03 mg GAE/g DW) than wild tuber (4.86 ± 0.03 mg GAE/g DW). Out of gallic acid (2) and hydroxybenzoic acid (4), later were found to be in much higher content in in vitro tuber (20.86 ± 0.07 mg/100 g DW) in comparison to raised tuber (7.56 ± 0.03 mg/100 g DW). Gallic acid (2) content was observed to be similar for both the cases, i.e., 5.35 ± 0.10 mg/100 g and 5.51 ± 0.05 mg/100 g in in vitro raised and wild tuber, respectively. Comparative reports on the phytochemical composition of *H. edgeworthii* and *H. intermedia* were also given by Rawat et al. (2014) in which the following phytochemicals were reported, i.e., alkaloids, tannins, phenolics, flavonoids, thiamine (15), riboflavin (16), mineral ash, fiber, and fat. It was observed that *H. edgeworthii* contained more fat content (6.27 ± 0.8 mg/g DW) followed by thiamine (15) (5.75 ± 0.06 mg/g DW), total phenolic content (5.31 ± 0.06 mg GAE/g DW), fiber (4.61 ± 0.1 mg/g DW), mineral ash (3.56 ± 0.3 mg/g DW), flavonoids (3.05 mg QE/ g DW), riboflavin (16) (3.03 mg/g DW), and tannins (2.24 mg TAE/ g DW). In *H. intermedia*, the thiamine (15) content (8.46 ± 0.01 mg/g DW) was found to be more followed by total phenolic content (6.89 ± 0.34 mg GAE/ g DW), mineral ash (4.57 mg/g DW), fiber (3.82 mg/g DW), flavonoids (3.26 mg QE/ g DW), fat (3.16 mg/g DW), tannin (2.89 mg TAE/g DW), and riboflavin (16) (1.67 mg/g DW). Quantitative evaluation of phenolic compounds was also carried out and the presence of only two phenolic compounds, namely gallic acid (2) and hydroxybenzoic acid (4) out of four tested phenolic compounds with a greater amount of both the acids in *H. intermedia* (gallic acid—2.48 ± 0.3 mg/100 g DW, hydroxybenzoic acid—18.5 ± 2.02 mg/100 g DW) as compared to *H. edgeworthii* (gallic acid—6.0 ± 1.0 mg/100 g DW, hydroxybenzoic acid—7.6 ± 0.04 mg/100 g DW). Phytochemical profiling of rhizomes of *H. edgeworthii* was determined by Sedai (2015) which exhibited the presence of phenolics, alkaloids, and coumarin glycosides with the 2.51% moisture content of rhizomes. Most of the compounds like tannins, cardiac glycosides, steroids, terpenoids glycosides, flavonoid glycosides, saponin glycosides, and anthraquinones glycosides were found absent in tubers of *H. edgeworthii*. Sagar (2014) also carried out a similar study on screening of active phytochemical constituents in tuber part of *H. intermedia* and *H. edgeworthii*, and it was perceived that both Riddhi and Vriddhi constituted minerals and starch with bitter substances along with anticancer

compound taxol (17) being found in *H. edgeworthii.* Phenolic compounds were found as active constituents in tuber part of *H. intermedia.* The presence of anticancer compound taxol in *H. intermedia* tubers was also reported by Chauhan et al. (2007) and Singh et al. (2018). Rawat et al. (2016) also got similar results on the phytochemical studies of various extracts of the tuber part of *H. intermedia* and found these orchids to be an active source of phenols and flavonoids. Maximum total phenolic content was found more in methanolic extract of *H. intermedia* (4.77 ± 0.10 mg/g GAE DW) followed by ethanol, acetone, hexane, and aqueous extracts with respective total phenolic contents as 4.01 ± 0.15 mg/g GAE DW, 3.49 ± 0.09 mg/g GAE DW, 2.55 ± 0.09 mg/g GAE DW, and 2.11 ± 0.15 mg/g GAE DW. On comparing the total flavonoids content, it was observed that among solvents, ethanolic extract (9.54 ± 0.03 mg/g QE dW) exhibited maximum value. Other extracts of *H. intermedia*, i.e., hexane, methanol, acetone, and aqueous contained 7.04 ± 0.02 mg/g QE DW, 6.56 ± 0.00 mg/g QE DW, 4.97 ± 0.01 mg/g QE DW, and 4.81 ± 0.03 mg/g QE DW of flavonoid content, respectively. Figure 9.1 presents the structures of key secondary metabolites obtained from the species.

Quantification of the phenolic compounds was also done using 80% ethanolic extract and the presence of hydroxyl benzoic acid (4) (18.51 ± 2.02 mg/100 g DW) and gallic acid (2) (2.48 ± 2.77 mg/100 g DW) was reported. Balkrishna et al. (2018) executed a phytochemical, biological, and botanical evaluation of various *Ashtavarga* plants among which Riddhi and Vriddhi were also screened, and it was found out that *H. intermedia* (Riddhi) was an effective source of total phenols, tannins, thiamine (15), calcium, hydroxybenzoic acid (4), catechin (3), gallic acid (2), p-coumaric acid (12) and scopoletin (18) that also supported it as a propitious source of antioxidants (Goudar et al. 2014; Giri et al. 2017). One of the major studies on phytochemical evaluation on tubers of *H. intermedia* was carried by Habbu et al. (2012) in which isolation of two major and active secondary metabolites, i.e., gallic acid (2) and scopoletin (18) was done. Gallic acid (2) is among the major constituents of many medicinal plants which has been used as a precursor for the preparation of psychedelic alkaloid named mescaline (Tsao 1951). The other active constituent scopoletin (18) is a naturally occurring component of coumarin which has also possessed a range of biological activities with anticancer (Liu et al. 2001) and antidepressant (Capra et al. 2010) properties. Singh et al. (2018) also reviewed the medicinal application of various *Ashtavarga* plants among which *H. intermedia* tubers specific compound, taxol (17) possessed anticancer properties in addition to the presence of starch and minerals as main constituents.

9.8 Nutritional Composition

Various crop plants are known for their nutritional importance, orchids also possess different nutritional constituents in them. Various scientists have reported data on the nutritional composition of *H. intermedia* and *H. edgeworthii*. Rawat et al. (2014)

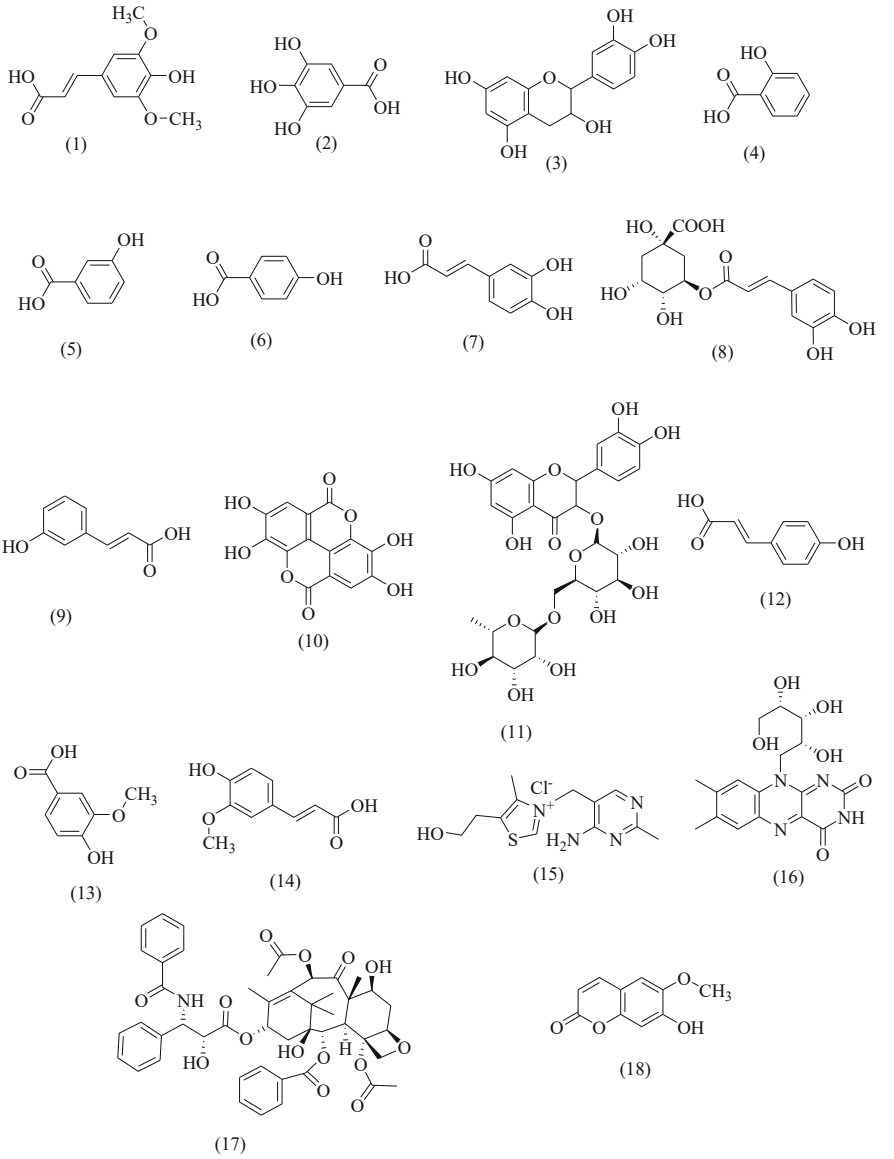


Fig. 9.1 Structures of key secondary metabolites obtained from the species

studied the mineral composition (mg/100 g DW) of tubers of *H. intermedia* and reported the presence of the following elements: sodium (31.35 ± 0.13), potassium (204.57 ± 0.21), calcium (792.41 ± 0.03), lithium (1.66 ± 0.01), copper (4.81 ± 0.01), zinc (3.55 ± 0.02), iron (82.70 ± 0.09), magnesium (1.29 ± 0.02), and cobalt (7.58 ± 0.03). Another species of *Habenaria*, i.e., *H. edgeworthii* was

also studied by Rawat et al. (2014), and similar results with elements viz. potassium (219.27 ± 0.04), calcium (158.65 ± 0.07), iron (84.54 ± 0.02), and sodium (62.90 ± 0.01) were reported. Other minerals were found in minute quantities, i.e., lithium (3.39 ± 0.01), copper (4.76 ± 0.02), zinc (4.51 ± 0.03), magnesium (6.69 ± 0.06), and cobalt (5.37 ± 0.12). Both the species were also evaluated for other nutritional components and the presence of fiber (4.61 ± 0.1 mg/g DW and 3.82 mg/g DW) and mineral ash (3.56 ± 0.3 mg/g DW and 4.57 mg/g DW) in *H. edgeworthii* and *H. intermedia*, respectively, were reported. *H. intermedia* also reported the presence of fat (3.16 mg/g DW). A similar study on toluene extract of *H. intermedia* was done by Virk et al. (2020) and found the presence of carbohydrates in addition to other phytochemical constituents viz. alkaloids, tannins, flavonoids, terpenoids, and phenolics.

9.9 Pharmacology

Basis of Ayurveda and Allopathy are the medicinal plants. The rural population of India depends on traditional medicine systems like Ayurveda and Homeopathic medicine. "Astavarga" in Ayurveda is a very common tonic that is useful in curing diseases like agalactia, seminal weakness, cough, bronchitis, burning sensation, hemorrhages, and general disability (Balkrishna et al. 2012). *H. intermedia* (Ridhi) and *H. edgeworthii* (Vriddhi) are medicinal plants, which are important components of traditional ayurvedic medicines called Ashtavarga (Dhyani et al. 2010). Both the species have high efficiency for curing a range of health problems like urinary disorders, heart disease, diabetes, fever, fractures, and polydipsia (Govindarajan et al. 2007).

H. intermedia tubers are sweet, depurative, anthelmintic, and revitalizing. These are useful in some diseases like asthma, cardiac disorders, and dermal diseases. *H. edgeworthii*, Orchidaceae, is also a Rasayana (meaning chemical) in Ayurveda. Its tubers are sweet, emollient, and used as revitalizing, intellect promoting, depurative, aphrodisiac, anthelmintic tonic. *H. intermedia* and *H. edgeworthii* form a major constituent of Chyawanprash, widely known to prevent aging and to maintain youthfulness, activeness, etc. (Dey 1988; Kirtikar and Basu 1994; Warrier et al. 1994; Govindarajan et al. 2007; Dhyani et al. 2010). These are also used in the treatment of skin, nervous disorders, pulmonary, inflammatory diseases, hyperplasia, and cough (Khare 2007). These two species are mainly found in India, Nepal, and Bhutan. In India, these are found in the states—Jammu and Kashmir, Sikkim and Himachal Pradesh, (Balkrishna et al. 2012). Various researchers have also reported in the pharmacological potential of *H. intermedia* and *H. edgeworthii* in terms of antistress, antianxiety, immunomodulatory, antioxidant, hepatoprotective, and antibacterial activities, which are summarized in Table 9.1.

Table 9.1 Pharmacological properties of *H. intermedia* and *H. edgeworthii*

S. No.	Habenaria extract used	Pharmacological activity	Reference
1.	Ethanol, ethyl acetate fractions	Antistress activity	Pannosian (2003), Krupavaram et al. (2007), Habbu et al. (2012), Sahu et al. (2013)
2.	*n*-hexane, chloroform, methanol, and aqueous extracts	Antianxiety activity	Kaur et al. (2014), Kumar and Kumar (2014)
3.	Methanol extract	Immunomodulatory activity	Kuttan (2000), Habbu et al. (2012), Sahu et al. (2013), Ukpo et al. (2013)
4.	Methanol extract	Antioxidant activity	Jagetia et al. (2004)
5.	Ethyl acetate and ethanol extracts of Habenaria	Hepatoprotective activity	Chaterjiee (2000) Bhandarkar and Khan (2004), Lee et al. (2004); Goudar et al. (2014)
6.	Ethanolic extract of the tuber of Habenaria	Antibacterial activity	Kaushik (2019)

9.9.1 Anti-stress Activity

Stress can be defined as a total of all the anabolic and catabolic reactions occurring in the body, which causes the abnormal physiological condition of the body and result in a disturbed homeostasis condition (Muruganandam et al. 2002; Rai et al. 2003). Stress is known to cause a variety of disorders viz. psychiatric disorders, endocrine gland disorders and suppression of immunity, diabetes mellitus, tension, male reproductive disorders, and other ulcers (Elliott and Eisdorf 1982). Indian Medicine System has given certification for several herbs, which are categorized as Rasayanas *H. intermedia* and *H. edgeworthii* are used as Rasayan herb adaptogens which can help in relieving the stress pathogenesis (Pannosian 2003). Stress responses in animals can be induced by the method of immobilization and more specifically, to explore the effects of drugs, on stress-related pathology of different organ systems. Immobilization can cause long-term immunological responses which affect both central and peripheral components of the brain (Singh et al. 2005). There are different methods that are used to study stress alterations. The most accepted and widely used method to study the stress-induced psychological and physical alterations and consequences of stress is immobilization (Al-Mohaisen et al. 2000; Krupavaram et al. 2007). Habenaria species fractions are good choice as antistress agents (Guan et al. 2005).

Habbu et al. (2012) used ethanol and ethyl acetate fractions of Habenaria tubers to study antistress activity of these species on induced acute stress through immobilization, chronically induced stress, and swimming induced stress in mice. These were found to restore the hypertrophy of the adrenal gland and deterioration of the spleen and thymus gland in acute stress and chronic stress. Increased swimming time was also noted in mice due to the administration of solvent fractions of *H. intermedia*. Acute stress increases the level of glucose due to the release of glucocorticoids

which stimulates the hypothalamic-pituitary-adrenal axis (HPA axis) to reimburse the requirement of energy (Deepak et al. 2003). This increased demand for glucose is overcome by the breakdown of glucose from the liver during acute stress. Pre-treatment with ethanol and ethyl acetate fractions of *H. intermedia* decreased the glucose level, and it had an influence on the HPA axis, which is highly responsible for stress and one of the important systems by which organism activate their defense against stress (McEwen 2000). The results of this experiment proved the antistress activities of *H. intermedia* in Ayurveda. Similar antistress activities have also been reported for Habenaria species in different studies (Sahu et al. 2013).

9.9.2 Anti-anxiety Activity

Anxiety is a human's mind and body's reaction to stress, danger, unfamiliar, and uncomfortable situations. It is a sense of uneasiness, dread, and distress before a significant event. Although anxiety helps us to stay alert and aware up to a certain level, its occurrence beyond normal is considered a disorder (Stein and Stein 2008; Craske et al. 2009). Examples of anxiety disorders include generalized anxiety disorder (Wittchen and Hoyer 2001), social anxiety disorder (social phobia) (Barrera and Norton 2009), specific phobias, and separation anxiety disorder (Bögels and Zigterman 2000). Antianxiety medications aid in decreasing the conditions like nervousness, spasms, fear, and are preferred in case of panic attacks or social anxiety disorders (Hollingworth et al. 2010). Benzodiazepines are used extensively for the treatment of anxiety-related problems (Shader and Greenblatt 1993). Regular use of benzodiazepines is known to cause compulsion, physical dependence, and depression (Baldessarini 2001). Therefore, research for finding effective treatment of anxiety disorders is the need of the hour. Compared to pharmaceutical drugs, herbal plants are safer, effective, and economical alternatives with fewer undesirable side effects. Herbs help in the improvement of overall health and can be combined with a vegan diet and good exercise. This has prompted the scientific fraternity to investigate plants employed in traditional and Ayurvedic systems of medicine for anxiety and sleep-related disorders. For the management of disorders related to anxiety, Habenaria species are the first choice. Kumar and Kumar (2014) reported that the methanol extract of *H. intermedia* showed anxiolytic activity. All the crude extracts (methanol, chloroform, and water extracts) of Habenaria were used in this study, but the methanol extract showed the highest anxiolytic activity. The number of entries and time spent in open arms were recorded as 5.00 and 8.88 s at 200 mg/kg rat for methanolic extract. The presence of therapeutically active constituents like phenolics, flavonoids, and coumarins in *H. intermedia* was reported to be responsible for their antianxiety activity. To assess the antianxiety activity of test drugs, the most commonly used model was the Elevated plus Maze Model (EPM) because this can be easily handled, less labor is required, and it also does not require any special training to the mice (Kumar and Kumar 2014). In this model, the animals are subjected to approach-avoidance encounters, which causes anxiety in them (Kaur

et al. 2014). All crude extracts of Habenaria were screened for its antianxiety activity by using different models (Kumar and Kumar 2014).

9.9.3 Immunomodulatory Activity

The immune system is a system of an organism that protects from disease. Autoimmune diseases result due to the disarray in the immunity of an organism which can result in, inflammatory diseases, cancer, and immunodeficiency (Ukpo et al. 2013). Immunomodulation is a process in which the immune system of an organism is said to be interfered; if it enhances the immune reaction is known as an immunostimulatory drug which primarily implies stimulation of a non-specific system (Kuttan 2000). Modulation of the immune system with the help of some chemical substances is one of the growing areas in pharmacology, where the immune system is undesirably suppressed by the use of therapies. Agents used for immunomodulation can increase or restrain the immune response of an individual by interfering with its normal regulatory mechanism. The agents for immunomodulation are highly favorable if it does not have any side effects. But major of them have side effects (Mohammaed et al. 1996). There are many medicinal plants and herbs in Ayurveda that possess good immunomodulatory activity (Patil et al. 2012). Most of the herbal plants belonging to Orchidaceae and various "Rasayana" are known to possess immunomodulatory activity (Mehrotra et al. 2004).

The immunomodulatory activity of *H. intermedia* was studied by Sahu et al. (2013) using its alcoholic extract in mice by observing delayed-type hypersensitivity response data. The hematological data (white blood cell count, total platelet count) in mice was recorded by giving two doses of Habenaria (300 and 600 mg/kg body weight). The higher dose of Habenaria showed increased white blood cells, red blood cells, hypersensitivity, and in turn increased immunostimulant properties in treated mice as compared to control. Jalal et al. (2008) also showed the immunomodulatory activity of *H. intermedia*.

9.9.4 Antioxidant Activity

Antioxidants are important compounds used to prevent the formation and action of free radicals, reactive oxygen, and nitrogen species, which are generated in vivo during the normal functioning of the body. Free radicals cause damage to vital biomolecules like DNA, lipids, proteins, etc., and result in the emergence of various disorders. Endogenous antioxidant defenses include superoxide dismutases, peroxidase, catalase, glutathione, which are inadequate to curb the complete damage; therefore, diet-derived antioxidants are important for maintaining well-being (Halliwell 1996). Nowadays, due to increased awareness, customers are more

attracted to natural antioxidants. In this context, medicinal plants are rich sources of natural antioxidants (Ramalakshmi et al. 2008). The high antioxidant potential of medicinal plants is attributed to the presence of polyphenols and other secondary metabolites in them (Tang and Halliwell 2010).

Jagetia et al. (2004) investigated the antioxidant activity of tubers of *H. intermedia* and *H. edgeworthii* in nitric oxide scavenging activity. Surveswaran et al. (2007) also reported an antioxidant activity in methanolic extract of Habenaria, while using different in vitro assays in different types of medicinal plants. *H. intermedia* and *H. edgeworthii* were tested for their antioxidant potential to strengthen the ayurvedic formulations in India. It was found that *H. intermedia* has more antioxidant potential as compared to *H. edgeworthii* (Rawat et al. 2014).

9.9.5 Hepatoprotective Activity

The liver performs important functions during the detoxification and biological equilibrium of the organisms. It helps in the removal of substances from the portal circulation, is subject to the toxicity from drugs xenobiotic, and oxidative stress, culminating in liver dysfunction. In spite of tremendous stride in modern medicine, there is no specific treatment to counter the menacing impact of dreaded liver diseases (Chaterjiee 2000). A large number of new drugs have evolved which enhance liver function, offer it protection and regeneration of hepatic cells. Due to this fact, attempts are being made to find suitable curative agents, less toxic and free from side effects than synthetic drugs, originating from the natural product of plants and minerals for the treatment of liver diseases (Bhandarkar and Khan 2004).

The Habenaria species are very helpful in preventing inflammation of the liver and various liver diseases and thereby protecting liver enzymes involved in fighting reactive oxygen species (Mukerjee 2002). Lee et al. (2004) in their study implicated a medicinal system by using Habenaria species to cure various hepatic disorders as there are no potential drugs to cure completely all liver damage. Goudar et al. (2014) conducted a study to assess the effects of ethanolic extracts of Habenaria against the carbon tetrachloride-induced hepatotoxicity in rats. Ethyl acetate and ethanol extracts of Habenaria showed significant hepatoprotective activity by restoration of increased levels of serum bilirubin, cholesterol, and enzymes. Histopathological studies of the liver also showed restoration of damage caused which further confirmed the hepatoprotective activity of Habenaria. To assess the hepatoprotective activity against carbon tetrachloride (CCl_4)-induced liver damage in albino rats (Chaterjiee 2000) secondary metabolites were isolated and characterized from *H. intermedia*. For this, ethanolic fraction of tubers of *H. intermedia* were used. The results of the present study justified that Habenaria can act as hepatoprotective formulations (Goudar et al. 2014).

9.9.6 Antibacterial Activity

Herbal plants are highly effective in fighting against microbial infections. Plant-based antimicrobials are a highly effective source as these have enormous therapeutic potential against many diseases (Vijayalakshmi et al. 2011). The therapeutic potential of Indian orchids is discussed in the Atharvaveda. Himalayan orchids particularly Habenaria species are also known for their antibacterial activity. Habenaria species have shown their antibacterial activity against the bacteria which has been studied by using filter paper discs immersed in different concentrations of alcoholic extracts to develop the bacterial lawns (Keerthiga and Anand 2015). The extract of Habenaria inhibited the growth of the bacteria thus showing antimicrobial potential. The antibacterial potential of *H. intermedia* was also studied by Kaushik (2013). The undiluted alcoholic extract and aqueous extract of root tubers of *H. intermedia were* tested for inhibitory zones against the cultures of *Escherichia coli, Salmonella typhi, Bacillus subtilis, Klebsiella pneumoniae*, and *Staphylococcus aureus* (Kaushik 2019). Habenaria species showed antibacterial potential signified by the formation of effective inhibitory zones. A large number of studies on medicinal plants indicate the immense potential of these plants for the treatment of various disorders.

9.10 Synthetic Strategies for Key Secondary Metabolite

H. intermedia and *H. edgeworthii* are some of the most important medicinal orchids of the Himalayan region. Their medicinal value is mainly due to the presence of different classes of secondary metabolites in them. Although these are not essential for the growth of the plant but are produced for their protection against abiotic, pathogenic, and herbivore attacks. These include alkaloids, terpenoids, phenolics, nitrogen, and sulfur-containing heterocyclic molecules, etc. Other than their biosynthesis in plants, these can also be prepared in the laboratory by following synthetic strategies. Synthetic pathways for the preparation of bioactive molecules of natural occurrence help synthesize bulk amounts of these compounds without posing any threat to the rare plant species. The presence of 18 different secondary metabolites has been reported in the *H. intermedia* and *H. edgeworthii* species in this chapter. Phenolic compounds like hydroxycinnamic acid (1), gallic acid (2), catechin (3), hydroxybenzoic acid (4), 3-hydroxy benzoic acid (5), 4-hydroxybenzoic acid (6), caffeic acid (7), vanillic acid (13), and ferulic acid can be synthesized by following different synthetic schemes (Michailof et al. 2008; Touaibia and Guay 2011; Masoud et al. 2012; Mouterde et al. 2013; Wang et al. 2017) and Pd-catalyzed C-H functionalization of arenes (Saha et al. 2019). Rutin (11) is a flavonoid compound found in many plants and fruits, with known antioxidant and anti-inflammatory activities (Guardia et al. 2001; Zielinska et al. 2010; Cosco et al. 2016; Jantrawut et al. 2017). Different reaction schemes have been reported by

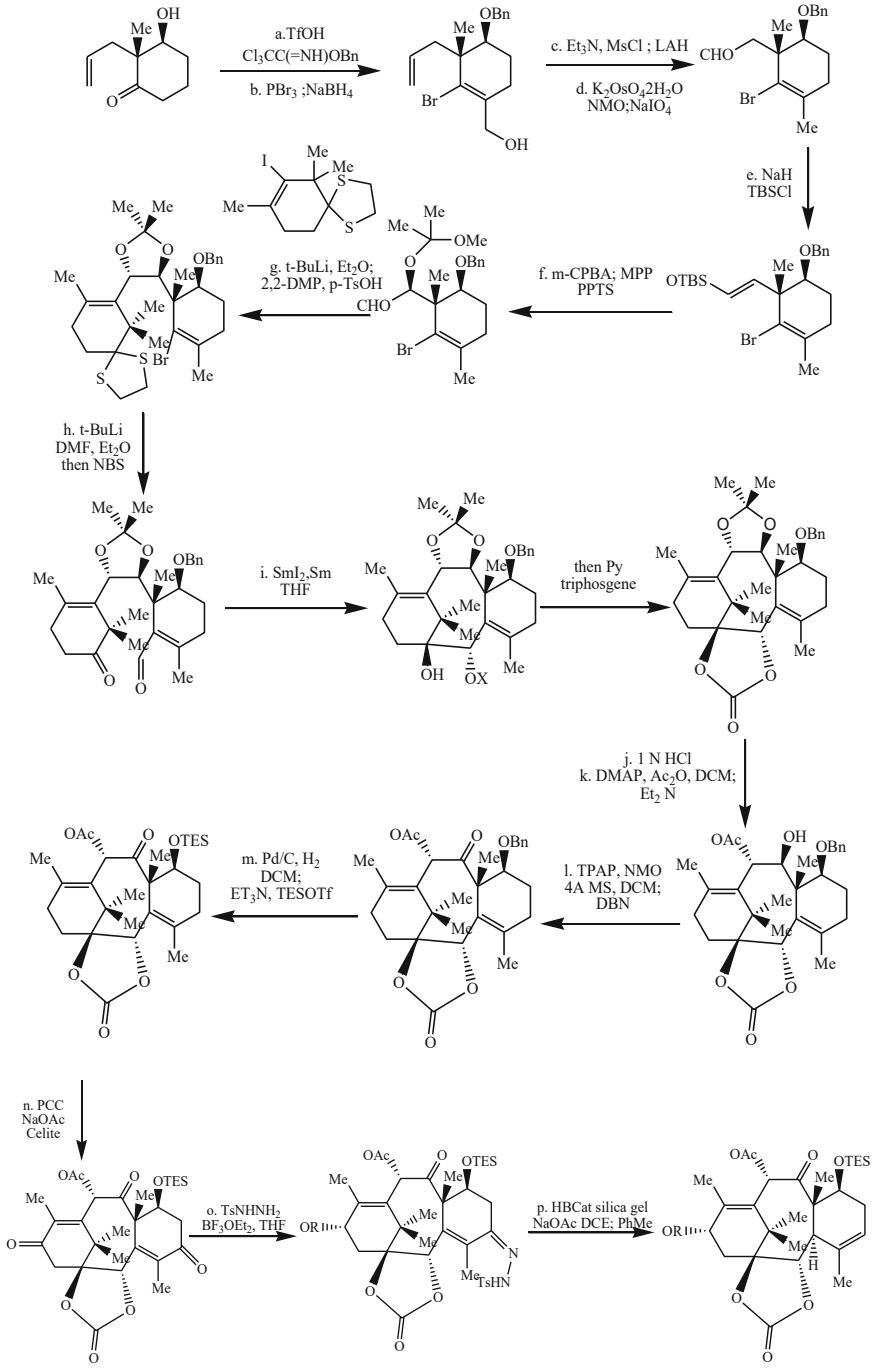
Khalifa et al. (1983) for the laboratory preparation of rutin (11). Taxol (17) is a natural diterpenoid compound with promising biological potential (Nicolaou et al. 1994) and high anticancer (Kingston et al. 1990; Malonga and Neault 2005; Malik et al. 2011) and anti-mitotic (Sato et al. 2000) activities. Synthetic procedure for the preparation of taxol (17) has been a challenge for chemists as it involves an eight-membered ring. Hu et al. (2021) have recently reported the asymmetric complete synthesis of taxol (17) and also isolated the involved intermediates (Scheme 9.1).

9.11 Cultivation Harvesting and Processing

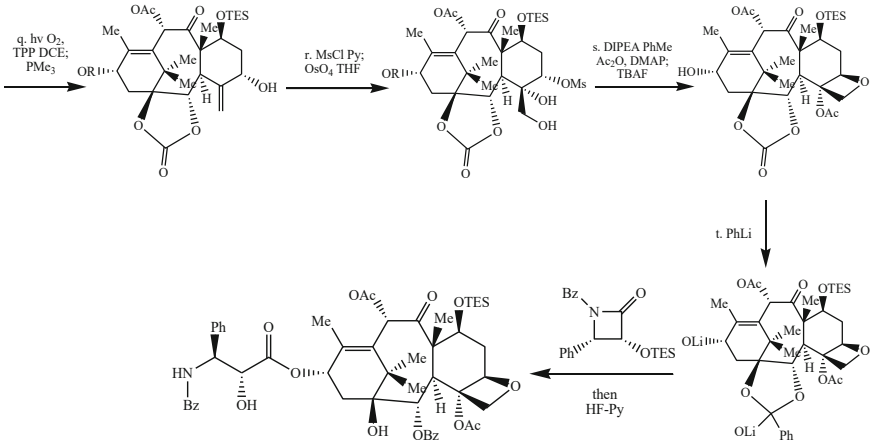
Habenaria species are distributed in open grassland Himalayan region at a height of 1500–2800 m from 10–15 °C temperature. It requires light for its propagation and hence is more cultivated in the eastern and southern slopes of the Himalayas. This species is cultivated along steep slopes and in open meadows. Sandy-loam soil with high humus content in raised beds with the inter-plant spacing of 20 × 20 cm is suitable for the cultivation of Habenaria species.

Manmohan et al. (2011) developed the propagation techniques for *H. intermedia* and concluded that raised and furrow beds were suitable for its cultivation. Soil is first prepared with ploughing, the addition of farmyard manure and leaf litter, and then keeping fallow for the winter season. For the proper pulverization of soil, another ploughing and addition of organic manure are carried out before sowing. Due to the endospermic nature, low germination rates, and less viability of seedlings, vegetative propagation with tubers (10–12 cm) is recommended for cultivation. For achieving the best results, the apical portion of tubers is kept intact and can also be treated with mercuric chloride fungicide. Planting is done in the month of May, which may sometimes be preponed in November due to the unavailability of tuber storage facilities. Under suitable storage conditions, tubers of *H. intermedia* remain viable for 6 months. Pit method and earthen pot method with sand were observed as best storage methods with 96–98% viability of tubers after 6 months (Manmohan et al. 2011). The estimated number of tubers required for cropping (with 20 × 20 spacing) in 1 ha of land is in the range of 2.0–2.5 lakhs. The addition of farmyard manure is recommended during planting stages for efficient nutrient uptake and mycorrhizal association. As planting is done just before the rainy season, irrigation is not required during normal conditions. However, twice a week irrigation is required during the initial stages of the crop in the absence of rain. *H. intermedia* and *H. edgeworthii* bloom in monsoon months with the flowering from July to August; and fruiting season from September to October (Balkrishna et al. 2012). Weed and pest control is most important during cultivation for achieving high yields.

Of these medicinally valuable species of orchids. For control of larval stages of insects (white grubs) in tubers, application of any broad-spectrum insecticide, preferably phorate is recommended during the time of planting. Harvesting is done in the months of October and November after the complete maturing and senescence of the crop. Harvesting is recommended once the stalk and leaves have dried



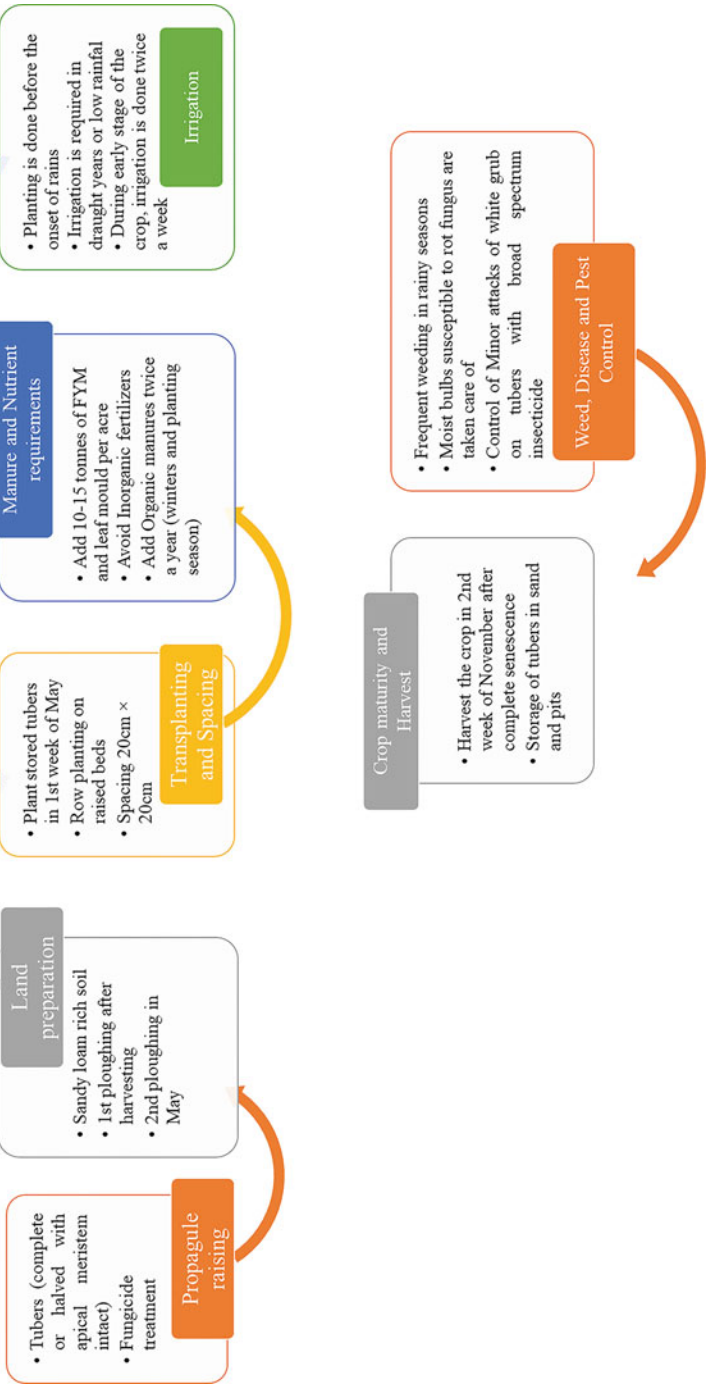
Scheme 9.1 Synthetic strategies for complete synthesis of taxol



Scheme 9.1 (continued)

(Sharma 2017). After harvesting, the tubers are dried under light for the minimization of fungal attack incidents. Tubers can also be preserved as germplasm under the soil in earthen pots and can be used in the next season for planting (De et al. 2016). Schematic representation for cultivation and harvesting of *H. intermedia* and *H. edgeworthii* is given in Scheme 9.2.

Tissue culture: Irrational harvesting and over-exploitation of medicinal orchids especially Habenaria species from their natural habitat have brought a threat of extinction to these valuable plants. The propagation of these plants through seeds is not favorable due to their low germination rates and fungal attack (Pathak et al. 2001). Vegetative propagation through tubers is also limited due to the poor availability of wild species. Therefore, there is an urgent need for the development of efficient propagation protocols and in vitro techniques. A preliminary study on in vitro seed germination of *H. edgeworthii* is presented by Vij et al. (1988). Giri et al. (2012b) conducted a study for determining the outcome inclusion of plant growth regulators on seed germination, rooting, callus induction, and organogenesis of *H. edgeworthii* and established an in vitro protocol using seed-derived callus. The highest seed germination was detected in Murashige and Skoog medium augmented with one micromolar α-naphthalene acetic acid. The maximum number of shoots (11.9 shoots/plant) was attained in medium augmented with 1/10 micromolar 6-benzyl adenine and 1/100 micromolar α-naphthalene acetic acid. Elongated shoots were then shifted to a ½-strength rooting medium with a different auxin concentration of induced roots and tubers, a maximum of 87.5% rooting was attained in a plant growth regulator-free MS medium. Rooted shoots were moved to a 1:1:1 mixture of soil: sand: perlite and a survival rate of 68% was observed. The genetic stability of restored plants was also assessed using inter-simple sequence repeat markers.



Scheme 9.2 Scheme for cultivation and harvesting of *H. intermedia* and *H. edgeworthii*

9.12 Formulation and Market Product

H. intermedia and *H. edgeworthii* are important medicinal plants of the Himalayan region and are acknowledged for their extensive range of biological and pharmaceutical activities. These are the important constituents of two important traditional Ayurvedic preparations namely, "Astavarga churn" and "Chayavanprash" (Balkrishna et al. 2012). Ashtavarga is a powder formulation consisting of eight species of medicinal plants including *H. intermedia* and *H. edgeworthii*, known for healing fractures, curing fever, weakness, and diabetics. Chyawanprash is a paste formulation of herbal plants which is famous in India for increasing immunity and providing relief from cold and cough. *H. intermedia* is known to have other formulations like Vachadi oil, Vajikaran ghrita, Asoka ghrta, Amrtaprasa ghrta, Dasamularista and Chagaladya ghrta. *H. edgeworthii* is present in formulations namely, Mahamayura ghrita, Ashtavarga churna, and Chyawanprash Rasayan (Rawat et al. 2014).

9.13 Conservation

Owing to the presence of a broad range of bioactive metabolites in *H. intermedia* and *H. edgeworthii* species of orchids, these have been used for the treatment of various disorders since ancient times. However, their natural habitats are very explicit in terms of ecosystem, and these arise in very small patches naturally (Giri et al. 2012a). On the one hand, natural factors like heavy landslides cause a huge fragmentation and loss of their habitats and on the other hand, human interventions are the dominant factors responsible for the decline in their natural populations. Threats to the natural populations of precious Himalayan plants have increased many times in recent years. Road constructions, overgrazing, fodder collection, irregulated tuber collection, irrational harvesting from the wild, etc. have contributed to the reduction in their natural populations. *H. intermedia* is counted in the list of endangered plants in IUCN's red list, and *H. edgeworthii* is also facing survival threats. (Kant et al. 2012; Balkrishna et al. 2012). *H. edgeworthii* is listed in CITES Appendix II, and its collection from the wild is totally banned (Giri et al. 2012b). Thus, immediate attention is required for taking necessary actions for the conservation of these gems of the Himalayas. We need to understand that to maintain the equilibrium between nature and humans, the conservation of valuable medicinal plant species is essential. Broadly, there are two strategies of conservation of any species: in situ and ex situ. "In-situ" aka "on-site" conservation signifies the conservation of endangered/threatened species in their natural environment and native habitat. "Ex-situ" aka "off-site" signifies the conservation of species in man-made habitats like a botanical garden, herbarium, etc. Bisht et al. (2016) has proposed the idea for in situ conservation of Himalayan medicinal plants in the Indian state of Uttarakhand. Emphasis was given on the usage and harvesting of aerial parts of plant instead of

underground portions. It was concluded that the extraction and isolation of bioactive compounds from aerial parts of medicinal plants without uprooting the whole plant will serve both purposes. Also, it was suggested to conduct awareness programs among plant collectors, harvesters, and buyers for harvesting aerial parts of the plant. (Bisht et al. 2016). Manmohan et al. (2012) also developed propagation practices and germplasm storage protocols for *H. intermedia* tubers and concluded that suitable agronomical techniques are the only option for fulfilling growing demands from users and pharmaceutical companies. According to Kant et al. (2012), it is very important to bring awareness among local people about the sustainable usage of medicinal plants through seminars, discussions, and workshops. Restrictions and punishments should be imposed strictly on tourists throwing non-biodegradable substances and tempering the natural resources of the Himalayan region. Government should make policies against the illegal collection of germplasm of endangered plant species. Grazing should not be allowed in the same area for more than 1 year, and it should also be properly regulated. Ex situ conservation can be accomplished through the advance of in vitro micropropagation followed by mass multiplication technologies. Also, studies can be conducted to search easily available natural or synthetic alternatives to these species in order to fulfill the growing demands of biologically active species of orchids (Manmohan et al. 2012; Rao 2019).

9.14 Conclusions and Future Prospective

The Himalayan region serves as a treasure to mankind having thousands of precious medicinal plants. Himalayan orchids have a wide range of pharmaceutical and nutraceutical properties. *H. intermedia* and *H. edgeworthii* are distributed from 1500–3000 m altitudes above sea level and are found in different countries-India, Tibet, Nepal, and Pakistan. Tubers of these orchid species are constituents of important Ayurvedic formulations like Chyawanprash. Various secondary metabolites viz. phenolics, flavonoids, and coumarins have been found present in these species. Over-exploitation of these valuable medicinal plants has brought a threat of extinction to these Himalayan gems. Strict steps are needed to be taken for the conservation of these plants in their natural habitat. More studies focusing on in situ and ex situ conservation methodologies, tuber storage techniques are required to be carried out. There is also a lot of scope for the development of tissue culture and agronomic techniques for their cultivation out of their natural habitat.

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Chapter 10
Humulus Lupulus L.: Beer Plant

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10.1 Introduction

Humulus lupulus known as common hop or hops, belongs to family *Cannabaceae* and the plant is a twinning dioecious herb which is spread worldwide in the temperate zones. The plant is named so as the yellow-colored resinous substance is filled in the membranous bracts of female inflorescence known as hops. The secondary metabolites produced in the glandular trichomes known as lupulin glands are responsible for the distinct tasting qualities. Numerous other components are also produced in these lands, but bitter acids and essential oils being produced are significant for unique bitter flavor and hoppy aroma, respectively. They also certify the foam formation and bacterial stability. Due to this stability the shelf life of beer was more and could be stored for longer duration and thus helped the brewers in marketing of beer. These hops are collected in late summer season and are processed for adding distinctive flavor, fragrance, and bitterness in the beer by breweries.

The hops (Fig. 10.1) in beers were used around 1750 BC when Sumerians exposed the fermentation process of barley to prepare beer with the help of yeast. The hops were cultivated by German monks in twelfth century for medicinal uses like treatment of insomnia as hops have sedative effects (Bocquet et al. 2018). Apart

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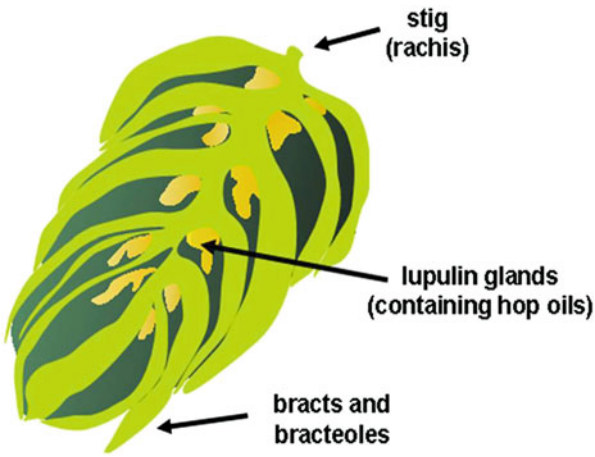


Fig. 10.1 Hop cone showing lupulin glands (Weiskirchen et al. 2015). Hop flowering plant

from a stress and anxiety reliever, it was also used in the stimulation of digestive tract, as diureticum for blood clarification, bacteriostatic, and anti-inflammatory agent. The flavoring in ales was done with herbs and spices before the advent of hops in fifteenth century. *Myrica gale* was used as major food additive in beer in primeval period but with the progressions in beer production in fourteenth century, the reputation of hops in the brewing of beer was more valued in northern central and North West Europe. Moreover, the rumors like *Myrica* used in beer is harmful and can cause blindness and even lead to death had weakened its use (Hadidi et al. 2017).

The hops were cultivated in Hallertau region of Germany, and the record has been documented in 736 although they were used in brewing there in 1079. The will written by Pepin of short who is the father of Chalemagne mentioned that hop gardens will be handed over to Cloister of Saint Denis in 768. Though hops were viewed as a malevolent and bad weed in late 1519, Britain imported hop beer from Holland about 1400. The manufacturing of beer, also known as brewing of ale, which is a fermented malt liquor flavored with hops, was outlawed in England in 1471. Thomas Tusser stated in 1557 "The hop for his Profit, I thus do exalt, it Strengthen drink and it flavored malt; and being well brewed long kept it will last, and drawing abide, if ye draw not too fast."

In sixteenth century, the use of hops was considered a political choice by Germany and had religious sentiments attached with the usage. There was a waiver of taxes on hops unlike gruits paid to catholic churches. Hops were imported from France, Germany and Holland by England and were under the bracket of import duty.

The enforcement of purity law "Reinheitsgebot" Wilhelm IV, the lord of Bayern has given a stamp to hops to be used as standard constituent beer and according to this law hops are mandatory in beer making and was adopted by several countries. Hops is considered the main ingredient in making beer since nineteenth century.

The majority of hops production is located north of the 48th parallel in damp temperate climates. The primary potato-growing states in the United States are also significant hops-producing states, but not all potato-growing regions can naturally produce high-quality hops: the soils of Canada's Maritime Provinces, for instance, lack the boron that hops need. Hops were originally imported from England rather than being grown in Ireland. More than 500 tonnes of English hops were imported in 1752, simply through Dublin.

10.2 Taxonomy and Distribution

Kingdom	Plantae
Superdivision	Embryophyta
Division	Tracheophyta
Class	Magnoliopsida
Order	Rosales
Family	Cannabaceae
Genus	*Humulus*
Species	*lupulus*

Distribution: Hops is distributed throughout the world but are native to Northern Hemisphere. The major producers of hops in 2021 are the United States, followed by Germany, China, Czech Republic and the United Kingdom. Hops is a perennial plant with dioecious flowers. These plants grow between latitudes of 38 and 51°. This is the reason for the production of hops in the areas of Oregon and Washington in the United States. They grow from April end to the start of July in the temperate zones. Hops are grown in Australia (37–43° latitude) and New Zealand (41–42° latitude) in the Southern Hemisphere.

The species *Humulus lupulus* has five varieties—*Neomexicanus, Lupuloides, Pubescens*, *H. lupulus* var. *cordifolius* and *H. lupulus* var. *lupulus* (Dodds 2017).

10.3 Morphology

Hop plant is herbaceous perennial having twin stems of 8 m in length, and they always wind in clockwise direction. All season new vines are produced which die on maturity. The support for vines should be a robust fence and the roots penetrate deep up to 5 m in the soil. Hops can grow in varied climatic conditions and on wide range of soils. Rich alluvial soils and well-drained loamy soils are more in preference to grow hops in general.

10.4 Flowering/Fruiting Season

It has separate male and female plants because it is a dioecious plant. Only flowers from female plants are harvested before ripening into fruits. An inflorescence known as strobiles is formed by these female flowers. It is made up of bracts and stipules joined by an axis. These bracts and stipules contain several polyphenols.

The cones of hops are get matured in the month of August for most of the regions of the world but at some regions they mature later. August through September are the ideal months for the Northern Region's hop harvest, but it is February in the Southern region. During harvesting, the whole aerial part is removed from the field and shifted for processing immediately. Then the hop cones are separated from the rest of the plant.

The cones of hops are harvested on full maturity and are usually picked up manually or machines can be used. The hops after harvesting should be kiln dried before brewing. The male and female flowers are developed separately, but pollination is not desirable in beer brewing so only female plants are allowed to grow in fields which prevents pollination and are thus propagated vegetatively and males are rejected if they grow from seeds. The plantation is done in rows which are 7 to 8 ft. apart. Hop harvesting is done in summer end.

10.5 Hops Cultivation and Harvesting

The photoperiodic requirements of the plant are the key factor limiting hop cultivation area (Neve 1991)**.** Optimal growth conditions are necessary for the successful cultivation of the hop plant, particularly in terms of daylight hours, summer temperatures, annual rainfall, and soil fertility (Verzele and Keukeleire 1991). Additionally, hop plants require specific climatic conditions for best growth, yield, and cone quality, including exposure to low temperatures during dormancy, warm temperatures in the spring, enough moisture from irrigation or rainfall throughout the season, and dry weather for harvest (Sirrine et al. 2010, Mozny et al. 2009**)**. Almost primarily for the brewing business, the female plant of the common hop species *Humulus lupulus L.* is grown for its hop cones. The majority (around 97%) of hops grown worldwide are used in brewing. Germany and the United States produce the majority of the world's hops. About 75–80% of the world's total output of hops is produced in both countries (Raiser 2011). The hop plant is cultivated in the majority of areas with mild climates, which are found between latitudes of 35° and 55° in the Northern and Southern Hemispheres. The farmer must deal with the infection immediately (Roberts and Wilson 2006). The Hallertau region of Germany and the US states of Washington, Oregon, and Idaho are home to the major hop-growing regions. The Czech Republic, Poland, Slovenia, England, Ukraine, China, South Africa, Australia, and New Zealand are additional nations that grow hops (Raiser 2011).

The hops are harvested in late summer or early fall when the cones have matured and the resin concentration is at its greatest. About 75–80% of the moisture in hops is present at harvest; with this level of moisture, not only do the hop compounds alter quickly, but they also swiftly begin to mould. To achieve a moisture content of roughly 10%, the hop cones are therefore carefully dried in the oast house or kiln at temperatures between 60 and 75 °C (Briant 1905). The hops are compressed and bundled into bales after chilling and conditioning. These bales are kept chilled until they are needed for sale or processing into hop-related goods.

Harvesting and Processing: Normal beer is created by first malting barley, which involves steeping, germination, and kilning stages; next crushing the malted barley and adding water to make the mash; next separating the resulting aqueous extract, known as "wort"; next boiling the wort with hops; next cooling and clarifying the wort; next fermenting the wort (in two stages); next filtering; next adding other additives; and finally pasteurizing the beer. (Sohrabvandi et al. 2012).

At various stages of the brewing process, hops are added to beer to give it flavor, aroma, and a distinctive bitterness. Hops were progressively used to beer primarily for flavor as the antibacterial beer-preserving feature gradually diminished around the end of the twentieth century. The antibacterial properties of hops were no longer important as brewing techniques, pasteurization, and storage rooms with consistent temperature and humidity all improved. Beer's shelf life can now be extended utilizing a variety of chemical treatment techniques (Gerhäuser 2005).

In brewing industry, hops are important for the bitterness and preservative effect due to the presence of alpha (α) acids and beta (β) acids. They are also important for flavor production in beer as the hops are rich in many essential oils. It has been studied that the concentration of essential oil in hops increase after traditional harvest dates. Harvesting time has a significant effect on the flavor and aroma in the beer. It has been observed that when a single variety of hop harvested at different times is used in beer produces different flavors in the beer. Thus in order to get the optimum flavor characteristics in the beer, the hops need to be harvested at proper time (Lizotte 2015).

Hop maturity is commonly determined in two ways. First is the sensory method which is based on look, feel, and smell parameters. The second is to measure the dry matter of the hops.

Harvesting of hops is very laborious work. It was extensively done manually before invention of mechanical harvesters. Due to higher labor costs, the manual harvesting is not economical process for hop growers. It is important to harvest the cones at proper maturity. After harvesting, these must be cleaned and dried immediately in order to maintain quality and increase the storage time. The length of the harvest season is determined by the number of grown types, and it is typically around a month (Dodds 2017).

The best time to harvest the hops in the Northern Region is from August to September, but it is February in the Southern region. During harvesting, the whole aerial part is removed from the field and shifted for processing immediately. Then the hop cones are separated from the rest of the plant.

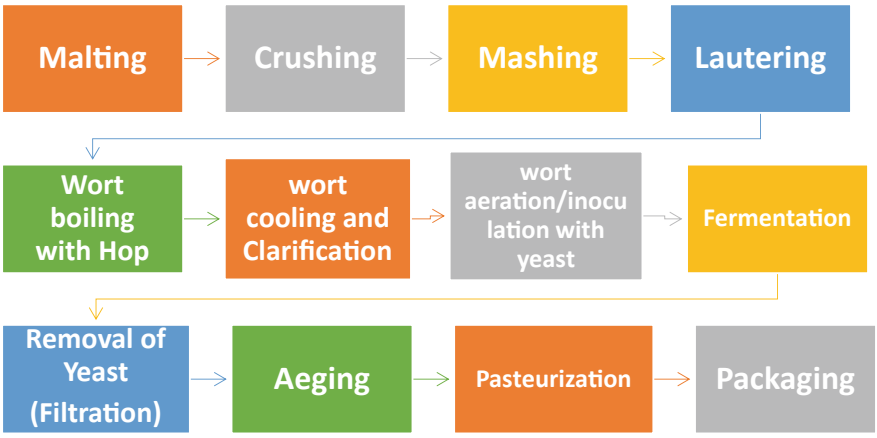


Fig. 10.2 Steps of beer processing with hops

The cones after harvesting are dried immediately to reduce the moisture content of about 8–12% since this leads to the better stability of the produce. The unfavorable environmental conditions may lead to the undesirable changes in the hops, and thus refrigeration is used throughout the processing stages. The bitter compounds present in the cones break down rapidly during storage resulting in a decrease in their concentration by 50–70% within a period of 6 months. The α-acids and β-acids are susceptible to oxidation resulting in their degradation. Temperature and other environmental factors also result in loss of these acid levels. In order to maintain optimal quality and storage life, the moisture content of the hops is reduced to safe levels of 8–12%. When the moisture in the hops is above this level, they are susceptible to breakdown and lower moisture level result in brittleness and oxidation of hops (Madden and Darby 2012).

Hop growers or processing companies make hops into pellets. Pellets can be prepared and sealed in many ways, but vacuum sealed pellets are widely prepared as they offer a number of benefits. These vacuum sealed pellets have a longer shelf life, require less space for storage and offer ease of handling during the brewing process (Dodds 2017). Figure 10.2 shows the various steps involved in beer processing with hops.

10.6 Traditional Uses

Apart from brewing, the hops were also consumed as sprouts in the first century AD and in medieval time period they were used as a substitute of asparagus by poor people. Also, the stems of hops were majorly used in the manufacturing of clothes and paper and other traditional items.

The female hops flowers were used to treat anorexia. It has also been used to prevent hair loss. They are used to purify blood, reduce fever, and purge yellow bile as a sedative, anti-rheumatic, analgesic, gynecological assistance for breast and womb issues, and for kidney-related ailments, it has also been utilized (Hamel and Chiltoskey 1975).

According to studies by Marcos et al. 2021, moderate beer drinking up to 16 g of alcohol in a day for women (1 drink per day) and approximately 28 g per day (1–2 drinks per day) for men is linked to a lower risk of heart-related disease and among other benefits to metabolic health, overall mortality.

Manipur tribal women of Sekmai and Payeng villages rub rice beer fermented with hops on their faces and skin as a beauty lotion (Nath et al. 2019). Epidemiological, experimental, and clinical studies have shown that drinking beer in moderation can have a variety of positive effects on a person's health. According to Bamforth 2009, there is a direct link between drinking alcohol and the majority of ailments. This translates to greater fatality rates for both abstainers and daily drinkers who consume more than six drinks. According to statistics, low alcohol consumption (10–15 g alcohol/day, or roughly one glass of standard beer per day), when compared to non-drinking and heavily drinking rabbits, lowers annual mortality (Yilmazer et al. 2001). Beer's medicinal properties have been somewhat linked to its alcohol levels, phenolic content, and protein composition.

Studies have shown that consuming alcoholic beverages (including beer fermented with hops) in moderation can have a number of positive effects on a person's health, apart from nutritional benefits other benefits include (Sohrabvandi et al. 2012)

(a) Beer possesses cancer-preventive and anti-mutagenic properties.
(b) It also has a cardioprotective effect, which lowers the risk of cardiovascular disease).
(c) Hypolipidemic effect.
(d) Aids in the immune system's stimulation.
(e) Reduction in the risk of osteoporosis.
(f) Lower risk of dementia.

Despite the dearth of studies particularly examining the health consequences of beer, the information that is now available points to a link between moderate beer drinking and a lower risk of total mortality and non-fatal cardiovascular diseases. When it comes to other health effects, such as those on general or abdominal obesity, data from studies have frequently been ambiguous, but a new small study demonstrates that moderate beer drinking, whether it be alcoholic or non-alcoholic, does not raise body weight in obese people. Additionally, mild beer drinking is also linked to lower diabetes risk (only in men) and higher BMD, which reduces the risk of senior fractures (Padro et al. 2018).

Since ancient times, *H. lupulus* has been used in folk medicine. There are several accounts of traditional uses, some of which are quite innovative, including storing fragile goods, removing blood stains, creating fabric and paper, and warding off demons at night. In addition to being used as a preservative, deodorant, and cow

feed, it is effective against anxiety, gastrointestinal issues, toothaches, fevers, and leprosy. Since the beginning of its cultivation, it has been known that *H. lupulus* has a sedative effect because it was seen that those who worked with the plants and collected hops became incredibly sleepy while doing so. Drinks have long contained hops as a sedative and sleep aid. Hop-filled soft cushions have likewise become more and more well-liked. Initially, they were heated and were replaced after roughly 3 days. Even now, a number of internet stores and sites provide pillows stuffed with lavender and hops. The European Scientific Cooperative on Phytotherapy and the German Commission E both support the use of hops as a treatment for agitation, anxiety, and sleep issues. Although hop plants are most commonly used for their strobili (sometimes known as "cones"), other components related to this plant have also attracted attention. Young shoots have been consumed as a vegetable in many parts of Europe since the dawn of time. The hop plant, which is closely related to hemp (*Cannabis sativa L.*), also possesses long fibers that have been used to make ropes, paper, and fabric that resembles linen. However, due to the paper's low cellulose content and low pulp production, it is not of exceptionally high quality (Korpelainen and Pietiläinen 2021). Table 10.1 represents the various traditional uses of *Humulus lupulus*.

10.7 Phytochemistry

The female hop cones are helpful in brewing and the peculiar zesty beer aroma results when these hop cones are incorporated at various beer processing steps like boiling of wort, secondary fermentation, and during ageing. The phytochemicals present in hops can be divided into primary and secondary metabolites. Primary metabolites observed in hops are sugars, lipids, amino acids, ketones and alkanes (Bocquet et al. 2018). Hop resins, hop oil, and hop polyphenols are the three types of secondary metabolites found in hops (Fig 10.3). The concentration of these phytochemicals in hops depend on variety, ripening stage, and climatic conditions (Astray et al. 2020).

Over 1000 different substances, including natural substances and their isomeric derivatives, can be found in hops. The most significant chemical groups produced by hops in terms of their historical commercial value are volatile oil and bitter acids (Wang et al. 2017). Prenylated flavonoids are also responsible for estrogenic action (Eri et al. 2000). The bitter principles, sometimes referred to as alpha- and beta-acids, are what set hops apart from other plants. Humulone, cohumulone, and adhumulone are alpha-acids found in plants. These molecules are isomerized to the group of compounds known as iso-alpha-acids., which have a bitter flavor, during the brewing process (Clarke and Hildebrand 1965). Lupulone and congeners belong to the beta-acid group of chemicals, which are eliminated during the brewing process. The amounts of bitter acids in hops determine their quality, and numerous methods for assessing hop acids in different kinds have been devised, like nuclear magnetic resonance (NMR) and high-pressure liquid chromatography (HPLC) (Česlová et al.

Table 10.1 Traditional uses of *Humulus lupulus* (Korpelainen and Pietiläinen 2021)

S. No.	Traditional use	Main region	Part of plant
1.	Vegetable	Mediterranean, the United Kingdom, Belgium	Young leaves
2.	Beer flavoring, preserving, and clarifying	–	Strobili
3.	Bread making (to cultivate yeast)	Europe, East Africa	Strobili
4.	Cattle fodder, manure preparation	Europe	Whole plant
5.	Cattle bedding	UK	Stems
6	Flavoring water, baked goods, tobacco	America	Strobili
7	Preservative in sausages	Germany	Strobili
8	Fiber (ropes)	UK	Stems
9	Paper		Stems
10	Bedding material for deceased		Whole plant
11	Cloth	Sweden	Stems
12	Packing fragile cargo		Inflorescences
13	Perfumes, skin lotions		Strobili
14	Deodorant (antimicrobial, fragrance)		Strobili
15	Hair rinse for brunettes	Russia	Leaves, flowers
16	Oil (food)		Strobili
17	Ornamental plant	Europe, USA	Whole plant
18	Dye (yellow and brown)		Leaves, flowers
19	Insulation		Stems
20	Sedative, sleep disturbances		Strobili
21	Antibiotic, anti-inflammatory		Strobili
22	Bleary eyes		Strobili
23	Gastric problems, indigestion, appetite	India, China	Strobili
24	Tenderness of limbs		Strobili
25	Headache, restlessness	China	Strobili
26	Delirium tremens, irritable bladder, aches		Strobili
27	Toothache, earache, neuralgia	America	Strobili
28	Clearing blood, flatulence		Strobili
29	Anthelmintic, antiparasitic	Northern Europe	Strobili
30	Cough, spasms, fever, anxiety		Strobili
31	Diuresis		Strobili
32	Leprosy, tuberculosis, asbestosis, silicosis	China	Strobili
33	Liver disorders (porphyria)	UK	Strobili

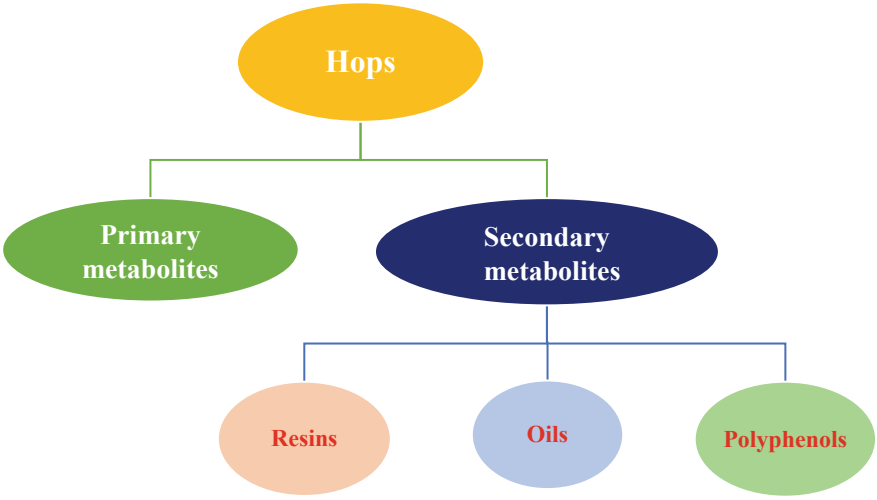


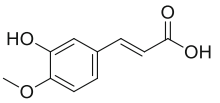
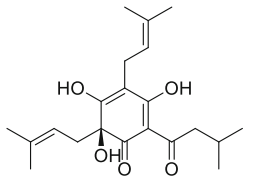
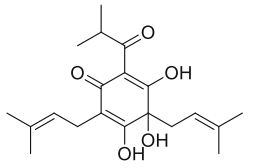
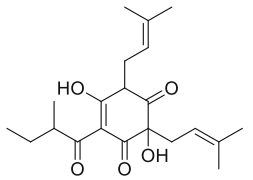
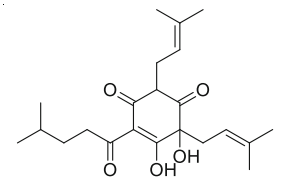
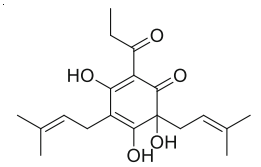
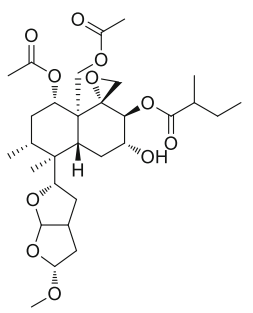
Fig. 10.3 Types of hop compounds

2009). Hop acids have a complicated profile that is influenced by genetics, cultivation, and storage conditions. Hops that are stored for an extended period of time lose a lot of their quality. Hops essential oils are less distinctive, yet they are still vital in determining hop quality. Gas chromatography-mass spectroscopy (GC-MS) and gas chromatography have been used to identify over 100 volatile chemicals. The most abundant elements of hops volatile oils are caryophyllene, beta-myrcene, and humulene. The prenylflavonoids are a third group of hops compounds. Hops' main prenylflavonoid is xanthohumol, but 8-prenylnaringenin is also important (Delmulle et al. 2006).

Alpha-acids and beta-acids, two types of bitter acids, make up the hops resins. Humulone, cohumulone, adhumulone, prehumulone, and posthumulone are components of alpha acids (Table 10.2). Similarly, there are five homologs of beta-acids (lupulones). Hop oils are produced by lupulin glands and result in the formation of the flavor development in the beer. More than 400 compounds have been reported in hop oils and are categorized into three different groups—hydrocarbons, oxygenated, and sulfur compounds. The flavanols, flavan-3-ols, phenolic carboxylic acids, and other polyphenolic chemicals can all be classified as hop polyphenols (Table 10.3). According to its content, quercetin and kaempferol are the two flavanols that are most prevalent (Astray et al. 2020).

The primary phenolic substances found in beer like benzoic acid derivative, isoferulic acid, indan, and catechin derivatives can be correlated with cancer chemoprevention (McCallum et al. 2019). Hops are a rich source of bioactive phytochemicals. The hops are put during brewing of beer in the form of cones or as a mix of bitter acids and can be put as alcoholic or supercritical fluid extraction. The isomerization product isoxanthohumol produced from prenylated chalcone xanthohumol has found to be having the potential to prevent cancer shown in a

Table 10.2 Key phytochemicals present in the *Humulus lupulus*

Common name	IUPAC NAME	Chemical Structures
Isoferulic acid	(E)-3-(3-Hydroxy-4-methoxyphenyl) prop-2-enoic acid	
Humulone (α-Lupulic acid; α-bitter acid)	(6S)-3,5,6-Trihydroxy-2-(3-methylbutanoyl)-4,6-bis (3-methylbut-2-en-1-yl)cyclohexa-2,4-dien-1-one	
Cohumulone	3,4,5-trihydroxy-2,4-bis(3-methylbut-2-en-1-yl)-6-(2-methylpropanoyl) cyclohexa-2,5-dien-1-one	
Adhumulone	2,5-dihydroxy-2,6-bis(3-methylbut-2-en-1-yl)-4-(2-methylbutanoyl)cyclohex-4-ene-1,3-dione	
Prehumulone	6-dihydroxy-2,6-bis(3-methylbut-2-enyl)-4-(4-methylpentanoyl)cyclohex-4-ene-1,3-dione	
Posthumulone	3,5,6-trihydroxy-4,6-bis(3-methylbut-2-enyl)-2-propanoylcyclohexa-2,4-dien-1-one	
Lupulin	(1*R*,2*S*,3*R*,4a*R*,5*S*,6*R*,8*S*,8a*R*)-8-(acetyloxy)-8a-[(acetyloxy)methyl]-3-hydroxy-5-[(2*S*,5*R*)-5-methoxyhexahydrofuro[2,3-*b*]furan-2-yl]-5,6-dimethyloctahydro-2*H*-spiro [naphthalene-1,2′-oxiran]-2-yl 2-methylbutanoate	

(continued)

Table 10.2 (continued)

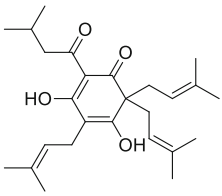
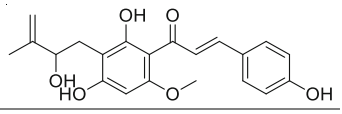
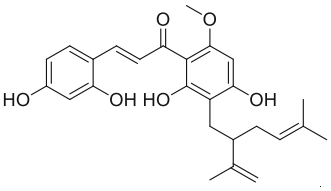
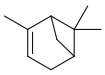
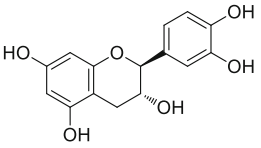
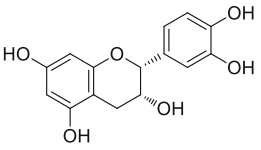
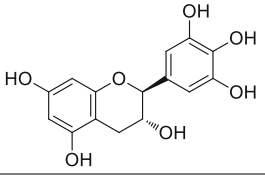
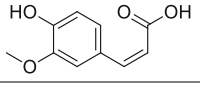
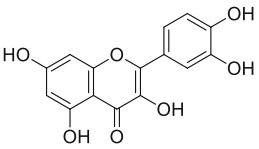
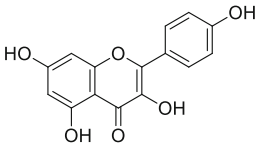
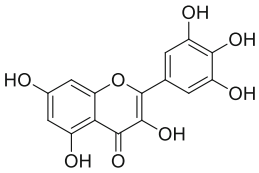
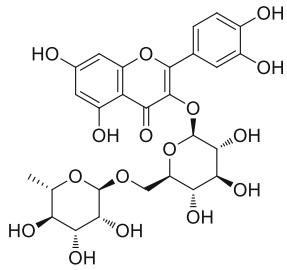
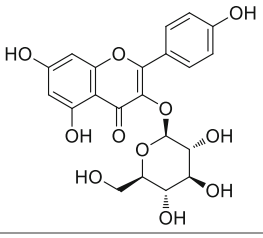
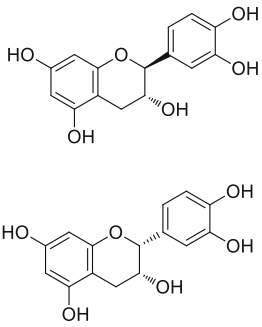
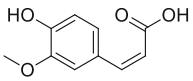
Common name	IUPAC NAME	Chemical Structures
Lupulone	3,5-dihydroxy-2-(3-methylbutanoyl)-4,6,6-tris(3-methylbut-2-enyl) cyclohexa-2,4-dien-1-one	
Xantholhumol	(*E*)-1-[2,4-dihydroxy-3-(2-hydroxy-3-methylbut-3-enyl)-6-methoxyphenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one	
Kuraridine	(*E*)-1-[2,4-dihydroxy-6-methoxy-3-(5-methyl-2-prop-1-en-2-ylhex-4-enyl) phenyl]-3-(2,4-dihydroxyphenyl)prop-2-en-1-one	
Alpha pinene	2,6,6-trimethylbicyclo[3.1.1]hept-2-ene	
(−) Catechin	(2*S*,3*R*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol	
(−) Epicatechin	(2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol	
(−) Gallocatechin	(2*S*,3*R*)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol	
Ferulic acid	(2*Z*)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid	

Table 10.3 Key polyphenols found in *Humulus lupulus*

Classification	Name	Description
Flavanols	Quercetin 	One of the primary compounds found and measured in hop shoot extracts was quercetin (Maietti et al. 2017)
	Kaempferol 	Hops contain Kaempferol, a member of the flavonoid subclass known as flavanols (Rajendran et al. 2014)
	Myricetin 	Myricetin is one of the main flavanols found in the hop plant (Dušek et al. 2021)
	Flavanol glycosides of quercetin and kaempferol	Various carbohydrate residues, flavanol glycosides are primarily related to the aglycones quercetin and kaempferol (Sägesser and Deinzer 1996)
	Rutin 	Higher plants, mosses, and ferns produce rutin, a UV-B-absorbing secondary plant metabolite, to shield themselves from disease and the damaging effects of UV-B radiation (Fabjan et al. 2003)
	Astragalin 	The inflammatory mediator's mRNA expression found significantly suppressed by Astragalin (Kou et al. 2008)

(continued)

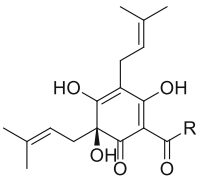
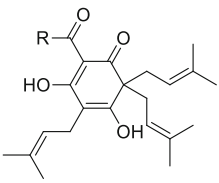
Table 10.3 (continued)

Classification	Name	Description
Flavan−3-ols	Catechin and Epicatechin 	The chemicals (+)-catechin and ()-epicatechin are very important in various industrial fields. These are widely employed as functional components in pharmaceutical and cosmetic formulations as well as functional foods, nutritional supplements, and nutraceuticals to improve consumer health, prevent various diseases, and lengthen product shelf lives (Alonso-Esteban et al. 2019)
	Flavanol dimers	Recent research has identified Glycosmis montana, Agrimonia pilosa, or cocoa liquor fermentation as sources of flavanol dimers connected by a methylene bridge and related unsymmetrical diaromatic methylene (Boyer and Ducrot 2005)
	Oligomeric flavanols	Flavanols are highly potent antioxidants that may scavenge a variety of free radicals and are biologically active compounds with a wide range of actions (Fernandes et al. 2012)
	Polymers(tannins)	After cellulose, hemicellulose, and lignins, tannins are the fourth most prevalent type of secondary metabolites found in all plants. (Sharma 2019)
Phenolic carboxylic acids	Ferulic acid 	The cell walls of grass, plants, nuts, and other organisms contain ferulic acid (FA), a type of aromatic acid molecule. These phenolic acids from the grape and malt can be released into the juice and wort, respectively, during the wine-making and beer-brewing processes (Xu et al. 2020)

research study. Hop cones or their alcoholic extracts when incorporated in the wort, furnish the beer with a variety of chemicals in addition to the bitter acids. Some of these substances, including isoxanthohumol and prenylated chalcone xanthohumol, have been said to have anti-cancer potential. (Klatsky 2009).

Bitter taste chemistry in beer: It is simpler to investigate the bitter elements of beer since hops only contain a small number of precursors. Hop acids, also known as humulones or lupulones and alpha-acids or humulones, are arguably the most significant class of hop chemicals. The side chain, which is formed from the hydrophobic amino acids' leucine, valine, and isoleucine for humulone, lupulone,

Table 10.4 Structure of major hop bitter acids (Caballero et al. 2012; Sanz et al. 2019; Zeković et al. 2007))

Acids	Structure	R	Synonym
α-Acids		$R_1 = CH_2CH(CH_3)_2$ $R_1 = CH(CH_3)_2$ $R_1 = CH(CH_3)CH_2CH_3$ $R_1 = (CH_2)_2CH(CH_3)_2$ $R_1 = CH_2CH_3$	Humulone Cohumulone Adhumulone Prehumulone Posthumulone
β-Acids		$R_2 = CH_2CH(CH_3)_2$ $R_2 = CH(CH_3)_2$ $R_2 = CH(CH_3)CH_2CH_3$ $R_2 = (CH_2)_2CH(CH_3)_2$ $R_2 = CH_2CH_3$	Lupulone Columulone Adlumulone Prelumulone Postlumulone

cohumulone, colupulone, and adhumulone adlupulone, respectively, differs in the two series' three members. Table 10.4. represents the structure of major Hop bitter acids.

A noteworthy quality of these chemicals is the exceptionally high concentration of hop acids—up to 25% or even more of the dry weight of the hop cones. The growing circumstances for a particular variety and the hop variety have a big impact on the relative quantities of the various elements. Hop acids are weak acids with very little water solubility and hardly any bitter flavor. In their purest state, they appear as solids that are pale yellow in color (De Keukeleire 2000).

10.8 Nutritional Composition

Whole hop cones are composed of proteins (15%), resins (15–30%), polyphenols (4%), essential oils (0.5–3.0%), waxes and amino acids (0.1%), ash (8%), moisture (10%). steroids (from traces to 25%), cellulose (43%), pectins (2%), monosaccharides (2%), and Hops are valued in the brewing industry for their flavor and bitterness and these characteristics in hops are due to the presence of resins produced by lupulin glands located inside the hop cones. The essential oil content in the hops is also essential for the fragrance and taste of the beer (Almaguer et al. 2014).

Various oils are present in hop cones such as lupulin which is waxy substance yellow in color, Oleoresins providing peculiar aroma and flavor. Lupulin comprises lupulone and humulone having antibacterial properties thus helping brewers' yeast to grow. The papery cones are discarded after the extraction of lupulin. The hops contain various components like alpha and beta acids, essential oils, and flavonoids which are responsible for nutrients as well as flavor. Hops are the richest source of

various kinds of polyphones. Between 3% and 6% of the dry weight of hop cones are hop polyphenolics (Moir 2000). Prenylflavonoids, bitter acids, and essential oils are released by lupulin glands together with the majority of polyphenols (also known as "leaf related polyphenols"), which are found in the string and bract (Almaguer et al. 2014).

10.9 Pharmacology

In addition to pharmacies in Europe and earlier in pharmacies in the United States, hop formulations are promoted in health-food stores in the United States for the treatment of anxiety and insomnia. Prenylnaringenin, one of the strongest in vitro estrogenic compounds known from the plant world, has been discovered in hops (Benkherouf et al. 2020). They are frequently described in popular writings as having sedative-like properties. Therefore, based on their long-term and present use in humans with no discernible negative effects, an early premise is that hops are safe (Chadwick et al. 2006).

Herbal treatments have been grown for hundreds of years or more, and as a result of human civilization, many genetic lineages have arisen. There are hundreds of cultivars and chemotypes of hops to choose from. In comparison to many other botanicals, hops are at a higher stage of evolution as an agricultural crop. This is partly owing to their prized organoleptic traits, which could be easily selected for in the quest for the standard beer. It was essentially a bioassay-guided selection process, with the demand for particular flavors among beer drinkers serving as the bioassay.

The chronic inflammation and infections in body are known to contribute cancers in the body and an enzyme cyclooxygenase (Cox) is the key element for the cause of inflammation. This enzyme generates endogenous substances like prostaglandins which resemble hormones which results in inflammation. Prostaglandins when produced in excess amount increases cell proliferation and enhances the new blood vessels formation which is the basis of cell injury and thus may cause carcinogenesis. The tumor tissues have more expression of the Cox 2 which is the inducible form of Cox. Hops have a potential chemopreventive mechanism of inhibiting this cyclooxygenase enzyme thus preventing cancers. Hop bitter acids (α- and β-acids) in various forms are discovered in hops (*Humulus lupulus*). Hop bitter acid mixtures have long been used as bitterness tastes or food additives in the brewing and culinary sectors. Hop bitter acids and their derivatives have recently been discovered to offer new medical and pharmacological applications in recent investigations. Manufacture initiatives for heterologous biosynthesis of objective hop bitter acids by designed microbial factories grew in response to rising demand for pure hop bitter acid (Karabín et al. 2016).

The antibacterial, antiseptic, and tuberculocidal capabilities of hops and its components have been the subject of extensive research. Hop bitter acids are particularly powerful against Gram-positive bacteria, according to research. They

function optimally in their undissociated state at low pH. Beta-acids (lupulone) and alpha-acids (humulone) are around 200-times and 700-times more powerful than phenol, respectively. The same compounds have little impact on Gram-negative bacteria while hardly inhibiting yeast and mould growth (Stavri 2004).

Since lupulone has the strongest in vitro activity of any hops component against Mycobacterium TB, it has been investigated as a tuberculosis therapy. Although the treatment was connected to gastrointestinal issues in men, early results were encouraging. At a clinical experiment, the adverse effects of the one drug administered in large quantities hindered the treatment.

Hops extracts (*H. lupulus*) were found to prevent obesity in mice fed a high-fat diet over the long term (Sumiyoshi and Kimura's 2013). It has been discovered that the lipophilic family of isohumulones generated from a modified hops extract has a special capacity to control the metabolic abnormalities that give rise to chronic metabolic inflammation. Numerous cell lines, animal model systems, and human intervention studies have consistently shown these effects. In humans, isohumulones reduce dyslipidemia and insulin resistance. In high fat-fed animal models, they have also been proven to decrease obesity, boost lipid metabolism, and reduce systemic inflammation. Last but not least, they have been demonstrated to improve gastrointestinal mucosal integrity and decrease post-prandial endotoxemia with high fat eating in animal models. The isohumulones position themselves as potentially useful as bioactive substances for inclusion in medical nutrition therapies for the management of chronic diseases associated with metabolic inflammation-related diabetes adiposity in high fat-fed animal models, according to these properties of this class of molecules (Kral 2014). Due to their distinct pleiotropic effects on the chronic diseases linked to metabolic inflammation, the isohumulones represent a family of chemicals that may likely find applications in medical nutrition therapy (Barabási 2007).

Hops' primary prenylflavonoid, xanthohumol, is highly effective at scavenging peroxyl radicals, one of the most prevalent reactive oxygen species in the body. Tests to measure the ability to absorb oxygen radicals show that xanthohumol is more effective than vitamins C and E in both hydrophilic and lipophilic conditions. The chemical is thought to provide a variety of possible health benefits because of its high antioxidant action. It primarily demonstrated antiproliferative, anticarcinogenic, antigenotoxic, anti-inflammatory, and anti-plasma glucose, lipid, and white adipose tissue weight reduction properties in in vitro studies with diabetic mice. Recent research have examined the efficiency of xanthohumol against certain viruses and the malaria protozoa (Plasmodium falciparum). For the health benefits in xanthohumol-enriched beers, special enriched xanthohumol extracts have been created (Gerhauser et al. 2002). Table 10.5 represents the various biological activities of *Humulus lupulus*.

Table 10.5 Different biological properties of *Humulus lupulus*

Biological activity	Source	Reference
Antioxidant activity	Hop cone, hop seeds, white hop shoots, hop essential oil	Vidmar et al. (2019), Sanz et al. (2019)
Anticarcinogenic activity	Hop extract	Julio et al. (2017), Gerhäuser (2005)
Sedative activity	Stem	Tyler (1987), Schiller et al. (2006)
Estrogenic activity	Hop cone	Koch and Heim (1953), Hansel and Schulz (1988), Keiler et al. (2013)
Antimicrobial activity	Leaves, stems, rhizomes, female cones	Bocquet et al. (2018), Cermak et al. (2015)
Anti-inflammatory activity	Hop extract	Karabín et al. (2016), Bocquet et al. (2018)
Antiproliferative activities and cancer-related bioactivities	Hop extract	Lee et al. (2007), Stevens and Page (2004), Roehrer et al. (2019)

10.10 Toxicology

Suzuki et al. (2018) reported that matured hop extract (MHE) prepared from heat treated hops did not produce or show any safety concern when administered in rats. They observed no mutation in Ames test and no abnormality in the rat micronucleus test. There were no deaths or toxicological signs recorded in their study.

10.11 Conclusion

Hops are important for flavor and preservation of beer and used since ages. The bitter, fragrant flavor of beer is primarily attributable because of the presence of hops. Hops extracts are also employed in the food industry for additional flavor applications. Hop is a plant that is being used in folk medicine for thousands of years. Polyphenols, essential oils, and resins are the three principal types of secondary metabolites found in hops. It's no surprise that these chemicals have a wide range of pharmacological effects due to their chemical diversity. Hops and lupulin are used in medicine to help with digestion, moderate sedation, diuresis, and menstruation difficulties. Hops has "generally regarded as safe" (GRAS) status as a historical food constituent although therapeutic doses of hops may offer a greater risk than usual levels of exposure in food.

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Chapter 11
Inula racemosa Hook. f. Pushkarmool: Its Ethnobotanical Uses, Phytochemicals, and Pharmacological Activities

Phurpa Wangchuk and Tenzin Jamtsho

11.1 Introduction

The Himalayan regions are the hotspot of biodiversity that harbours 17,500 plant species, of which 6000 of them are used in modern and traditional medical practices (Schippmann et al. 2002; Prajapati 2003). Their flowers, roots, fruits, saps, leaves, and whole plants are used for multiple purposes (Wani et al. 2020; Jamtsho et al. 2021). *Inula racemosa Hook. f.* is one of these important medicinal plants used in Indian Ayurvedic medicine, Chinese Traditional Medicine (CTM), Bhutanese *Sowa Rigpa* Medicine (BSM), and European Homeopathy (Lokhande et al. 2007; Wangchuk 2009). The extract of *Inula racemosa* is used against cough, dyspnoea, asthma, tuberculosis, pains, acute enteritis, dysentery, and as an expectorant (Kumar 2014; Marisetti 2018; Soni and Sharma 2018). It is also prepared with other plant extracts for treating angina, hyperlipidaemia, hepatic ischaemia and ischaemic heart diseases (Tripathi et al. 1988; Dwivedi et al. 1989; Manipuri et al. 2013; Wang et al. 2017). Since this plant is used in many cultures or traditional medicines for treating various ailments, it bears significant economic, commercial, and conservation values. This chapter discusses the distribution, taxonomy, ethnobotany, pharmacognosy, phytochemistry, pharmacology, and the trade and industry of *Inula racemosa*.

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11.2 Ecology and Distribution

Inula racemosa grows in the riverine and grasslands of the temperate and alpine Himalayas within the altitudinal ranges of 1500–4200 m above sea level (masl). It is distributed in China (Tibet, Qinghai, Sichuan, Xinjiang, and Gansu province), Pakistan (Baluchistan, Astor, and Chitral), India (Ladakh, Uttarakhand, Jammu, and Kashmir), Afghanistan, Nepal, Bhutan, and also in the East Asiatic Region (Rinchen et al. 2019; Firdous et al. 2018; Kimothi 2014) (Fig. 11.1). Its vernacular names vary between regions and are sometimes indicated by more than one name (Khare 2007; Mangathayaru 2009; Wang et al. 2017; Sastry and Nesari 2018). For example, it is commonly known as sun spear/orris root/elecampane in English. In Sanskrit, (liturgical language of Hinduism, Buddhism, and Jainism), it is known as pushkaramula. While it is called pushkaramula in Hindi (India) and Nepali (Nepal), it is known as manu in Dzongkha (Bhutan) and Zong zhuang tu mu xiang in Chinese (China).

I. racemosa has been introduced in many European countries (mostly cultivated species) including Sweden, Netherland, France, Czech Republic, the USA, Finland, Canada, Belgium, Russia, and Norway (Shah and Coulson 2021; de Vries and Lemmens 2021; Ueda 2020; Swinnen et al. 2018; Vanreusel et al. 2021; Svirin et al. 2021; Barstow 2021) (Fig. 11.1).

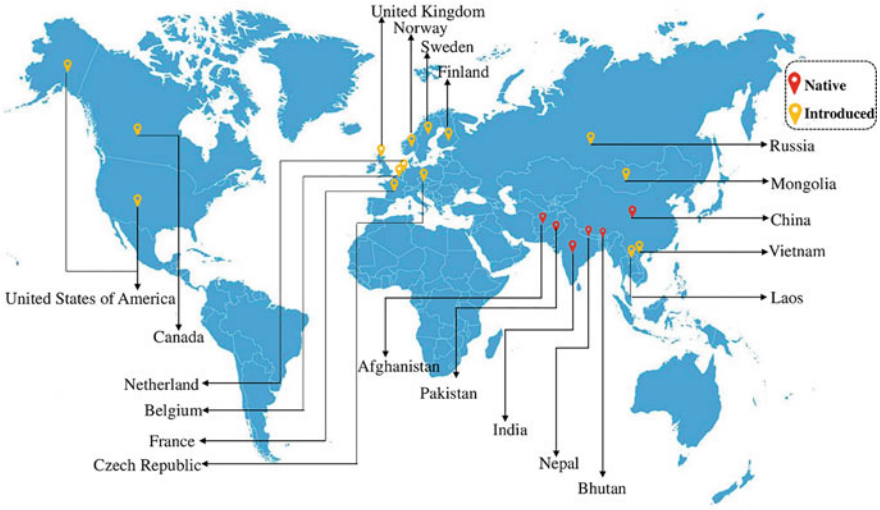

Fig. 11.1 Distribution of *Inula racemosa* as native and introduced species

11.3 Taxonomy and Botanical Description

Inula racemosa Hook. f. (Asteraceae) is a stout herbaceous perennial plant (up to 1.75 m tall) with basal leaves arranged in a racemose manner (Fig. 11.2a, b) (Sharma et al. 2016). While the lower leaves are winged, upper leaves are lanceolate (20–50 × 10–20 cm long petioles) (Wangchuk 2009; Yousheng and Anderberg 2011). The stem is 60–200 cm tall, grooved, and densely pubescent. The plant flowers in between July and September and has short-stalked flower heads (4–8 cm in diameter). Ray florets are slender (2.5 cm long) and outer involucre bracts are broad-triangular in shape. Fruiting occurs from October to November. The fruits are slim, hairless with achenes 0.4 cm long and hairy. Fresh roots are unequally fusiform with brown skin and camphoraceous odour.

11.4 Crude Drug Description and Ethnobotanical Uses

Most herbal medicines use dried plant roots of *I. racemosa* for treating various diseases. On drying, the crude drug, which is mostly the roots, becomes greyish in colour, coarse, irregularly wrinkled/cylindrical or slightly bent with many small-sized rootlets still intact. The root is aromatic and taste bitter. The diameter of the roots when cut horizontally ranges between 1 and 7 cm in diameter. Figure 11.2a, b shows the appearances of the fresh root and the crude drug (when dried) of *I. racemosa*.

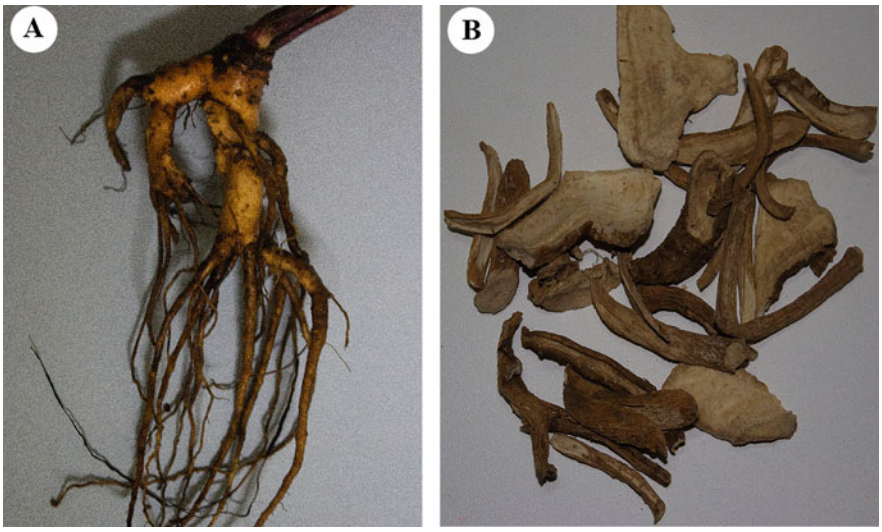

Fig. 11.2 *Inula racemosa* crude drug. (**a**) Fresh root; (**b**) crude drug (sliced and dried roots)

I. racemosa is used in Indian Ayurvedic and Unani medicines, BSM, Tibetan medicine (TM) and CTM (Shabir et al. 2013). In European and Asian countries, roots are famous for treating cardiac disorders and tuberculosis (Lokhande et al. 2007). In Indian Ayurvedic medicine, most parts of *I. racemosa* including flowers, leaves, and roots are used as ingredients for manufacturing as many as 18 polyherbal formulations (Bhūtyā 2011). For example, the water extract of *I. racemosa* root is used for treating rheumatic pains, liver problems, gonorrhoea, asthma, cardiac disorders, obesity, skin diseases, inflammation, anorexia, bronchitis, anaemia, diabetes, wounds, and ulcers (Shishodia et al. 2008; Mangathayaru 2009; Kumar 2014; Khurana et al. 2015; Choudhary 2012; Kapoor 2018). When taken with lukewarm water, the root powder also has rejuvenating, anti-ageing, anthelmintic, aphrodisiac and appetising properties (Kumar 2014; Sastry and Nesari 2018; Grimaud 2009). Likewise, the extracts of *I. racemosa* leaves and flowers are used against diarrhoea, dysentery, jaundice, eye infections, and asthma in India (Vadnere et al. 2009; Kimothi 2014). In Nepal, the root component is prescribed for treating cardiovascular diseases and cancer (Firdous et al. 2018). In CTM, *I. racemosa*, which is known as "Zong dmini or tumu xiang" is prescribed for treating acute enteritis, abdominal pain, and bacillary dysentery (Xu and Shi 2011; Arumugam and Murugan 2013; Wang et al. 2017). In TM, its root is used for treating stomach disorders and abdominal swelling, stimulating the spleen, boosting liver functions, and preventing miscarriage (Liu et al. 2006).

In Bhutan, local people use *I. racemosa* (locally known as Manu) for treating indigestion, loss of appetite, nausea, stomach inflammation, and premature cough and cold (Dorji et al. 2017). In addition, its root forms one of the important ingredients of the officially recognised scholarly BSM, which is provided freely through hospitals and basic health units in the country (Wangchuk 2014). *I. racemosa* is procured annually from Bumthang (Central Bhutan) and Paro (Western Bhutan) by Manjong Sorig Pharmaceuticals Corporation Ltd. for producing as many as 23 poly-ingredient formulations (Wangchuk 2009). Ethnobotanical uses of *I. racemosa* by different cultures and countries are summarised in Table 11.1.

11.5 Anatomical Features of Stem, Root, and Powder

The transverse sections (TS) of stem and root, and the microscopical features of powder of *I. racemosa* was described previously in the *Monograph on Medicinal Plants of Bhutan* (Wangchuk 2009).

TS microscopy of stem: The microscopical characteristics of stem, root and powder were determined using our previous methods (Wangchuk et al. 2020). The epidermis consists of oval or rectangular cells with thin cuticles. The surface has multicellular protective and glandular hairs. Several collenchymatous (hypodermis) and parenchymatous (cortical or general cortex) layers with conspicuous intercellular space are present beneath the epidermis. The vascular bundles are conjoint, collateral, and open with end arch protoxylem, covered by sclerenchyma

Table 11.1 Uses and the mode of administration of *Inula racemosa* in different countries

Ailment/Use	Plant Part	Country	Mode of administration	References
Abscess	Root	India	The powder is applied to the affected parts	Soni and Sharma (2018)
Angina	Root	India	The powder with honey is taken orally	Soni and Sharma (2018)
Anorexia	Root	India	2 g of powder is taken with honey for 3 days	Kumar (2014)
Asthma	Root Leaf	India	3 g of powder is taken with hot water for 5 days. The powder is taken with "dash mool kasaya" (Ayurvedic medicine in liquid form). Leaves (dry) are smoked	Kumar (2014), Kimothi (2014), Soni and Sharma (2018)
Boil	Root	India	Decoction of boiled root is taken daily	Lal and Singh (2008)
Colic	Root	India	Decoction of root powder mixed with other ingredients is taken orally twice a day	Bhūtyā (2011)
Cough	Root	India, Bhutan	3 g of powder is mixed in the decoction of dasamoola (10 different plants) and taken orally for 5 days. The powder is taken with dash mool kasaya	Kumar (2014), Wangchuk (2009), Soni and Sharma (2018)
Diarrhoea and dysentery	Leaf	India, China, Bhutan	Juice extracted from fresh leaves is taken orally	Kimothi (2014), Wangchuk (2009), Liu et al. (2006)
Diuretic	Root	India	Root powder prepared in lukewarm water is taken orally twice a day	Soni and Sharma (2018)
Headache	Root	India, Bhutan	Mixture of its powder (2 g), *Mesua ferrea* (2 g), raw sugar, and butter is taken in the morning before sunrise, and at bedtime for 2 weeks	Bhūtyā (2011), Wangchuk (2009)
Hiccups	Root	India	Root powder (3 g) is taken orally with lukewarm water for 2 days	Kumar (2014)
Hypercholesterolaemia	Root	India	Its powder (2 g) is mixed with Triphala and *Commiphora mukul* and taken orally with warm water—twice a day	Bhūtyā (2011)
Pruritis and ribs pain	Root	India	Root paste is prepared with cow's urine and applied on affected parts	Bhūtyā (2011)
Relieving pain	Root		The liniment is applied on affected parts till reclamation	Soni and Sharma (2018),

(continued)

Table 11.1 (continued)

Ailment/Use	Plant Part	Country	Mode of administration	References
		India, China, Bhutan		Wangchuk (2009), Liu et al. (2006)
Renal pain	Root	India, Bhutan, China	Root decoction (1 g) is mixed with other ingredients and taken orally—thrice a day	Bhūtyā (2011), Liu et al. (2006)
Rheumatic pains and liver problems	Root	India, China, Bhutan	The water extract of the root is taken orally	Wang et al. (2017), Kimothi (2014), Wangchuk (2009)
Skin diseases	Root	India	20 g of powder is boiled, concentrated, and taken orally for 1 week	Kumar (2014)
	Root	India	Root paste mixed with ghee is applied on the affected skin two times a day—for 7 days	Lal and Singh (2008)
Swellings	Root	India, China, Bhutan	Root paste is applied on the affected area for minimum 3 days	Wang et al. (2017), Kimothi (2014), Wangchuk (2009)
Toothache	Root	India	Turmeric powder and honey mixed with 2 g of root powder are externally applied to the affected gum area	Soni and Sharma (2018), Kumar (2014)

fibre, and arranged in a ring fashion. In between vascular bundles, a few layers of radial parenchymatous cells constitute medullary rays. The pith comprises thin-walled and rectangular parenchymatic cells with conspicuous intercellular spaces (Fig. 11.3a, b).

TS microscopy of root: It is irregular in outline due to the development of rhytidome. The outermost tissue, the cork, is composed of 8–12 layers of elongated rectangular cells with thick-walled, filled with reddish-brown material (Fig. 11.3c, d). Below these cells, 3–4 layers of cortex cells are present, composed of oblong parenchymatous cells. The xylem and phloem are clearly defined and consist of tracheids, parenchyma, and vessels. The starch grains are absent or rarely present in the cortex region.

Microscopical features of powder: The powdered crude drug appears reddish-brown under a microscope with distinct pitted vessels, fibre, and tracheids. Calcium oxalate crystals are also present. Starch grains are rare. Parenchyma cells and fragments of bark cells are also visible (Fig. 11.4).

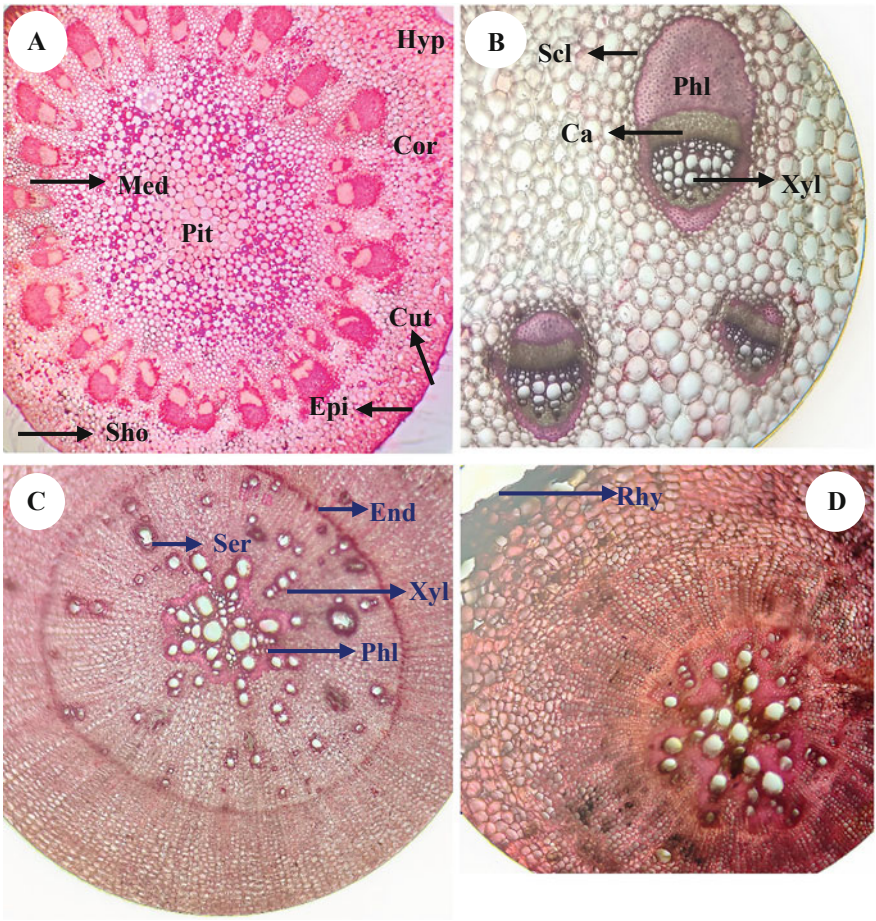

Fig. 11.3 Microscopic characteristics of *Inula racemosa*. (**a**, **b**) TS of fresh stem. (**c**, **d**) TS of fresh root. *Epi* Epidermis, *Cor* Cortex, *Pit* Pith (parenchymatous cells), *Sho H* Shoot Hair, *Hyp* Hypodermis, *Xyl* Xylem, *Phl* Phloem, *Cam* Cambium, *Rhy* rhytidome, *Ser C* secretory cavity, *Sci fib* sclerenchyma fibres, *Med R* Medullary Ray. Observed using Novex Microscope B-Range (Holland) under 10×. Photographs were taken with a Canon 1200D digital camera

11.6 High-Performance Thin Layer Chromatography (HPTLC) Profile

HPTLC profile of *I. racemosa* was developed using the instrument and method described previously for other medicinal plants (Wangchuk et al. 2020; Jamtsho et al. 2021; Reich and Schibli 2006). HPTLC profile shown here is used at Manjong Sorig Pharmaceuticals Corporation Ltd. in Bhutan for monitoring the quality of the crude drug of *I. racemosa*. The crude drug extract is applied to a TLC plate (5µl dilution), air-dried the plate, and let it run for few minutes under a solvent mixture of

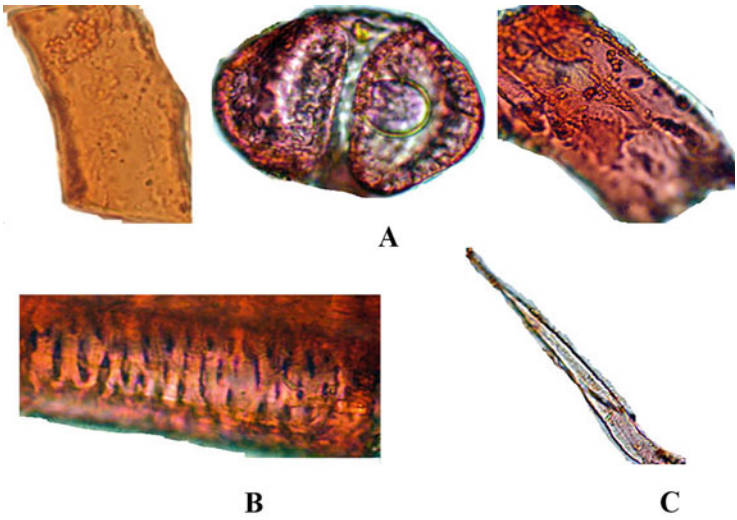

Fig. 11.4 Isolated elements from powdered crude drug. (**a**) Different types of tracheids. (**b**) Pitted vessel. (**c**) Fibre

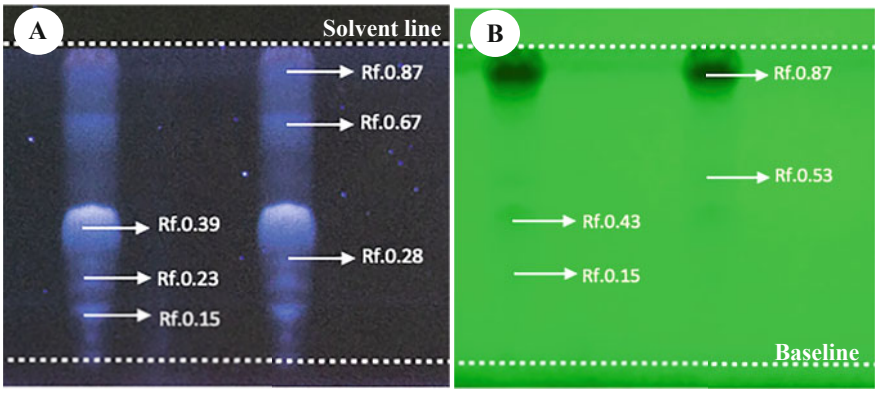

Fig. 11.5 HPTLC profile of methanolic extract of *Inula racemose* (5µl loading). (**a**) Visualisation under 366 nm UV light of wavelength. (**b**) Visualisation under 254 nm UV light of wavelength. Solvent system used was butonal: acetic acid: water (4:1:5)

butanol: acetic acid: water (4:1:5). While some of these bands are visible under daylight, other bands can be visualised only under certain UV wavelengths (254 and 366 nm) (Fig. 11.5a, b). The bands represent the molecular signature/profile of a plant, and they can be distinguished by colours and the retention factor (Rf) values. The formula for calculating the Rf value of each band on a TLC plate is given below:

$$\mathrm{Rf} = a/b$$

Where a is distance travelled by the solute band from the baseline; b (solvent front) is a distance travelled by the solvent from the baseline.

The HPTLC profile of *I. racemosa* (5μl dilution) shows six major bands under 366 nm UV light spectrum (Rf 0.15, 0.23, 0.28, 0.39, 0.67, and 0.87). Only four major bands are visible under 254 nm wavelength (Rf 0.15, 0.43, 0.53, and 0.87). Bands with Rf 0.15 and 0.87 are observed commonly under 366 nm and 254 nm wavelengths.

11.7 Physico-chemical and Extractive Values

Physico-chemical properties including unwanted foreign materials, percentage ashes content (total, acid-insoluble, alcohol-soluble), water-soluble component, and loss on drying have been determined for *I. racemosa* in accordance to the standards described in Thai Herbal Pharmacopoeia (Anonymous 1990, 2000), World Health Organization Monographs on Selected Medicinal Plants (WHO 2021), Bhutanese Medicinal Plant Monographs (Wangchuk 2009). The minimum percentage or acceptable limit of each physico-chemical parameter for *I. racemosa* sample/crude drug is given in Table 11.2. These are the standard parameters established by Manjong Sorig Pharmaceuticals Corporation Ltd. in Bhutan for monitoring the quality of crude drug/raw material. Any variations in the standard limits are considered adulteration. For example, the crude drug of *I. racemosa* should not contain more than 2% foreign matter related to a plant (not contaminant) and 14% loss on drying (Anonymous 1990, 2000, 2004). Loss on drying determines the shelf life of the dried plant material (Table 11.2). Higher the moisture content of the dried plant material, the higher the risk for microbial contamination especially fungal growth. On the other hand, the water and alcohol-soluble extractives should not be less than 61% and 18%, respectively.

Table 11.2 Acceptable physico-chemical limits for monitoring the quality of *I. racemosa*

Parameters	Acceptable physico-chemical limits (≤%)
Foreign materials (adulterants)	2.0
Total ash content	5.0
Acid-insoluble ash content	0.6
Alcohol-soluble extractive content	18.0
Water-soluble extractive content	61.0
Loss on drying	14.0

11.8 Phytochemical Contents and Their Groupings into Different Classes

I. racemosa was reported to contain terpenoids, phytosterol, flavonoids, glycosides, starch, lipids, and essential oil (Jamna et al. 2012; Mohan and Gupta 2017). While Mohan and Gupta (2017) suggested the presence of alkaloids and reducing sugar in the roots of a plant, no pure alkaloid has been isolated to date. The comprehensive literature search revealed that 67 compounds have been isolated from their root parts (Table 11.3) and most of them belong to the chemical class of terpenoids. For example, alantolactone and isoalantolactone were isolated as the major terpenoids from its root (Kalsi et al. 1988) along with *n*-decanyl docosdienoate (fatty acid), daucosterol (glycosides), and *β*-sitosterol (phytosterols) (Tan et al. 1998; Khan et al. 2014; Nengroo and Rauf 2020). Ma et al. (2012) isolated 24 sesquiterpene lactones from the Chinese grown *I. racemosa* roots and they showed that seven of these compounds possess significant antiproliferative activities. Khan et al. (2014) isolated aliphatic and eudesmanolide esters from the Indian grown *I. racemosa* roots. The representative chemical structures of most common bioactive molecules isolated from *I. racemosa* roots are given in Fig. 11.6.

In addition to the isolated components, more than 15 additional sesquiterpenes with heptadeca-1,8,11,14-tetraene (apiotaxene) as the most abundant compound were also detected in the *I. racemosa* root extracts (Bokadia et al. 1986). Sesquiterpene lactones such as 4*β*, 5*α*-epoxy-4,5-cis-inunolide, 4β-5α-epoxy-10 α, 14-Hinuviscolide, 2d-OH alantolactone, tomentosin, 8-epiisoivangustin, 9*β*-OH costunolide, 9 *β*-propionyloxy costunolide, 1- deoxy-8-epi-ivangustin, 9 *β*-(2-methylbutaryloxyl) costunolide, ivalin acetate and carborne were detected in the aerial parts of a plant (Firdous et al. 2018).

11.9 Pharmacological Activities

The crude extracts of *I. racemosa* have been widely studied for their biological properties (Table 11.4). The pharmacological activities of compounds isolated from this plant are listed in Table 11.3. Chemical structures of common bioactive molecules isolated from *I. racemosa* are given in Fig. 11.6. The pharmacological activities of both crude extracts and isolated compounds are discussed in detail under each sub-heading as an analgesic, anti-inflammatory, antimicrobial, antiparasitic, anticancer, antiasthmatic, antiallergic, antidiabetic, antiapoptotic, cardioprotective, and hepatoprotective activities. While the crude extracts demonstrated antidiabetic, antiatherogenic, cardioprotective, and immunomodulatory properties, none of the compounds isolated from *I. racemose* possessed these biological activities. On the contrary, isolated compounds possessed anticancer, antiproliferative, and antiplatelet activities, whereas the crude extract did not.

Table 11.3 Major pure compounds isolated from *I. racemosa* roots and their reported pharmacological activities

Isolated compound	Chemical class	Reported pharmacological activities	Model organism, Cell/cell line used	Effective dosage (IC_{50} values unless stated)	References
Alantodiene	Terpenoid	NA	NA	NA	Kalsi et al. (1988)
Alantolactone	Terpenoid	• Inhibited LPS-induced NO production • Antiproliferative • Antibacterial	• RAW264.7 • A549 • HepG2 • HT1080 • HUVEC • *Bacillus cereus* and *Pseudomonas aeruginosa*	7.39μM 0.55μg/mL 0.13μg/mL 0.696μg/mL 2.5μg/mL MIC value of 100μg/mL	Zhang et al. (2012a), Ma et al. (2012), Lokhande et al. (2007)
Alloalantolactone	Terpenoid	Antiproliferative	• A549 • HepG2 • HT1080	2.67μg/mL 3.57μg/mL 3.87μg/mL	Ma et al. (2012)
Aplotaxene	Alkatetrienes	NA	NA	NA	Bokadia et al. (1986)
Daucosterol	Lignan glycosides	NA	NA	NA	Tan et al. (1998)
Dihydroepoxyalantolactone	Terpenoid	NA	NA	NA	Kalsi et al. (1988)
Dihydroisoalantolactone	Terpenoid	NA	NA	NA	Kalsi et al. (1988)
Dihydro-4(15)-α-epoxyisoalantolactone	Terpenoid	NA	NA	NA	Kalsi et al. (1988)
Dehydroivangustin	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	15.97μM	Zhang et al. (2012a)
Disesquicin	Terpenoid	Anticancer	• HeLa • MDA MB-231 • A549	5.99μg/mL 9.10μg/mL 12.47μg/mL	Tyagi et al. (2021)
Epoxy alantolactone	Terpenoid	NA	NA	NA	Shah et al. (2009)

(continued)

Table 11.3 (continued)

Isolated compound	Chemical class	Reported pharmacological activities	Model organism, Cell/cell line used	Effective dosage (IC_{50} values unless stated)	References
Epoxyisoalantolactone	Terpenoid	Larvicidal	Asian tiger mosquitoes	LC_{50} values of 35.13μg/mL	He et al. (2014)
Inunal	Terpenoid	NA	NA	NA	Kaur and Kalsi (1985)
Isoalantodiene	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	14.06μM	Zhang et al. (2012a)
Isoalantolactone	Terpenoid	Inhibited LPS-induced NO production Antifungal Anticancer Antiproliferative Antiplatelet	RAW264.7 • *Aspergillus flavus* • *A. niger* • *Geotrichum candidum, Candida tropicalis* • *C. Albicans* • A549 • Bel 7402 • BGC 823 • HCT-8 • A2780 • A549 • HepG2 • HT1080 • HUVEC Inhibited β-glucuronidase in rat's polymorphonuclear leukocytes	12.05μM MIC values of 50μg/mL 50μg/mL 25μg/mL 25μg/mL 25μg/mL 8.1μM 12.4μM 24.6μM 21.2μM 22.5μM 0.38μg/mL 1.77μg/mL 0.79μg/mL 2.40μg/mL 80.5% inhibition at 10μm	Zhang et al. (2012a), Tan et al. (1998), Zhang et al. (2012b), Ma et al. (2012), Zhang et al. (2010)
Isoalantolactone	Terpenoid	NA	NA	NA	Kaur and Kalsi (1985)
Isoinunal	Terpenoid	NA	NA	NA	Kalsi et al. (1988)

Isotelekin	Terpenoid	Antiproliferative	• A549 • HepG2 • HT1080	1.47μg/mL 4.19μg/mL 2.44μg/mL	Ma et al. (2012)
Macrophyllilactone E	Terpenoid	Larvicidal Antiplatelet	Early fourth-instar larvae of *A. albopictus* Inhibited β-glucuronidase in rat's polymorphonuclear leukocytes	LC_{50} values of 18.65μg/mL 64.5% inhibition at 10μm	He et al. (2014), Zhang et al. (2010)
n-Decanyl docosdienoate		NA	NA	NA	Khan et al. (2014)
n-Hexadecanyl behenate		NA	NA	NA	Khan et al. (2014)
Phenylacetonitrile	Benzene and substituted derivatives	NA	NA	NA	Bokadia et al. (1986)
Racemolactone I	Terpenoid	Anticancer	• HeLa • MDA MB-231 • A549	LC_{50} values of 0.9μg/mL 4μg/mL 3.8μg/mL	Alam et al. (2021)
Racemosalactone A		Antiproliferative	• A549 • HepG2 • HT1080	1.51μg/mL 2.76μg/mL 1.88μg/mL	Ma et al. (2012)
Racemosalactone B	Terpenoid	NA	NA	NA	Ma et al. (2012)
Racemosalactone C	Terpenoid	NA	NA	NA	Ma et al. (2012)
Racemosalactones D	Terpenoid	NA	NA	NA	Ma et al. (2012)
Racemosalactones E	Terpenoid	NA	NA	NA	Ma et al. (2012)
Racemosin A	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	40.16μM	Zhang et al. (2012a)

(continued)

Table 11.3 (continued)

Isolated compound	Chemical class	Reported pharmacological activities	Model organism, Cell/cell line used	Effective dosage (IC_{50} values unless stated)	References
Septuplinolide	Terpenoid	Anticancer	• A549 • Bel 7402 • HCT-8 • A2780	36.8μM 30.7μM 29.7μM 35.9μM	Zhang et al. (2012b)
Telekin	Terpenoid	NA	NA	NA	Kalsi et al. (1988)
5-*α*-epoxyalantolactone	Terpenoid	Larvicidal Antiproliferative	Asian tiger mosquitoes • A549 • HepG2 • HT1080	29.37μg/mL 1.59μg/mL 2.73μg/mL 1.32μg/mL	He et al. (2014), Ma et al. (2012)
4(15)-*α*-epoxy isoalantolactone	Terpenoid	NA	NA	NA	Kalsi et al. (1988)
5-*α*,6-*α*-epoxy alantolactone	Terpenoid	NA	NA	NA	Kalsi et al. (1988)
(4R,5S,10S)-5-hydroxy-11,12,13-trinoreudesm-6-en-8-one	Terpenoid	NA	NA	NA	Ma et al. (2013)
(4R,5R,10S)-5-hydroxy-11,12,13-trinoreudesm-6-en-8-one	Terpenoid	Antiproliferative	• A549 • HepG2 • HT1080	3.71 mg/mL 5.94 mg/mL 3.95 mg/mL	Ma et al. (2013)
(4R,5R,10R)-4,15-epoxy-11,12,13-trinoreudesm-8-one	Terpenoid	NA	NA	NA	Ma et al. (2013)
11,12,13-trinoreudesm-5-en-7-*β*,8-*α*-diol	Terpenoid	NA	NA	NA	Ma et al. (2013)
3-oxoalloalantolactone	Terpenoid	NA	NA	NA	Kalsi et al. (1988)

3-β-hydroxy-11-α,13-dihydroalantolactone	Terpenoid	NA	NA	NA	Zhang et al. (2010)
11-α-hydroxyeudesm-5-en-8-β,12-olide	Terpenoid	Anticancer	• BEL-7402 • HCT-8	9.6μg/mL 9.2μg/mL	Zhang et al. (2010)
11,13-dihydroalantolactone	Terpenoid	NA	NA	NA	Khurana et al. (2015)
11,13-dihydroisoalantolactone	Terpenoid	Larvicidal activity	Early fourth-instar larvae of *A. albopictus*	LC_{50} values of 21.86 μg/mL	He et al. (2014)
11α,13-dihydro-2-α-hydroxy-alantolactone	Terpenoid	Anticancer	• A549 • Bel 7402 • HCT-8 • A2780	28.3μM 32.0μM 27.1μM 39.3μM	Zhang et al. (2012b)
11,13-dihydroivalin	Terpenoid	Anticancer	• A549 • Bel 7402 • BGC 823 • HCT-8 • A2780	25.2μM 25.4μM 34.3μM 27.2μM 28.5μM	Zhang et al. (2012b)
11-β-H-2-α-hydroxyeudesma-4(15)-en-12,8-β-olide	Terpenoid	NA	NA	NA	Kalsi et al. (1988)
11,12,13-trinoreudesm-5-ene-7-β,8-α-diol	Terpenoid	NA	NA	NA	Kalsi et al. (1988)
(7R,8R,10R)-8-hydroxyeudesma-4(5),11(13)-dien-12-oic acid	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	52.06μM	Zhang et al. (2012a)
(4S,8R,10R)-13-dimethoxyeudesma-5(6),7(11)-dien-12,8-olide	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	45.99μM	Zhang et al. (2012a)
(4S,8S,10R)-12-hydroxyeudesma-5(6),7(11)-dien-12,8-olide	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	12.03μM	Zhang et al. (2012a)

(continued)

Table 11.3 (continued)

Isolated compound	Chemical class	Reported pharmacological activities	Model organism, Cell/cell line used	Effective dosage (IC_{50} values unless stated)	References
13-hydroxy-5,7(11)-eudesmadien-8,12-olide	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	11.07μM	Zhang et al. (2012a)
4-*α*-H-eudesma-11(13)-en-4,12-diol	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	20.39μM	Zhang et al. (2012a)
3-oxo-eudesma-4,11-dien-12,8-*β*-olide	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	18–90μM	Zhang et al. (2012a)
11-*α*,13-dihydroalantolactone	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	10.13μM	Zhang et al. (2012a)
[1(10)E]-5-*β*-hydroxygermacra-1(10),4(15),11-trien-8,12-olide	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	6.35μM	Zhang et al. (2012a)
2-*α*-hydroxyeudesma-4,11(13)-dien-12,8-*β*-olide	Terpenoid	Inhibited LPS-induced NO production	RAW264.7	5.39μM	Zhang et al. (2012a)
1-one-4-epi-alantolactone	Terpenoid	NA	NA	NA	Zhang et al. (2012b)
4-*α*,13- dihydroxy-5,7(11)-eudesmadien-12,8-olide	Terpenoid	NA	NA	NA	Zhang et al. (2012b)
13-acetyloxy-5,7(11)-eudesmadien-12,8-olide	Terpenoid	NA	NA	NA	Zhang et al. (2012b)
15-tricosterienyl eudesmanolide	Sesquiterpenic ester	NA	NA	NA	Khan et al. (2014)

15-nonadecenyl eudesmanolide	Eudesmanolide ester	NA	NA	NA	Khan et al. (2014)
15-tetracosdienyl eudesmanolide	Sesquiterpenic ester	NA	NA	NA	Khan et al. (2014)
15-tetracosenyl eudesmanolide	Eudesmanolide ester	NA	NA	NA	Khan et al. (2014)
4,(15)-α-epoxyisotelekin	Terpenoid	NA	NA	NA	Goyal et al. (1990)
4,(15)-α- Epoxytelekin	Terpenoid	NA	NA	NA	Goyal et al. (1990)
7-hydroperoxy-11-α-H,13-dihydroisoalantolactone	Terpenoid	NA	NA	NA	Goyal et al. (1990)
β-sitosterol	Steroid derivatives	NA	NA	NA	Tan et al. (1998)

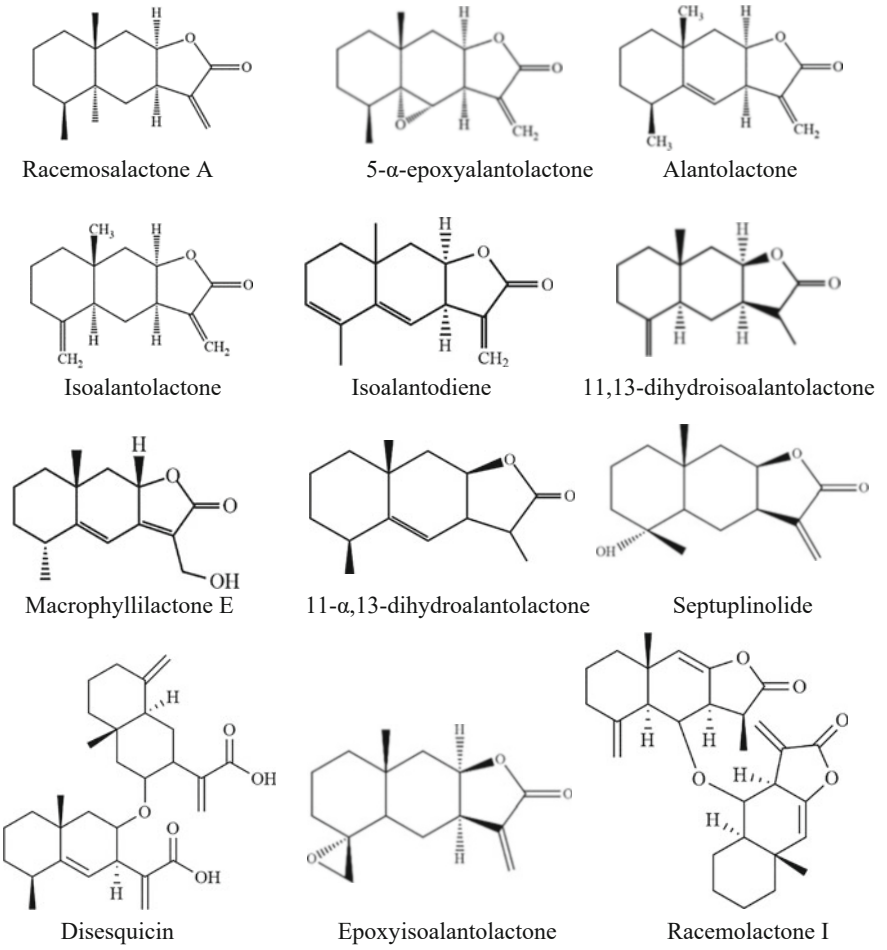

Fig. 11.6 Chemical structures of common bioactive molecules isolated and identified from roots of *I. racemosa*: anti-inflammatory (alantolactone), antimicrobial (alantolactone, isoalantolactone), antiparasitic (macrophyllilactone E), anticancer (racemosa lactone A, isoalantolactone, macrophyllilactone E, disesquicin, alantolactone, 5-α-epoxyalantolactone, 11-α,13-dihydroalantolactone, racemosa lactone I and septuplinolide), hepatoprotective (isoalantolactone), and antiplatelet (macrophyllilactone E and isoalantolactone)

11.9.1 Anti-inflammatory Activities

Crude ethanol extract of *I. racemosa* roots significantly protected Wistar albino rats against the carrageenan-induced paw oedema and hot plate-induced inflammation (Khan et al. 2010). Out of 14 sesquiterpenes (isolated from the *I. racemosa* root) tested against LPS-induced RAW264.7 macrophages, three of them including alantolactone, (1(10)E)-5β-hydroxygermacra-1(10),4(15),11-trien-8,12-olide, and

Table 11.4 Reported biological activities of the crude extract of *I. racemosa*

Bioactivity	Extracts tested	Screening targets and results	References
Antiallergy	Ethanol	Protective against egg albumin-induced passive cutaneous anaphylaxis.	Srivastava et al. (1999)
Antiapoptotic	Aqueous	Reduced total apoptotic cells from 12% to 3.5%.	Arumugam and Murugan (2013)
Antiasthmatic	Hydroethanolic, petroleum ether	Protective against mast cell degranulation, histamine-induced contractions, and milk-induced eosinophilia in mice.	Srivastava et al. (1999), Vadnere et al. (2009)
Antiatherogenic (in vivo)	Hexane and ethanol extracts	The hexane extracts increased plasma HDL-C levels. The ethanol extract reduced cholesterol and the atherogenic index	Mangathayaru (2009)
Antidiabetic (in vivo)	Methanolic extract	Alloxan-induced hyperglycaemia animals showed a significant reduction in blood sugar level	Ajani et al. (2009)
	Root power	Diabetic patients treated with root powder showed reduced glucose level	Singh et al. (1985)
Anti-inflammatory	Ethanol extract	Significantly reduced carrageenan-induced paw oedema	Khan et al. (2010)
Antimicrobial	Ethanol and aqueous extract	Significantly inhibited *Escherichia coli* and *Staphylococcus aureus*	Sharma et al. (2016)
	Ethyl acetate extract	Inhibited the growth of *Klebsiella pneumoniae, Salmonella typhi, Escherichia coli,* and *Pseudomonas aeruginosa*	Lokhande et al. (2007)
Cardioprotective (in vivo)	Ethanol and methanolic extracts	Both extracts exhibited a cardioprotective effect against isoproterenol-induced myocardial injuries	Ojha et al. (2010), Ojha et al. (2011)
Cytotoxicity (in vitro)	Ethanol and hexane extracts	It induces apoptosis with an IC50 value ranging from 10.25 to 17.89 mg/l in the tested cell lines	Pal et al. (2010)
		SRB (Sulphorhodamine-B) and MTT assay methods determined the extracts to be mildly toxic on human liver cells	Gnanasekaran et al. (2012)
Hepatoprotection	Ethanol extract	Showed significant hepatoprotection against carbon tetrachloride-induced human liver cells	Moerman (1986), Manipuri et al. (2013)
Immunomodulatory	Aqueous extract	Exhibited immunomodulatory action by stimulating phagocytic function	Mishra et al. (2016)
Larvicidal activity	Ethanol extract	Showed larvicidal activity against *A. albopictus*	He et al. (2014)

2α-hydroxyeudesma-4,11(13)-dien-12,8β-olide demonstrated strong anti-inflammatory activity with IC_{50} values of 7.39, 6.35, and 5.39μM, respectively (Zhang et al. 2012a).

11.9.2 Antimicrobial Activity

The ethanol extract demonstrated inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* with MIC (minimum inhibitory concentration) values of 6.25 mg/mL and 12.5 mg/mL, respectively (Yang et al. 2009). Isoalantolactone isolated from the root showed moderate antifungal activity against *Candida albicans, Candida tropicalis, Geotrichum candidum, Aspergillus flavus* and *Aspergillus niger* (Tan et al. 1998). The ethyl acetate extract of the dried root and their isolated compounds also showed mild antibacterial activity against *P. aeruginosa* and *B. cereus* (Lokhande et al. 2007).

11.9.3 Antiparasitic/Larvicidal Activity

The ethanol extract of *I. racemosa* root exhibited moderate larvicidal activity against the Asian tiger mosquitoes (*Aedes albopictus*) with LC_{50} value of 25.23μg/mL (He et al. 2014). Similarly, four isolated compounds of the roots (epoxyisoalantolactone, macrophyllilactone E, 5*α*-epoxyalantolactone and 11,13-dihydroisoalantolactone) showed moderate larvicidal activity against the fourth-instar larva of Asian tiger mosquito (He et al. 2014).

11.9.4 Anticancer and Antiproliferative Activity

Both crude extracts and isolated compounds of *I. racemosa* have been extensively tested for anticancer and antiproliferative activities (Table 11.3). For example, ethanol root extract demonstrated significant anticancer activities against prostate, ovary, colon, lung, CNS, and leukaemia cancer cell lines (HL-60 cell line). The isolated compound, racemolactone I, showed anticancer activity against different cancer cell lines (cervical cancer HeLa, breast cancer MDA MB-231, lung cancer A549) (Alam et al. 2021). Similarly, a dimeric sesquiterpene, disesquicin, showed marked anticancer activity against different human cancer cell lines (MDA MB-231, HeLa, and A549) with IC_{50} values of 5.99, 9.10 and 12.47μg/mL, respectively (Tyagi et al. 2021). Of many compounds tested, racemosalactone A, alloalantolactone, 5-α-epoxyalantolactone, 4(15)*α*-epoxyisoalantolactone, alantolactone and isoalantolactone exhibited antiproliferative activity against

different human cancer cell lines (A549, pG2, and HT1080 cells) with IC_{50} values ranging from 0.38 to 5.94μg/mL (Zhang et al. 2012b; Ma et al. 2012).

11.9.5 Hepatoprotective Activity

Male Wistar rats with hepatic ischaemic/reperfusion injury when treated with hydroalcoholic root extract showed reduction in the levels of alkaline phosphatase, alanine transaminase, lactate dehydrogenase, and aspartate transaminase, which is an indication of significant hepatoprotective activity (Manipuri et al. 2013). Further, the early period of hepatic ischaemia/reperfusion injury rat showed a notable increase in free radicals scavenging activity (Manipuri et al. 2013; Wang et al. 2017). When normal human liver cells were treated with ethanolic root extract, they showed higher percentage viability (78%) in comparison to toxicant CCl_4-treated cells (42% viability) indicative of significant hepatoprotection (Gnanasekaran et al. 2012). When Wistar rats with CCl_4-induced liver injury was treated with the isolated compound, isoalantolactone, it significantly reduced the level of bilirubin, oxaloacetate transaminase, serum glutamate, and pyruvate transaminase, indicative of hepatoprotection (Kalachaveedu 2015).

11.9.6 Antiasthmatic Activity

The ethanol, aqueous, and petroleum ether extracts of *I. racemosa* roots were tested for their antiasthmatic activity in Wister rats. Among the three extracts, petroleum ether extract exerted better: (i) antagonistic effect on histamine-induced contraction, (ii) antiasthmatic effect against milk-induced eosinophilia, (iii) adaptogenic activity on milk-induced leukocytosis, and (iv) protection against mast cell degranulation as compared with the control group (Singh et al. 1980; Vadnere et al. 2009).

11.9.7 Antiallergic Activity

When a different dose of *l. racemosa* ethanol extract was administered to albino rats with egg albumin-induced passive cutaneous anaphylaxis and mast cell degranulation, the crude extract protected them against type-I hypersensitivity, which confirmed its antiallergic property (Srivastava et al. 1999).

11.9.8 Cardioprotective Activity

The herbal formulation consisting of *I. racemosa* and *C. mukul* (1:1 ratio) improved the precordial pain and dyspnoea, and also reduced the triglycerides, cholesterol, and total lipid levels of the ischaemic heart disease patients (Singh et al. 1993). The petroleum ether extract and the isolated compound—alantolactone, reduced the glutathione levels and lipid peroxidation in Wistar albino rats with isoproterenol-induced myocardial ischaemia, confirming their cardioprotective effect (Chabukswar et al. 2010). Isoproterenol-induced myocardial infarction rats when treated with the hydroalcoholic and ethanol root extracts barred the outflow of cardiomyocyte-specific enzymes (lactate dehydrogenase and creatine phosphokinase isoenzyme) and restored the depleted endogenous antioxidant enzymes (catalase, peroxidase, glutathione, and superoxide dismutase), which suggested the cardioprotective effect of *I. racemosa* (Ojha et al. 2010; Ojha et al. 2011; Shirole et al. 2013).

11.9.9 Antidiabetic Activity

Diabetic patients (Diabetes mellitus) when treated with one tablespoonful of root powder of *I. racemosa* thrice a day for 3 months, their blood glucose levels became normal (Singh et al. 1985). The crude root extract also lowered the blood sugar level and improved plasma insulin and liver glycogen level in rats (Tripathi and Chaturvedi 1995). When corticosteroid-induced hyperglycaemic Swiss albino mice were treated with the alcohol root extract, it decreased the serum concentrations, suggesting their hypoglycaemic effect (Chaturvedi et al. 1995; Gholap and Kar 2003; Gholap and Kar 2004). The alloxan-induced hyperglycaemic rats when treated with the methanol root extract, it reduced blood sugar level and improved other pathological parameters (Ajani et al. 2009). None of the compounds were tested for their antidiabetic properties.

11.9.10 Antiapoptotic and Antiplatelet Activities

Arumugam and Murugan (2013) investigated the protective effect of crude aqueous root extract (pre-treatment) against apoptosis in Swiss albino mice. Apoptosis was induced by intraperitoneal injection of 4-nitroquinoline-1-oxide and allowed to develop in mice for 24 h. The crude aqueous extract of *I. racemosa* root reduced the percentage of total apoptotic cells (micro-nucleated polychromatic erythrocytes in bone marrow cells) from 12% to 3.5% (Arumugam and Murugan 2013). While the crude extract of *I. racemosa* was not studied for the antiplatelet property, the isolated compounds (macrophyllilactone E and isoalantolactone) inhibited β-glucuronidase

production in rats with the inhibitory ratios of 65.4% and 80.5%, respectively (Zhang et al. 2010).

11.10 Toxicity

Many plant species containing sesquiterpenoid lactones are known to cause contact dermatitis in farm workers and animals (Seca et al. 2014). *Inula racemosa* also contains sesquiterpenoid lactones (alantolactone and isoalantolactone) (Marc et al. 2008), which causes acute and chronic dermatitis at the exposed sites (Seca et al. 2014; Paulsen 2002; Ventura et al. 2006; Aberer 2008). The ethanol root extract displayed cytotoxicity on the normal human liver cell with CTC50 (common toxicity criteria 50/50% of cytotoxicity inhibition) values of 666.14, 690.14μg/mL (Gnanasekaran et al. 2012). The hexane extract of the root was also found cytotoxic (Manipuri et al. 2013). However, the detailed toxicological profile of the plant is lacking (Rathore et al. 2022) and needs to be thoroughly characterised.

11.11 Conservation and Cultivation Approaches

I. racemosa is associated with many medicinal properties, and therefore it is in high demand in many countries. Owing to this reason, this plant has been over-exploited in many Asian countries (Wani et al. 2006) and classified as vulnerable in India (Walter and Gillett 1998). Owing to the fragile habitat and destructive collecting practices, the plant has been identified as a critically endangered species not only in India but all across western Himalayan range (Wani et al. 2006). However, data for a global status is scarce (Molur and Walker 1998).

11.11.1 Cultivation Practices and Harvesting

I. racemosa is co-cultivated along with cash crops such as barley and wheat in Kashmir and Lahaul valley (Himachal Pradesh) (Bano et al. 2019). It is also cultivated in Bhutan, Sweden, Netherland, France, Czech Republic, the USA, Finland, Canada, Belgium, Norway, Russia, and China (Xinjiang) (Jabeen et al. 2007; Yousheng and Anderberg 2011; Shah and Coulson 2021; de Vries and Lemmens 2021; Ueda 2020; Swinnen et al. 2018; Vanreusel et al. 2021; Svirin et al. 2021; Barstow 2021) (Fig. 11.1). *I. racemosa* propagation is possible both sexually and vegetatively. It grows better in sandy loam and alluvial soils, but the produce is better when grown in black sandy loam soil (Das 2008; Yashwant 2011). Propagation through seeds has shown only 50–60% germination successes (Das 2008; Yashwant 2011) and takes 2–2.5 months to germinate. However, the

germination rate is enhanced to 80% when seeds were pre-treated with chill temperature (3–4 °C) for 40 days. The application of gibberellic acid can reduce the germination to less than a month (Bano et al. 2019). In root cutting propagation, the root sprouts survive better in late autumn (October) and early spring (May) (Arora et al. 1980). The best time to harvest the roots is in October or November (Arora et al. 1980). However, there is a decline in cultivation due to a long life cycle, scarce land for cultivation, and regular fluctuations in the market price (Yashwant 2011; Kumar 2014)

11.11.2 Tissue Culture: In vitro Micropropagation

The in vitro method to regenerate multiple shoots from leaf and nodal segments of a parent plant (*I. racemosa*) is faster than propagations through seeds and root cutting (Jabeen et al. 2007). In the tissue culture techniques, Murashige and Skoog medium (MS) is the most used culture media. The MS medium augmented with 6-benzylaminopurine is the ideal culture media to inoculate explants. Before regenerated shoots (micropropagules) are transferred to the field, they are acclimatised in the greenhouse (Jabeen et al. 2007) to enhance the survival rate in the field. For in vitro shoot multiplication, the best supporting medium is the MS medium stimulated with 1.00 mg/L BA (benzyl adenine) is (Kaur et al. 2010). Split rhizome cuttings treated with 100 ppm indole acetic acid sprouts better (88%) and forms the root faster (77%). Seed germination achieved 90% when scarification and gibberellic acid are applied together (Sharma and Malav 2017)

11.12 Trades, Product Registrations and Patents

11.12.1 Trade

I. racemosa is a popular medicinal plant found in many countries, and it is widely traded across the world. For instance, India annually trades about 200–500 metric tons of *I. racemosa* at a price of Indian Rupee (Rs) 150–220/kg (3.5 USD/kg) (Goraya and Ved 2017). As a raw material, it is in demand in many Asian countries. Few countries including India and Bhutan also use this plant as one of the raw materials to formulate many poly-ingredient herbal drugs. In Bhutan alone, *I. racemosa* is used by Menjong Sorig Pharmaceuticals Corporation Ltd. for producing as many as 23 poly-ingredient commercial medicines (Wangchuk 2009). Various commercial products/formulation names, dosage, mode of administration and their traditional uses are given in Table 11.5

Table 11.5 Various commercial products/formulation names, dosage, mode of administration, and their traditional uses

Herbal product names	Country	Manufacturer	Dosage form	Traditional therapeutic indications	References
a-gar-15	Bhutan	MSPCL	400 mg capsule	Useful for blood (*Khrag*) and air (*rlung*) disorders, pain, kidney injury, dysuria, and stiffness of the leg	Wangchuk (2009), Tenzin and Tashi (2015)
a-gar-20	Bhutan	MSPCL	350 mg capsule	For treating insanity, paralysis, and high blood pressure (*khrag-rlung*)	Wangchuk (2009), Tenzin and Tashi (2015)
b.de-byed-snyoms-ldan	Bhutan	MSPCL	500 mg capsule	Indicated for the treatment of all phlegm (*bad-kan*) diseases	Wangchuk (2009), Tenzin and Tashi (2015)
b.dud-rtsi-g.sum-sbyor	Bhutan	MSPCL	500 mg capsule	Used for treating fracture, stomach, liver, and gall bladder diseases (*bad-mkhris*)	Wangchuk (2009), Tenzin and Tashi (2015)
cong-zhi-6-pa	Bhutan	MSPCL	500 mg capsule	Useful for chest pain, digestive diseases, and watery vomiting	Wangchuk (2009), Tenzin and Tashi (2015)
d.vags-sman-15	Bhutan	MSPCL	500 mg capsule	Useful for stomach and liver disorders and colic pain	Wangchuk (2009), Tenzin and Tashi (2015)
shing-kuen-25	Bhutan	MSPCL	450 mg capsule	Used for treating wind/air (*Rlung*) disorders	Wangchuk (2009), Tenzin and Tashi (2015)
thang-chen-25	Bhutan	MSPCL	400 mg capsule	Appetiser and detoxicant. Also useful for treating fever and stomach disorders	Wangchuk (2009), Tenzin and Tashi (2015)
zhi-byed-6-pa	Bhutan	MSPCL	400 mg capsule	Good for indigestion, constipation, colic pain, abdominal distension, removing placenta, and dead foetus	Wangchuk (2009), Tenzin and Tashi (2015)
zhi-byed-11	Bhutan	MSPCL	500 mg capsule	Used for inducing labour, relieving pain, clearing blood	Wangchuk (2009), Tenzin

(continued)

Table 11.5 (continued)

Herbal product names	Country	Manufacturer	Dosage form	Traditional therapeutic indications	References
				clots, and cleaning the birth canal	and Tashi (2015)
rga-lo-sman-d.mar	Bhutan	MSPCL	500 mg pill	Heals lung diseases, bronchitis, fever, liver, and stomach disorders	Wangchuk (2009), Tenzin and Tashi (2015)
rta-zi-d.mar-po	Bhutan	MSPCL	500 mg capsule	Useful for arthritis, fever, and cough and cold	Wangchuk (2009), Tenzin and Tashi (2015)
b.sam-phel-nor-bu	Bhutan	MSPCL	500 mg pill	Used for treating gout, arthritis, paralysis, leprosy, and kidney disorder	Wangchuk (2009), Tenzin and Tashi (2015)
zla-shel-b. dud-rtsi-ma	Bhutan	MSPCL	500 mg pill	Useful for treating liver and stomach disorders, gastritis, fever, and eye infections	Wangchuk (2009), Tenzin and Tashi (2015)
a-gar-35	Bhutan	MSPCL	500 mg tablet	Good for fever, heart disease, insomnia, and high blood pressure (*khrag-rlung*)	Wangchuk (2009), Tenzin and Tashi (2015)
g.tso-bo-25	Bhutan	MSPCL	500 mg tablet	Alleviates bones and lung inflammation, nausea, pain, and lung bleeding	Wangchuk (2009), Tenzin and Tashi (2015)
ko-byi-13	Bhutan	MSPCL	500 mg tablet	Useful for arthritis, hypertension, oedema, and bouchut respiration	Wangchuk (2009), Tenzin and Tashi (2015)
skyu-ru-25	Bhutan	MSPCL	500 mg tablet	Useful for poor eyesight, chest pain, digestive disorders, and hypertension	Wangchuk (2009), Tenzin and Tashi (2015)
g. ser-thang-	Bhutan	MSPCL	Brown coarse powder	For relieving pain in the upper part of the body due to blood and bile disorders and recuring leg swelling	Wangchuk (2009), Tenzin and Tashi (2015)
ko-by-Inga-thang	Bhutan	MSPCL	Coarse powder	Useful for blood related disorders,	Wangchuk (2009), Tenzin

(continued)

Table 11.5 (continued)

Herbal product names	Country	Manufacturer	Dosage form	Traditional therapeutic indications	References
				including hypertension and acute fever	and Tashi (2015)
ma-nu-b.zhi-thang	Bhutan	MSPCL	Coarse powder	Used for treating blood diseases caused by air/wind (*rlung*) humour	Wangchuk (2009), Tenzin and Tashi (2015)
nor-bu-b. dun-thang	Bhutan	MSPCL	Coarse powder	Useful for blood (*khrag*) and air (*rlung*) disorders. Often given to a patient before the bloodletting procedure	Wangchuk (2009), Tenzin and Tashi (2015)
ko-la-11	Bhutan	MSPCL	Tablet	Indicated for spleen-related diseases	Wangchuk (2009), Tenzin and Tashi (2015)
Pushkarmool or Orris Root	India	Bioayurveda Pvt. Ltd.	Tablet	Useful for respiratory discomfort, cough, and chest pain. It nourishes lung, purifies blood, and improves circulation	Bioayurveda (2021)
Pushkarmool	India	Heilen Biopharm Pvt. Ltd.	Powder	Anti-ischaemic. Heals dental problem and used for treating sprain and bruises	Heilen Biopharm (2021)
Pushkarmool powder	USA	Herbs Forever	Powder	Supports respiratory, pulmonary, and nervous systems. Useful for a cough-related chest pain, flank pain, and back pain	Herbs Forever (2021)
Pushkarmool powder	India	Planet Ayurveda	Powder	Keeps respiratory and nervous system healthy and improves blood pressure and cholesterol level	Planet Ayurveda (2020)
Pushkarmool churna	India	Tansukh Herbals Pvt. Ltd.	Syrup or Tablet	Effective against asthma, bronchitis, and cough	Tansukh Herbals (2021)

(continued)

Table 11.5 (continued)

Herbal product names	Country	Manufacturer	Dosage form	Traditional therapeutic indications	References
Amlycure	India	Aimil Pharmaceuticals Ltd.	Capsule	Protects liver from toxins and acts as a detoxifier	Aimil pharmaceutical (2021)
Pushkarmool capsules	India	Chakrapani Ayurveda	Capsule	Useful for skin disorders, amenorrhoea, dysmenorrhoea, dyspnoea, oedema, and fever	Chakrapani Ayurveda (2021)
Khadiradi gutika	India	Dabur India limited	Tablet	Reduces pain in swallowing food, relieves cough and hoarseness in the throat	Dabur India limited (2021)
Cardiwin DS	India	Baidyanath Ayurvedant	Capsule	Supports healthy cholesterol levels through dietary supplement and ensures a better circulatory system	Baidyanath Research Foundation (2021)
Breathe free	India	Organic India	Capsule	Relieves asthma, shortness of breath, and coughing	Organic India (2021)
Divya mukta vati	India	Patanjali Ayurved Ltd.	Tablet	Useful for high blood pressure and heart diseases	Patanjali Ayurved limited (2021)
Epilac tablet	India	Ayursun Pharma Company	Tablet	Activates neuromuscular communication	Ayursun Pharma (2021)
Inula racemosa extract (powder)	China	Shaanxi Iknow Biotechnology Co. Ltd.	Power	Used as food additives and health supplements	Shaanxi Iknow Biotechnology Co., Ltd. (2021)

11.12.2 Patents

The Convention on Biological Diversity and Nagoya Protocol and the federal biodiversity protection acts regulate the collection of *I. racemosa* (Greiber et al. 2012). The intellectual property and patent laws differ from countries to countries. While the plant cannot be owned or patented by any individual company or countries, their derivatives such as products, processes and isolated bioactive compounds have been patented in many countries. For example, several *I. racemosa*-containing formulations and products, and processing and propagation techniques have been patented (Table 11.5) (Modi et al. 2000; Doshi et al. 2003; Banerjee et al.

2014). In India, the antidiabetic formulations (for preventing, treating, and managing diabetes) has been patented by Muniyal Ayurvedic Research Centre, Manipal, India (Patent No.—US 2018/0236018 A1). This formulation is used for treating hypoglycaemic and hypolipidaemic, and it is believed to be good for pancreatic cell regeneration (Shetty 2020).

Another product containing *I. racemosa* crude extract, which is believed to prevent diabetes, has been also patented by Conopco, Inc., D/B/A Unilever (US 2015/0352164 A1) (Banerjee et al. 2015). Amlycure (herbal formulation) that contain *I. racemosa*, which is used for treating chronic respiratory disorders and histamine/pollen-induced bronchial spasms, is patented by J.B. Chemicals and Pharmaceuticals, Ltd., Bombay (patent number: US 2003/0228383 A1) (Doshi et al. 2003). Similarly, an Ayurvedic formulation containing *I. racemosa* and other three medicinal plants, which is used for treating coronary heart disease, has been also patented in India (Patent number: EP2393503 B1) (Dubey and Rajamanickman 2016). In the USA, the antidiabetic formulation consisting of *I. racemosa* and naringin has been patented (a patent number: US 2015/0343.007 A1) (Singh et al. 2015). In Bhutan, 23 poly-ingredient products have been officially registered by Menjong Sorig Pharmaceuticals Corporation Ltd. (MSPCL) (Table 11.5) as the essential traditional medicines with the Drug Regulatory Authority of Bhutan. These products are manufactured at MSPCL and then distributed to different hospitals and Basic Health Units in the country, where they are used by Traditional Physicians (locally known as Drungtshos) for treating various diseases.

In addition to the product/formulation patents, there are few process patents approved for novel techniques and processes involving *I. racemosa*. The *Inula racemosa* extraction method for vasodilation and microcirculation has been patented by India (Patent number: WO 2014/124803 A1). A standard procedure for making antidiabetic formulation using *I. racemosa* root and sitagliptin was patented in the USA by Conopco, Inc. D/B/A Unilever, New Jersey (Patent number US 2016/0193269 A1). This formulation can significantly reduce blood sugar levels and alleviate the adverse effects of allelopathic medication (Banerjee and Singh 2016).

Zhang et al. (2013) proposed the synthesis of sesquiterpene lactone from *I. racemosa,* which is high yielding, and this biosynthetic method/process has been patented in China by the Northwest Institute of Plateau Biology of Chinese Academy of Sciences (patent number: CN103251666A). The tissue culture method using *I. racemosa* root was also patented in China (patent number: CN101647359A). This patented tissue culture method gives a better seed germination rate (95%) when treated with gibberellin solution and a greater germchit maintaining rate (95%) when treated with potassium permanganate solution or a carbendazol solution (Ma et al. 2010). The method for preparing anti-cough product using aqueous *I. racemosa* extract has been patented in Russia by the Russian Agency for Patents and Trademarks (Patent number: RU2157226C2) (Modi et al. 2000).

11.13 Conclusions and Future Perspective

I. racemosa is a native plant of the temperate and the sub-alpine region of the Himalayas including Nepal, Pakistan, Afghanistan, Bhutan, Tibet, and China. The plant is also distributed in the European countries mostly as introduced species. This plant is used in Asian countries for treating abscess, angina, anorexia, asthma, boils, colic, cough, diarrhoea and dysentery, headache, hiccups, hypercholesterolaemia, pruritis and ribs pain, rheumatic pains, liver problems, skin diseases, swellings, toothache, and also as a substance to promotes diuresis. Several countries, especially Bhutan, have set up several quality parameters including anatomical, morphological, organoleptic, physico-chemical, and HPTLC profiles for monitoring the quality and authenticity of *I. racemose*.

To date, a total of 67 phytochemicals have been isolated from *I. racemose* with major phytochemicals being sesquiterpene lactones (alantolactone and isoalantolactone). Both crude extracts and the isolated phytochemicals of the root component have been extensively studied for their pharmacological properties such analgesic, anti-inflammatory, antimicrobial, antiparasitic, anticancer, antiproliferative, hepatoprotective, antiasthmatic, antiallergic, cardioprotective, antidiabetic, antiatherogenic, cytotoxic, immunomodulator, and antiapoptotic activities. However, the phytochemicals and the biological activities of leaves, stems, and flowers remain less explored.

I. racemosa is widely traded across the world, mostly as a raw material for formulating many poly-ingredient herbal drugs. There are more than 35 poly-ingredient herbal drugs that are manufactured commercially. A number of these drugs along with the processes of manufacturing have been either registered or patented in Bhutan, India, the USA, China, and Russia. Due to broad-spectrum pharmacological properties and the production of many herbal drugs, there is high demand for *I. racemosa* worldwide.

Owing to high demand, *I. racemosa* has been over-exploited in many Asian countries. Anthropogenic activities and the fragile nature of its habitat add to a speedy decline in its wild population. While its wild population in the western Himalayan range is already experiencing a rapid deterioration in both population density and range, the information for a global conservation status is scarce. Studies have shown that plant tissue culture, including the micropropagation technique, could protect their wild population from extinction or becoming critically endangered. The cultivation of this plant species has been already proven successful, especially in Bhutan where it is grown in surplus to what is required by Menjong Sorig Pharmaceuticals Corporation Ltd. for producing as many as 23 commercial products. The surplus produce of *I. racemosa* from Bhutan can be exported to other countries in need, thereby promoting trade.

Future endeavours should focus on: (1) protecting the wild population of *I. racemosa* wherever it is considered critically endangered, (2) promoting sustainable trade and industry development around the cultivated plant species, (3) isolating bioactive components from the less explored components of a plant including leaves,

stems, seeds and flowers, and (4) rigorous toxicity studies of a plant to confirm their anecdotal health risk.

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Chapter 12
Juniperus recurva Buch.-Ham. ex D. Don and *Juniperus squamata D. Don*: Himalayan Juniper

Deeksha and Monika Hans

12.1 Introduction

Plants are utilized as a source of medication from prehistoric times for a wide range of ailments. In the Indian Himalaya, there are over 8000 different species of vascular plants, with 1748 of them having pharmacological significance (Singh and Hajra 1996; Samant et al. 1998). Since ancient times, a variety of wild and cultivated plants have been used for healing purposes, and medicinal plants have lately gained popularity as natural components for cosmetics as well as herbal medications (Joshi et al. 2016).

Juniperus recurva Buch.-Ham. ex D. Don. is a medicinal plant that can grow to be a 40 m high shrub or a tree. This plant is endemic to the Himalayas where it is distributed at altitudes ranging from 2400 to 4500 m above the sea level, from north Pakistan extending upto west Yunnan in southwestern China. It has needle-shaped six-rank leaves in three alternating whorls and curved branchlets. In Western Europe, *J. recurva* and related species are frequently grown as ornamental plants (Adams 2004; Farjon 2005). The natives of the Rasuwa region in central Nepal make use of this herb for the treatment of common ailments such as fever, headache, cough, and cold (Uprety et al. 2010). Juniper species may thrive in continental temperatures, rocky formations, and improperly formed soil, making them an important part of the xeric forest ecosystem (Olano et al. 2008). Six juniper species (*J. communis* L., *J. excelsa* Bieb., *J. macropoda* Boiss, *J. pseudosabina*, *J. recurva* Ham., *J. recurva* Ham. var. *squamata*) grow wild in the Himalayan region's dry interior, from north-west to north-east at an altitude of 1600–5000 (Weyerstahl et al.

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1988). *J. recurva* differs from other types in that its distal twigs are pendulous and recurved, leaves are appressed and overlapping, glaucous-green, and with or without flaccidity. Four to six to nine times longer than wide, with ragged edges; mature female cone is ovoid-elongate (Gautam et al. 2020).

Juniperus squamata, commonly known as Himalayan Juniper, is a gymnosperm, belonging to the family Cupressaceae, grows at a high altitude of 2700–4200 m in the Himalayan region. *J. squamata* is distributed in China, North India, Bhutan, Myanmar, Pakistan, and Afghanistan. It is important ecologically as well as economically (Farjon 2005; Adams 2004). It can grow in semi-arid climatic conditions therefore it can be employed for soil and water conservation. It also serves as a food source and shelter for birds and other animals. It is quite popular as an ornamental shrub in Europe and North America (Farjon 2005). It is quite popular traditionally as a medicinal plant. Essential oil can be extracted from dried berries, leaves, or twigs. Juniper berry is a popular diuretic in ayurvedic medicine known to improve digestion. It can also be used externally to relieve arthritic pain and swelling while ash of the bark is employed in treating skin problems. Bhutanese Sowa Rigpa medicine (BSM) is a traditional medical system that employs medicinal plants and their derivatives in formulations for the treatment of several ailments. Yeshi et al. (2019) characterized a total of 12 medicinal plants as priority species among 50 alpine medicinal plants studied and employed them to produce 48 different medicines at Menjong Sorig Pharmaceuticals. One of them is *Juniperus squamata*, which is employed in approximately seven different formulations. Plant parts that can be used whole or in portions include aerial parts, leaves, flowers, seeds, roots, and bark (Yeshi et al. 2019).

Song et al (2019) decoded the chloroplast genome of *J. recurva* and discovered that it was 127,602 bp long comprising 82 protein-coding genes, 33 transfer RNA genes, and cytotoxic ribosomal RNA genes. Hundred and fifteen genes were present in a single copy, except two genes (trnl-CAU and trnQ-UUG) that existed in double copies. There were eight genes with only one intron and two genes with two introns. Inverted repeat sequences were absent in *J. recurva* chloroplast genome. The whole cp genome had a 35.0% GC content. *J. recurva* was discovered to be closely related to *J. tibetica* in accordance with a maximum likelihood tree based on the concatenated amino acids of 78 protein-coding genes from 11 juniper species.

Juniperus species are a promising candidate for generating innovative medications using natural ingredients because it is a diversified genus (75 Juniperus species) with a wide range of medical uses.

12.2 Taxonomy

The Juniperus genus, which contains 68 species, is separated into three groups: *Caryocedrus* having one species, *Juniperus* with 9–10 species, and *Sabina* Approx. 50 species (Adams 1999; Rawat and Everson 2012) (Fig. 12.1).

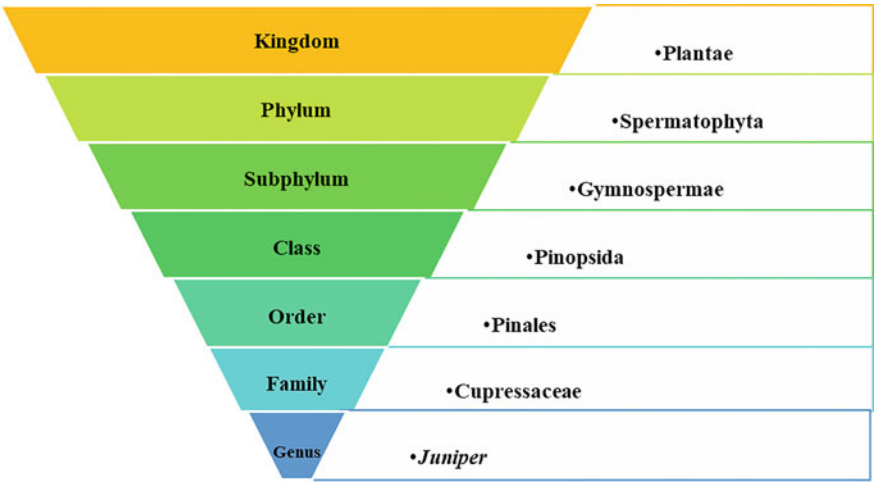

Fig. 12.1 Taxonomical classification of Genus Juniper

Dar and Christensen (2003) studied the Juniperus genus from the Western Himalayas in detail and discovered that the taxonomy of western Himalayan junipers has been perplexing. A total of seven taxa have been identified: one belongs to Juniperus Sect. Juniperus, *J. communis var. saxatilis*, and the other six to Juniperus Sect. Sabina. *J. squamata* and *J. recurva* were included in the latter, along with *J. semiglobosa*, *J. polycarpos*, *J. wallichiana,* and *J. pseudosabina*.

12.3 Vernacular/Common Names

Vernacular/Common names of *J. recurva and J. squamata* are given in the figure below.

12.4 Geographical Distribution

Conifers in India are confined in North-west and Western Himalayas. Several coniferous species that thrive well in different climatic zones in Himachal Pradesh include *Pinus roxburghii, Pinus wallichiana, Pinus gerardiana, Picea smithiana, Abies pindrow, Abies spectabilis, Cupressus torulosa, Cedrus deodara, Juniperus macropoda, Juniperus recurva, Juniperus squamata, Juniperus communis, Juniperus indica,* and *Taxus wallichiana* (Bhardwaj et al. 2020).

Juniperous pseudosabina and *Juniperus recurva* flourish in bushy formations above 3600 m, where the tree line terminates, in North and East Sikkim, particularly on the exposed sunny hill slopes near Thangu (4200 m) and Chhangu (4300 m) (Hajra 1996).

12.5 Morphology

J. recurva is a medium-sized tree with irregular branching that grows to a height of 6–20 m and a diameter of 2 m. It has three whorls of loosely overlapping 6–8 mm long scaly leaves. Female cones are 5–10 mm long and have 4–6 scales, while male cones are 3–4 mm long and contain imbricated scales. The flowers are dioecious and are pollinated by wind. The fruits are sub-globose and resinous, purplish-brown to black when ripe (Adams 2004; Adams et al. 1998; Farjon 2010; Bhardwaj et al. 2020).

12.6 Traditional Uses

Juniper recurva has myriad uses in traditional folk remedies. The plant is generally bitter and pungent but it possesses several properties, for instance, carminative, anthelmintic, and laxative properties. It is beneficial in diarrhea and other abdominal illnesses, piles, bronchitis, indigestion, constipation, and vaginal discharges. The fruit is useful as an aphrodisiac, and a generic remedy for stomach issues. The oil obtained from the fruit possesses abortifacient, tonic, and anthelmintic properties and is useful for earaches, toothaches, piles, and brain cooling (IUCN 2000). In addition, the cones of *Juniperus communis* and *Juniperus recurva* are commonly used as food, while the berries are used to flavor gin (fermented drink from barley) (Vichi et al. 2005). Table 12.1 summarizes the ancient uses of species belonging to the genus Juniper.

The heartwood powder of *Juniper recurva* Buch. has traditionally been used as a pesticide, especially against domestic insects by the natives in Nepal and surrounding regions. This is a proven fumigant which is quite effective and also devoid of any harmful impact on the environment or nontarget organisms (Oda et al. 1977).

Table 12.1 Traditional uses of various species of the Genus *Juniper*

	Plant part	Traditional use	References
Juniper recurva	Berries	• Used to treat skin diseases	Rokaya et al. (2010)
	Leaves	• Used to reduce fever	Nawang (1996), Khan et al. (2009)
	Aerial parts	• Burnt as incense	Bhardwaj et al. (2020)
	Fruits and oil	• Flavor and fragrance, diuretic, carminative, stimulant, dropsy, and rheumatic diseases	Bhardwaj et al. (2020)
Juniper squamata	Fruit and leaf	• Aids in the clearance of accumulated body fluids, sleep disorders, kidney issues, bleeding, skin issues, wound infection • Used as incense	Pandey (2006)
Juniper indica	Fruit and leaf	• Disorders associated with kidneys, lungs, gall bladder, liver, skin, lymph • Fever, cough, limb paralysis, uterine disease, and gum swelling treatment • Used as incense and for flavoring cosmetics and drinks	Pandey (2006)
Juniper communis	Seeds	• Dried seeds used as culinary ingredient	Rezvani et al. (2009)
	Berries	• Dried berries used for flavoring foods and alcoholic beverages (beer and gin) • Used as diuretic, antiseptic, and treating gastrointestinal issues	Rezvani et al. (2009), Baytop (1999)
	Aerial parts	• Used as incense	Rezvani et al. (2009)

Thujopsene and 8-cedren-13-ol were discovered to be the important insecticidal components found in the heartwood of *Juniperus recurva*. In the neutral portion of the ether extract, an additional 12 sesquiterpenes were discovered (Oda et al. 1977).

Juniperus recurva Buch.-Ham. ex D. Don and *Juniperus squamata* Buch.-Ham. ex D. Don may be used to prevent and treat cancer in Nepalese traditional medicine (Graham et al. 2000; Lechner-Knecht 1982). Juniper is revered as a sacred tree in the Himalayan region. Woods, leaves, and twigs are utilized as incense in Nepali and Tibetan culture as it is believed that they help to restore household energies both indoors and out (Dahal and Borthakur 2017). *J. recurva var.* coxii, or drooping Juniper, is a popular decorative plant, suitably grown in moderate and damp climates throughout Europe. Large trees were frequently used for coffin construction in its natural habitat (Li et al. 2013). Coffins are made from the wood of huge trees in Myanmar (Burma), for example, *J. recurva* is used as a source of wood and as an ornamental tree near monasteries and temples. In Buddhist temples, the wood and foliage are burnt for incense (Farjon 2010).

Juniperus squamata aids in reducing kidney inflammation as well as excess buildup of watery fluid in the joints (Wangchuk et al. 2008; Yeshi et al. 2019).

12.7 Phytochemistry

Terpenoids, cardiac glycosides, alkaloids, and many phenolic compounds have been found in *Juniperus* species. Leaf extract of *Juniper squamata* Buch. Ham ex D. Don possessed secondary metabolites such as alkaloids, carbohydrates, flavonoids, glycosides, proteins, phenols, saponins, terpenoids, resins, and starch (Sati and Kumar 2015). Methanolic and ethanolic extracts of *J. squamata* comprised of a total of 13 and 11 phytochemicals, respectively. Both extracts were devoid of phlobatannin and anthraquinones. Only the methanol extract contained tannins, volatile oils, and fat, whereas quinones were found exclusively in the ethanol extract. These phytochemicals are pharmacologically significant compounds that could be utilized to treat a variety of ailments.

Essential oil extracted from the leaves of *J. recurva* had major components such as α-pinene, sabinene, δ-3-carene, limonene, terpinen-4-ol, γ-cadinene, δ-cadiene, elemol, cubenol, epi-α-cadinol, epi-α-muurolol, α-cadinol, and 4-epi-abietal (Adams et al. 1998). Wedge et al. (2009) used hydrodistillation to extract essential oil from leaves and branches of *Juniperus squamata* D. Don var. fargesii Redh. et. Wils. (Jsq), yielding 0.52% oil. The key components obtained were oxygenated monoterpenes (66.5%), oxygenated sesquiterpenes (13.9%), oxygenated monoterpenes (10.0%), and diterpenes (7.2%). The chemical composition of essential oil, in general, is impacted by the season, variety, age of the tree, and its environment. For example, oil from the leaf of *J. squamata* var. fargesii from China had chief components such as α-pinene, sabinene, limonene, cis- and trans-thujone, cis-thujopsene, and 8-α-acetoxyelemol, while limonene was found in abundance in leaf oil from *J. squamata* from India, followed by sabinene, α-pinene, and δ- and γ-cadinenes (Adams et al. 1996).

Juniperus communis L. and *Juniperus indica* Bertol. leaf and berry essential oils from Uttarakhand showed qualitative and quantitative differences. *J. communis* leaf and berry oil comprise of major components such as α-pinene (35.35% and 10.78%), limonene (23.75% and 15.06%), and terpinen-4-ol (0.93% and 8.76%), respectively. Whereas the key compounds present in the leaf and berry essential oil of *J. indica* were sabinene (27.75% and 23.17%), terpinen-4-ol (16.11% and 23.61%), α-pinene (6.34% and 8.82%), and γ-terpinene (6.05% and 6.58%), respectively (Lohani et al. 2010).

Kuo et al. (1987) discovered the key compounds in heartwood extract of *Juniperus squamata* Lamb., viz., 8,14-cedranoxide, 8,14-cedranolide, cedrol, β-sitosterol, widdrol, 7-oxototarol, procerin, 8,14-cedranediol, honokiol, sugiol, hinokiic acid, cedrolic acid, and five new sesquiterpenoids, epicedranediol, 4-ketocedrol, 3β-hydroxycedrol, isocedrolic acid, and β-chamigrenic acid.

Podophyllotoxin is one of the most desirous aryl tetralin lignan found in some plant species. It is employed as a starting material in the manufacture of anticancer medicines. Due to limited natural sources and restrictions in synthetic approaches, podophyllotoxin is particularly limited. Kour et al. (2008) discovered that the endophytic fungus *Fusarium oxysporum* found in *Juniper recurva* produces

Table 12.2 Essential oil and its constituents

Species	Plant part	Major constituents	References
J. recurva	Wood	α-Cedrene β-Cedrene Thujopsene Acoradiene β-Chamigrene Cuparene Cedrol 8,14-Cedranoxide Widdrol 8-Cedren-13-ol acetate	Adams et al. (1998); Bhardwaj et al. (2020)
J. recurva	Leaves	α-Pinene Sabinene δ-3-Carene Limonene Terpinen4-ol γ-Cadinene δ-Cadiene Elemol Cubenol Epi α-cadinol Epi-α-muurolol α-Cadinol 4-epi-abietal 4β-Hydroxygermacra-15-diene β-Oplopenone Oplopanone 8-Acetoxy elemol Manool	Adams et al. (1998); Bhardwaj et al. (2020)
J. squamata	Leaves	α-Pinene Sabinene Limonene Cis- and trans-thujone Cis-thujopsene 8-α-acetoxyelemol	Adams et al. (1996)
J. communis	Leaves	α-Pinene Limonene	Lohani et al. (2010)
J. communis	Berries	α-Pinene Limonene Sabinene	Lohani et al. (2010)
J. indica	Leaves	Sabinene Terpinen-4-ol	Lohani et al. (2010)
J. indica	Berries	Terpinen-4-ol Sabinene	Lohani et al. (2010)

podophyllotoxin. HPLC and LC-MS were carried out to confirm the production of podophyllotoxin. It was observed that the highest production (μg/g dry weight mycelia) was obtained on day 4.

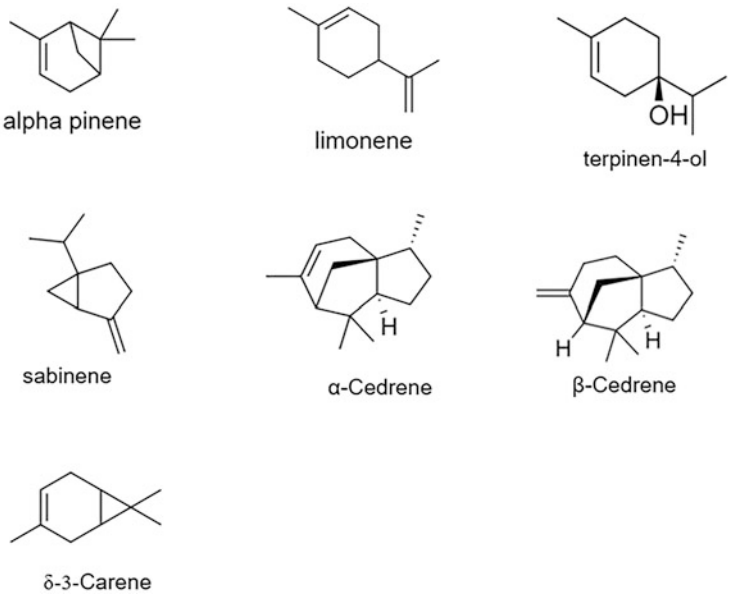

Fig. 12.2 Structures of important compounds present in Juniper species

Table 12.2 summarizes the essential oil constituents present in *Juniper* species. Figure 12.2 depicts the structures of important compounds found in essential oils of juniper species.

12.8 Pharmacology

Juniper extracts and essential oils have exhibited several biological activities in various studies such as antioxidant, antibacterial, antiviral, antifungal, antiproliferative, and immunomodulatory (Raina et al. 2019).

Antibacterial: Juniper species have been shown to have antimicrobial action against a variety of diseases in several investigations and are of medical importance. For instance, bark essential oil from *Juniperus recurva* demonstrated significant antimicrobial activity (Rashid et al. 2016). Monoterpene hydrocarbons such as α-pinene (20.7%), p-cymene (15.0%), and γ-terpinene (14.4%) were the major components of essential oil. In addition, oxygenated monoterpenes (5.2%), oxygenated sesquiterpenes (3.3%), and sesquiterpene hydrocarbons (traces) were found in the oil. Essential oil exhibited strong antibacterial activity against *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus faecium*, and *Staphylococcus epidermidis* with a minimum inhibitory concentration in the range of 125–250 μg/mL. Further, it was studied that essential oil from *J. recurva* impeded the growth of

several fungal strains such as *Candida albicans*, *Candida albicans,* and *Aspergillus niger* with minimum inhibitory concentration ranging from 500–1000 μg/mL (Rashid et al. 2016).

Similarly, antibacterial efficacy of *J. communis* oil against a variety of bacterial species has been widely investigated and verified, for instance, *B. cereus*, *E. coli*, *L. monocytogenes*, *Corynebacterium species, and S. aureus* (Filipowicz et al. 2003; Pepeljnjak et al. 2005; Glisic et al. 2007). The phytochemicals, such as alkaloids, flavonoids, anthraquinones, polyphenols, phytosterols, and saponins, present in them are believed to be linked to the antibacterial effect (Raina et al. 2019).

Antifungal: Several studies have shown the antifungal action of *Juniperus* essential oils. For instance, essential oil from Tibetan *Juniperus squamata* var. fargesii possessed antifungal activity against *Colletotrichum acutatum, C. fragariae, and C. gloeosporioides* (Wedge et al. 2009). Leaf oil of *J. oxycedrus* ssp. *oxycedrus* possessed appreciable antifungal action against *Candida*, *Aspergillus,* and dermatophytes, with minimal inhibitory concentration and minimum lethal concentration of 0.08–0.16 μL/mL and 0.08–0.32 μL/mL, respectively. The key components found in this oil included α-pinene (65.5%) and δ-3-carene (5.7%). The antifungal activity was ascribed to δ-3-carene found in the essential oil (Cavaleiro et al. 2006). Juniper leaf extracts in different organic solvents inhibited aflatoxigenic *Aspergillus flavous* with varying percentage of inhibition. Methanol extract was most potent in inhibiting the growth of *Aspergillus flavous* at 400 ppm (Pankaj et al. 2010). It is plausible that essential oil from Juniper species can be used to cure fungal infections, based on the findings of several investigations.

Anti-platelet and vasorelaxing: Thrombosis, which occurs when blood forms a clot in a blood vessel and inhibits blood flow, is the major cause of death from myocardial infarction. Interaction between platelets and blood vessel wall plays a significant role in thrombosis and atherosclerosis. Anti-platelet and vasorelaxing effects were found in 14-acetoxycedrol, which was synthesized from 8,14-cedranediol isolated from *Juniperus squamata* Hayata. Acetylation of 8,14-cedranediol (a sesquiterpene from *J. squamata*) yields 14-acetoxycedrol. 14-acetoxycedrol inhibits the production of thromboxane and leads to phosphoinositide breakdown. This is believed to be the basis for its anti-platelet activity, whereas its vasorelaxing impact is ascribed to its reduction of Ca^{2+} influx through the voltage-dependent Ca^{2+} channel (Teng et al. 1994).

Xanthine oxidase inhibitory activity: Xanthine oxidase inhibitory activity was determined in *Juniper recurva* plant extract (Rashid and Kuchay 2016). Xanthine oxidase, a cytoplasmic enzyme, catalyzes purine degradation by converting hypoxanthine and xanthine to uric acid, producing reactive oxygen species in the process. The reactive oxygen species and the uric acid can have harmful effects on the body. Gout is a clinical condition caused by elevated concentration of uric acid, and xanthine oxidase inhibitors can be used to treat it. Methanol fraction of *J. recurva* possessed maximum inhibitory activity against xanthine oxidase activity present in samples treated with different concentrations of hypoxanthine on the second, third, fourth, and fifth weeks in comparison to other plant extracts (Mahajan et al. 2012).

Xanthine oxidase inhibitors derived from plants can be further explored to check the efficacy and dose required to treat the disease.

Antioxidant: Antioxidant activity of Juniper species has been verified in various studies. Methanol extract of *J. recurva* was evaluated for in vitro antioxidant activity. Diphenyl picryl hydrazine scavenging, nitric oxide scavenging, metal chelating, and reducing power activities were all used to validate the antioxidant activity in vitro. The key component found in *J. recurva* extract was gallic acid. Gallic acid, demonstrated to be a very powerful antioxidant in vitro and in vivo studies, could be responsible for the extract's significant antioxidant capacity. The extract enhanced memory and increased motor coordination performance in mice in in vivo trials. Following the administration of the extract, serum acetylcholinesterase activity was reduced. The memory-enhancing action of *J. recurva* extract is attributed to the suppression of the acetylcholinesterase enzyme (Kumar et al. 2019).

Juniperus communis also possess significant antioxidant activity which was demonstrated in vitro. The peroxidation of a linoleic acid emulsion was inhibited by up to 92%, with the help of aqueous and ethanol extracts of Juniper fruit. At concentrations of 20, 40, and 60 μg/mL, it had significant reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating properties. It can be deduced that juniper possesses good in vitro antioxidant activities, which is the foundation for its lipid peroxidation inhibition properties (Elmastaș et al. 2006). Juniper berry essential oil showed antioxidant potential at a concentration of 10 ppm in *Caenorhabditis elegans*. Worms treated with Juniper berry oil had increased survival rate and improved resistance to stress. Juniper berry oil can be commercialized for the formulation of anti-aging products (Pandey et al. 2018).

Cytotoxic: *J. recurva*, a medicinal plant from Nepal, was evaluated for its cytotoxic effect against breast cancer cell lines. Different plant parts were checked for cytotoxic activity, for instance, leaf and seed extract was taken from two different parts of the tree, viz., JRL1, JRL2, JRSO (with outer seed coat), and JRSib (inner black seed without the coat). According to the findings, the percentage inhibition was dose sensitive. The IC_{50} values obtained were 40.38 μg/mL for JRL1 and 50 μg/mL for JRL2, JRSO, and JRSib respectively (Bhandari et al. 2015). Further research work is needed to establish the cytotoxic activity of *J. recurva* against various cell lines. The literature contains several examples of essential oils from other Juniperus species which have cytotoxic activity. Essential oils from *Juniperus excelsa* and *J. oxycedrus* showed cytotoxic activity CEM/ADR5000 leukemia cells (Saab et al. 2012). Berry and leaf essential oil derived from *Juniperus phoenicea* exhibited cytotoxic activities against several human cell lines, viz., brain tumor, cervix carcinoma, lung carcinoma, liver tumor, and breast carcinoma. The presence of high amounts of monoterpenes might be responsible for high cytotoxic activity in them (El-Sawi et al. 2007).

Anticancer: The triggering of mitotic catastrophe by the ethanolic extract of *Juniperus squamata* suppressed cell proliferation in human oral cancer cell lines. Enlarged multinucleated cells, disrupted microtubule formation, and elevated phosphorylation of histone H3 at Ser^{10} were among the several characteristics that signal

the start of mitotic catastrophe. The decreasing levels of Mcl-1 protein indicated apoptotic cell death in multinucleated cells. The ethanolic extract of *Juniperus squamata* has been suggested as a possible treatment for oral cancer by triggering mitotic catastrophe and apoptotic cell death (Jung et al. 2021). Further, *J. recurva* has been shown to possess remarkable anti-proliferative activity as well (Rashid and Kuchay 2016; Ivanova et al. 2020).

Juniper indica Bertol extract suppressed cell cycle progression and caused apoptosis in oral cancer cells, demonstrating anticancer potential. It can be combined with chemotherapeutic medications as an adjuvant to reduce pharmacological adverse effects. Oral cancer cells were more effectively killed by a combination of *Juniper indica* Bertol extract and cisplatin than by either treatment alone. Our findings confirmed that *Juniper indica* Bertol extract could be a safe drug for reducing the likelihood of adverse effects, making it a promising choice for future clinical trials with cisplatin (Huang et al. 2021).

Immunomodulatory and antioxidant activity: *Juniper squamata* was demonstrated to possess antioxidant and immunomodulatory activity. The phenolic content of the methanolic extract was the highest, whereas that of the petroleum ether extract was the lowest. Methanolic extract also demonstrated maximum DPPH radical scavenging at 700 g/mL concentration. Furthermore, IC_{50} values were determined for methanol extract which suggests that it is an effective scavenger of hydrogen peroxide radical and lipid peroxidation. *J. squamata* plant extract significantly improved the delayed-type hypersensitivity response and phagocytic carbon clearance in treated animals. The findings suggest that the methanolic leaf extract of *Juniperus squamata* possesses powerful antioxidant and immunomodulatory properties (Ali et al. 2018).

Neutrophils being the most abundant white blood cells function in innate immunity and aids in the human inflammatory response (Malech et al. 2020). Hence, neutrophils are a promising pharmacological focus for drug development. There are several examples wherein essential oils from plants can modulate immune responses based on neutrophils (Ozek et al. 2017). Essential oil derived from Juniper species, viz., *J. horizontalis*, *J. scopolorum*, *J. communis*, *J. seravschanica*, *J. sabina*, *J. pseudosabina*, *J. pseudosabina* subsp. *turkestanica*, and *J. sibirica*, contained cedrol, which is believed to play a role in immunomodulatory impact on neutrophils (Ozek et al. 2021). Juniper essential oils and pure cedrol were found to cause intracellular mobilization of Ca^{2+} in human neutrophils. Human neutrophils and N-formyl peptide receptor 1 and 2 (FPR1 and FPR2) transfected HL60 cells were pretreated with juniper essential oil or pure cedrol led to a decrease in agonist-induced Ca^{2+} mobilization, implying that these responses were desensitized (Fig. 12.3).

Fig. 12.3 Pharmacological uses of *Juniper recurva*

12.9 Key Secondary Metabolites

Seca and Silva (2006) have described the compounds found in *Juniperus* species, viz., coumarins, flavonoids, lignans, sterols, terpenoids (sesquiterpenoids, bicyclic diterpenoids, tricyclic terpenoids, etc.). Several of these compounds possess biological properties which are well documented.

Cupressuflavone: Hameed et al. (1973) identified biflavones in dried leaves of *Juniperus* plants. A total of three bands were identified which corresponded to amentoflavone, mono-, and dimethyl ethers, respectively. Band named JR1 was considered to be a mix of amentoflavone and cupressuflavone, while band JR2 contained a mixture of amentoflavone monomethyl ether, cupressuflavone

monomethyl ether, and hinokiflavone. Band, JR3, had amentoflavone dimethyl ether and cupressuflavone dimethyl ether.

Hinokiol: Hinokiol, found in several *Juniperus* species including *Juniperus squamata* Buch.-Ham. ex D. Don, possesses pharmacological properties (Seca and Silva 2006; Seca et al. 2015). It inhibits the synthesis of nitric oxide, tumor necrosis factor (TNF-α), and pro-inflammatory enzymes from lipopolysaccharide-stimulated macrophages. It also possesses antioxidant and hepatoprotective in addition to anti-tumor properties (Alqasoumi and Abdel-Kader 2012; Gaspar-Marques et al. 2008; Wang et al. 2002).

Deoxypodophyllotoxin (DPT): Deoxypodophyllotoxin (DPT) is an aryltetralin cyclolignan which is found in several Juniperus species, for example, *J. squamata* and *J.recurva* Buch.-Ham. ex D. Don. (Seca and Silva 2006; Seca et al. 2015). DPT is highly cytotoxic in addition to anti-inflammatory and anti-angiogenic activity (Jiang et al. 2013; Wang et al. 2015).

Cedrol: Several biological properties have been documented in case of Cedrol, a sesquiterpene. For instance, anti-inflammatory, analgesic, anticancer are a few bio-activities shown by cedrol in various investigations (Chang et al. 2020; Mishra et al. 2020; Zhang et al. 2016). Ozek et al. (2021) reported the immunomodulatory potential of pure cedrol or Juniper essential oil samples containing a high concentration of cedrol.

12.10 Tissue Culture

Seed germination is typically ineffective and inconsistent for *Juniperus* species (Wesche et al. 2005; Al-Ramamneh et al. 2012). Several factors that contribute to ineffective germination are insufficient pollination, pest-infested cones, and a low percentage of viable and developed seeds at harvest (Ortiz et al. 1998; Wesche et al. 2005). Vegetative propagation via stem cuttings has been documented in *J. squamata* (Kentelky 2011).

12.11 Patent

Hazan and Lucassen (2015) evaluated composition of extracts from gymnosperms belonging to the Cupressaceae family which were associated with therapeutic value. Several examples of species belonging to the Cupressaceae family include *Tetraclinis articulata, Cupressus sempervirens, Cupressus finebris, Cupressus goveniana, Cupressus forbesii, Cupressus guadalupensis, Cupressus marcrocarpa, Cupressus abramsiana, Juniperus communis, Juniperus conferta, Juniperus rigida, Juniperus phoenicea, Juniperus cedrus, Juniperus deltoides, Juniperus navicularis, Juniperus oxycedrus, Juniperus macrocarpa, Juniperus chinensis, Juniperus excelsa, Juniperus polycarpos, Juniperus indica, Juniperus komarovii, Juniperus*

procera, Juniperus procumbens, Juniperus pseudosabina, Juniperus recurva, Juniperus Sabina, Juniperus Saltuaria, Juniperus semiglobosa, Juniperus squamata, Juniperus thurifera, Juniperus tibetica, and Juniperus wallichiana. Pharmaceutical formulations for the treatment of neurodegenerative disorders were created. Formulations were derived from plant material which included resin, bark, fruits, leaves, roots, twigs, wood, or seeds. These formulations could help in the treatment of neurological disorders, prevention and treatment of fibrotic conditions, reduction of scar at wounds, and tissue repair.

12.12 Conservation

Juniperus recurva var. *coxii* has been categorized as Near Threatened on the IUCN (International Union for Conservation of Nature) Red List of Threatened Species (Li et al. 2013). Juniperus is important to the livelihoods of poor and inaccessible mountain communities who endure extreme climatic conditions and have limited access to alternative fuel and energy sources. Excessive harvesting of juniper wood and leaves, overgrazing, habitat fragmentation, and poor regeneration capability are some of the major threats to juniper forest conservation. Several species of Juniperus genus are ideal for afforestation programs in cold deserts because of their capacity to survive in arid situations, such as *Juniperus polycarpos* C. Koch, *Juniperus indica* Bertol., and *Juniperus communis* L. var. saxatilis (Pallas). These are major species occurring in the Lahaul valley in the north-western Himalayas (Rawat and Everson 2012).

12.13 Conclusion and Future Perspective

Plants have historically provided bioactive compounds that have been used to cure ailments. Herbal remedies used for the prevention and treatment of several disorders are becoming quite popular. The Indian Himalayas offer a diverse range of medicinal plants that are being researched and have a lot of potential for commercialization in the form of plant-based pharmaceuticals. Juniper species possess pharmacological properties such as antibacterial, antifungal, antioxidant, anticancer, cytotoxic, xanthine oxidase inhibitory activity, immunomodulatory, anti-platelet, and vasorelaxing. Further research, particularly clinical trials, is required to establish pharmacological, toxicological, and safety aspects.

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Chapter 13
Lancea tibetica Hook.f. & Thomson: Ethnobotany, Phytochemistry, and Pharmacology

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13.1 Introduction

Lancea tibetica Hook. & Thomson (Fig. 13.1) belonging to the family Mazaceae, (Mazaceae), is a small perennial herb mostly found in the mountains of India, China, Bhutan, and Tibet at an altitude of 2000–4500 m. The taxonomic history of the plant species has long debatable issues due to its controversial systematic position in different families. Earlier, it was in the family Scrophulariaceae and then transferred to the family Phrymaceae and now, it is in Mazaceae. Generally, flowers are coming in the month of May to July and fruit in the month of July to September. It has 3–5 fascicles of purple or blue flowers or flowers are arranged in a raceme inflorescence with 6–10 rosulated leaves. Corolla with a yellow throat with purple dots. Fruits are red to dark purple in an ovoid shape. Different phytochemical studies on *L. tibetica* reveal that it possess approximately 71 compounds having various pharmacological properties such as an antitumor, antifungal, antimalarial, antioxidant, hypoglycemic-inhibiting, and hepatoprotective properties (Song et al. 2011; Liu et al. 2014, 2015). Most of these compounds belong to phenylpropanoids, flavones, and triterpenoides. This chapter summarizes all the related information associated with the plant and illuminates current advancements associated with the plant.

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Fig. 13.1 *Lancea tibetica* Hook. & Thomson

13.2 Botany

First the genus *Lancea* was described in 1857 by Hook.f. and Thomson while their botanical excursion in India. They have described many new species of Indian Scrophulariaceae along with Lancea in their Hookers Journal of Botany and Kew Garden Miscellany. They collected it from the vicinities of Sikkim Himalaya and named it in the honor of the Bengal Service sir Mr. Lace. The plant is annual with a height of 3–7(−15) cm and the leaves are arranged in rosulate form having 6–10 numbers; the leaf blade is obovate or spathulate with 2–8 cm length, margin is entire or obscurely or sparsely toothed, apex obtuse and usually apiculate. Flowers are arranged in 3–5 flowered fascicles inflorescence or in a raceme inflorescence with subulate-lanceolate bracts. Calyx is about 1 cm long and leathery, with subulate-triangular lobes. Corolla dark blue to purple, 1.5–2.5 cm long, tube 0.8–1.3 cm long, throat yellowish or with purple dots; lower lip middle lobe entire; upper lip erect, deeply two-lobed, rarely shallowly two-parted. Stamens are inserted near the center of the tube, and the filaments are glabrous. The fruit is red to dark purple, ovoid in shape, and about 1 cm long, with a persistent calyx. Seeds are numerous, brownish yellow, oblong in shape, and about 1 mm long. Generally, the flowering time of the plant is May to July and the fruiting time is from July to September.

13.3 Habitat and Distribution

The plant species is mainly present in the pasture and scattered forest lands at an altitude of 2000–4500 m and is distributed in different regions of China, Nepal, Bhutan, India (Jammu & Kashmir-Nubra, Ladakh, Leh, Rupshu, Zanskar), Pakistan, Baltistan, East Himalaya, and Mongolia.

13.4 Traditional Uses of *L. tibetica* Plant

In Tibetan Traditional medicine, the plant is used to treat leukemia, intestinal angina, heart disease, pneumonia, asthma, carbuncles, colic, heart disease, and cough. L. tibetica roots are known as "depgul" in Kashmir India and are used in powdered form along with tobacco after being roasted over a fire. This mixture is known to act as a stimulant when combined with milk or smoked with tobacco. Its roots are also used in the healing of the lungs and cough. It draws out any pus that has formed in the lungs and dries up vitiated blood and *chu-ser* (serum) caused by wound formation in the lungs. Leaves are known to have healing properties for wounds and skin diseases. Due to its significant uses in the medicinal world and its pharmacological activities, the plant has received much attention throughout the world.

13.5 Phytochemistry

The plant consists of many chemical compounds, viz., lignans, glycoside phenylpropanoids, triterpenoids, flavonoids, amino acids, propanediols, and glycosides which are responsible for various pharmacological activities. Other compounds such as steroids (ergosterol, β-sitosterol), alkaloids, and pigments (lycopene) are also present in trace amounts in various parts of the plant. Figure 13.2 presents the structures of some key secondary metabolites obtained from *L. tibetica.*

Phenylpropanoids: Phenylpropanoids are important plant secondary metabolites derived from phenylalanine. Several numbers of phenylpropanoids were identified in *L. tibetica*. The major phenylpropanoids present are:

Lignans: Lignans are the important constituent of the *L. tibetica*. Total 16 lignan compounds were identified till now after years of research (Su et al. 1999; Li et al. 2008; Liu et al. 2014). These lignan compounds were proven to have various health beneficial properties such as antioxidant and anticancerous properties.

Neolignans: The fruits of *L. tibetica* have yielded a number of neolignans however only five have been identified so far by Duan (2005).

Nonanones: Few nonanones were identified from fruit of *L. tibetica* plants including Tibeticone A, Tibeticone B, and Tibeticone C.

Phenylpropanoid glycosides: Till now, a total of nine phenylpropanoid glycosides have been identified in *L. tibetica* plants namely Parvifloroside A, Acteoside, Isoacteoside A, Leucoseptoside A, Styraxjaponoside C, 1-*O*-Feruloyl-β-D-glucopyranose, Phillyrin, Lantibeside, Lantibeside B (Wang et al. 2019).

Propanediol: In the fruits of *L. tibetica* four propanedoils were found so far from ethanol extracts by duan et al., 2005: R-(+)-3-(3,4,5-trimethyxyphenyl)-1,2-propanediol, R-(+)-3-(5-methoxy-3,4-methylenedioxyphenyl)-1,2-propanediol, (+)-3-(3,4-Methylenedioxyphenyl)-1,2-propanediol, 1-(3-Methoxy-4-hydroxyphenyl)-1,2-propanediol.

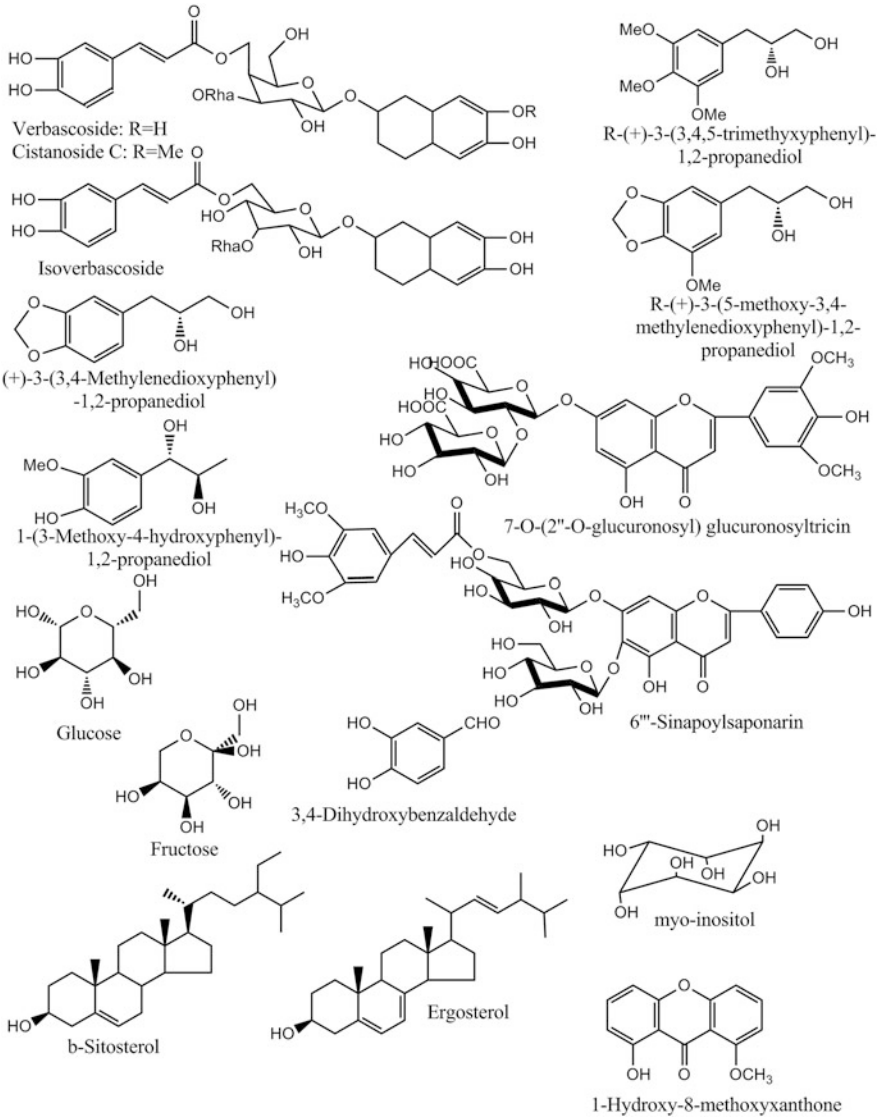

Fig. 13.2 Chemical structure of compounds found in *Lancea tibetica*

Triterpenoids: Triterpenoids are natural compounds abundant in the plant kingdom. They impart a role in defense mechanism against pathogens and herbivores (Sawai and Saito 2011). Having diverse functions in plants and health aspects of human, they have been extensively studied in different plants. In *L. tibetica*, total of six triterpenoids have been identified in the whole plant till now. Oleanolic acid, ursolic acid, betulinic acid, (3β)-Lup-20(29)-en-3-yl stearate, 2α, 3-α-Dihydroxyolean-12-en-28-oic acid, dillenic acid B (Zheng et al. 1985; Zhang

et al. 1987; Liu et al. 2014). Ursolic and oleanolic are the primary triterpenoids with 0.96 and 0.75%, respectively of the total content (Lu and Ma 2009; Liu et al. 2015).

Flavonoids: Flavonoids are important plant-specialized metabolites present ubiquitously in the plant kingdom. These metabolites perform an important role in plants like pigmentation to flowers and seeds, and protection against various biotic and abiotic stresses (Winkel-Shirley 2001). Flavonoids also have various health beneficial properties and provide protection against diseases such as anti-apoptotic, anticancerous, immunosuppressive, anti-inflammatory, protection against cardiovascular disease (de Pascual-Teresa and Sanchez-Ballesta 2008). In *L. tibetica,* the major flavonoids present are Apigenin, 5,40—Dihydroxyflavone, chrysoeriol, luteolin, selagen, tricin, 5-Hydroxy-40—methoxyflavone, 7-O-(200-O-glucuronosyl) glucuronosyltricin, 6”-Sinapoylsaponarin 3 which are isolated from ethanolic extract of the whole plant (Li et al. 2008; Liu et al. 2014).

13.6 Pharmacological Activities

Due to the presence of important chemical compounds, *L. tibetica* possess various pharmacological properties. It has been proven that the species has multiple potential effects towards different diseases. Its traditional values, especially in Tibetan system of medicine, signify its importance in the medicinal drug world. Some of the important properties are mentioned as follows and explained in Table 13.1.

Antioxidative activity: Antioxidants are important compounds which prevent cell against damage by inhibiting oxidation and free radicals production. The extracts of the plant showed antioxidant activity due to the presence of certain compounds like verbascoside, tibeticone A, cistanoside C, and isoverbacoside performed by DPPH tests in vitro (Duan 2005; Li et al. 1997). A recent study by Wang et al. (2019) has established an online HPLC-DPPH method for isolating compounds with antioxidant activity from *L. tibetica* plant. Seven phenylpropanoid compounds were isolated which shows antioxidant activity as (+)-pinoresinol-β-D-glucoside, isoacteoside, acteoside, tibeticoside, epipinoresinol, anthelminthicol, and phillygenol. Two lignans namely Sylvaesmin and Lantbeside from *L. tebitica* plant were found to possess oxidized free radicals scavenging property when tested using pulse radiolysis techniques (Miao et al. 2004).

Antimalarial effects: Due to the abundance of different secondary metabolites such as phenylpropanoids, flavonoids, and alkaloids in the *L. tibetica* plant, it is reported to have antimalarial activity against *Plasmodium falciparum*. Studies using extract from different plant parts revealed that the compounds like 1-(l,3-Dihydroxyphenyl)-9-(4-hydroxyphenyl) nonan-1-one and (+)-Erythro-(7S,8R)-D80-7-hydroxy-3,4,5,30,50-pentamethoxy-8-O-40—neolignan shows antimalarial activity against *Plasmodium falciparum* at different concentrations (Pham et al. 2000; Duan 2005; Zhang et al. 2001).

Antimicrobial and Antifungal effects: *L. tibetica* plant possesses antimicrobial and antifungal activity due to the presence of phenolic and lignan compounds. Different

Table 13.1 Chemical compounds of *Lancea tibetica* and their therapeutic uses

S. No.	Compound name	Health beneficial properties	References
1.	Phenylpropanoids		
	(a) lignans	Antioxidant, anticarcinogenic	Su et al. (1999), Li et al. (2008), Liu et al. (2014)
	(b) Neolignans	Anti-carcinogenic, antimalarial, antifungal, and antioxidative	Duan (2005)
	(c) Nonanones	Anti-carcinogenic, antimalarial, antioxidant, and antimicrobial	Duan (2005)
	(d) Phenylpropanoid glycosides	Antioxidant	Wang et al. (2019)
	(e) Propanediols	Antioxidant	Duan (2005)
2.	Triterpenoids	Anti-diabetic and antimicrobial	Sawai and Saito (2011), Zheng et al. (1985), Zhang et al. (1987), Liu et al. (2014), Lu and Ma (2009), Liu et al. (2015)
3.	Flavonoids	Anti-carcinogenic, cardiovascular, immunosupresive, anti-inflammatory	de Pascual-Teresa and Sanchez-Ballesta (2008)
	(a) Flavonols	Anti-apoptotic, anti-osteoporotic	Abotaleb et al. (2018), Patel et al. (2018)
	(b) Anthocyanin	Anti-carcinogenic, anti-inflammatory, antioxidative	Lim and Song (2017), Abotaleb et al. (2018)
	(c) Proanthocyninidin	Antioxidative, anti-carcinogenic, anti-diabetic	Abdulkhaleq et al. (2017), Abotaleb et al. (2018)

compounds when tested for antimicrobial activity against microorganisms nonanones were found to have antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* (Orabi et al. 1991; Duan 2005). Neolignans compound (+)-Erythro-(7S,8R)-D80-7-hydroxy-3,4,5,30,50-pentamethoxy-8-O-40—neolignan was reported to contain antifungal activity against *E. floccosum* in an agar dilution assay (Zacchino et al. 1998; Duan 2005).

Hypoglycaemic effects: Because of the presence of triterpenoids like oleanolic acid which is reported to increase insulin level in pancreatic β-cells by Teodoro et al. (2008), *L. tibetica* plant may have a role in diabetes prevention.

Antitumor effects: Certain compounds from *L. tibetica* have been shown to have strong antitumor activities. Verbascoside and isoverbascoside were found to have antitumor properties in pharmacological studies. Compound sylvatesmin had shown antitumor activity on mouse melanotic carcinoma cells (B16 cells) by MTT assay (Su et al. 1999). Lignan compounds Lantebiside B and Lantebiside D when tested for cytotoxic activity with MTT method against HL-60 and MOLT-4 cells have very less cytotoxicity against HL-60 cells (Human leukemia) (Li et al. 2008).

13.7 Conclusion and Future Prospectives

Lancea tibetica is a potential plant species having a narrow niche, especially in alpine region of the Asian region. Because of the presence of multiple compounds, particularly phenolpropanoids, flavones, and triterpenoids, it possesses multiple health beneficial properties such as antioxidant, antitumor, antimalarial, antifungal, and antimicrobial. In this chapter, we have summarized the precise botany, phytochemistry, and pharmacology of *Lancea tibetica* plant. Further detailed picture of the signaling pathways and chemistry of different compounds present in the plant is needed to study. Earlier, a few studies are based on its chemistry and ethnopharmacological activities but no systematic review is available so far. The plant is widely used in various systems of medicine and hence its diverse chemical composition needs to be understood and explored. Comprehensive animal studies to determine pharmaceutical and medicinal properties could help us learn more about this plant. Taken together, these studies have suggested its potential for future research work in the fields of pharmacy, chemistry, botany, and conservation.

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Chapter 14
Malaxis muscifera (Lindley) O. Kuntze. and *Malaxis acuminata* D. Don.: Jeevak

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14.1 Introduction

The history of the genus *Malaxis* is dated back to the year 1788. In that year, Solander first mentioned it in a research paper (Szlachetko and Margonska 2006). *Malaxis* is a genus of land-loving orchids. More than 200 species of this genus are found on the earth, and they dominantly occupy tropical montane ecosystems which are found in the footsteps of mountains. Some species of genus *Malaxis* are able to grow in rocks and also in epiphytic conditions. The root of the plant is found on the base of a cylinder shaped, not hard and herbaceous stem. The peduncle is not covered and small flowers of different colours grow on it in very high numbers and very narrow space is available between two flowers. The origin of the word "Malaxis" is from Greek literature which represents the soft appearance of plant leaves of genus *Malaxis* (Teoh 2016). *Malaxis acuminata* is found in many Asian countries which include Bhutan, China, India and Nepal (Lama et al. 2013). Himalayan region is the best area in India which is dominantly occupied by this plant species. *Malaxis acuminata* is not a very tall plant; it grows to a height close to 29 cm and always grows in normal soil conditions. The stems have pseudobulb on them and the base of the stem also contains foliose scale. The leaves are found in three to five groups, and it also has margins on the leaf surfaces. The flowering session of this plant is in the months of July, August and September (Gupta 2015). The chromosome number of *Malaxis acuminata D. Don* is $2n = 42$ (Löve 1980). *Malaxis muscifera* is commonly known as an adder mouth orchid. It is a land plant and it

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obtains a height almost between 14 and 44 cm. In Asia, *Malaxis muscifera* is found in a country like Nepal, Bhutan, Pakistan, China, etc. In India, it is found in a western Himalayan state like Uttarakhand and an eastern Himalayan state like Sikkim. The stem of *Malaxis muscifera* obtains a good height, and the pseudobulbs are found on the base of the stem. The plant body produces a very low number of leaves which is often close to two. Flowers are greenish yellow in colour which is found in terminal raceme inflorescence. The pedicle is often longer than the bract of the flower. Many important biochemicals like alkaloid and flavonoids are present on the pseudobulb of *Malaxis muscifera* (Balkrishna et al. 2012). Indian traditional medicine has a very long history. The oldest documentation about the use of orchids is dated back to the Vedic era, when many traditional medicines were prepared from Orchids (Ming et al. 2003). Four types of orchids including *Malaxis muscifera (Lindley) O. Kuntze, Malaxis acuminata D. Don* and other plants are used in "Astavarga" plants; there are eight medicinal plants in this group that are used to prepare various traditional medicine. Tuberculosis is a very harmful disease; it can kill people very easily. In Ayurveda, *Malaxis acuminata* is used to treat this deadly disease (Kant et al. 2012). This traditional knowledge of orchid oriented medicine is passing through the different generations of ethnic people, and many of them do not even have any written evidence (Khajuria et al. 2017).

In this book chapter, we will discuss two medicinally important orchid species known as *Malaxis muscifera (Lindley) O. Kuntze* and *Malaxis acuminata D. Don* which are included under genus *Malaxis*. Both orchids are found in the western Himalayan region and both have separate uses in Ayurvedic treatment. Due to morphological similarities, many people confused these two orchids as one; so here we will try to separate both species as much as possible through our detailed analysis on various parameters embedded in the upcoming points.

14.2 Morphology and Taxonomy

Scientific name: *Malaxis acuminata* D. Don.

Synonyms: *Crepidium acuminatum* (D. Don) Szlach, *Microstylis acuminata* Ridl.

Malaxis acuminata D. Don belongs to the kingdom Plantae. Later, it is included in division Tracheophyta. *Malaxis acuminata* D. Don is classified under class Liliopsida (Gupta et al. 2015). The order Asparagales includes the family Orchidaceae in old systems of classification (Kim et al. 2012). *Malaxis acuminata* D. Don is a plant classified under the family Orchidaceae. It is further classified under subfamily Epidendroideae. This orchid plant is included under tribe Malaxideae. *Malaxis acuminata* D. Don is a plant under genus *Malaxis* (Jalal et al. 2008a).

There are a few very notable taxonomic characters observed in *Malaxis acuminata* D. Don which helps in the identification and classification of this particular plant. In *Malaxis acuminata* D. Don, most part of the stem is found

below the soil. From the lower parts of the stem hairy roots move towards the lower direction. Leaves are usually close to 3 in number and leaves are often fixed in one place. Leaves also have petioles on them. Leaves are 8–13 cm in length and ovoid lanceolate in shape. Clear margins are visible on the leaf surface of *Malaxis acuminata* D. Don. Leaves are present in clusters and the basal leaves are also found in the stem which forms a tube-shaped structure. Greenish flowers are found in *Malaxis acuminata* D. Don which have a stalk on it. The length of the flower is 8–21 cm. Flowers are arranged in terminal raceme inflorescence which is close to 11 mm in diameter. Bracts of the flower are spread smaller than the ovary. Sepals are shorter than the petals. Petals are not thick and five lips are also found in the flower. Four numbers of pollinia are found in this flower. Capsules are chambered and six capsules are found in one fruit. Numerous small powders like seeds are formed in *Malaxis acuminata* D. Don. Pseudobulbs are 2–8 cm in length and in diameter; it is close to 2 cm. Pseudobulbs are not found singly and it is greenish in colour, not hard and slimy in appearance. Flowers bloom in the month of August in normal conditions, and fruits are formed after one to 2 months of flowering (Gupta et al. 2015).

Scientific name—*Malaxis muscifera* (Lindley) O. Kuntze.

Synonyms: *Malaxis muscifera* (Lindley) O. Kuntze are Microstylis muscifera (Lindl.) Ridl., and Dienia muscifera Lindl.

A detailed classification of *Malaxis muscifera* (Lindley) O. Kuntze is not added here due to unavailability of authentic data. The plant family Orchidaceae is classified under order Asparagales (Kim et al. 2012). *Malaxis muscifera* (Lindley) O. Kuntze is included under the plant family Orchidaceae. *Malaxis muscifera* (Lindley) O. Kuntze is a species belonging to subfamily Epidendroideae. This plant species is classified into tribe Malaxideae. *Malaxis muscifera* (Lindley) O. Kuntze is classified under genus *Malaxis* (Jalal et al. 2008b).

Few characters are used to identify and classify *Malaxis muscifera* (Lindley) O. Kuntze. The stem of *Malaxis muscifera* (Lindley) O. Kuntze is not short, upright and tube-shaped. At the lower part of the stem, pseudobulb is found. Usually, a number of leaves are close to two with different shapes, and petiole is found in the leaves of *Malaxis muscifera* (Lindley) O. Kuntze. The length of the leaf is close to 4–9 cm and the width is close to 3–5 cm. The shape of the leaf is ovate-lanceolate or rounded and a thin petiole is attached at the lower part of the leaf. Flowers of *Malaxis muscifera* (Lindley) O. Kuntze are very small only 3 mm in length. Pale yellowish-green flowers have 4–5 mm long pedicles and terminal raceme inflorescence is found in *Malaxis muscifera* (Lindley) O. Kuntze. Pedicles are longer or equal to the bract in length. Petals are linear and sepals are lanceolate in shape and sepals are longer than petals. The labellum is found in *Malaxis muscifera* (Lindley) O. Kuntze flowers and it serves as an attraction for insects during pollination. The column is very small with a small spreading arm. Four pollinia are found in the flower of *Malaxis muscifera* (Lindley) O. Kuntze. Appearance of round and white pseudobulb is a characteristic feature of this plant. Then the length of the pseudobulb is close to 3–8 cm and the diameter is close to 1 cm. Slimy substances are found in the pseudobulb of *Malaxis muscifera* (Lindley) O. Kuntze. Flowers bloom in the month of July to August month and fruits are developed after 1–2 months (Balkrishna et al. 2012).

14.3 Common Names

Many tribal communities all over the world use *Malaxis acuminata* D. Don for medicinal purpose, but common name used by them to point out this plant is yet to be studied; that is why we are able to add only limited data in this field. *Malaxis acuminata* D. Don is very popular as Jeevak in Ayurvedic medicine (Khajuria et al. 2017). This plant is used as a part of Astavarga in Ayurvedic medicine. Sanskrit which is an ancient language; this plant is known as Cirajivi, Jivya and Dirghayu. Hindi is the most popular language in India. In Hindi *Malaxis acuminata* D. Don is known as Jeevak. In the southern part of India, this plant is also used in medicine. In Tamil and Malayalam, *Malaxis acuminata* D. Don has the same name, i.e. Jeevakam. In Telugu, this Orchid plant is known as Jeevakamu (Sharma et al. 2014). Besides, few other common names of this orchid are Dirghayu, Svadu, Chirnjivi, Shringaka, Harsanga, Pranda, Kurchashira, Madhurak, Ksveda, Madhur and Kurchkakaar (Kumar et al. 2018).

Malaxis muscifera (Lindl.) O. Kuntze is also included under Astavarga of Ayurvedic treatment. In nervous diseases, it is used very extensively. In Ayurveda, it is known as Rishbhake (Khajuria et al. 2017). In the Garhwal region of Uttarakhand, it is also known as Rishbhake. In Nepali, it is known as Jivaka (Teoh 2016). There are various common names used for *Malaxis muscifera* (Lindl.) O. Kuntze. Few common names are Dheera, Bandhura, Inderaksa, Gopati, Durdhara, Kakuda, Vrishshringvat, Lashunkand, Vrisha, Matrika, Vishan, Nissar, Varishnabha and Suksampatrak (Kumar et al. 2018).

14.4 Flowering/Fruiting Season

When we compared the flowering season of *Malaxis acuminata* D. Don and *Malaxis muscifera* (Lindley) O. Kuntze with eastern Himalayan orchids, we observed differences in flowering months. Eastern Himalayas cultivated terrestrial plants like *Calanthe chloroleuca* Lindley which bloom between the month of April and May; *Calanthe masuca* (D. Don) Lindley blooms during August and September; *Calanthe plantaginea* Lindley blooms in the month of March and April, *Calanthe puberula* Lindley blooms between July and August, *Esmeralda cathcartii* (Lindley) Reichenbach blooms in the month of March and April, *Paphiopedilum hirsutissimum* (Lindley) Stein blooms in the month of April and May, *Phaius flavus* (Blume) Lindley blooms in the month of March and April, *Phaius tancervilliae* (Aiton) blooms in the month of April to June and *Tainia latifolia* Bentham ex Hooker blooms in the month of March and April (Deb and Imchen 2011).

Previously, we mentioned that both *Malaxis acuminata* D. Don and *Malaxis muscifera* (Lindley) O. Kuntze are dominantly found in the western Himalayan region. The flowering season of *Malaxis acuminata* D. Don is July and August while fruits are developed almost after 2 months. The fruiting season of *Malaxis*

acuminata D. Don is in the month of September and October. *Malaxis muscifera* (Lindley) O. Kuntze also blooms in the month of July and August, and the fruiting season of this plant is between September and October; almost 2 months after blooming season (Balkrishna et al. 2012).

14.5 Distribution

For the study and conservation of threatened and rare plant diversity, pattern of distribution and study of the surrounding environment are very important (Djordjević et al. 2016). Here we mention the distribution of *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don in three steps. First, we describe the distribution of both orchid species in different countries; in the second step, we mention all the states of India where these two species of *Malaxis* are found. In the last step, we mention a specific area in western Himalayan states where these two orchid species are found.

Malaxis muscifera (Lindley) O. Kuntze is found in many Asiatic countries. *Malaxis muscifera* (Lindley) O. Kuntze is found in Nepal, Afghanistan, China, Pakistan, Bhutan and India etc. All of these countries are situated very close to each other (Rajashekhar et al. 2015). *Malaxis muscifera* (Lindley) O. Kuntze is found in Indian states like Jammu and Kashmir, Sikkim, Uttarakhand and Sikkim (Vij et al. 2019). It is also found in North-East Indian states like Arunachal Pradesh (Kant et al. 2012) and Sikkim (Vij et al. 2019). Different areas of western Himalayan states where *Malaxis muscifera* (Lindley) O. Kuntze is found are as follows—in Jammu and Kashmir, *Malaxis muscifera* (Lindley) O. Kuntze is found in Leh and Gulmarg; in Uttarakhand, it is found in places like the Jumma area, Bakariudiyar, Dunagiri, Pithoragarh, Bajmora, Ralam Valley, Chamoli, Vasuki tal, Palangarh, Dhanaulti, Ralam, Almora, Deoban Tehri, Masar Tal, Dehradun chakrata; in Himachal Pradesh, *Malaxis muscifera* (Lindley) O. Kuntze is found in Sangla, Rahala forest, Chamba, Dhanchoo and few places of Shimla like Fagu, Hatu forests, Mashobra, etc. (Balkrishna et al. 2018). The range of distribution of *Malaxis muscifera* (Lindley) O. Kuntze in Himachal Pradesh is very close to 1799–3649 m (Kant et al. 2012).

Malaxis acuminata D. Don is distributed in countries like Thailand, Bhutan, Myanmar, China, Nepal and India (Kant et al. 2012) and also found in Cambodia (Balkrishna et al. 2012). It is found in North-East Indian states such as Assam, Mizoram, Manipur, Meghalaya, Tripura and Arunachal Pradesh (Kant et al. 2012). One of the most notable points in the distribution pattern of *Malaxis acuminata* D. Don is that this orchid species is found in Andaman Island. It is also found in Sikkim and Nilgiris (Gupta 2015). *Malaxis acuminata* D. Don is also distributed in the Southern part of India in Anaimalai Hills and Travancore. This plant also grows in the Madhya Pradesh, India (Balkrishna et al. 2012). Different places in western Himalayan states where *Malaxis acuminata* D. Don is found are as follows—in Uttarakhand, it is found in Ranikhet, Down to the Mussoorie bypass road,

Chaubatia, Dehradun—Camelback road, Almora, the upper side of Barlow ganj, Ramgarh, Chakrata, Bhowali, Jaunsar, Nainital, Tehri, Thal Kedar, Pauri-Pode khal, Berinag, Nagnath, Chamoli, Sarju Valley, Ukhimath, Gopeshwar; in Himachal Pradesh, *Malaxis acuminata* D. Don is found in Hatu, Elysium Hill, Chail, Shimla-Glen. Chamba, Boileauganj, Khajjiar, etc. (Balkrishna et al. 2018). Range of distribution of *Malaxis acuminata* D. Don in Himachal Pradesh is close to 1749 m to 2299 m (Kant et al. 2012).

It is very easily observable that *Malaxis acuminata* D. Don is more widely distributed in comparison to *Malaxis muscifera* (Lindley) O. Kuntze. *Malaxis acuminata* D. Don is found in the northeastern, southern part of India; it is even found in Andaman but *Malaxis muscifera* (Lindley) O. Kuntze is distributed only to a limited area of the western Himalayan state dominantly. But the distribution range of *Malaxis muscifera* (Lindley) O. Kuntze is higher than *Malaxis acuminata* D. Don.

14.6 Traditional Uses

Malaxis muscifera (Lindley) O. Kuntze is used to treat body weakness and the powder of the pseudobulb is used as tonic (Bisht et al. 2013). In Chyawanprash preparation, *Malaxis muscifera* (Lindley) O. Kuntze is used as a major component and it is also used as a rejuvenating product (Jalal et al. 2008a). This plant is used to treat fever and burning sensation. It is also used to increase sexual desire. The root of *Malaxis muscifera* (Lindley) O. Kuntze is used as a tonic and it is also able to increase the flow of mother's milk (Hossain 2011). This plant is also helpful in the treatment of dysentery which is a common disease in India (Khajuria et al. 2017).

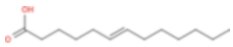
Malaxis acuminata D. Don is also used as a tonic. This orchid plant is also used in the treatment of tuberculosis. *Malaxis acuminata* D. Don has the ability to increase the sperm count. *Malaxis acuminata* D. Don is a prominent part of Astavarga medicine (Jalal et al. 2008a). It is used to increase body strength. Rhizome of *Malaxis acuminata* D. Don is used as a nutritional tonic which also helps in the treatment of tuberculosis. The pseudobulb of this orchid is used to treat burning sensation, bleeding diathesis, etc. For the treatment of fever, special products are produced from the pseudobulb of *Malaxis acuminata* D. Don. It is used to provide high sexual desire. *Malaxis acuminata* D. Don is used to treat thin body problems (Hossain 2011). The tuber of this plant shows analgesic and anti-inflammatory characters (Khajuria et al. 2017). This plant is usually used for fertility and treat sperm-related issues. It promotes the vitality of the human body and increases strength, body power, skin brightness and enhances other body characters. *Malaxis acuminata* D. Don is also helpful in gaining bodyweight. Extract of the pseudobulb of this plant has antioxidant properties. *Malaxis acuminata* D. Don with other herbs is used to treat diseases like asthma, aphasia, vaginal disease and other blood-related problems in the classical medicinal system. Honey, sugar and *Malaxis acuminata* D. Don plant with other herbs is used to treat heart problems and cough. Jivaniya Ghrita which is prepared with *Malaxis acuminata* D. Don and other plants is an

important treatment of vata-related diseases. From this plant, Mahamayur Ghrita is produced which is very beneficial for sensory organ disease of the human body. Ghrita of *Malaxis acuminata* D. Don and other herbs are beneficial for small babies also. Chitrakadi taila produced from this plant is used to cure many diseases like gout, limping, kyphosis, etc.. Production of Jivaniya ghrita, Mahapadma ghrita, etc. generally involves *Malaxis acuminata* D. Don plant, and these types of traditional medicines are used to treat fever, gout and other chronic disorders. In Malaria and anaemia, one famous medicine prepared from this plant is used generally which is commonly known as Asthapana Vasti. In traditional medicine, snake bite is treated with a powdery preparation of *Malaxis acuminata* D. Don and other herbs; in this process, powder and honey mixture is applied in the beaten part to reduce the toxic capacity of snake venom (Gupta et al. 2015).

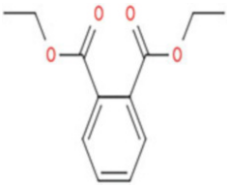
When we talk about traditional medicinal techniques, Ayurveda is the most famous among them. In ancient Indian literature, lots of plant species are described mainly in Veda (Jaiswal and Williams 2017). Sushruta Samhita, Charaka Samhita and Bhavaprarkasha Samhita describe the primitive use of orchids in the ayurvedic treatment method (Vaidya et al. 2000). There is no proper formula available for the kind of process used in the preparation of Chyawanprash in ancient ayurvedic time. Different preparation techniques are used by various production industries. According to Ayurvedic Pharmacopoeia of India 2007, both *Malaxis acuminata* D. Don and *Malaxis muscifera* (Lindley) O. Kuntze are important components of Chyawanprash preparation. More than 45 plant components are used in the preparation of Chyawanprash and both the studied orchids are two of them. Pseudobulb of *Malaxis acuminata* D. Don and root or another plant part of *Malaxis muscifera* (Lindley) O. Kuntze is used in Chyawanprash. Here most important point is that a root part of *Malaxis muscifera* (Lindley) O. Kuntze gets more importance than pseudobulb in Chyawanprash preparation (Wagh et al. 2013). Chyawanprash has been more nutritious from ancient times in India. Many nutritional substances like fibres, Cu, Fe, Zn, Mn, vitamins, tannic acid, etc. are found in Chyawanprash which have very good nutritional values. Chyawanprash can increase digestion properties in humans and it is very good in gastrointestinal diseases. It is helpful in increasing immunity and mainly boosts the production of immunoglobulin E. It is clinically proven that Chyawanprash can increase haemoglobin levels in infected persons (Sharma et al. 2019).

14.7 Phytochemistry

According to Singh and Duggal (2009), there is no phytochemical information of both *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don available, but here we can mention some of the very rare and authentic phytochemical information of both of these orchids. *Malaxis muscifera* (Lindley) O. Kuntze is included under "Astavarga" plants in Ayurvedic medicine. This plant contains various important phytochemicals which are very helpful for the treatment of deadly

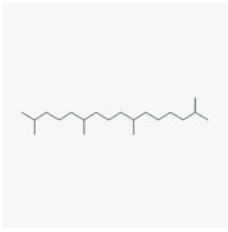


a) 6-octadecenoicacid



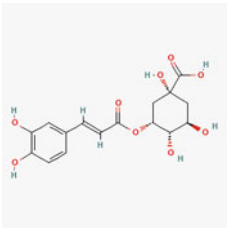
b) Diethyl phthalate

c) Thiirane

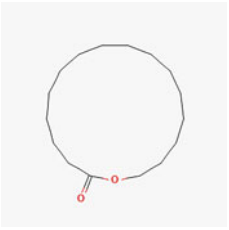


d) Hexadecane-2,6,10,15tetramethyl

e) Heneicosane

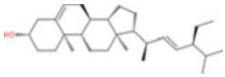


f) Neochlorogenicacid



g) Oxacyclohexadecan-2-one

h) Methyl ricinoleate



i) Stigmastanol

Fig. 14.1 (**a**–**i**) Structures of few important phytochemicals found in *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don

diseases. Few important chemicals (Fig. 14.1) present in *Malaxis muscifera* (Lindley) O. Kuntze are as follows—N-isopropyl palmitamide, 5-O-caffeoylquinic acid and stigmastanol. Pseudobulb of *Malaxis muscifera* (Lindley) O. Kuntze contain calcium oxalate crystals (Chinmay et al. 2011), and they also contain flavonoid, glycoside and alkaloid (Balkrishna et al. 2012).

Malaxis acuminata D. Don is a very popular medicinal plant used in various ayurvedic preparation. There is a various phytochemical substance present in this plant. Chemicals present in *Malaxis acuminata* D. Don are as follows—Methyl ricinoleate; Heneicosane; Diethyl phthalate; Docosane; Hexadecane 2,6,10,15-tetramethyl; Pentadecanoic acid; Heptadecanoic acid methyl ester; 6-Octadecenoic acid; Methyl ester, (Z)-; Heneicosane; Cyclohexadecanolide; Methyl tetradecanoate; Octadecanoic acid; Thiirane, octyl; l-(+)-Ascorbic acid 2,6-dihexadecanoate; 4-Hydroxy-2-butanone; 1-Butanol, 3-methyl-, acetate; 9-Octadecenal, (Z)-; Heptadecanoic acid, 16-methyl-, methyl ester; 1,2-Oxathiane, 6-dodecyl-,

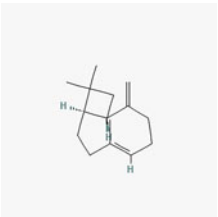
2,2-dioxide; Oxacyclohexadecan-2-one; Methyl margarate; Lignoceric acid; 6-Octadecenoic acid, methyl ester, (Z)-; Octadecanoic acid; 11-Hexadecenoic acid, methyl ester; tert-Hexadecanethiol; Oxacyclohexadecan-2-one; Methyl tetradecanoate; Butyl oleate; Hydrofol Acid 150; 9-Tetradecenal, (Z)-; Eicosanoic acid, methyl ester; Octadec-9-enoic acid; Heptadecanoic acid, methyl ester; Heneicosanoic acid; Thiirane, octyl-; Octadecanoic acid; 9,9-Dimethoxybicyclo [3.3.1]nona-2,4-dione; Tridecanoic acid, methyl ester; 2,6-Diisopropylnaphthalene; Cyclohexadecanolide DB5-2595; Margaric acid; Cyclopentane tridecanoic acid, methyl ester; Methyl (6E)-6-octadecenoate; 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester; Triarachine; Hexadecanoic acid, methyl ester; 8-Octadecenoic acid, methyl ester; Univol U 316S; Cerasynt Special; Oxirane, tetradecyl-; E-8-Hexadecen-1-ol acetate; Hydrofol Acid 150; Hexadecanoic acid, 15-methyl-, methyl ester; cis-Oleic Acid; Methyl tetradecanoate; n-Octadecanoic acid; Cyclopentadecanolide; Stearic acid; Methyl tetradecanoate; 8-Octadecenoic acid, methyl ester; Octadecanoic acid, 2-(2 hydroxyethoxy) ethyl ester; 13-Octadecenal, (Z)-. All of these chemicals are isolated and identified from *Malaxis acuminata* D. Don by applying various methods (Raval et al. 2016).

14.8 Nutritional Composition

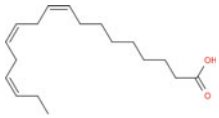
Both *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don are taxonomically and morphologically different, but we cannot separate them phytochemically due to the presence of similar phytochemical and that is why they both show similar therapeutical characters such as antioxidant and antimicrobial activity. An overview of nutritional compound present in *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don are mentioned below as well as in Fig. 14.2.

Malaxis acuminata D. Don is a very important medicinal plant and it has very good nutritional value as well. There is very important mineral nutritional compound present in this plant such as iron, zinc, manganese, copper, calcium, magnesium, potassium, boron, aluminium, barium, chlorine and molybdenum. *Malaxis acuminata* D. Don also contains fatty acids such as α-linolenic acid, palmitic acid, linoleic acid, oleic acid, eicosanoic acid, stearic acid, eicosadienoic acid and γ-linolenic acid. Vitamin E and γ-tocopherol is also present in *Malaxis acuminata* D. Don (Lohani et al. 2013). Nutritional molecules like carbohydrate, crude fibre, saponin, fat substance, tannin, alkaloid and resin are also present in the plant body of *Malaxis acuminata* D. Don (Arora et al. 2019). There are various oils extracted from *Malaxis acuminata* D. Don pseudobulb which show both therapeutic and nutritional value; so they are very beneficial for the human body. Therapeutic oil content found in *Malaxis acuminata* D. Don is as follows: Caryophyllene protects against neurological problem and alcohol addiction; Eugenol is very good for the heart and contains antioxidant properties; Caryophyllene oxide is helpful against fungal infection; Humulene has anti-inflammatory properties; Phenol, 2,4bis(1,1dimethylethyl)

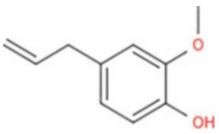
a) 2, 5 octadecadiynoicacid



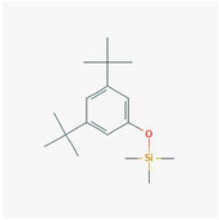
b) Caryophyllene



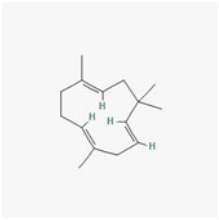
c) Linoleic acid



d) Eugenol



e) Phenol, 2,4bis(1,1dimethylethyl)



f) Humelene

Fig 14.2 (**a**–**f**) Structures of few nutritionally important compounds found in *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don

can prevent various microbial infections; 2,5Octadecadiynoic acid and methyl ester also have therapeutic properties (Arora et al. 2017).

14.9 Pharmacology

The scientific study of medicine is commonly known as "pharmacology". The word pharmacology comprises two Greek words. The word "Pharmacon" means medicine and "Logos" means study or knowledge in Greek. In a wider view, we can say that pharmacology is the study of the reaction of chemicals deliberately inserted inside a biotic system. Mainly pharmacology focused on valuable medicine used to cure and control a disease (Tripathi 2013). In recent times, pharmacology is one of the most dynamic topics for researchers.

In *Malaxis acuminata* D. Don, the pseudobulb part carries some antioxidant properties which are proved by various laboratory studies. This property is very important against deadly chronic diseases because it can block processes related to these diseases. In *Malaxis acuminata* D. Don, pseudobulb also has anti-ageing properties. It is used to increase fertility and is effective against sterility-related issues. Pseudobulb of *Malaxis acuminata* D. Don is also very effective against skin-related issues. The leaf tonic of this orchid is also helpful in maintaining structural composition in humans. Both natural and laboratory-grown plants of *Malaxis acuminata* D. Don is effective for protection against UV light which can cause

serious problems like a damage in DNA. The leaf of *Malaxis acuminata* D. Don has anti-inflammatory properties. This plant has the ability to prevent inflammatory symptoms in the body. *Malaxis acuminata* D. Don has the ability to show responses against different kinds of microorganisms. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are highly affected by methanol and ethanol extract of *Malaxis acuminata* D. Don. Those bacteria which show negative results during Gram staining are negatively affected by *Malaxis acuminata* D. Don. The pseudobulb extract with different chemicals shows activeness against various groups of bacteria. The most important pharmacological properties of *Malaxis acuminata* D. Don is that chloroform extract of *Malaxis acuminata* D. Don can prevent *Bacillus subtilis* and *Escherichia coli*; butanol extract is also found to be effective against *Escherichia coli*. *Malaxis acuminata* D. Don also shows antifungal properties mainly against *Candida albicans* (Suyal et al. 2020).

Malaxis muscifera (Lindley) O. Kuntze is known to be used in male sterility-related problems and it is also used to cure fever (De 2020). In the case of *Malaxis muscifera* (Lindley) O. Kuntze, we are not able to find more data about its pharmacology. We need more clinical research on this plant in future because this plant contains medicinal properties and is used as the most important traditional medicinal plant in India from the ancient times of Ayurveda, and it is well known that this plant is used in different traditional formulations.

14.10 Synthetic Strategies for Key Secondary Metabolite

Flavonoid and phenolic compounds are found as a key secondary metabolite in *Malaxis acuminata* D. Don. *Malaxis muscifera* (Lindley) O. Kuntze contains secondary metabolites such as glycosides and flavonoids. A different area of the plant body of *Malaxis acuminata* D. Don is cleaned under running water and excess water is removed with tissue paper at normal temperature. 0.1 g of dry tissue paper is homogenized in 100 ml of acetone, acetonitrile, methanol, water and chloroform solvent. Extraction was continuously shaking at almost 180 rpm for 1 day on an Orbital shaker. The mixture of plant material and the solvent was then put in a centrifuge machine and centrifuged at 10,000 rpm for the quarter of an hour. The remaining part of the mixture was filtered by using Whatman No. one filter paper. Cary-100 Bio double beam spectrophotometer was used to measure the quantity of all the phytochemical present in the plant material (Bose et al. 2017).

14.11 Tissue Culture

In tissue culture, a small fragment of plant tissue is put in the artificial environment for a definite time where it can increase in mass and size. In the case of *Malaxis muscifera* (Lindley) O. Kuntze, there is still no sufficient and authentic data available

to describe tissue culture. But in the case of *Malaxis acuminata* D. Don, a detailed description of tissue cultural study was available.

Tissue culture techniques in the plant have adapted to a new height after the modernization of cultural techniques. For tissue culture, both laboratories grown and freely growing *Malaxis acuminata* D. Don plant samples are used. About 1 cm long part of almost 9 weeks old plant's pseudobulbs is used for tissue culture. The part of the leaves attached with the studied part is removed inside the laminar airflow chamber, and agar from the previous culture is removed by using distilled water. For the natural sample, almost 2 cm long 4 weeks old pseudobulb parts are used for the study. All the unnecessary part like leaves attached with the pseudobulb is removed. Later the sample is washed with labolene and moving water. Later the sample was sterilized by applying chemical substances like ethanol. Sterilized samples are put inside sterile water until the next processes start. The pseudobulb of both samples of *Malaxis acuminata* D. Don is cut in the horizontal and vertical direction. In tissue culture, a gelling substance like agar and three different helping substances is used as an alternative such as forest leaf litter, polyurethane foam and coconut coir used in tissue culture. These supporting materials are sized according to the requirements of the tissue culture. The substrata are then sterilized by using an autoclave machine at more than 120 °C in 1.05 kg/cm^2 for 60 min. In the starting of tissue culture pseudobulb of *Malaxis acuminata* D. Don is poured in Murashige and Skoog medium. Later citric acid, sucrose and casein hydrolysate are added to the medium for further enrichment. Later plant growth regulator N6 benzyl adenine and α-naphthalene acetic acid is added to the medium. Agar was used to gelling the medium and the pH of the medium is adjusted slightly acidic than 6. A part of the medium is separated in the test tube and put in the autoclave machine at close to 120 °C for more than a quarter of an hour at 1.05 kg/cm^2. In one test tube, close to 19 pseudobulb segments are used. The temperature of the medium is maintained between 23 and 27 °C and maintained light photo-cycle of half a day time period. Sub-culture is developed from the previously cultured sample after almost 4 weeks. PLB or protocol like bodies are developed in the culture. When initial leaflets are developed in PLB, the young meristematic loci with leaflets are moved to MS medium enriched previously with plant growth regulator and sucrose individually or in the mixture. In the agar-based medium and any other previously mentioned helping mediums are sterilized and used in the development of the cultured sample, and an artificial environment is continuously provided to the PLBs. When more than three leaves and roots are developed, it moved to MS medium containing sucrose but without a single trace of plant growth regulators for more than 6 weeks. For further hardening of plants, they are transferred to community plotting mixture and water is provided for two times in every 7 days for next 60 days; now the newly formed *Malaxis acuminata* D. Don plants are ready to be grown in the natural environment (Deb and Arenmongla 2014).

14.12 Formulation and Market Product

Formulation of *Malaxis acuminata* D. Don are Brahini gutika, Chyawanprash rasayan, Vajikarana ghrita, Chitrakadi taila, Jivaniya ghrita, Vachadi taila, Mahapadma taila, Maha kalyanaka ghrita. Mahamayura ghrita, Himvana agada, Astavarga churna, etc. The formulation of *Malaxis muscifera* (Lindley) O. Kuntze are Vajikaran ghrita, Chyawanprash rasayan, Jivaniya ghrita, Chitrakadi taila, Himvana agada, Mahapadma taila, Astavarga churna, Maha kalyanaka ghrita and Mahamayura ghrita (Rajashekhar et al. 2015).

Chyawanprash is the most popular market product where both *Malaxis acuminata* D. Don and *Malaxis muscifera* (Lindley) O. Kuntze are used as a key ingredient in ancient times. In ancient Chyawanprash formulation, both of these orchid plants are used as a part of "Ashtavarga". Ashtavarga is a combination of eight medicinally important plant species. Due to government policies for conservation and protection of threatened medicinal plants, the use of *Malaxis acuminata* D. Don and *Malaxis muscifera* (Lindley) O. Kuntze is strictly prohibited in industrial Chyawanprash production. Few replacements of these eight plants in commercial production of Chyawanprash are reported recently; these substitute plants are *Asparagus racemosus*, *Withania somnifera*, *Ipomoea digitata* and *Dioscorea bulbifera* (Sharma et al. 2019).

14.13 Conservation

Malaxis acuminata D. Don and *Malaxis muscifera* (Lindley) O. Kuntze are two threatened medicinally important orchid species. For their survival in future, we have to develop various strategies to minimize their destruction in natural habitat. *Malaxis muscifera* (Lindley) O. Kuntze is a vulnerable species according to IUCN (Chandra et al. 2021). Many plants of *Malaxis muscifera* (Lindley) O. Kuntze are eaten by domestic herbivores and small snail-like organisms. Development of infrastructure reduced the natural habitat of this orchid plant in many areas. Due to the movement of domestic animals in many hilly areas during the rainy season, movement of soil causes a reduction in the plant population of *Malaxis muscifera* (Lindley) O. Kuntze. In the plantation of woody plants, lots of *Malaxis muscifera* (Lindley) O. Kuntze plants got reduced because these plants need a large area to grow. Reduction of habitat and anthropogenic movement (mainly tourists) also boosts the reduction of *Malaxis muscifera* (Lindley) O. Kuntze (Kant 2015). We have to prevent the entry of domestic animals by putting a net surrounding the plant. Besides that, we must have to teach local people about the benefits and therapeutic importance of this medicinal orchid plant, and we have to be careful about the destruction caused by cow. Before building any kind of infrastructure (mainly house and road), everyone should have to take responsibility not to harm the natural habitat of *Malaxis muscifera* (Lindley) O. Kuntze plant. The habit of plantation of a large

tree in the habitat zone of *Malaxis muscifera* (Lindley) O. Kuntze should be stopped soon because *Malaxis muscifera* (Lindley) O. Kuntze is a rare medicinal plant, and it is much more valuable than some common woody plants. During the flowering season of this plant, entry of humans into their natural habitat should be stopped because this will reduce the destruction of the flower by anthropogenic means and will give this plant more chance to live for a long time. Collection of fodder is harming the regeneration of *Malaxis muscifera* (Lindley) O. Kuntze. Hydroelectric works are responsible for the reduction of *Malaxis muscifera* (Lindley) O. Kuntze orchid plants (Kant et al. 2012). This type of project should not start in the habitat zone of *Malaxis muscifera* (Lindley) O. Kuntze. For on-the-spot conservation, forest department in many areas did fencing to protect *Malaxis muscifera* (Lindley) O. Kuntze from the herbivores. Spiny plants were planted in the surrounding to protect *Malaxis muscifera* (Lindley) O. Kuntze from herbivores during flowering and maturation of seeds. These plants were removed from the surroundings of *Malaxis muscifera* (Lindley) O. Kuntze after this particular period is over. Micropropagation of the seed of *Malaxis muscifera* (Lindley) O. Kuntze is used as laboratory-based conservation technique (Kant 2015).

Malaxis acuminata D. Don is reduced due to the destruction of its natural habitat and human interference. Overuse of this medicinally important plant boosts the reduction of the population of *Malaxis acuminata* D. Don. Illegal selling of this plant is a very great issue. Along with another plant, *Malaxis acuminata* D. Don is also often consumed by herbivores mainly domestic animals of local people. *Malaxis acuminata* D. Don is used in various Ayurvedic medicines and due to that this plant is often collected in huge quantities from its natural habitat. *Malaxis acuminata* D. Don is often found in a very small area and the collection of complete plant body before the releasing of seed causes very high reduction (Lohani et al. 2013). The natural habitat of this terrestrial orchid should be protected and human entry into the habitat should be prohibited. The use of *Malaxis acuminata* D. Don in medicine is banned at present time and this rule is very important for the conservation of this plant. The illegal business of natural recourses should be stopped with immediate effect. Government should take initiative to teach local people about the therapeutic importance of *Malaxis acuminata* D. Don which will be a very effective way of conservation of *Malaxis acuminata* D. Don. The plant should always be collected after releasing its seed and collection of the entire plant is not an appropriate method. Collection of plant material in an illegal way and over-collection cause rapid reduction in population. Development of urban areas destroys the habitat of *Malaxis acuminata* D. Don (Kant et al. 2012). Industrial development and urbanization should be more beneficial for the plant community and threatening medicinal plants should be given more preference.

14.14 Conclusion and Future Prospective

During the study of works related to *Malaxis acuminata* D. Don and *Malaxis muscifera* (Lindley) O. Kuntze, we found many loopholes in the works done by previous workers. Many taxonomic workers considered these two plants as one species but in reality; they are completely two different species. Many workers mistook their ayurvedic names "Jeevak" and "Rishbhake" which makes the study more difficult, and it is a serious problem in the scientific study at the grass-root level. Separation of both of these terrestrial orchids by scientific means with enough authenticated data is necessary. In the taxonomic study of *Malaxis acuminata* D. Don and *Malaxis muscifera* (Lindley) O. Kuntze, lack of data causes a big problem. There is no reliable classification data available for these two plants. Many workers added the systematic position of *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don in their work, but they do not describe the possible reason behind their systematic position or they are not able to show all the characters, which are used in the classification of this terrestrial orchids. A very little amount of morphological data is added in the case of these two orchids which are not enough in the identification of this plant in their natural environment. Almost all taxonomists failed to show the differences between both *Malaxis acuminata* D. Don and *Malaxis muscifera* (Lindley) O. Kuntze.

Only a few common names are available for both species which indicate that we need a detailed study on the common name and traditional use of *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don by different communities of the world and the importance of these plants as a source of ethnic medicine. Different stages of flower and fruit development are not added by previous workers, and no one tries to differentiate these two plants on the basis of floral structure and characters. All the data about the distribution of *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don is described in different regions of India and very few neighbouring countries of India. We lack sufficient detailed data about the growth and development of these plants outside India except for one or two countries like Nepal. Very few research works from foreign countries regarding this plant is available in the research database which demands an immediate measure for conservation of this plant. Many workers do not even mention the longitude and latitude from where they collected their materials; this makes it more difficult for later workers to study about *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don.

Only a few Ayurveda-based traditional use are mentioned by many workers repeatedly; besides this, there is a detailed study on the traditional use and medicinal importance of these plants is very much needed. Accumulation of all traditional data about *Malaxis acuminata* D. Don and *Malaxis muscifera* (Lindley) O. Kuntze will be very helpful for future research on traditional orchid-based medicines. Only a very less amount of data is available on the phytochemical part. A detailed phytochemical study of these plants will be a very dynamic topic for research because these plants contain substances that are medicinally important. Phytochemical study

of *Malaxis muscifera* (Lindley) O. Kuntze is very negligible and future workers can analyse these chemicals in depth. Many workers used plant materials for phytochemical analysis without a detailed taxonomic study and they confused *Malaxis muscifera* (Lindley) O. Kuntze with *Malaxis acuminata* D. Don. The nutritional properties of these orchids are a topic of analysis because these plants can become a substitute for our present nutritional sources.

A detailed biochemical analysis of pharmacological properties of *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don should be done as soon as possible because we are unable to find any authentic pharmacological data about *Malaxis muscifera* (Lindley) O. Kuntze, and we are able to find very few data on pharmacological properties of *Malaxis acuminata* D. Don. In the time of Covid-19, a detailed pharmacological study about this traditional medicine may provide us with a great source of immunity. There is no cultivation record found on these plants in recent times because they are threatened species. Many local people harvest them in an illegal way which causes more harmfulness because they collect the entire plant body from the soil surface. There is very little work done on tissue culture in the case of *Malaxis muscifera* (Lindley) O. Kuntze; so in the future, tissue culture technique may become very helpful for in vitro production of this species and the large number of plants produced from tissue culture will play a major role in the conservation of this species.

Conservation of *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don is still a subject of concern. Due to various reasons, the number of individuals of these plants in the environment is decreasing day by day. Conservation biologists in future must focus on more scientific methods of biodiversity conservation. The methods described by previous workers are more traditional than scientific; so future workers are much focused on more scientific methods of conservation. There is only a very few publication available in the case of conservation of *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don which is a big area of concern. Ministry of AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy) have to focus on the conservation, clinical study of *Malaxis muscifera* (Lindley) O. Kuntze and *Malaxis acuminata* D. Don, and with the help of local people and research workers they can easily prevent the harvesting of these precious Indian traditional therapeutic plants.

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Chapter 15
Picrorhiza kurroa Royle ex Benth.: Kutki

Kamalpreet Kaur, Gurpreet Kaur, and Vijay Singh

15.1 Introduction

Immunotherapy is a modern-day treatment paradigm which is redefining the progressive therapeutic line for the immunosuppressive illnesses. Discovery of immunomodulatory agents which could alleviate the rates of mortality and morbidity caused by different fatal infections with no toxicity profile is the need of the hour. The trend of research is projecting more towards finding a natural product as they serve healthy and effective remedies against many clinical manifestations (Ji et al. 2019; Al Nasr 2020). Currently, the research is going on for the expansion of immunostimulatory herbal formulations to induce a robust immune response for the immunodeficiency diseases like pulmonary disease, diabetes, and tumor. As a result, medicinal plants have been regarded as the superior immunomodulators, economic and non-toxic therapeutics against deadly infections (Pressnall et al. 2020). *Picrorhiza kurroa* Royle ex Benth is a medicinal plant found at an elevation 3000–5000 m in Northern-Western Himalayas. This perennial plant belongs to Plantaginaceae family and is frequently identified as Kutki, Gentian, and Kurro (Kumar et al. 2015). *Picrorhiza kurroa* has extensively utilized in traditional Chinese and Indian medicinal herb for the prevention of fever, hepatic and respiratory problems, diarrhea, and dyspepsia. This plant is used for the remedy of scorpion stings. *P. kurroa* showed hepatoprotective efficacy as it normalizes the deranged liver function markers as well as adjusts the levels of various involved cytokines in

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standard range. It has pronounced effect as an anti-asthmatic, antioxidant, anti-inflammatory, and anticancer. These characteristics of *P. kurroa* are mainly accredited to the existence of active phytoconstituents, most preferential iridoid glycosides (Viljoen et al. 2012; Pandit et al. 2013). This plant has unique iridoid glycosides like bartsioside, picroside, kutkoside, and kutkin. These glycosides possess beneficial effects in the cure of liver, viral, bacterial, mutagenic, and other infections (Bhattacharjee et al., 2013; Kumar et al. 2016a, b). *P. kurroa* stem has marked amount of picroside I and II, which possesses broad spectrum of medicinal properties like anti-inflammatory and hepatoprotective (Ma et al. 2020). About 2000 herbal formulations contain the major portion of active phytoconstituents like picroside and kutkoside (Bhandari et al. 2009; Sah and Varshney 2013). In India, *P. kurroa* is one among the 15 plant species marketed due to its economic value. (Ved and Goraya 2007). The worldwide yearly claim for the *Picrorhiza* is 500 tons, whereas the export is about 375 tons, with India contributing just 75 tons per year. The demand of the *P. kurroa* are augmented worldwide because of the existence of a variety of potent secondary metabolites (Thani 2018). Several pharma trades like Livomyn, Picroliv by BACFO Pharma, Livplus, TTK Pharma, Dindayal Aushadhi, and DIL Limited are commercialized and sold formulation based on the content of picrosides (Thakur et al. 2021).

Due to overexploitation, *P. kurroa* was listed as a susceptible species in the Red Data Book (Nayar and Sastri 1990) and as an endangered plants by the IUCN (International Union for Conservation of Nature and Natural Resources). For retaining the synthesis of secondary metabolites in *P. kurroa*, several in vitro culture techniques and micropropagation methodologies have been explored (Patial et al. 2012; Rawat et al. 2013; Rawat et al. 2021).

15.2 Taxonomy and Common Names

Picrorhiza kurroa Royle ex Bentham is the scientific name for this plant (Fig. 15.1). In Greek, picros implies bitter, whereas rhiza indicates root. *P. kurroa* is also referred to as bitter root. The plant's precise label is derived from the Punjabi word "karu," which signifies bitter. Common vernacular names of *Picrorhiza kurroa* are kadu, hunglen, katuka, Kavi, honkadu, katukaa, katu, Kaurohini, huhuanglian, katki, kadukrohini, karru, kadugurohini, kalikutki, karupicrorhiza, katuka, katukaa, and Kauka (Sharma 2020) (Table 15.1).

15.3 Distribution

P. kurroa is predominantly distributed in dry western Himalayas whereas *P. scrophulariiflora* is found largely in moist eastern Himalayas (Bantawa et al. 2011). *P. kurroa*, also identified as "kutki" shows its distribution in West China,

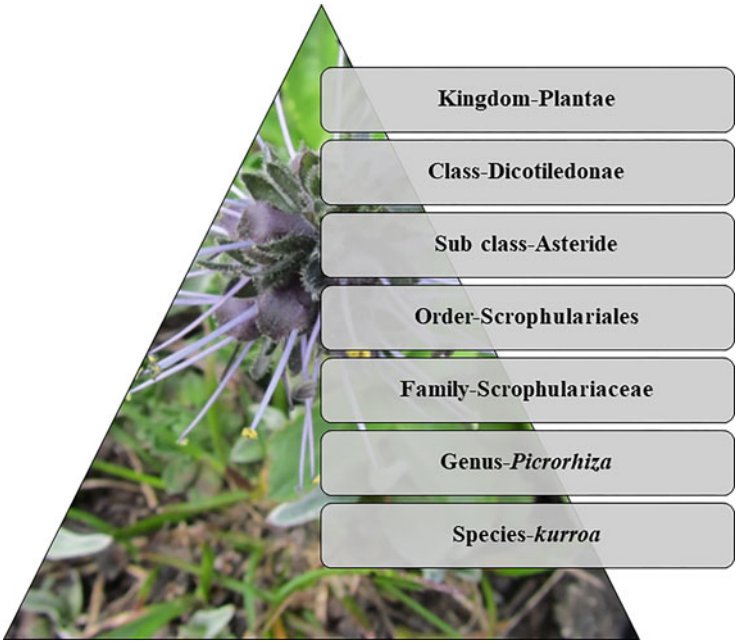


Fig. 15.1 Taxonomy of *Picrorhiza kurroa* (Mannan et al. 2021)

Table 15.1 Regional common names of *P. kurroa* (Raina et al. 2021)

Language	Regional Common Name
Sanskrit	Katuka, Krishna, Katurohini
Bengali	Kattki
Urdu	Kutki
Hindi	Katuka
Gujrati	Kadu
Telugu	Katukarohini
Punjabi	Kaundd
Tamil	Katukarogini
Marathi	Kali katuki

Tibet, North Burma, and North-Western Himalayas stretching from Kashmir to Garhwal and Sikkim territories of India, Nepal, Bhutan, and Pakistan. It grows well in rocky crevices of the alpine region and in organic soils too in the spring season.

In Nepal, kutki grows abundantly in the alpine Himalayan area at the height of 3500–4800 m and occupies cliffs and turfs of glacial and rock fissures of the north facing slopes in Western Nepal (Masood et al. 2015; Sultan et al. 2017). In India, *P. kurroa* exhibits its distribution predominantly in Uttarakhand, Jammu and Kashmir, Arunachal Pradesh, Ladakh and Himachal Pradesh, (Table 15.2) and Sikkim (Arya et al. 2013; Panda 2021).

Table 15.2 Distribution of *P. kurroa* in India

State	Location	Reference
Himachal Pradesh	Chhitkul, Chandrokani, Rohtang Pass, Manimahesh, Pieri Pass, Lahaul valley, Pangi valley, Kulu, Bharmour valley, Dhauladhar valley, Spiti, Kinnaur, Rohru, Chhakinal watershed, Chepuwa, Kullu, Talla Johar, Eastern Kumaon, Changla Pass, Kugti Wildlife Sanctuary, Himalayan National Park, Dhauladhar	Pennell (1943), Uniyal et al. (1982), Chauhan (1988), Mehta et al. (1994), Dobriyal et al. (1997), Uniyal et al. (2006)
Jammu and Kashmir	Zoji La Pass, Burzil Pass, Sonsa Nag, Pahalgam, Kamri Pass, Lipper Valley, Jammu, Kishanganga Valley, Kolahoi, Zojpal, Simthan, Pir Panjal Range, Upper Lidder valley	Sharma (2021), Pennell (1943), Sharma (2021), Coventry (1927), Kapahi et al. (1993)
Uttarakhand	Ponwati, Harsil, Sukhi, Tali, Sayara, Tehri-Garhwal, Raithal, Harsil, Bhilangna Valley, Damodar Valley, Sahastra Tal, Deodi Ramani, Gangotri, Tungnath, Kali Valleys, Kedarnath Wildlife Sanctuary	Uniyal et al., (2002), Semwal et al., (2007), Arya et al., (2013)

15.4 Morphology

P. kurroa is a perennial herb characterized by 6–10 inches length and hard roots. Roots are approximately 5–10 cm in length, 0.3–1.0 mm width, straight or a bit curved, dusty greyish in color, associated with dotted scars or a small number of longitudinal wrinkles and are generally fixed to the rhizomes (Mannan et al. 2021; Sultan et al. 2017). Root stacks resemble little fingers in thickness and are unevenly curved (Kirtikar and Basu 1999). The rhizome is sub-cylindrical, zig-zag, joined, curved erratically due to rooting and branching at jointed nodes, greyish brown in color, 4–8 mm thick and 2.5–8 cm in length (Mannan et al. 2021). The stem is small, delicate, slightly hairy and creeps on the ground and is erect at flowers and leaves (Debnath et al. 2020; Bhardwaj et al. 2021). *P. kurroa* leaves are sub-radical, oval, and unevenly toothed. It bears oval shaped, pale, purplish color fruits of 1.3 cm length (Fig. 15.2). The rhizome and root of *P. kurroa* tastes bitter but has a pleasant aroma (Kar 2007; Elizabeth 2002).

15.5 Flowering

Flowers of *P. kurroa* are around 8 mm in diameter, with a five-lobed center, bisexual and longer filaments. The fruit is tapering at the topmost which dehisces into four valves and having numerous, ellipsoid seeds, with a very thick seed coat. The

Fig. 15.2 *Picrorhiza kurroa Royle ex Benth*

flowers are actinomorphic with calyx divided into five parts, with a corolla that is 10 mm long. Color of flowers is dark blue-purple and flowers bloom from July through August. Flowering ensues in one or two periods according to the height of the growing place, and the flowers originate in compact terminal spikes coming from a rosette of prominently serrated leaves. The first phase of flowering begins in the first week of May and lasts until the third week of June at a relatively lower elevation of 2500–3500 ft. above mean sea level, and the second phase begins in August and lasts until the end of September (Fig. 15.3). Flowering happens just once in alpine locations (>4000 m), and seeds grow in September (Sultan et al. 2017; Prakash et al. 2020).

15.6 Traditional Uses

Picrorhiza kurroa is an imperative remedial herb in Ayurvedic medicine, and it has been utilized for the treatment of hepatic and bronchial problems. Among the Bhotiya tribal groups of the Niti valley in the Central and Western Himalaya, *Picrorhiza* is given as a traditional treatment for fever, cold, and cough (Phondani et al. 2010; Nand and Naithani 2018; Singh and Negi 2019).

The rhizomes of *Picrorhiza* are used to make medicines in Bhutan's hospitals. The Gaddi and Gujjar tribes of Himachal Pradesh utilize *Picrorhiza* roots to increase their appetite. *Picrorhiza* in combination with ajwain is employed as an efficient remedy for the dermal infections to disinfect blood (Guleria and Vasishth 2009). The extract of *Picrorhiza* is employed for the therapy of digestive disorders, diarrhea, dysentery, and jaundice in China. *Picrorhiza* extract has a hepatoprotective effect

Fig. 15.3 Flower of *Picrorhiza kurroa*

due to its choleretic action, and it has been used traditionally for hepatic infections in Himachal Pradesh's Hamirpur district. The rhizome extract of *Picrorhiza* is authenticated as an antibiotic in India and has great usage in Ayurvedic and Unani medicine sector. It is a major constituent in Ayurveda medicine Arogyavardhini for the relief of many hepatic complications. *Picrorhiza* extract is utilized for the cure of jaundice, asthma, and hepatitis in Nepal and Kashmir. The rhizome of *Picrorhiza* has been utilized as an antidote for scorpion bite in Kathmandu. It is also employed for the treatment of hypertension, eye infections, gastrointestinal problems, gastritis, and aching throats (Kumar and Choyal 2012; Salma et al. 2017). In Northern part of Himachal Pradesh particularly in Lahaul and Spiti, various parts of *Picrorhiza* have been exploited for the treatment of fever and are also used as a blood cleanser and pain killer (Sharma et al. 2014; Dutt et al. 2014). The tribal society of Kupwara district in Kashmir use stems and roots of *Picrorhiza* as a purgative, tonic, and stomachic. *Picrorhiza* extract is widely exploited as antipyretic, diuretic, anti-inflammatory, relaxant, analgesic, and antistress in Garhwal Himalayas. The roots of this plant have been used traditionally as a remedy for the sores, wounds, and lesions in the West Sikkim (Lone et al. 2012; Tamang et al. 2017). In Sikkim, the *Picrorhiza* roots and leaves is employed for the cure of malaria, jaundice, respiratory problems, and as appetizer (Bharti and Sharma 2009; Chhetri 2004; Idrisi et al. 2010).

15.7 Phytochemistry

The chemical composition of *P. kurroa* has been comprehensively explored and a huge amount of bioactive constituents have been purified from different portions of *P. kurroa* like rhizomes, seeds, roots, stem, and leaves (Zhang et al. 2005; Wang et al. 2008; Kumar et al. 2016a, b; Sultan et al. 2017; Ali et al. 2017). The large number of secondary metabolites (more than 65) isolated from *Picrorhiza kurroa* can be alienated into four groups: cucurbitacins, phenolic compounds, iridoid glycosides, and acetophenones (Bhardwaj et al. 2021). The major iridoid glycosides includes picrosides (I-III) which are separated from the bitter rhizome of *P. kurroa*. Cucurbitacins exhibit anticancer activity and apocyanin displays anti-inflammatory effect and inhibits neutrophil oxidative burst (Van den Worm et al. 2001). "Picroliv" or "kutkin" is a combination of two glycosides: "kutkoside" and "picroside-I" present in 2:1 ratio and is isolated from the rhizome and roots of *P. kurroa* (Bhardwaj et al. 2021; Verma et al. 2009). The active component of the dried rhizome of *P. kurroa* is a mixture of picroside-I and picroside-II (about 60%) present in 1:1.5 ratio and the remaining 40% includes components like cucurbitacin glycosides and iridoids. The biological importance of *P. kurroa* is predominantly due to the existence of picrosides-I, II, III, IV, and kutkosides which belong to iridoids class of compounds. Additionally, *P. kurroa* contains iridoids such as vernicoside, 6-feruloyl catalpol and minecoside, cucurbitacin glycosides, phenolics such as apocyanin, vanillic acid, androsin and picein (Thani 2018). The important phytoconstituents isolated from *P. kurroa* have been indicated in Table 15.3.

15.7.1 Iridoid Glycosides

The iridoids are monoterpenoid compounds bio-synthesized from isoprene and occur mostly as their glycosides. The iridoid glycosides are used by plants for defense and protection against infections caused by insects and other microorganisms (Soni and Grover 2019). *P. kurroa* contains seven unique iridoid glycosides including picroside-V, kutkin, kutkoside, mussaenosidic acid, pikuroside, boschnaloside, and bartsioside (Soni and Grover 2019; Kumar et al. 2016a, b). The active component of *P. kurroa* is a bitter crystalline phytoconstituent named "kutkin" a blend of two C9 iridoid glycosides, kutkoside, and picrosides present in a fixed proportion of 1:2 (Kumar et al. 2004). This herbal medicine is used in treating liver and spleen diseases, as immune-modulator and in conditions of asthma and fever. Additionally, picroside-III, picroside-V, minecoside, 6-feruloyl catalpol, veronicoside, and pikuroside also exist in smaller amounts (Kumari et al., 2021) as indicated in Fig. 15.4. Win et al. (2019) reported isolation of novel iridoid glycosides namely abeloside A (**19**), abeloside B (**20**), sylvestroside IV dimethyl acetal (**21**), sweroside (**22**), 8-epi-loganin (**23**), and saungmaygaosides A-D (**15–18**) from the stem of *P. kurroa*. Morikawa et al. (2020) extracted seven novel iridoid glycosides

Table 15.3 Phytoconstituents present in *Picrorhiza kurroa*

Parts/Extract	Chemical Constituents	References
Subterranean part (mostly rhizome), Methanolic extract	Picrosides I (**1**)	Kitagawa et al. (1969)
	Picrosides II (**2**)	Weinges et al. (1972)
	Picrosides III (**3**)	Weinges et al. (1972)
	Picrosides IV (**4**)	Li et al. (1998)
	Picroside–V (**5**)	Simons et al. (1989)
	6-Feruloyl catalpol (**6**)	Stuppner and Wagner (1989)
	Minecoside (**7**)	Sticher and Afifi-Yazar (1979)
Roots/petroleum ether	Kutkin (**65**)	Rastogi et al. (1949), Singh and Rastogi (1972), Morikawa et al. (2020)
	Kutkoside (**66**)	
Rhizome, methanolic extract	Picrorhizaosides A (**8**)	
	Picrorhizaosides B (**9**)	
	Picrorhizaosides C (**10**)	
	Picrorhizaosides D (**11**)	
	Picrorhizaosides E (**12**)	
	Picrorhizaosides F (**13**)	
	Picrorhizaosides G (**14**)	
Stems/n-butanol	Saungmaygaoside A (**15**)	Win et al. (2019)
	Saungmaygaoside B (**16**)	
	Saungmaygaoside C (**17**)	
	Saungmaygaoside D (**18**)	
	Abeloside A (**19**)	
	Abeloside B (**20**)	
	Sylvestroside IV dimethyl acetal (**21**)	
	Sweroside (**22**)	
	8-epi-loganin (**23**)	
Roots/$CHCI_3$-MeOH	Picein (**56**)	Stuppner and Wagner (1989), Kant et al. (2013)
	Androsin (**57**)	
Seeds/ethyl acetate	Gallic acid (**58**)	Zhang et al. (2004); Stuppner and Wagner (1989)
	Ellagic acid (**59**)	
	Isocorilagin (**60**)	Zhang et al. (2004)
	1-O-galloyl-β-D-glucose (**61**)	Zhang et al. (2004), Stuppner and Wagner (1989)
	1-O,3-O,6-O-trigalloyl-β-D-glucose (**62**)	
	1-O,2-O,3-O,4-O,6-O-pentagalloyl-β-D-glucose (**63**)	Zhang et al. (2004)
	Vanillic acid (**64**)	Rastogi et al. (1949)
	Apocyanin (**67**)	Basu et al. (1971)
Rhizome, methanolic extract	Myristyl picraldehyde (**68**)	Ali et al. (2017)
	Lauryl picraldehyde (**69**)	

(continued)

Table 15.3 (continued)

Parts/Extract	Chemical Constituents	References
	Capryl vanillic acid (**70**)	
	Picrortetraglucoside (**71**)	
	Picraldehyde diglucoside (**72**)	
Seeds, ethyl acetate	(−)-Shikimic acid (**73**)	Wang et al. (2004)

named picrorhizaosides A-G (**8**–**14**) and six already known iridoid gylcosides: picroside I-V (**1**–**5**), 6-feruloyl catalpol (**6**), and minecoside (**7**) from the methanolic extract of *P. kurroa*. The structures of the compounds as well as their stereochemistry were determined by NMR and mass spectrometry analysis. Out of these compounds, picrorhizaosides D (**11**), picrorhizaosides E (**12**), picrosides I (**1**), picrosides II (**2**), picrosides IV (**4**), and minecoside (**7**) exhibited strong hyaluronidase inhibitory activities. Attri et al. (2021) carried out quantification of the active phytoconstituents of *P. kurroa* (picroside I and picroside II) from different parts of the plant such as stolons, roots, and leaves by using chromatography. NJ cluster analysis indicated that leaves acted as a better source of total picrosides in comparison to stolons and roots. The total picrosides obtained from leaves (4.237–6.894%) were found to be maximum, followed by stolons (2.134–7.444%) and roots (2.154–7.400%). The picroside-I in leaves, stolons, and roots was found to be 3.540–5.677%, 0.183–0.684%, and 0.138–0.493%, respectively. Similarly, picroside-II was found to be maximum in roots (2.014–7.048%); followed by stolon (1.954–6.777%) and minimum amount in leaves (0.697–1.787%).

15.7.2 Steroidal Compounds

Steroids are triterpenic, tetracyclic compounds with a peculiar bitter taste and contain 9-β-methyl-19-norlanosta-5-ene as its basic nucleus which is also oxygenated at many positions. The ethyl acetate and methanol/chloroform extract of *P. kurroa* comprises a steroidal glycoside such as cucurbitacin B, cucurbitacin D (Stuppner et al. 1991; Stuppner and Moller 1993). The isolation as well as structural elucidation of novel cucurbitacins (**41**–**49**) from the root extract of *Picrorhiza* was reported (Stuppner and Wagner 1989; Stuppner et al. 1991). In continuation with their work on *P. kurroa,* Stuppner et al. (1991) reported isolation of four cucurbitacins (**24**–**27**) by fractionating the ethyl acetate extract of the roots of *P. kurroa* followed by semipreparative high performance liquid chromatographic (HPLC) technique, whose structures were the newly discovered compounds (**24**–**27**) possessed a side-chain which was presumably cyclized by means of ether linkages to yield six membered cyclic compounds (Fig. 15.5). The chloroform and ethyl acetate extract of roots of *P. kurroa* exhibited seven cucurbitacins (**28**–**34**) and six more novel cucurbitacin glycosides (**35**–**40**) were purified from the methanolic extract of the

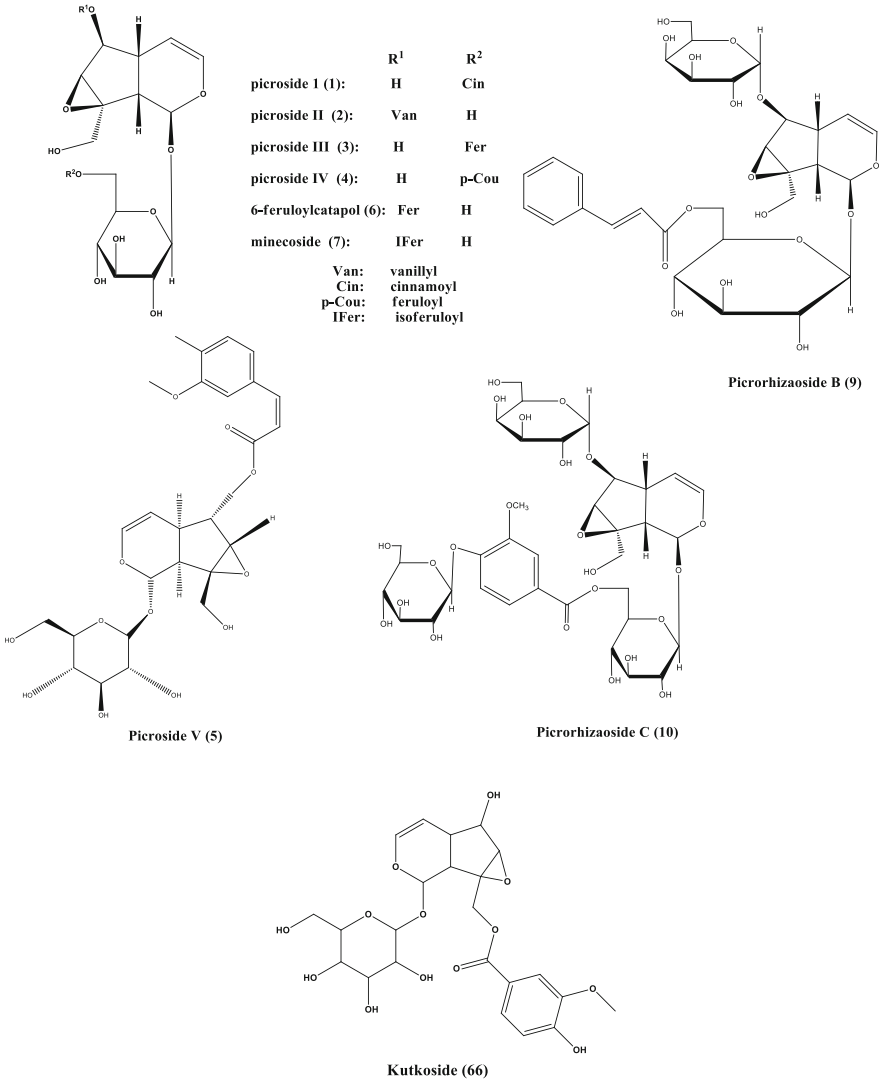


Fig. 15.4 Structures of iridoid glycosides and bis-iridoid glycosides of *P. kurroa*

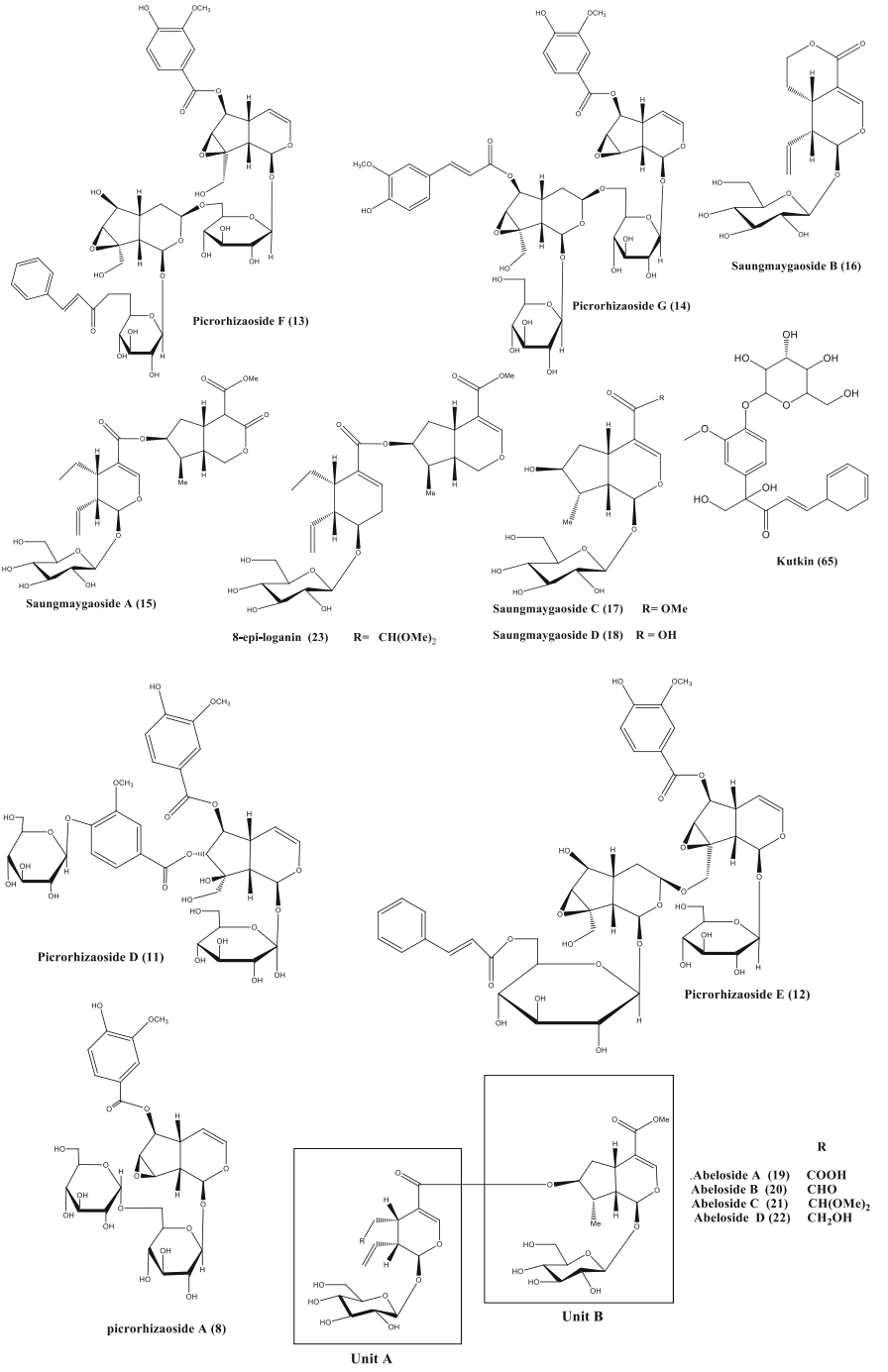
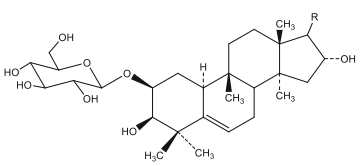
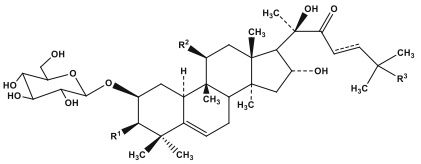


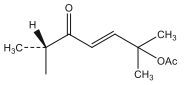
Fig. 15.4 (continued)



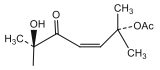


	R^1	R^2	R^3	Other
41	O=	O=	OAc	Δ^{23-24}
42	O=	O=	OAc	-
43	OH	H_2	OAc	Δ^{23-24}
44	OH	H_2	OH	Δ^{23-24}
45	OH	H_2	OH	-
46	OH	O=	OAc	Δ^{23-24}

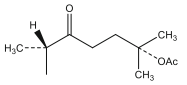
R =



(23E)-25-acetoxy-2-β-D-glucosyloxy-3β-16α-dihydroxy-9β-methyl-19-nor-l0α-lanosta-5,23-dien-22-one (24)



(20R,23Z)-25-acetoxy-2-β-D-glucosyloxy-3β, 16α, 20-trihydroxy-9β-methyl-19-nor-l0α-lanosta-5,23-dien-22-one (25)



2-β-D-glucosyloxy-3β, 16α-dihydroxy-4,4,9β,14α-tetramethyl-19-nor-l0α-pregn-5-en-20-one (26)

2-β-glucosyloxy-3,16,20,25-tetrahydroxy-9-methyl-19-norlanosta-5,23-diene-22-one (41)

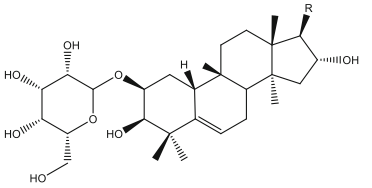
2-β-glucosyloxy-3,16,20,25-tetrahydroxy-9-methyl-19-norlanost-5-ene-22-one (42)

25-acetoxy-2-β-glucosyloxy-3, 16,20-trihydroxy-9-methyl-19-norlanosta-5,23-diene-11,22-dione (2-O-glucoside cucurbitacin) (43)

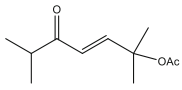
2-β-glucosyloxy-I 6,20-dihydroxy-9-methyl-19-norlanosta-5,24-diene-3, 11,22-trione (2-O-glucoside deacetoxy cucurbitacin B) (44)

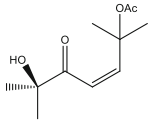
2-O-glycolside of cucurbitacin B: 25- acetoxy-2-β-glucosyloxy-16,20- dihydroxy-9-methyl-19-norlanosta-5,23-diene-3,11,22-trione (45)

25-acetoxy-2-β-glucosyloxy-16,20- dihydroxy-9-methyl-19-norlanost-5- ene-3,11,22-trione (2-O-glucoside of 23,24-dihyro cucurbitacin B) (46)



R=



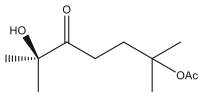


(20R)-25-acetoxy-2-β-D-glucosyloxy-3β-16α, 20-trihydroxy- 9α-methyl-19-norlanost-5-en-22-one (27)

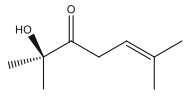
25-(acetyloxy)-2-(β-D-glucopyranosyloxy)-3,16-dihydroxy-9-methyl-l9-norlanosta-5,23-dien-22-one (28)

25-(acetyloxy)-2-(β-D-glucopyranosyloxy)-3,16 trihydroxy-9-methyl-l9-norlanosta-5,23(Z)-die one (29)

R=



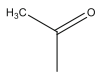
25-(acetyloxy)-2-(β-D-glucopyranosyloxy)- 3,16,20-trihydroxy-9-methyl-19-norlanost-5-en-22-one (30)



2-(β-D-glucopyranosyloxy)-3, l6,20-trihydroxy-9-methyl-l9-norlanosta-5,24-dien-22-one (31)

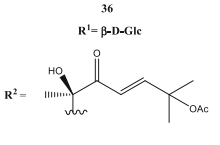
R= H

2,3,l6,20,25-pentahydroxy-9-methyl-l9-norlanost-5-en-22-one (33)

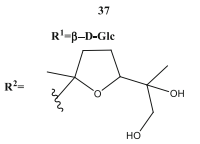


2-(β-D-glucopyranosyloxy)-3,l6-dihydroxy-4,4,9,14-tetramethyl-l9-norpregn-5-en-20-one (32)

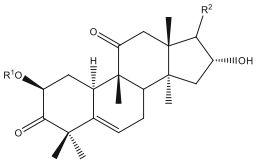
Fig. 15.5 Structures of cucurbitacin glycosides of *P. kurroa*

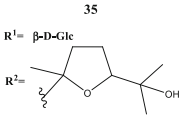


(E)-6-hydroxy-2- ((2S,9R,10R,13R,14S,16R)-16- hydroxy-4,4,9,13,14-pentamethyl-3,11-dioxo-2-((3S,4R,5S,6R)-4,5,6- trihydroxy-2-(hydroxymethyl)- tetrahydro-2H-pyran-3-yloxy)-2,3,4,7,8,9,10,11,12,13,14,15,16,17- tetradecahydro-1H-cyclopenta[a] phenanthren-17-yl)-6-methyl-5- oxohept-3-en-2-yl acetate

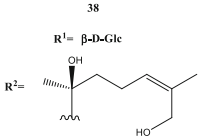


(2S,9R,10R,13R,14S,16R)-17-(5-(1,2- dihydroxypropan-2-yl)-2-methyl-tetrahydrofuran-2-yl)-16-hydroxy-4,4,9,13,14-pentamethyl-2- ((3S,4R,5S,6R)-4,5,6-trihydroxy-2- (hydroxymethyl)-tetrahydro-2H-pyran-3-yloxy)-1,7,8,10,13,15,16,17-octahydro-2H-cyclopenta[a]phenanthrene-3,11(4H,9H,12H,14 H)-dione

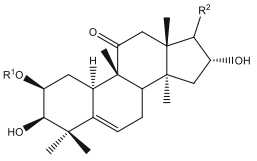


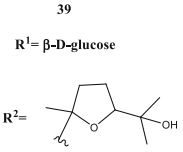


(2S,9R,10R,13R,14S,16R)-16- hydroxy-17-((5S)-5-(2-hydroxypropan-2-yl)-2-methyltetrahydrofuran-2-yl)-4,4,9,13,14-pentamethyl-2-((3S,4R,5S,6R)-4,5,6-trihydroxy-2-(hydroxymethyl)-tetrahydro-2H-pyran-3-yloxy)- 1,7,8,10,13,15,16,17-octahydro-2H-cyclopenta[a]phenanthrene-3,11 (4H,9H,12H,14 H)-dione

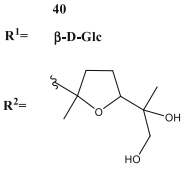


(2S,9R,10R,13R,14S,16R)-17-((S,Z)- 2,7-dihydroxy-6-methylhept-5-en-2- yl)-16-hydroxy-4,4,9,13,14- pentamethyl-2-((3S,4R,5S,6R)-4,5,6- trihydroxy-2-(hydroxymethyl)-tetrahydro-2H-pyran-3-yloxy)- 1,7,8,10,13,15,16,17-octahydro-2H-cyclopenta[a]phenanthrene-3,11 (4H,9H,12H,14 H)-dione



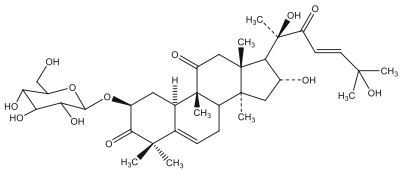


(2S,3R,9R,10R,13R,14S,16R)-3,16- dihydroxy-17-((5S)-5-(2-hydroxypropan-2-yl)-2-methyl-tetrahydrofuran-2-yl)-4,4,9,13,14-pentamethyl-2-((3S,4R,5S,6R)-4,5,6- trihydroxy-2-(hydroxymethyl)-tetrahydro-2H-pyran-3-yloxy)- 1,3,4,7,8,10,13,15,16,17-decahydro-2H-cyclopenta[a]phenanthren-11 (9H,12H,14 H)-one

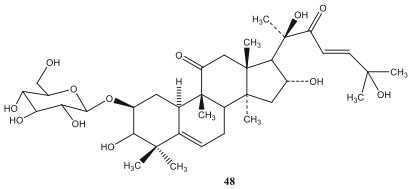


(2S,3R,9R,10R,13R,14S,16R)-17-(5- (1,2-dihydroxypropan-2-yl)-2-methyl-tetrahydrofuran-2-yl)-3,16-dihydroxy-4,4,9,13,14-pentamethyl-2-((3S,4R,5S,6R)-4,5,6-trihydroxy-2- (hydroxymethyl)-tetrahydro-2H-pyran-3-yloxy)- 1,3,4,7,8,10,13,15,16,17-decahydro-2H-cyclopenta[a]phenanthren-11 (9H,12H,14 H)-one

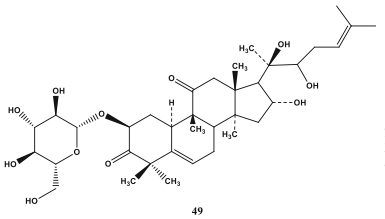
Fig. 15.5 (continued)



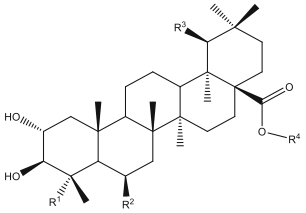
47 Arvenin-III



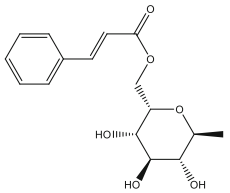
48 (2S,3R,9R,10R,13R,14S,16R)-17-((R, E)-2,6-dihydroxy-6-methyl-3-oxohept-4-en-2-yl)-3,16-dihydroxy-4,4,9,13,14-pentamethyl-2-(3,4,5- trihydroxy-6-(hydroxymethyl)- tetrahydro-2H-pyran-2-yloxy)- 1,3,4,7,8,10,13,15,16,17-decahydro-2H-cyclopenta[a]phenanthren-11 (9H,12H,14 H)-one



49 (9R,10R,13R,14S,16R)-17-(2R)-2,3- dihydroxy-6-methylhept-5-en-2-yl)- 16-hydroxy-4,4,9,13,14-pentamethyl-2-(3,4,5-trihydroxy-6- (hydroxymethyl)-tetrahydro-2H-pyran-2-yloxy)-1,7,8,10,13,15,16,17- octahydro-2H-cyclopenta[a]phenanthrene-3,11(4H,9H,12H,14 H)- dione



	R^1	R^2	R^3	R^4	
50	CH_2OH	H	OH	Glucose	2α, 3β, 19β, 23-tetrahydroxyolean-12-en-28-O-h- β -glucoside
51	CH_2OH	H	OH	H	2α, 3β, 19β, 23-tetrahydroxyolean-12-en-28-oic acid
52	CH_2OH	H	OH	Glucose	2α, 3β, 23-trihydroxyolean-12-en-28-O-β-D-glucoside
53	CH_2OH	H	H	H	2α, 3β, 23-trihydroxyolean-12-en-28-oic acid
54	CH_3	H	OH	H	2α, 3β, 19β, trihydroxyolean-12-en-28-oic acid
55	CH_2OH	OH	H	H	2α, 3β, 6β, 23-tetrahydroxyolean-12-en-28-oic acid



(34) 2-(6-O-cinnamoyl-β-D-glucopyranosyloxy)-3,16,20,25-tetrahydroxy-9-methyl-l9-norlanost-5-en-22-one

Fig. 15.5 (continued)

rhizome of *P. kurroa*. Mallick et al. (2015) detected cucurbitacin B, D, E, and betulinic acid from dichloromethane (DCM) fraction of the rhizome of *P. kurroa*. In another studies, Sindhu et al. (2011) isolated 20 different components including cucurbitacins from *P. kurroa* extract by employing reverse phase-HPLC method. The detailed metabolic profiling of *P. kurroa* extract of the leaves and rhizomes was performed by a combination approach using HPTLC, NMR, and LC-MS/MS analysis. The secondary metabolites were significantly different in rhizomes and leaves. The amount of cucurbitacin and flavonoids was found to be more in leaves whereas rhizomes were rich in iridoids Kumar et al. (2016a, b). The ethyl acetate seeds extract of *P. kurroa* reported one novel (**50**) and five already known triterpenoids (**51–55**).

15.7.3 Phenolic Compounds

Rhizome and root of *P. kurroa* exhibited a large number of monocyclic phenolics like ellagic acid and vanillic acid (**64**), androsin, and the corresponding glycosides like androsin and picein. Stuppner and Wagner (1989) isolated two phenolic compounds picein and androsin from the roots of *Picrorhiza*. Picein was reported to be purified from the leaves of *P. kurroa* by means of column chromatography by Kant et al. (2013). Phenolic compounds and their glycosidic derivatives such as gallic acid (**58**), ellagic acid (**59**), isocorilagin (**60**), 1-O-galloyl-β-D-glucose (**61**), trigalloyl β-D-glucose (**62**), and pentagalloyl-β-D-glucose (**63**) (Fig. 15.6) were detected also in the seeds of the plant (Zhang et al. 2004). The rhizome of *P. kurroa* also has flavonoids like apocynin (**67**) which is associated with anticancerous potential (Mallick et al. 2015).

15.7.4 Other Chemical Constituents

Other phytoconstituents like cinnamic acid, vanillic acid, ferulic acid, D-mannitol; aliphatic compounds like shikimic acid, (Fig. 15.7) acyl picraldehydes including lauryl and myristyl picraldehyde; glucovanillin, capryl vanillic acid, picrortetraglucoside, and picraldehyde α-D-diglucoside have also isolated from the methanolic extract of the rhizomes *of P. kurroa* (Ali et al. 2017; Bhardwaj et al. 2021).

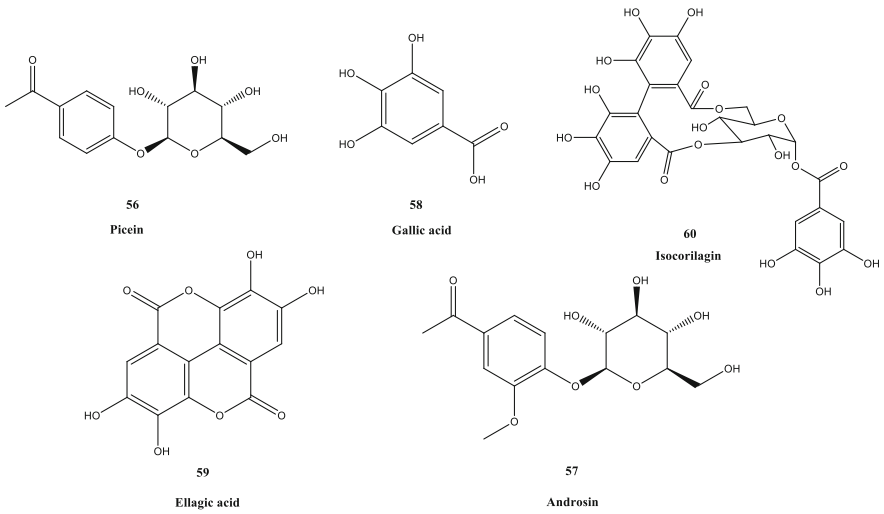


Fig. 15.6 Phenolic compounds present in *Picrorhiza kurroa*

15.8 Pharmacology

Picrorhiza kurroa has long been used for the cure of liver complications, lung infections, fever, scorpion stings, indigestion, and chronic diarrhea (Sandhiya 2020). *Picrorhiza*, like many other medicinal plants, is endangered by overharvesting. This plant is a key herb in several ayurvedic hepatic preparations in India. Picroliv is an iridoid glucoside combination extracted from *P. kurroa*'s roots and rhizomes. It has been utilized as a potent hepatoprotective drug in conditions like jaundice as it contains a significant amount of picroside I and kutkoside (Ansari et al. 1988). This plant extract has also been revealed to show properties like cardioprotective, antidiabetic, nitric oxide scavenger, anticancer, and anti-viral (Sandhiya 2020). It displays antiallergic, anti-asthmatic, choleretic, hypoglycemia, hypolipidemic, anti-viral, antimalarial, anti-phosphodiesterase, neuritogenic, molluscicidal, and leishmanicidal effects (Singh and Banyal 2011). In addition to it, parts of *P. kurroa* like shoots, stolons, and roots are utilized in traditional and modern medicine for urinary infections, snake bites, leukoderma, scorpion stings, inflammatory infections (Fig. 15.8), antiperiodic (Sturm and Stuppner, 2000), cholagogue, stomachic, and laxative activities (Bhandari et al. 2008; Dharmender et al. 2011). Livokin, Picroliv, Livomap, Picrolax, Tefroliv, and other health-promoting herbal tonics are made from the *P. kurroa* extract (Sud et al. 2013).

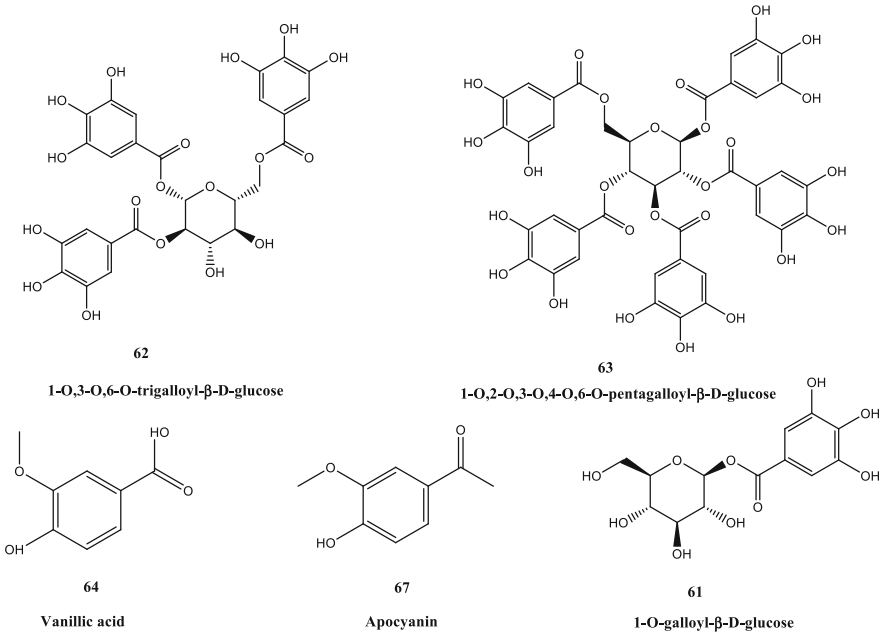


Fig. 15.6 (continued)

15.8.1 Hepatoprotective

Hepatocytes are cells that form the 70–85% of the liver's part and are found in the primary parenchymal tissue. When the concentration of serum transaminase enzymes increases, hepatocytes die and subsequently cause hepatic damage (Navarro and Senior 2006). Hydroalcoholic extract of *Picrorhiza kurroa* reversed the fatty alterations in the liver of rats which are induced by high fat diet. Histopathology studies revealed that the plant extract declined the hepatic fatty intrusion and reduced the amount of lipids in comparison to the only high fat diet control group (Shetty et al. 2010). Moreover, in another study, hydroalcoholic extract of *Picrorhiza kurroa* exhibited protective effect in albino rats against hepatic oxidative damage induced by potassium dichromate. Administration of potassium dichromate caused a remarkable enhancement in the concentration of liver enzymes like alanine transaminase, alkaline phosphatase, and aspartate transaminase and increased biomarkers like thiobarbutric acid reactive species and carbonyls and declined antioxidant enzymes like superoxide dismutase, catalase in serum. However, *Picrorhiza kurroa* extract enhanced the significant restoration of the concentration of hepatic markers and antioxidant enzymes (Navya et al., 2018). Moreover, Jia et al. (2015) demonstrated that Picroliv, a bioactive component of *Picrorhiza kurroa* decreased the elevated hepatic enzymes in mice which were enhanced due to liver damage. Thakur et al. (2021) detected many peptides in the *P. kurroa* hydrolysate, and these

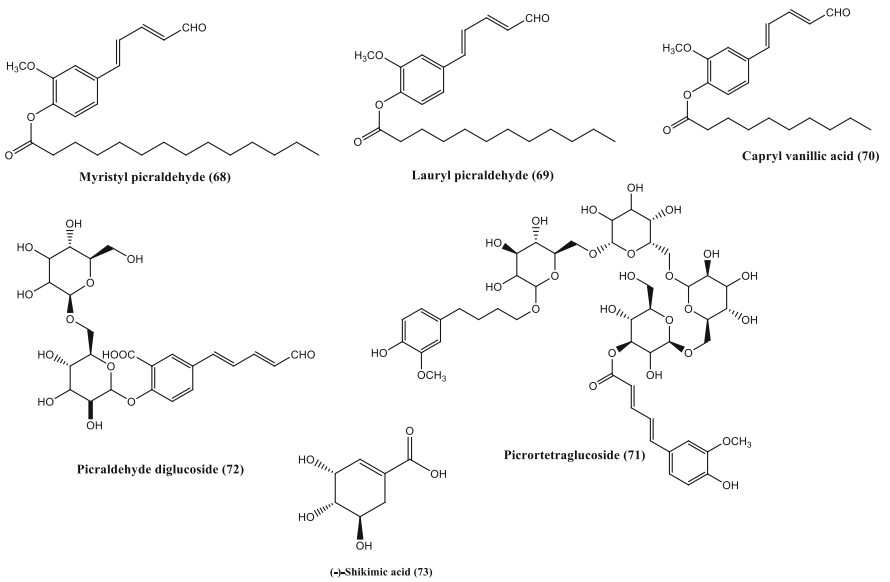


Fig. 15.7 Other constituents present in *Picrorhiza kurroa*

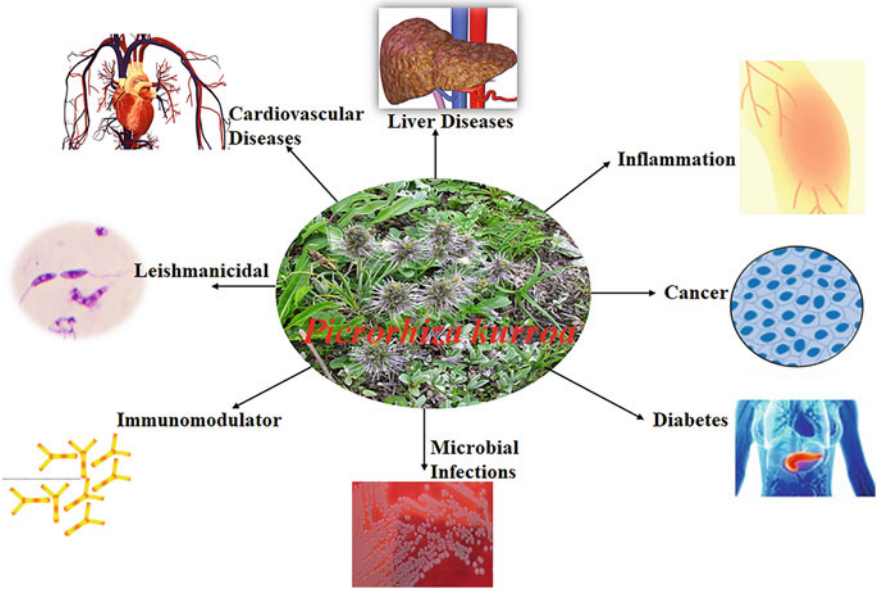


Fig. 15.8 Pharmacological potential of *Picrorhiza kurroa*

peptides revealed antioxidant characteristics and hindered dipeptidyl peptidase and angiotensin-converting enzyme. BP1 displayed reduction in the ROS (reactive

oxygen species) content, malondialdehyde, and stimulating the innate antioxidant defense activity. Treatment with *Picrorhiza kurroa* extract displayed significant decline in liver impairment in numerous animals and for hepatitis clinically. Picroside II remarkably lessened free fatty acids stimulated lipotoxicity. These results advocate a prerequisite to grow a new drug candidate of picroside II for hepatic infections (Dhami-Shah et al. 2021).

15.8.2 Neuroprotective

Picrorhiza kurroa extract showed improvement in the cognitive function, neuroinflammation, and morphology of microglia in Alzheimer's disease. Moreover, the mechanisms associated with the beneficial activity of herbal extract in Alzheimer might comprise regulation of NLRP3 inflammasome-mediated microglia stimulation and downregulation of expression of secretase (Kim et al. 2020).

15.8.3 Immunomodulatory

An immunomodulatory agent is a kind of medicine which might serve as an immunosuppressor immunostimulatory dependent on its impact on the immune response. The bioactive fraction from *Picrorhiza kurroa* rhizomes boosted the immunity of mice by enhancement in the concentration of lymphocytes and cytokines (IFN-γ) in serum, phagocytic index, and $CD4^+/CD8^+$ T cells (Gupta et al. 2006). Hussain et al. (2013) revealed protective action of *Picrorhiza kurroa* (200 mg/kg body weight) on the humoral immunity (Fig. 15.9). Administration of *Picrorhiza kurroa* extract caused remarkable induction of cellular and humoral antibody response. However, the alcoholic extract of *Picrorhiza* induced a strong delayed type hypersensitivity in comparison to the water extract. Pre-treatment with *Picrorhiza kurroa* stem extract repressed macrophage-associated cytokines like TNF, IL-1, IL-10, IL-6, and by the decrease of NF-κB signaling, resulting in anti-inflammatory action (Kumar et al. 2017a, b). Picroside II, active component of *Picrorhiza scrophulariiflora*, also revealed anti-inflammatory characteristics in cells and animals by depressing the concentration of cytokines. It hampered the induction of p65 NF-κB signaling pathway in comparison to the lipopolysaccharide (LPS) stimulation (Shen et al. 2017).

15.8.4 Anti-asthmatic Potential

The anti-asthmatic action of *Picrorhiza kurroa* root extract was examined in guinea pigs in in vivo as well as in vitro models. The histamine-stimulated

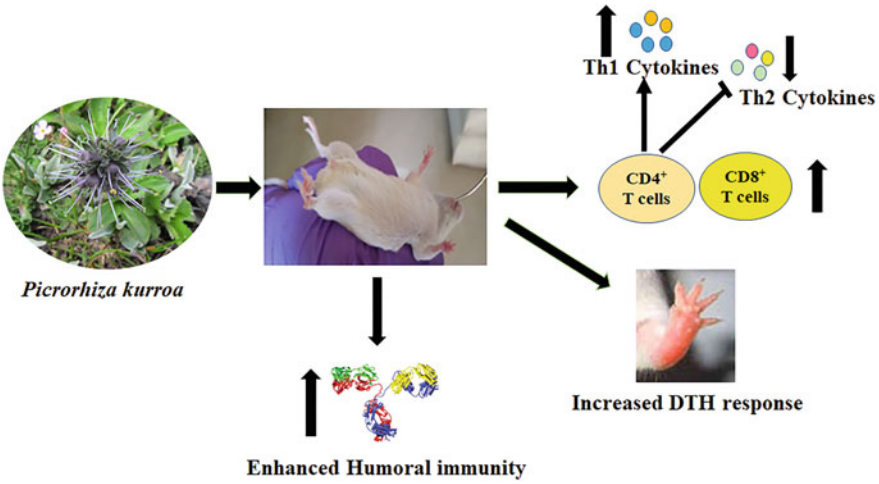


Fig. 15.9 Immunomodulatory potential of *Picrorhiza kurroa*

bronchoconstriction in guinea pigs was employed in animal research. The *Picrorhiza kurroa* extract showed an extreme level of protection of 52%, which was near to the 66% caused due to salbutamol. The effect of the herbal alcoholic extract on guinea pig ileum was determined to monitor how the extract displayed muscular relaxant effect. The extract was observed to be active against histamine and acetylcholine-stimulated contractions in the study. The extract revealed a relaxing action against histamine and acetylcholine-stimulated contractions. The anti-asthmatic potential of herbal extract might be attributed to the existence of saponins and flavonoids (Sehgal et al. 2013).

15.8.5 Anticancer Activity

Stem of *P. kurroa* showed potent anticancer activity as it contained potent active constituent like cucurbitacins, glycosides like picroside and apocynin (Kong et al. 2014). Combination of *P. kurroa* extract with other plants repressed the development of breast cancer cell lines (Suryavanshi et al. 2019). The dichloromethane extract of *P. kurroa* established active anticancer effect (Mallick et al. 2015). Antimetastatic activity of picroside was determined in mammalian breast cancer cells in in vitro as well as in animal models. Picroside II caused a significant reduction in the metastasis of MDA-MB-231 cancer cells. Picroside treatment induced a noticeable deterioration in the concentrations of metalloproteinase in the cancer cells. Picroside II exhibited significant decrease in the amount of CD31, a marker of angiogenesis, depicting that picroside has anti-angiogenic action. Moreover, treatment of picroside decreased the umbilical vein endothelial cell motility, invasion, and matrix metalloproteinase 9. Picroside II triggered a noticeable decline

in the angiogenesis in the chorioallantoic membrane of a chick embryo (Lou et al. 2019). The active components of *Picrorhiza kurroa* revealed anti-invasive efficacy against breast cancer cells, and this repressive outcome was accredited to the suppression of collagenases and gelatinases activity (Rathee et al. 2013). *P. kurroa* extract hindered the sarcoma and extended the lives of mice with tumor and lessened the amount of solid tumors. It showed significant inhibition in the proportion of topoisomerase I and II in *S. cerevisiae* mutant strains (250 g/mL) (Joy et al. 2000). The anticancer potential of Picrosides is due to the deactivation of TNF, IL-6, IL-1, and NF-κB gene. Picrosides suppressed the cancer growth by impeding the levels of cell cycle check points. Thus *P. kurroa* and its active components might be a worthy candidate for the therapy of cancer (Soni and Grover 2019).

15.8.6 Anti-inflammatory

Inflammation is a limited tissue defense response to infection, characterized by redness, puffiness, discomfort, and, in few cases, loss of function. Administration of stem extract of *P. kurroa* caused marked reduction in joint inflammation (Kumar et al. 2016a, b). It possesses robust activity against edema stimulated by chemicals and might be considered as a superior natural analgesic. *P. kurroa* displayed protective efficacy in murine models against arthritis induced due to formaldehyde and adjuvant. *P. kurroa* extract showed marked decline in the expression of TNF receptor-1, IL-6, and IL-1 in the arthritic rats as compared to the control. Treatment of *P. kurroa* extract reduced the levels of nitric oxide, malonaldehyde, and TNF-α and enhanced the concentration of superoxide dismutase, glutathione, and catalase activities. Moreover, plant extract inhibited the matrix metalloproteinases-9 and metalloproteinases-3 in arthritic rat. Mathias et al. (2020) described the anti-inflammatory potential of formulations comprising of *P. kurroa, Zingiber officinale, B. diffusa*, and *Phyllanthus niruri*. These formulations caused reduction in the TNFα, NFκB, lipoxygenase, and cyclooxygenase and depicting the protective activity of these herbal formulations against inflammatory diseases.

15.8.7 Antimicrobial Activity

The protective potential of *P. kurroa* root extract was tested against *Trichophyton rubrum, Penicillium marneffei, C. albicans*, and *Candida tropicalis*. Ethanolic extract of *P. kurroa* were found to be active in the inhibition of these experimental fungal isolates (Shubha et al. 2016). In addition to it, acetone and methanolic extracts of *P. kurroa* exhibited microbicidal action against the pathogenic microbes like *Fusarium oxysporum, Rhizoctonia solani, Sporisorium scitamineum*, and *Erwinia chrysanthemi* (Laxmi and Preeti 2015). In another study, dichloromethane extract of *P. kurroa* unveiled maximum growth inhibition (39.06 ± 1.0 mm) against

Staphylococcus aureus bacteria; however, methanol extract displayed the inhibitory activity 6 ± 4.1 mm against *Escherichia coli*. Hexane and dichloromethane extract of *P. kurroa* showed a potent activity against breast cancer MCF7 and ovarian cancer cell lines (Tabassam et al. 2021).

15.8.8 Nephroprotective

The protective efficacy of *P. kurroa* was monitored in mice against nimesulide-induced toxicity. Nimesulide (750 mg/kg body weight) caused renal toxicity in mice which is documented by the raised abnormal concentration of serum creatinine and urea. However, administration of *P. kurroa* declined the levels of functional serum biomarkers of kidney within the normal range in nimesulide-treated animals. Further histopathological analysis also confirmed that treatment of *P. kurroa* reversed the damage to the kidney (Siddiqi et al. 2018). *P. kurroa* root extract administration caused marked decrease in fasting glucose concentration in diabetic rats induced by streptozotocin-nicotinamide which depicts the protective potential of this plant against diabetes (Husain et al. 2009). In another study, *P. kurroa* enhanced the concentration of insulin and GLUT levels of muscle of diabetic rats. Histological studies indicated that the treatment of plant induced the renewal of pancreatic β-cells. Administration of plant extract boosted the GLUT-4 levels that leads to facilitated uptake of glucose by skeletal muscles in diabetic animals (Husain et al. 2014). Moreover, *P. kurroa* extract caused the β-cell renewal with an increase in the quantity of insulin and displayed the anti-hyperglycemic activity by upgrading the insulin-induced transfer of GLUT 4 from cytoplasm to membrane. It indicated the improved glucose uptake by muscle cells and a better-quality glycemic regulation in diabetes (Kumar et al. 2017a, b). Moreover, Ibrahim et al. (2021) reported that hydroethanolic extract of *Picrorhiza kurroa* showed potent antidiabetic activity. The UPLC/MS studies showed the 23 most bioactive constituents. Hydroethanolic extract of *Picrorhiza kurroa* displayed remarkable inhibitory activity against α-amylase and glucosidase. It also consisted of huge quantity of phenols and flavonoids and displayed strong free radical scavenging action. Further, hydroethanolic extract of *Picrorhiza kurroa* restored the glucose-stimulated rise in hepatocytic enzymes, aldehyde dehydrogenase, and hexokinase. Moreover, administration of *Picrorhiza kurroa* remarkably upgraded the glucose-induced cytotoxicity in HepG2 cells. It indicated the potential of *Picrorhiza kurroa* in the management of hyperglycemia, diabetes, and oxidative stress.

15.8.9 Cardioprotective

P. kurroa extract showed efficacy against cardiomyopathy in rats induced due to adriamycin. Treatment with *P. kurroa* displayed a remarkable waning in the enzyme

levels like creatine phosphokinase, lactate dehydrogenase, alanine aminotransferase, and peroxidation of fats in myocardial tissue. The efficacy of plant extract was due to its membrane stabilizing effect or its capability to sustain the typical levels of antioxidant enzymes which shield the myocardial membrane from peroxidative impairment by diminishing the lipid peroxidation (Rajaprabhu et al. 2007). Root extract of *P. kurroa* displayed recovery of hemodynamic and left ventricular dysfunctioning which was induced due to the exposure of isoproterenol. Treatment of plant extract exhibited reduction in oxidative stress by repairing the activity of various markers like catalase, myocardial superoxide dismutase, and glutathione. The concentration of creatine kinase and lactate dehydrogenase was observed to be low after the treatment of *P. kurroa.* All these studies depicted that *P. kurroa* extract can be a potent cardioprotective agent, and it might lead to its extensive use in the future (Nandave et al. 2013).

15.8.10 Analgesic and Healing Activity

The ethanolic *P. kurroa* root extract was monitored for analgesic activity in albino mice. Hot plate and chemical stimulated-writhing technique was used for determining the pain-relieving activity. The *P. kurroa* displayed comparable efficacy in comparison to the standard drug pentazocin (Shid Rupali et al. 2013). *P. kurroa* extract caused healing in the indomethacin-induced ulceration in gastrointestinal wall. It augmented the levels of mucin and prostaglandins along with enhancement in the activity of growth factors and cyclooxygenase enzymes (Banerjee et al. 2008).

15.8.11 Antioxidant Potential

Antioxidant compounds serve as radical scavengers which hinder the human health from several fatal infections (Kalaivani and Mathew 2010). Deshpande et al. (2015) described that *P. kurroa* administration showed significant decline in the liver enzymes among the hepatic cirrhosis patients. Treatment with *P. kurroa* displayed potent DPPH radical scavenging and metal chelating potential and IC_{50} was observed to be 75.16 ± 3.2 and 55.5 ± 4.8 μg/mL, respectively (Rajkumar et al. 2011; Krupashree et al. 2014). Antioxidant potential of *Picrorhiza* extract specifies its vigorous part to diverse oxidative stress associated ailments, as a food additive and reservoir of natural antioxidants.

15.8.12 Other Therapeutic Effects

The active components of *Picrorhiza* like picrorhizaosides D and minecoside displayed a robust hyaluronidase inhibitory action as compared to those of the antiallergic medicines (Morikawa et al. 2020). The protective efficacy of *Picrorhiza kurroa* was determined against anemia in rats induced due to phenylhydrazine. The alcoholic leaves extract of *Picrorhiza kurroa* at 100–200 mg/kg/day amplified the levels of red blood cells, hemoglobin, and reticulocytes in anemic rats (Turaskar et al. 2013). In another investigation, Picroliv in combination with sodium stibogluconate (SSG) exhibited positive leishmanicidal reaction in infected hamsters. It showed higher leishmanicidal activity in infected hamsters in contrast to the SSG alone. It caused marked drop in the parasite load in infected golden hamsters. Moreover, treatment of Picroliv also presented hepatoprotective activity by averting the changes of vital enzymatic markers (Mittal et al. 1998). Moreover, the active component of this plant Picroliv showed good activity against DSS (dextran sulfate sodium)-induced ulcerative colitis. Treatment of picroliv revealed enhancement in the levels of myeloperoxidase, and MDA along with a decrease in concentration of IL-1, TNF, and NF-κB in mice (Zhang et al. 2012).

15.9 Clinical Studies

There are wide range of experimental and clinical trials conducted on the antiallergic potential of *Picrorhiza* (Vaidya et al. 1996; Thomas et al. 2007). Baruah et al. (1998) described that active component Picroliv also possess antiallergic action by the suppression of secretion of chemical mediator from mast cells. Picroliv is an authenticated for therapeutic use as hepatoprotectant in India and has the ability to shield the liver from paracetamol and carbon tetrachloride toxicity (Gupta 2001; Guliani et al. 2016). A clinical study of Arogyavardhini (medicinal formulation of *P. kurroa*) was conducted on patients with viral hepatitis. This herbal formulation showed good efficacy against hepatitis (Antarkar et al. 1980). Another herbal formulation, AYUSH-64 showed potent activity against malaria and filariasis and 76% patients displayed no reversion of the infection after 1 month of follow-up duration (Chari et al. 1985). Moreover, stem extract of *P. kurroa* at higher concentration caused profound reduction in the respiratory complications in patients with pulmonary disorders. Roots and stem extract of *P. kurroa* demonstrated marked decline in the concentration of cholesterol in 80% patients with hypercholesterolemia (Tiwari and Jain 1980; Bikshapati et al. 1996).

15.10 Toxicity

Picrorhiza has more solubility in ethanol as compared to water; therefore, it can be taken as a essence (bitter flavor). However, it is commonly given as a standardized extract (picroliv/kutkin). The typical concentration of plant for adult is 0.4–1.5 g/day though regular concentration of *Picrorhiza* is 3500 mg/day for fevers. *Picrorhiza* roots are extensively employed in India with less negligible side effects. *Picrorhiza* extract displayed non-toxicity in in vivo models and free of mutagenic and teratogenic activity (Jain and Sethi 1992; Verma et al. 2009). Oral studies indicated that lethal dose (LD_{50}) of *Picrorhiza* extract was observed to be more than 2600 mg/kg in murine models; however, LD_{50} of picroside and kutkoside was observed to be more than 1000 mg/kg. In another study, *P. kurroa* showed no adverse impact in murine models at the concentration of 2 g/kg body weight and according to preclinical investigations; this plant was observed to be safe for use in herbal formulations (Krishna et al. 2016).

15.11 Threats and Conservation

About 90% of market demand of *Picrorhiza* is met from the wild populations and haphazardly harvested for raw materials (Rai et al. 2000). For example, for 1 kg of the dry weight of the species 300–400 individuals are uprooted (Uniyal et al. 2011). So more demand, high use value and small population size, has been figured *Picrorhiza* among the priority species for cultivation and conservation and in Western Himalayas. This plant species has been listed as endangered by IUCN (Nayar and Sastri 1990). Further, the species is under depletion and in Himachal Pradesh and Jammu and Kashmir (Chauhan 1988), where its assigned endangered status and also in Uttarakhand (Shah 1975), where it was declared critically endangered.

15.11.1 Conservation through Cultivation

Seeds of *P. kurroa* has a very thick coat and are covered by a hyaline reticulate sac that has a vital role in seed dispersal (Raina et al. 2010). The normal seed germinated within 20 days and complete its life span in 5 months in temperate conditions (Patial et al. 2012). The high percentage of seed germination (98%) is found in water heated seeds at 40–45 °C for 60 s, which is a requisite temperature required for the germination of seeds, which naturally is obtained by sun rays after melting of snow (Körner, 2003). Similarly, high germination of seeds was obtained in fresh seeds, which get reduced after 5–6 months (NMPB 2008). For vegetative reproduction, the plant perennates through underground runners with active growth confined

to the summers, and also through seeds in nature. The unorganized harvesting and lack of cultivation policy/strategies resulted in the substantial diminution of natural populations of the species and results in its listing in endangered species of IUCN criteria (Nayar and Sastri 1990; Rai et al. 2000). To overcome this situation, cultivation practices are the only option to save the wild populations. Sustainable farming of such medicinal herbs will diminish the pressure on the diversity of plants and also confirm a consistent availability of raw materials (Muhammad et al. 2006, Sahoo et al. 2010). *P. kurroa* needs a suitable climate like that of Himalayan ranges, with the example of Garhwal Himalayas, where some industries have been implanted and also adopted by the local inhabitants as a curative option of livelihood (Nautiyal and Nautiyal 2004). Further, for the cultivation, of *P. kurroa*, High Altitude Plant Physiology Research Centre (HAPPRC) in district Rudraprayag of Uttarakhand has developed the agro-techniques and cultivation cum demonstration nursery, where different morpho/eco-variants from different localities are cultivated. Farmers of this region have shown great interest and are provided with planting material with proper field training for cultivation on large scale (Chand et al. 2016).

15.11.2 Micropropagation

The use of conventional propagation methods like vegetative propagation and seed germination, etc. are not enough to encounter the growing demand (Nadeem et al. 2000). Therefore, to meet the increasing demand and reduce collection pressure on wild species, micropropagation/tissue culture techniques is an alternative for the extensive plantlets production and or rapid cloning of desired genotypes. Micropropagation of selected geno/ecotypes helps in mass multiplication of high yielding ones (Chandra et al. 2006, Jan et al. 2010); there is a significant disparity in the regeneration response that makes it impractical to grow all populations with one method. In the current situation, micropropagation in both basic and practical aspects is much more organized than it was in the beginning. The most commonly used explant is shoot tips, however, regeneration potential of other explants like axillary buds, floral stalks, inflorescence segments, stem discs, leaves, leaf peels, perennating organs (pseudobulbs, rhizomes, tubers), and roots have also been utilized successfully (Arditti 2008). But the usage of leaf explants holds tremendous potential for numerous shoot formation and consequent use for mass multiplication and regeneration as there is no damage to the plant and constitute a vital phase towards the ex situ conservation. Furthermore, the adaxial of the leaf is favored over the abaxial surface, as it can be correlated to more palisade parenchyma in the earlier (Welander 1988). However, there are few reports of shoot formation and callus formation with tissue from abaxial surface (Sujatha et al. 2008). Exposure to Thidiazuron also promotes high-efficiency shoot proliferation as reported in *Phaseolus lunatus* (Mok and Mok 1985). Further, Thidiazuron inhibits cytokinin oxidase and modulates the concentration of hormones in plants (Hutchinson et al. 1996; Murthy et al. 1998). Cytokinin supplemented medium was also used for vitrification of shoots in

P. kurroa (Upadhyay et al. 1989). However, upon transfer medium with cytokinins and auxins, a normal shoot formation was observed from vitrified shoots (Chandra et al. 2006), but, the opposite result was observed upon transfer to agar medium (Zimmerman et al. 1991; Chandra et al. 2006). The highest germination (95%) of *Picrorhiza* seeds have been reported in sandy soil (1:1) at pH 5.5–5.8 (Patial et al. 2012). Similarly, a pH range of 5.0–5.7 favors seed germination and also helps in the colonization of vesicular-arbuscular mycorrhiza (Singh et al. 2008), which also increases seedling.

15.12 Cultivation

Since we depend on the wild population to meet the market demands of *P. kurroa*, for which, cultivation is useful for its conservation and sustainable supply. So its experimental cultivation in mountain villages of temperate Himalayas by local inhabitants as a curative option of livelihood from 2001 onwards (Nautiyal and Nautiyal 2004). During 2001, farmers of Ghes village (Chamoli, Uttarakhand), situated at an altitude of 2300–2500 m, were motivated by the HAPPRC institute. Farmers of this region have shown great interest and are provided with planting material with proper field training for cultivation on large scale (Chand et al. 2016). Similar initiatives were taken by Horticulture Department, Forest Department of Uttarakhand, Herbal Research and Development Institute, Gopeshwar, a nodal agency to promote cultivation activities in medicinal plants as cash crops and providing financial aids to cultivate medicinal plants. For example, in the years between 2007 and 2010, an average productivity of *P. kurroa* is around 500 kg/ha (estimation), and about 1.43 tons were marketed @ Rs 243.25/kg from Uttarakhand (Kuniyal et al. 2013). The yield was encouraging for 2015–16, where a total 253.00 kg @ Rs 1500.00/kg was exported collective efforts of an innovative farmer and government (Kuniyal et al. 2015).

15.13 Formulation and Market Product

A large number of drug formulations of *Picrorhiza kurroa* are available in Ayurveda. A drug formulation named "Aarogyavardhini gutika" containing *P. kurroa* in more than 50% amount predominantly improves the digestive system, constipation, improves liver functioning, reduces cholesterol, prevents *atherosclerosis and is associated with* anti-viral and anti-inflammatory properties (Verma et al. 2009). "Mahatiktaka ghrita" is another poly-herbal medicated formulation with ghee base and is used in a preparatory procedure called "Snehakarma" for treating various skin diseases such as eczema, boil, pus discharge, leprosy, leukoderma, bleeding disorders, and peptic ulcers. One more herbal formulation of *P. kurroa* named "Picroliv" acts as an efficient hepatoprotective compound for treating a number of liver

Table 15.4 Different drug formulation of *P. kurroa*

Drug Formulation	Picroside-I (%)	Picroside-II (%)
Katuki	1.29	1.16
Arogya	1.01	0.55
Kutaki	4.17	3.25
Livocare	0.06	0.14
Livocare	0.12	0.06
Livomyn	0.07	0.01
Livplus	0.22	0.49
Pravekliv	0.15	0.12
Vimliv	0.001	Traces

ailments (Verma et al. 2009; Sud et al. 2013). "Kutaki" powder has excellent phototoxic properties and is recommended for managing vitiligo and has beneficial properties in controlling asthma and rheumatoid arthritis. Every 1.585 g of "Picrolax" herbal supplement, an extract of *Picrorhiza kurroa* containing picroside I and picroside II in 45 mg and 1.585 g amount, respectively, is used as a laxative (Bhardwaj et al. 2021; Verma et al. 2009). Additionally, "Livomyn" herbal formulation (Table 15.4) is helpful in regulating liver enzymes and maintaining proper functioning of liver cells (Thani 2018; Bhandari et al. 2009). Likewise, "Virulina," the polyhedral formulation of *P. kurroa* is a blend of many immune-stimulating herbs and acts as an efficient therapy for immune system improvement.

15.14 Conclusion and Future Perspectives

From the above discussion, *P. kurroa* extract is an essential therapeutic which includes several phytoconstituent that balance the body's metabolic function and cure a variety of illnesses. This herb has tremendous usage in the traditional medical system. *P. kurroa* extract is widely used against a number of malignancies and a wide range of ailments. This plant has huge potential as hepatoprotective, anti-asthmatic, immunomodulatory, and anticancer agent. *P. kurroa* and has massive marketable potential and profits for the healthiness of the society. Furthermore, researches are required to explore the chemical composition and biological activities of *P. kurroa*. It is vital to detect the composition and structure of many phytocomponents in *P. kurroa* extract to figure out its bioactive properties for the production of drug candidates against several fatal infections. More clinical studies should be conducted to determine the biological activity of *P. kurroa.* Moreover, continuous efforts are required for the conservation of endangered herb *P. kurroa.*

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Chapter 16
Species of the Genus *Polygonatum* found in the Western Himalayas

Khushbu Gumber and Heena Barmota

16.1 Introduction

Polygonatum, universally recognized as the Solomon's seal, is decumbent recurrent herb that refers to more than 50 species of the biosphere. It is extensively disseminated in the northern temperate region of the hemisphere, especially the Himalayas (Saboon et al. 2016). It is basically characterized by its thick, fleshy slinking sympodial rhizomes that refers to its general name, *Polygonatum,* meaning yovi, a knee, that refers to many little knees as its main characteristic (Miller 1754).

The extraordinary features of these plants make them a valuable medicinal herb. All plant parts have significant properties, like roots that are eaten as raw vegetable in ancient period to treat menstrual troubles, anaemia, urino-genital disorders, gastric problems, nerve tonic, general weakness, wounds, appetizer, backache, etc. The other important part is rhizomes that are consumed as syrup or by mixing with dairy products to cure wounds, general body weakness, kidney trouble. However, its tuber part is taken as vegetable and used for anorexia, promote body heat, urinary problems, general debility, fever, appetizer, and nerve tonic. Further, another part bulb is used in powdery form to cure problems like tuberculosis, dampness, and diabetes. The seed of *Polygonatum* and its members are used to treat indigestion while whole herb is utilized to cure appetite, kidney distress, strengthen body, and as a nerve tonic (Suyal et al. 2021).

Among the various important species of *Polygonatum*, *P. cirrhifolium*, *P. multiflorum*, and *P. verticillatum* are vital Himalayan herbs (Fig. 16.1)

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P. cirrhifolium

P. multiflorum

P. verticillatum

Fig. 16.1 Himalayan *Spp.* of *Polygonatum*

(Balkrishna et al. 2012; Ballabh et al. 2008). These three species are widely being utilized in various ayurvedic formulations like Chyavanprash, Dhanwantharam Thailam, Dhanwantharam Kuzhambu, Dasamoolarishtam, etc. Their bio-efficacies are owed to the secondary metabolites that are the main constituent of these plants. These secondary metabolites play preventive role against the ageing processes, molecular damage, and increased longevity, etc. But the overexploitation of these species due to unrestrained harvesting, degradation of the plant habitats, over-grazing, and various anthropogenic pressures leads to enormous loss to the herbal industry (Suyal et al. 2020). Though, no precise data on *P. multiflorum* is available yet, but the two other species viz. *P. cirrhifolium* and *P. verticillatum*, are said to be vulnerable (Ved et al. 2003).

This chapter therefore emphasis on complete data about taxonomy, distribution, phytochemistry, pharmacology, traditional uses, agro-techniques, biotechnology, toxicology, conservation, and sustainable harvesting of all the three important species of *Polygonatum*.

16.2 Taxonomy

The botanical description of the plant *Polygonatum* species is given below in Fig. 16.2.

16.3 Common Names

The common nomenclature for *Polygonatum,* used in various regions of origin of plants is different. *P. cirrhifolium,* King's Solomon's seal is associate of Ashtawarga also said to be *Convallaria cirrhifolia*, the coiling leaf or the tendril leaf Solomon's seal, or coiling leaf *Polygonatum* (Singh and Patra 2019). It is locally known as Khakan, in Ayurveda said to be meda and in Sanskrit called Manichhidra, Dhara, and Svalpaparni (Gaur 1999).

The other important species that is more pronounced in China is *P. multiflorum,* the Eurasian Solomon's seal. It is commonly referred to as David's harp, ladder-to-heaven or Shou Wu Pian, He Shou Pian, Fo-Ti, and Chinese knotweed.

P. verticillatum is recognized as whorled Solomon's seal and commonly called Kantula and mitha dudhia (Nautiyal and Nautiyal 2004; Gaur 1999). It is also known as mahameda in Ayurveda; Devamani, Tridanti, and Vasuchhidra in Sanskrit.

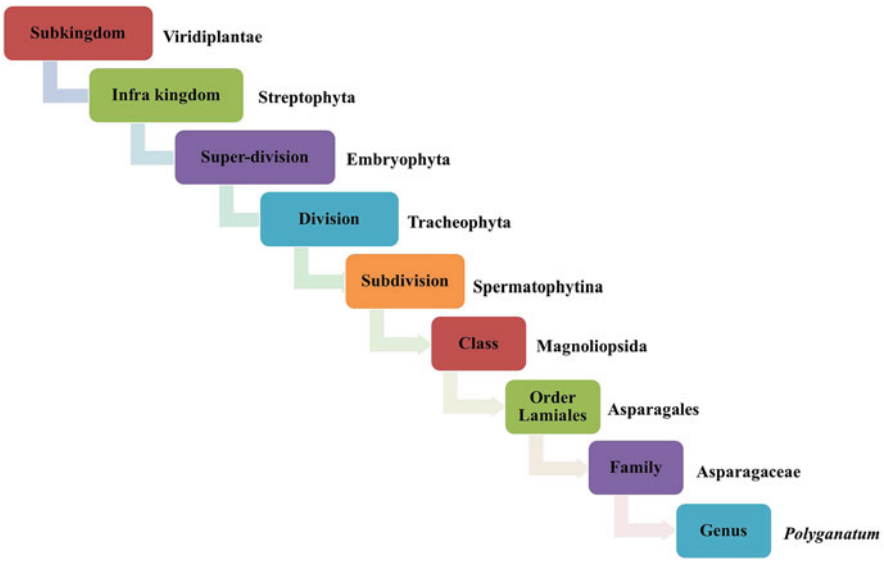


Fig. 16.2 Botanical description

16.4 Flowering and Fruiting Seasons

The flowering of the plant takes place for several weeks from the month of June to October followed by fruiting of spherical, blue-black grapes like fruits that are often eaten by birds.

16.5 Distribution

The distribution of *Polygonatum* sp. is worldwide. It is widespread in Europe, China, and Himalayas. *P. cirrhifolium* are majorly found in forests, shrubberies, and open slopes of eastern Asia, ranging from Asia to China. The species are present in the temperate region around 1200–4200 m high and 1500–3700 m westwards from Himachal Pradesh and Uttarakhand (Bhatt et al. 2014).

P. multiflorum is native to Europe along with temperate Asia whereas *P. verticillatum* is present in the temperate region at height of 1800–3900 m, it is distributed from Kashmir to Northeast States of India, Southeast Tibet, Nepal, Bhutan, China, Russia, Afghanistan, Pakistan, and Garhwal Himalaya (Naithani 1984) and from montane to alpine Himalaya (Vashistha 2006) and Europe (except Mediterranean region) (Gaur 1999; Chauhan 1999).

16.6 Morphology

P. cirrhifolium is tall erect, perennial herb that is up to 120 cm high with sessile, whorled, and linear tendril tips leaves. Flowers are pink, white, or greenish purple over small stocks. The fruits are small round blue-black berries. Rhizomes of the plant are thick and fleshy (Ali 2007; Zhengyi et al. 2013).

P. multiflorum is a rhizomatous perennial herb, 90 cm tall and 25 cm wide. The stems are arching with alternate leaves with pendent tubular greenish white flowers hanging under the stems. It colonize in the area of shade as required for woodland planting.

P. verticillatum is tall, erected herb with 60–120 cm height. Leaves of this specie is also sessile, whorled, long, with acute tips and glaucous beneath, that are ciliolate near the ends and veins. Flowers of the plant are light pink or green in the form of whorled racemes. Rhizomes are shortly branched, tuberous terete, 0.7–1.5 cm thick. Stems are 20–80 cm long, erect, glabrous (Ali 2007; Zhengyi et al. 2013).

16.7 Traditional Uses

P. cirrhifolium is wild tasty leafy vegetable eaten roasted, young shoots are also added to salads (Wujisguleng et al. 2012). Its dried rhizomes and bulbs show higher protein content, macro- and micronutrients with some fats and fibre content (Sharma et al. 2014). In Lahaul valley, the plant is considered suitable for curing kidney pain and waist area pain; retention of fluid in joints and restoration body strength (Sharma et al. 2011). In Almora district of Uttarakhand, India, the tubers mixed with water is used for removing weakness, leaves are cooked as vegetable, root infusion is taken with milk as an aphrodisiac and for purifying blood, paste of roots is utilized for healing wounds, cuts, skin irritation, and inflammations. Tea is consumed to cure different symptoms related to indigestion, diabetes, infertility, kidney pain, and insomnia and to heal broken bones (Kumari et al. 2012). In Garhwal region, *P. cirrhifolium* is utilized as tonic and whole plant is used for curing dermal problems, fever, cough, bronchitis and general debility. In Himachal Pradesh's Parvati valley, the plant is utilized in treating cough, rheumatism, and debility from old age (Sharma et al. 2004). In China, the plant has reported to show hypoglycaemic, hypertensive, antibacterial and antifungal effects (Singh 2006). Locals in Kinnaur uses leaves as vegetable and tonic (Negi and Chauhan 2009). Local amchi suggested that the powdered roots of the plant mixed with milk act as tonic, to cure headache, skin irritation, and healing burns.

P. multiflorum is utilized for the treatment of lung complaints, swelling, and to dry out tissues. It is sometimes applied as paste to the skin for treatment of bruises, ulcers, boils, haemorrhoids, skin redness, and oedema. It is also found to be useful for the reduction of blood sugar levels.

Various plant parts of *P. verticillatum* is widely used directly or indirectly to cure different diseases. In Gilgit, plant roots are taken with milk and ghee as general tonic (Khan and Khatoon 2008). Oral administration of plant material is done for treating gastric flatulence and allergies. The fresh roots of the plants are used to cure spermatorrhoea and piles (Kaur et al. 2011), whereas the dried powdered roots taken with water is helpful for leucorrhoea (Phondani et al. 2010), paste of the roots are used for curing the wounds. In other areas, the entire herb is reported to be utilized for curing appetite, trouble of kidney, and restoration of body strength (Sharma et al. 2011). In Himalayan region, it is considered as a vegetable and the plant roots are consumed raw or cooked (Radha et al. 2013; Pant and Samant 2006) (Table 16.1).

16.8 Phytochemistry

Various chemical constituents have been reported by different scientists from the genus *Polygonatum* (Fig. 16.3).

Table 16.1 Traditional uses of the *Polygonatum spp.*

Species	Plant part	Uses
P. cirrhifolium	Whole plant	• Treatment of kidney pain and pain in waist region, retention of fluid in joints, restoration of body strength (Sharma et al. 2011) • Cure bronchitis, cough, fever, wounds, skin diseases, ulcers, general debility of old age and rheumatism (Sharma et al. 2004)
	Tubers	• Mixed with water is used for removing weakness (Kumari et al. 2012)
	Leaves	• Eaten as vegetable (Kumari et al. 2012) • Tonic (Negi and Chauhan 2009) • As tea to cure diabetes, indigestion, insomnia, infertility, menopause, kidney pains, and broken bones (Kumari et al. 2012)
	Root infusion with milk	• Aphrodisiac and blood purifier (Kumari et al. 2012) • Tonic, cure headache, skin irritation, and healing burns
	Roots paste	• Healing cuts and wounds, skin irritations, and inflammations (Kumari et al. 2012)
P. multiflorum	Whole plant	• Lung disorders, swelling • Applied to the skin directly for treatment of bruises, ulcers, boils, haemorrhoids, skin redness, and oedema • Reduction of blood sugar levels (Chevallier 1996)
P. verticillatum	Roots with milk and ghee	• General tonic (Khan and Khatoon 2008)
	Whole plant	• Gastric flatulence and allergies • Cure appetite, kidney trouble and restores body strength (Sharma et al. 2011)
	Fresh roots	• Cure spermatorrhoea and piles (Radha et al. 2013; Kaur et al. 2011; Pant and Samant 2006)
	Dried roots	• Helpful for leucorrhoea • Applied on wounds (Phondani et al. 2010)

Fig. 16.3 Chemical constituents classes found in the genus *Polygonatum*

The detailed studies on the same indicated that the plants of Himalayas are great source of different phytochemicals that are said to acquire various antioxidant, antibacterial, anti-inflammatory, and antifungal properties. Other reported work explained the various antitumor, antimutagenic, antidiabetic, and anti-ageing properties as well of the isolated molecules (Guo et al. 2016; Wang et al. 2011; Suyal et al. 2019).

Singh and Patra (2019) reported the complete account of the phytochemicals extracted from *P. cirrhifolium.* The preliminary studies of the rhizome extracts indicated the existence of alkaloids, polyphenolics, saponins, tannins, and phytosterols. The confirmation about the existence of various biologically active molecules like diosgenin, sitosterol (2,3-bis-[(tri-methylsilyl)oxy]propyl ester), 9,12,15-octadecatrienoic acid, 1-mono-linoleoylglycerol trimethylsilyl ether, 13-docosenamide, 6,7-dichloro-5-[(1-ethylpyrrolidin-2-yl)-methyl-amino]-1,3-dimethyl-pyrido[2,3-d]-pyrimidine-2,4(1H,3H)dione, dodecane, 9,12,15-octadecatrienoic acid, 2,3-bis[(trimethylsilyl)-oxy]propyl ester, hexadecanoic acid, pentafluoropropionate, 3,5-dicyclohexyl-4-hydroxy-benzoic acid-methyl ester, ethyl ester, cannabinol is done by GC/MS studies of different plant (Zhao et al. 2018; Liu et al. 2018; Chen et al. 2017; Khan et al. 2016).

γ-sitosterol is a phytosterol that act as an antioxidant, anticancer, and anti-inflammatory molecules (Zhao et al. 2018). This phytosterols is also said to have anti-stress activity that helps to normalize adrenal hypertrophy, hyperglycaemia, and other chronic stress conditions. Diosgenin, another important compound is known to have anti-inflammatory, antifatigue, hepatoprotective, antistress, estrogenic, mastogenic, and hypocholesterolaemic potential (Khan et al. 2016).

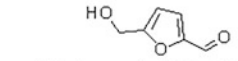
Khan et al. (2011) isolated two chemical compounds viz. diosgenin and 5-hydroxymethyl-2-furaldehyde from rhizome of *P. verticillatum*. In another study, it was reported that the major component present in the rhizome of the plant is diosgenin and santonin along with threonine, aspartic acid, β-sitosterol, glucose, lysine, serine, and sucrose (Shanker et al. 1970; Srivastava et al. 1969; Khan et al. 2015; Sagar 2014). While the study of Khan et al. (2010) reported the good amount of alkaloid and saponin from the crude and solvent extract of rhizome of this plant which was responsible for its bioactivity. Another study on rhizomes of *P. verticillatum* showed that it contains compounds β-sitosterol and 2-hydroxybenzoic acid (Khan et al. 2013a). The rhizomes of the plant are also known to have significant amount of flavonoids and phenols (Khan et al. 2012a). Anthraquinones and terpenoids were also reported in the rhizome. The two chemical derivatives viz. propyl- and 2,3-dihydroxypropyl pentadecanoate were isolated and confirmed by the use of one- and two-dimensional NMR and high-resolution mass analysis (Khan et al. 2013b). The aerial part of this plant contains favourable concentration of alkaloids, terpenoids, flavonoids, saponins, phenols, sterols, and tannins. The high proportion of asparaginic acid was responsible for the pure form of lectin which was extracted from the roots of *P. verticillatum* (Antoniuk 1978). The other plant parts are known to contain pentacosane, α-bulnesene, piperitone, linalyl acetate, calarene, eicosadienoic, and docosane.

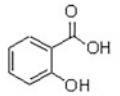
The phytochemical investigation of *P. multiflorum* leads to the isolation of two novel glycosidic steroidal molecules viz. polmultoside A and polmultoside B with three known molecules viz. huangjiangsu A, protodeltonin, and protobioside. The confirmation of the structures was made by one- and two-dimensional NMR studies and high-resolution mass analysis (Gvazava et al. 2019).

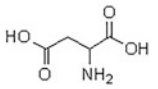
The new marker compounds are the other much needed molecules used to identify the authenticity of the commercial formulations. Virk and coworkers reported two new compounds viz. Quinine and Ferulic acid isolated from *P. verticillatum.* Phytochemical analysis of the methanolic extract of rhizomes of the plants indicated the presence of carbohydrates, alkaloids, terpenoids, flavonoids, polyphenolics along with quinine, the pure white crystalline powder of (6-methoxyquinolin-4-yl-8-vinylquinuclidin-2-yl) methanol that is reported as a marker compound for checking the adulteration, substitution, and standardization parameters of different components of *P. verticillatum* (Virk et al. 2016a).

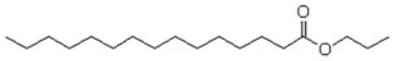
In another study, column chromatography leads to an isolation of novel 4-hydroxy-3-methoxy-cinnamic acid (Ferulic acid) that makes up white crystalline solid along with other reported components. Ferulic acid is also utilized as a marker compound for standardization of *P. verticillatum* (Virk et al. 2016b).

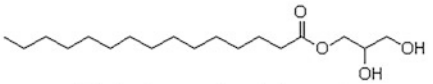
The structure of key chemical molecules isolated from *Polygonatum* are given here below:


5-hydroxymethyl-2-furaldehyde

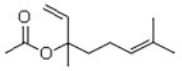

2-Hydroxybenzoic acid

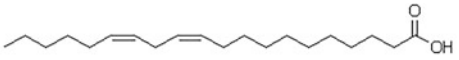

2-Aminobutanedioic acid

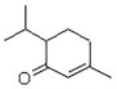

Propyl pentadecanoate

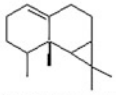

2,3-dihydroxypropyl pentadecanoate

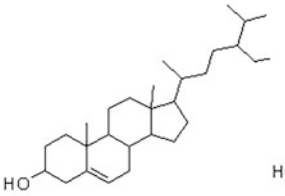

Pentacosane

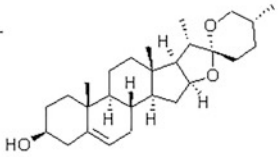

3,7-dimethylocta-1,6-dien-3-yl acetate

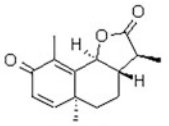

Eicosadienoic acid

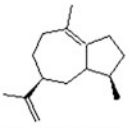

Piperitone

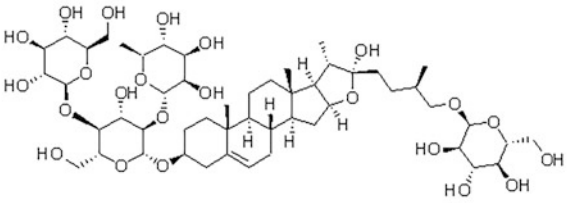

(7aS)-1,1,7,7a-tetramethyl-2,3,5,6,7,7b-hexahydro-1aH-cyclopropa[a]naphthalene

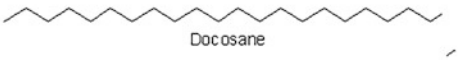

Beta-sitosterol

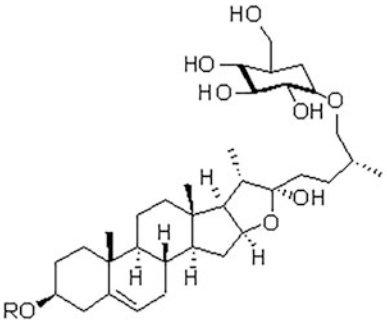

Diosgenin


Santonin

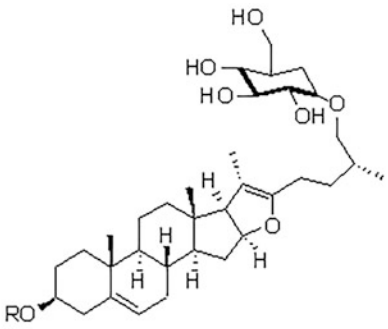

Alfa-bulnesene


Protodeltonin

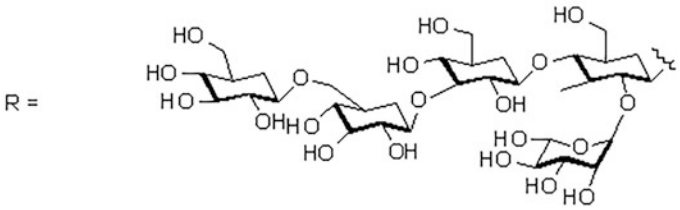

Docosane



Polmultoside A



Polmultoside B



16.9 Nutritional Composition

Sharma et al. (2021) reported the comprehensive study on nutritional composition of *Polygonatum spp.*, having enormous uses in food and medicine. The complete list of all the constituents in fruits, leaves, and rhizomes indicated the total flavonoid, phenolic, carbohydrate, fat, and protein content in the range of 101.40–109.50, 24.50–27.64, 58–200, 5–56, and 99–100, μg/mg, respectively, while antioxidant potential of fruits is highest (456.30 μg/mL). *P. verticillatum* in particular contains essential amino acids, polyphenols and various micro- and macronutrients in its all parts. Leaves enclosed the maximum amount of amino acids and polyphenols, trailed by rhizomes and fruits of the plants. The important micronutrients (Fe, Cu, Pb, Cd, Zn, Co, Ni, Sb, Cr, and Mn), macronutrients (Na, K, and Ca) along with various other natural constituents viz. proteins, fats, and carbohydrates are also found to be the active constituents of different plant parts (Khan et al. 2012b; Sharma et al. 2014; Saeed et al. 2010a).

16.10 Pharmacology

The extensive range of pharmacological properties of *Polygonatum* species is reported (Fig. 16.4). The complete description of the same is given in the following section:

16.10.1 Antioxidant Activity

P. cirrhifolium was found to have remarkable adaptogenic property that owed to the antioxidant potential of various secondary metabolites (triterpenoids, flavonoids, and phenolic compounds) present in the plant. The detailed studies of the extracts showed the potential nature of the molecules that can be utilized well in the development of molecules for stress management condition and utilization for the development of novel molecules as potential candidates (Singh and Patra 2019).

The aerial plant parts and its rhizomes showed the highest free radical scavenging activities investigated by utilizing 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Khan et al. 2012c). The compounds viz. santonin and diosgenin obtained from *P. verticillatum* are the main source with antioxidant potential.

Suyal et al. (2021) reported the content specific potential of all the test species. The antioxidant properties of polyphenolics in *P. verticillatum* and flavonoids in

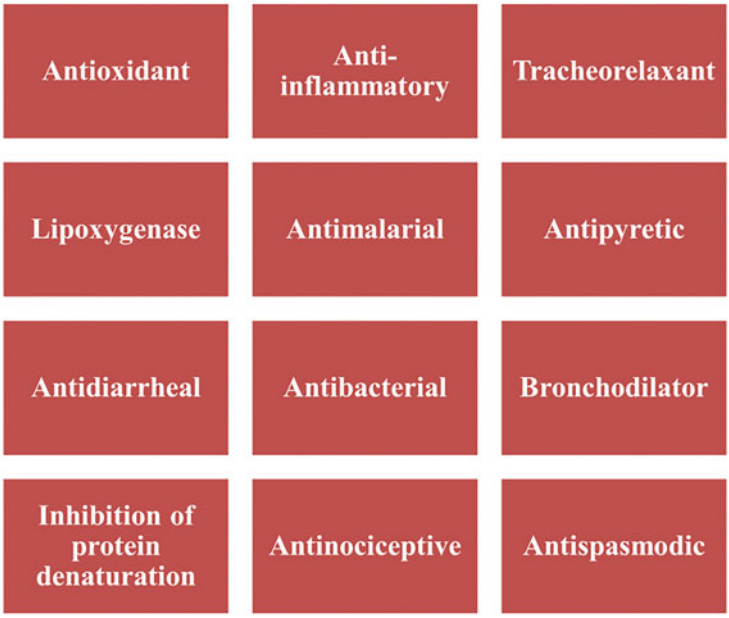


Fig. 16.4 Various pharmacological activities of *Polygonatum Spp.*

P. multiflorum and *P. cirrhifolium* are considered to be very pronounced. *P. multiflorum* among these showed the highest antioxidant efficacy thus appeared as prospective candidate to be promoted for use in herbal formulations as a substitute of the other two more vulnerable species viz. *P. verticillatum* and *P. cirrhifolium.*

16.10.2 Anti-inflammatory Activity

The anti-inflammatory properties of P. multiflorum were explored by plum-blossom needle taping clinical studies on around 150 patients. The clinical studies indicated the good therapeutic signs of the *plant* and its biologically active constituents (Bounda and Feng 2015).

The anti-inflammatory potential of the rhizomes of *P. verticillatum* was studied in rats at three different concentrations viz. 50, 100, and 200 mg/kg doses in which the carrageenan encouraged rat paw oedema (Khan et al. 2013).

Singh and Patra (2018) studied rhizomes extract of *P. verticillatum* that showed a remarkable anti-inflammatory and antioxidant activities attributed to different phenolic compounds of the plant whereas the flavonoids along with the other components leads to the anticancer activity of the extracted molecules.

16.10.3 Tracheorelaxant Activity

The tracheorelaxant activity of *P. verticillatum* rhizome was observed in guinea-pig tracheal tissues. The result indicated that inhibition of large K^+ and carbachol-prompted contractions in the variable concentration range of 0.01–10 mg/mL. These results were in accordance with verapamil that also leads to the relaxation of tissues (Khan et al. 2013).

16.10.4 Lipoxygenase Activity

In another study, the aerial parts of plant, *P. verticillatum* were tested in dissimilar solvent extracts that exhibited lipoxygenase activity of plant in all extracts. Among such extracts, ethyl acetate one was found to have the best potential inhibitors of the enzyme with IC_{50} value 97 μg/mL, trailed by aqueous part (IC_{50}: 109 μg/mL) and crude extract that exhibited an inhibition value (IC_{50}) of 125 μg/mL (Khan et al. 2013).

16.10.5 Antimalarial Activity

The use of n-hexane fraction along with chloroform fraction showed maximum antimalarial activity of *P. verticillatum* against *Plasmodium falciparum*. This result showed significant antimalarial action of the crude part and its non-polar fractions (Khan et al. 2012c).

16.10.6 Antipyretic Activity

Khan et al. (2013c) analysed the rhizome and aerial plant parts in Wistar rats and Albino NMR imaging mice, separately. The rhizome part of plant displayed 82.20% antipyretic activity at 200 mg/kg while its aerial plant parts showed 64% activity at 200 mg/kg.

16.10.7 Antidiarrhoeal Activity

The alcoholic rhizome extract of *P. verticillatum* showed antidiarrhoeal activity in test organism, mice that induce diarrhoea by using castor oil. At 1000 mg/kg, the reduction in diarrhoea was found to be 80% that was similar to drug loperamide (Khan et al. 2013).

16.10.8 Inhibition of Protein Denaturation

At 500 mg/mL, attenuation of heat-induced protein denaturation was shown by santonin and diosgenin, isolated compounds of *P. verticillatum,* in a concentration subordinate way with most extreme impact of 61.55% and 67.90%, respectively (Khan et al. 2015).

16.10.9 Bronchodilator Activity

The methanolic extract of aerial parts of plant, *P. verticillatum* were verified against carbachol and K^+ at concentration of 80 mmol/L that showed Ca^{2+} channel blocker-like activity and induced more contractions resulting in strong bronchodilator activities in the tracheal tissues of rabbits (Khan et al. 2013d).

16.10.10 Antibacterial Activity

The two extracted compounds of *P. verticillatum* rhizomes, i.e. diosgenin and santonin were tested against different Gram-positive and -negative bacteria viz. *Bacillus subtilis, B. cereus, Staphylococcus aureus, S. epidermidis*, *Escherichia coli*, and *Salmonella typhi*. The results indicated noteworthy inhibition zone against both the positive and negative bacteria (Khan et al. 2015). In another study, agar well diffusion technique was used to assess the different crude and solvent extracts of plant aerial parts against *B. subtilis*, *E. coli*, *S. typhi*, *S. aureus*, *Shigella flexneri*. The Gram-positive bacteria (*B. subtilis)* were found to be effectively inhibited by the test extract whereas the extract was ineffective against *P. aeruginosa*. Similarly, different extract of rhizome investigated against same bacteria indicated an antibacterial activity against *E. coli*, *S. typhi*, *S. flexneri*, and *S. aureus* whereas ineffective against *P. aeruginosa* (Khan et al. 2013).

16.10.11 Antifungal Activity

The antifungal potential of aerial plant parts was studied by Khan et al. (2013e) by agar tube dilution method against the same six fungal strains in which extract was found to be unaffected against all fungal strains except *Microsporum canis* (Khan et al. 2013). The santonin and diosgenin were the same isolated compounds of *P. verticillatum* rhizome which were studied for antifungal activity *Aspergillus flavus*, *A. niger*, *Trichoderma harzianum*, and *Fusarium oxysporum* fungal strains. This study revealed only santonin showed significant antifungal activity. The agar tube dilution technique was utilized to find the efficacy of various rhizome extracts of plant against all the six fungi, i.e. *A. flavus*, *Candida albicans, C. glabrata, F. solani, Microsporum canis, Trichophyton longifusus,* in which results were only restricted toward the *M. canis* and *F. solani* (Khan et al. 2015).

16.10.12 Antispasmodic Activity

The plant rhizome extract of *P. verticillatum* were dose dependent which were used to study the antispasmodic activity in the isolated rabbit jejunum. In this study, there was thorough relaxation found at 10 mg/mL which was almost similar to inhibitory action of cromakalim and verapamil. Further, it was checked against low and high K^+ prompted contractions that results in inhibition at low K^+ induced contractions, but partial inhibition in high K^+ induced contractions (Khan et al. 2013).

16.10.13 Antinociceptive Activity

The results of aerial plant extract were tested in various pain models for antinociceptive activity. From the result, it was concluded that obstruction or the release of endogenous substances like arachidonic acid derivatives, the pharmacologically active compounds of plant leads to the excitation of pain nerve endings (Khan et al. 2011). At 50, 100 and 200 mg/kg concentrations, methanolic extract of the *P. verticillatum* rhizomes were also evaluated over the various pain models of rodents. This showed significant antinociceptive activity (Khan et al. 2010) (Table 16.2).

16.11 Toxicology

The toxicological profile of *P. verticillatum* was defined on the basis of its phytotoxic, cytotoxic, insecticidal, and anti-leishmanicidal activities by various in vitro paradigms. The results indicated the strong phytotoxic effect of the plant material and its solvent extract at variable doses of 5, 50, and 500 μg/mL with 100% inhibition at highest test dose (Saeed et al. 2010b).

16.12 Tissue Culture

Kumari and Saggoo (2017) studied the two samples of *P. cirrhifolium* of the family Asparagaceae for male meiosis was studied from the cold desert area of Kinnaur. Both the samples displayed the existence of 16 bivalents (based on $x = 8$) at diakinesis and metaphase. The other reports are on the floristic diversity of medicinal and aromatic plants but the cytological effect along with the meiotic abnormalities in the species was not well studied that increases the necessity to create awareness among the local people about the proper preservation and application of medicinal plants.

16.13 Patent

Qazi and coworkers filled a patent on methods for in vitro germination of *P. cirrhifolium* along with compositions (Qazi et al. 2001). The disclosure described the culture media containing MS basal culture media and plant hormones, preferably chosen from the group comprising of gibberellic acid (GA3), 6-benzyl-aminopurine (BAP), and naphthalene acetic acid (NAA). The method of in vitro cultivation of

Table 16.2 Plant species, bioactivities, and active constituents

Species	Plant part	Phyto-constituent	Test organisms and methods	Bioactivity	References
P. cirrhifolium		Flavonoids		Antioxidant	Singh and Patra (2019), Suyal et al. (2021)
P. multiflorum		Flavonoids		Antioxidant	Suyal et al. (2021)
			Plum-blossom needle taping	Anti-inflammatory	Bounda and Feng (2015)
P. verticillatum	Aerial parts and rhizomes	Diosgenin, santonin, and other polyphenolics	1,1-diphenyl-2-picrylhydrazyl (DPPH)	Antioxidant	Khan et al. (2012c), Singh and Patra (2018), Suyal et al. (2021)
	Rhizomes		Rats (the carrageenan-induced rat paw oedema)	Anti-inflammatory	Khan et al. (2013)
	Rhizomes	Phenolic compounds and flavonoids		Anticancer	Singh and Patra (2018)
	Rhizomes		Guinea-pig tracheal tissues	Tracheorelaxant	Khan et al. (2013)
	Aerial parts			Lipoxygenase	Khan et al. (2013)
	Rhizomes and aerial parts		Wistar rats	Antipyretic	Khan et al. (2013)
			Plasmodium falciparum	Antimalarial	(Khan et al. 2012c)
	Rhizomes		Mice	Antidiarrhoeal	Khan et al. (2013)
	Aerial parts		Tracheal tissues of rabbits	Bronchodilator	Khan et al. (2013)
	Aerial parts and rhizomes	Diosgenin and santonin	*B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, *S. flexneri*	Antibacterial	Khan et al. (2013), Khan et al. (2015)
		Diosgenin and santonin	*M. canis* *A. flavus*,	Antifungal	Khan et al. (2013),

(continued)

Table 16.2 (continued)

Species	Plant part	Phyto-constituent	Test organisms and methods	Bioactivity	References
	Aerial parts and rhizomes		*A. niger*, *T. harzianum*, and *F. oxysporum*		Khan et al. (2015)
	Rhizome		Rabbit jejunum	Antispasmodic	Khan et al. (2013)
	Aerial plant parts and rhizomes	Arachidonic acid	Rodents	Antinociceptive	Khan et al. (2011), Khan et al. (2010)

Polygonatum seeds with culture medium and GA3 was also disclosed. The primary explant was transferred to second medium consisting of MS basal culture medium, BAP, and NAA, and on appearance of a first foliage leaf, and further transferred to third medium containing MS basal culture medium, BAP, NAA, and GA3. The results indicated an even germination in less than about 90 days along with growth of epicotyl, coleoptile, and radicle. The method is known to have a novel culture medium compositions leading to extraordinary fast and coordinated in vitro induction of germination and release of epicotyl dormancy with fast in vitro propagation.

16.14 Formulation and Market Products

The market products are flooded with derivatives of *P. cirrhifolium* plant. It is present in number of revitalizing ayurvedic formulations. The drawback faced in the field include the inadequate quantities, lack of resources but ever rising demands that prompted the utilization of various officially approved substitutes suggested by the Department of AYUSH. But this leads to the adulterations of the formulations but substandard/spurious substitutes by the manufacturers. It was supported by the fact that 60% Ayurvedic parameters as well as pharmacological actions of the real ingredients of Ashtawarga plants do not match the standards of their substitutes leading to reduced efficiency of the drugs with loss of faith in the field of herbal drugs. The situation is further being exploited by manufacturers because regulatory authorities lack the specified marker compounds required for the identification of authentic plants (Virk et al. 2017).

16.15 Conservation

Rana and Kumar (2017) described the overexploitation of the important *Polygonatum* species viz. *verticillatum* and *cirrhifolium* that made them vulnerable or endangered species according to IUCN red-list criteria. The major reason for the same is constricted distribution, disturbances in land-use, alterations in habitat, climate changes, over-grazing by livestock, population bottleneck, and genetic drift. To overcome the problem, it is necessary to establish the gene banks and repositories under in situ and ex situ conditions that can serve as conservation and preservation blocks for valuable genetic materials and wild relatives.

On the basis of the above reported studies, a Gene Bank was established in Forest Research Institute's herbal garden of Dehradun, India. It consists of 149 accessions collected from 31 geographical sites of three Himalayan States of Jammu and Kashmir, Himachal Pradesh and Uttarakhand. The results showed that the species propagate through rhizomes and grow well in altitudes ranging from 1600 to 3600 m. The rhizomes are known to be a worthy source of noteworthy phytochemicals like flavonoids, phenolics, lectins, terpenoids, allantoin, diosgenin, β-sitosterol and quinine. The ultimate aim is thereby focused on evaluating genetic diversity of the species and capturing encouraging genotypes for carrying of the future research (Rana 2018).

Qadir et al. (2020) reported the novel conservation method to overcome the existing problems of older propagation techniques for *P. verticillatum* by utilizing seeds as explant. The study involved the evaluation of the suitable concentration of plant growth regulators on induction of callus and regeneration of rhizomes and shoots.

16.16 Conclusion and Future Prospects

This chapter included a complete description on taxonomy, distribution, phytochemistry, pharmacology, traditional uses, agro-techniques, biotechnology, toxicology, conservation, and sustainable harvesting of three significant *Polygonatum* spp., i.e. *P. cirrhifolium, P. multiflorum*, and *P. verticillatum*. These important medicinal plants are growing in high altitudes of Himalayas. The reported pharmacological potential of these important herbs is found in an area of anti-inflammatory, antioxidant, antibacterial, antimalarial, tracheorelaxant, lipooxygenase, antipyretic, antinociceptive, antispasmodic, etc. The active bioagents isolated from them include diosgenin, santonin, 2-hydroxybenzoic acid, β-sitosterol, etc.. These compounds are considered to be responsible for most of the bioactivities of this plant. Various commercial revitalizing ayurvedic formulations of these herbs are already flooding in the market. But due to overexploitation, under-developed cultivation, poor harvesting, loss of natural habitat and less awareness to the local people, the biodiversity of these significant medicinal plants are confronting risk of extinction.

Therefore, there is a robust need for the promotion of effective in situ conservation and cultivation techniques. Also, more concentration should be directed to discover the unidentified biological prospective of *Polygonatum multiflorum* as it is less vulnerable in comparison to the other two species viz. *P. verticillatum* and *P. cirrhifolium*. Though the biopotential of the *P. multiflorum* is less explored, the presence of similar constituent can make it a good alternative to the existing endangered species.

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Chapter 17
Species of the Genus *Rhodiola* Found in the Western Himalayas

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17.1 Introduction

The use of immunomodulators is the robust approach for the treatment of chronic infectious diseases. Plants have the potential to preserve the homeostasis of the immune system during the progression of prolonged treatment (Gutiérrez-Rebolledo et al. 2017). They are acquiring more consideration as they decrease the risk of long-term diseases by employing as immunostimulators. From ancient to modern era, many plants have been utilized as booster of immunity. Approximately 210,000 types of herbal products revealed and recognized for human usage by means of Dictionary of Natural Products (Ji and Zhang 2009; Khanna et al. 2017). *Rhodiola* also known by the name of golden root or stone root and is one such immunomodulator which has been identified for around 2000 years. Massive work is being done on it to reveal its significant contribution in increasing immunity and other applications. It has an extensive past of usage as an important herb in several diseases and also serve as good source of enhancing energy and mental ability. Moreover, *Rhodiola* is recognized as "Adaptogen" which assist in the acclimatization of the

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body to fight against stress. Old Chinese remedy system reflects this plant to be a medication of "foundation of adaptation to environment" (Li et al. 2007). The *Rhodiola* genus has greater than hundred species, of which approximately 20 species are employed as remedies like *Rhodiola crenulata* (*R. crenulata*), *R. sacra*, *R. heterodonta*, *R. rosea*, and *Rhodiola kirilowii* (Li et al. 2019; Kosakowska et al. 2018). Several species of *Rhodiola* offers a good shield against whole-body fatal gamma radiation tempted variation in hemopoietic organization in mice (Goel et al. 2006). *R. imbricata* is extensively utilized as a nutraceutical complement in the Himalayan area and has enormous capacity for mitigation of biotic mutilation in a radiation atmosphere (Arora et al. 2008). This plant has good preventive activity as a biotic protector in nuclear-powered crises. This plant is given as capsules which can be engaged as a preventive or remedy for radiation (Chawla et al. 2010). *R. crenulata* possess enormous pharmacological and nourishing status and traditionally also known by the name of "Snow Ginseng" and "Plant Gold" (Zhao et al. 2012). *R. crenulata* has been described to reveal remarkable neuroprotective, anti-hypoxia, anti-fatigue, and radioprotective properties (Lin et al. 2018). In the market, this plant has great usage in the production of extensive diversity of medicines with significant curative properties, and also employed for the formulation of tea, wine, and other types of food (Yang et al. 2012). Diverse medicinal value of *Rhodiola* plants is associated to their phytoconstituents. Both roots and rhizomes contain several phytocompounds like phenylpropanoids, monoterpene alcohols, phenylethanoids, glycosides, gallic acid and its derivatives, flavonoids, cyanogenic aryl glycosides, and flavonolignans. The pharmacological properties of *Rhodiola* varies with its species. The chief active phytoconstituents of *Rhodiola* are rosin, tyrosol, salidroside, rosarin, rhodionin, catechin, and gallic acid (Liu et al. 2017). *R. heterodonta* (Hook f. & Thomson) is consider as good adaptogen (Grace et al. 2009). *R. tibetica* is known as anti-stress agent, and it reinstates memory as health stimulant (Bhadrecha et al. 2017). Due to a wide range of functional value of *Rhodiola*, it is utilized in various products like food additives, drinks, medicines, food supplements, and cosmetics (Cunningham et al. 2020). The responsibilities of conservation, the expansion of health foods, additives, and nutraceuticals in India has been taken up by the Defence Research and Development Organisation of Government of India (Khanum et al. 2005). *Rhodiola* spp. has huge therapeutic potential, and this review covers four species, i.e., *R. imbricata*, *R. crenulata*, *R. tibetica*, and *R. heterodonta* which would give stimulus to conduct novel research for the expansion of *Rhodiola*-based health remedies (Khanna et al. 2017).

17.2 Taxonomical Status and Common Names

Rhodiola is a herbaceous plant, commonly recognized as rose root (due to rose like aroma of freshly cut roots) or arctic root. In addition to it, this plant is also called golden root, shrolo (Common in Ladakh region), Solo, stone crop (Lakey 2016; Ballabh and Chaurasia 2007; Singh et al. 1996). The taxonomical position of the

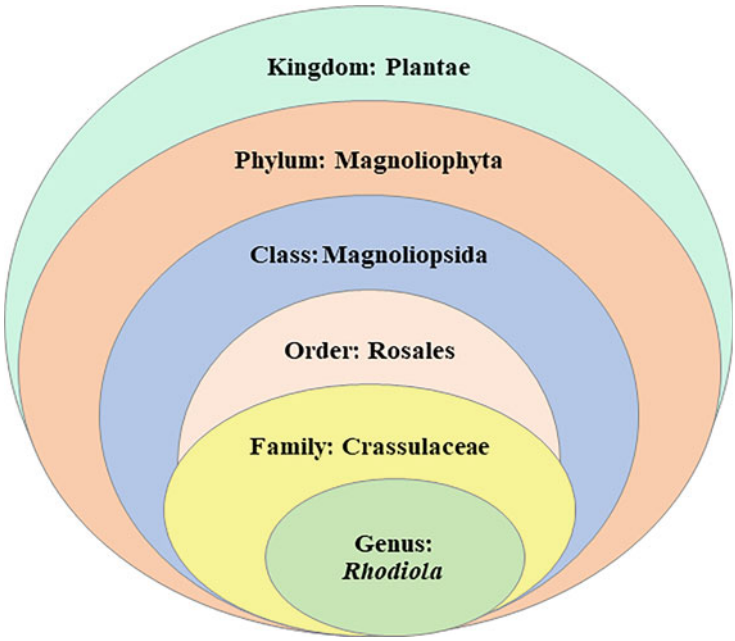


Fig. 17.1 Taxonomy of *Rhodiola* sp. (Ghiorghita et al. 2015)

genus *Rhodiola* is fairly multifaceted. Earlier taxonomists famed among diverse species of *Rhodiola* as independent genus (Hegi 1963). Ghiorghita et al. (2015) gave the systematic classification (Fig. 17.1) of *Rhodiola* sp.:

17.3 Distribution

The genus *Rhodiola* consists of around 1400 species which are further distributed in 33 genera. *Rhodiola* grows mainly in mountainous regions of low temperature like tundra, precipices, rock ledges, and riverbanks in the northern hemisphere (Grech-Baran et al. 2015). It has a world-wide distribution though it is found in Africa, Asia, Europe, and Northern hemisphere (Gupta et al. 2007; Khanna et al. 2017). The high-altitude cold regions of Northern Hemisphere such as Altai Mountains, Iceland, Tibet, Far East area, and Alaska contain about 90 species of *Rhodiola* (Lei et al. 2003; Xia et al. 2005; Hillhouse et al. 2008; Zhu and Lou 2010) whereas Qinghai-Tibet Plateau in China comprises 73 species of it (Fu and Fu 1984). Six species of *Rhodiola* specifically *R. imbricata*, *R. quadrifida*, *R. heterodonta*, *R. tibetica*, *R. sinuate*, and *R. wallichiana* are found in India (Chaurasia and Gurmet 2003). The species *Rhodiola crenulata* is an inhabitant of Qinghai-Tibet Plateau which is chiefly distributed in high cold areas of Hengduan Mountains of Tibet, China, and Yunnan (Lei et al. 2003) and at high altitudes in the Arctic region. In India, it is

prevalent at an altitude of 9186–18,372 ft. in the Grassland and Mountain slopes, rocky places, and rock crevices in Sikkim and Lahaul region (Bhardwaj et al. 2018a, b). Whereas *Rhodiola imbricata* (lately recognized as a sanjivani) (Bhardwaj et al. 2018a, b) is a dioecious perennial herb indigenous to Trans-Himalayan region (Pundir et al. 2019) and Northern hemisphere, also grows in mountain areas of Southwest China and has shown distribution in Sinai Himalayas, Xizang, Pakistan, Nepal, Qinghai, India, China, and Tibet. In India, it is predominantly found in wet, rocky slopes and higher passes of western Himalayas at an altitude of about 14,000–18,500 ft. mainly in Zanskar, Indus, and Changthang valleys situated in the cold dessert of Ladakh region and in Kumaon (Chaurasia et al. 2007; Tayade 2015; Kanupriya et al. 2005). It also shows its distribution in high latitude and mountainous areas of Eurasia (Khanum et al. 2005). *Rhodiola heterodonta* species are widespread in the Asia and East Europe (Grace et al. 2009). In India, it is endemic to Khardungla, Changla, and Penzila regions of Ladakh and Kumaon and slopes and gorges of Kashmir at an altitude of 9186–18,500 ft. (Chaurasia et al. 2007). *Rhodiola tibetica* is a herbaceous plant chiefly distributed in the Tibet Autonomous region (Jin 2020; Lu et al. 2021) and rocky slopes and sides of the gorges of Himachal Pradesh and Kashmir at a height of 13,451–17,716 ft. (Lu et al. 2021; Bhardwaj et al. 2018a, b).

17.4 Morphology

Morphologically, *Rhodiola*, a perennial plant has a fleshy and stout rhizome or caudex which is covered with brown or black membranous and scale-like leaves and can be easily differentiated from its slender leafy flowering-dimorphic stems (Liu et al. 2013). The species *R. crenulata* is a perennial dioecious herb which is roughly 20 cm in height and has a thick cylindrical rhizome. It has a robust main root and a slender lateral root. The stem is 5–20 cm tall and is erect or flabellate. Leaves are shortly pseudo-petiolate and alternate and are elliptic oblong to suborbicular in shape and the apex is obtuse to mucronate. Inflorescence is corymbiform and many flowered. Flowers are unisexual and large. Sepals are narrow triangular, oblong, or lanceolate, petals are red to purplish red in color (Li and He 2016; Bhardwaj et al. 2018a, b).

R. imbricata is a perennial herb with a subcylindrical rhizome, 10–35 cm in length and is scented when freshly cut; stem is 10–20 cm long and glabrous, leaves are 1.3–3 cm long, oblong to narrowly elliptic, and are densely arranged. The flowers are arranged in the form of congested clusters and are surrounded by involucre of leaves. The petals are angular-oblanceolate, and the stamens are noticeably longer than the petals, anthers are purplish red in color and are 3–5 mm long, filaments are 5–8 mm in length, fruits are 4–5 in number and are many seeded (Chaurasia et al. 2007; Chaurasia and Brahma 1996; Bhardwaj et al. 2018a, b). Both *R. heterodonta* and *R. tibetica* are glabrous herbs having thick rootstock, which is rarely branched in *R. heterodonta* and is generally branched in *R. tibetica*. Stem of both of these is

smooth, erect, and simple. *R. heterodonta* has alternate green/glaucous leaves which are fleshy, thickly toothed along the margins, triangular to ovate, and are loosely attached to the stem. Whereas the leaves of *R. tibetica* are 1 inch long, glaucous but pale on the ends, sessile, longer than the internodes and have lanceolate to oblong shape. Inflorescence is terminal in both the species. Flowers of *R. heterodonta* species are pentamerous, male flowers have blunted, linear and greenish sepals and yellow or red petals and widespread stamens and steaked red filaments. However, the female flowers of *R. heterodonta* have same sized petals and sepals which are blunt and green or purple colored, with erect, large, and green carpels and small sized style. Flowers of *R. tibetica* have dark purple shade. Calyx of female flowers is saucer shaped and the petals are twice the size of sepals, are dark purple and lanceolate whereas the carpels have erected and purple tips.

17.5 Traditional Uses

Rhodiola imbricata is a wild edible herb, and it has been employed conventionally as a folk medicine in India and several neighboring countries such as Nepal, Tibet, China, and Pakistan since centuries for the cure of several fatal infections (Han et al. 2002). The young buds of *Rhodiola* spp. are warmed and added in the yogurt to formulate a local Ladakh dish "Tantur" (Chaurasia and Brahma 1996; Gupta et al. 2012). Traditionally, it has been extensive usage in the cure of several epidemic infections, traumatic wounds, and burns. It plays significant role in the reduction of swelling of legs and for the removal of contaminants from the body. Moreover, it is recognized to decrease the impacts of hypoxia and weakness. The roots of R. imbricata is known in Amchi and Tibetan traditional medicine system for the cure of lungs infections, cough cold, and contagious illnesses (Chaurasia et al. 2007). In addition to it, *R. heterodonta* is employed as herbal remedy in Tibetan medicinal system for the cure of respiratory infections (Kala 2006). In view of traditional Chinese folk medicine, *R. crenulata* help in the improvement of depression, altitude sickness, fatigue, insomnia, and infertility (Pooja et al. 2009; Chiu et al. 2013).

17.6 Phytochemistry

The genus *Rhodiola* comprises a wide range of phytochemicals which are of bioactive nature. Their presence results in the pharmacological potential of *Rhodiola* species. A number of research groups have investigated the phytochemistry of different *Rhodiola* species. In this chapter, we are going to study the phytochemistry of four *Rhodiola* species including *R. imbricata*, *R. heterodonta*, *R. crenulata*, and *R. tibetica*. Extensive work has been done on the phytochemical profile of *R. crenulata* and *R. imbricata* whereas *R. heterodonta* and *R. tibetica* have not been explored thoroughly.

17.6.1 *Phytochemistry of* Rhodiola imbricata

The pharmaceutical potential of *Rhodiola imbricata* is due to the presence of phenylethanoid and phenylpropanoid metabolites (Chaurasia et al. 2003; Gupta et al. 2007; Kumar et al. 2011; Bhardwaj et al. 2018a, b; Kapoor et al. 2018, 2019; Tao et al. 2019). The detailed study on the phytochemistry of *Rhodiola imbricata* was performed by Tayade et al. (2013) who investigated and quantified 63 phytoconstituents present in the root extract of *R. imbicata* in different solvents including chloroform, ethyl acetate, dichloromethane, n-hexane, 60% ethanol and methanol by GC/MS analysis. Various classes of compounds including phytosterols, alcohols, phenols, fatty acids, and their esters and terpenoids were investigated as the main group of compounds present in the root extract of *Rhodiola imbricata*. Out of the 63 phytoconstituents (Tayade et al. 2013), 1-pentacosanol (1); stigmast-5-en-3-ol (2); teracosanol (3); hentriacontanol (4); 13-tetradecen-1-ol acetate (5); bis (2-ethylhexyl) phthalate (6); hexadecanoic acid (7); campesterol (8); 3-methoxy-5-methylphenol (9); methyl tri-butyl ammonium chloride (10); 7,8-dimethylbenzocyclooctene (11); ethyl linoleate (12); 17-pentatriacontene (13); benzenemethanol, 3-hydroxy, 5-methoxy (14); α-tocopherol (15); thujone (16); camphor (17); 1-dotriacontane (18); 1,3-dimethoxybenzene (19); dodecanoic acid, 3-hydroxy (20); 1,3-benzenediol, 5-pentadecyl (21); cholest-4-ene-3,6-dione (22); ethanone, 1-(4-hydroxyphenyl) (23); octadecane, 1-chloro (24); ascaridole (25) and heptadecane, 9-hexyl (26) were obtained as the major components.

Choudhary et al. (2015) described the purification and identification of four novel and 15 already known phenolic compounds and the phenolic glycosides from the ethyl acetate extract of *R. imbricata*. The structures of the compounds were established by the combined use of chemical and spectroscopic methods. Among the isolated compounds, 3,5-dihydroxybenzyl alcohol (27); 3-methoxy-5-hydroxybenzyl alcohol (28); orcinol (29); tyrosol (30); O-methylorcinol (31); *p*-hydroxybenzaldehyde (32); hydroxybenzylalcohol (33); *p*-hydroxyacetophenone (34); methoxyphenethyl alcohol (35); 3-methyl-5-methoxyphenyl-β-D-glucopyranoside (36); 2-hydroxymethyl-6-methoxyphenyl-β-D-glucopyranoside (37); 3-hydroxy-5-methylphenyl-β-D-glucopyranoside (38); 3,5-dimethoxyphenyl-β-D-glucopyranoside (39); phenyl-β-D-glucopyranoside (40); 3,4,5-trimethoxyphenyl-β-D-glucopyranoside (41) were the already known compounds whereas 3-hydroxy-2-(3-methyl-2-buten-1-yl)-benzoic acid (42); 2-(hydroxymethyl)-6-methoxy-3-acetylphenyl-β-D-glucopyranoside (43); 2-(hydroxymethyl)-6-methoxyphenyl-β-D-glucopyranoside (44); and 2-hydroxy-4-methylphenyl-β-D-glucopyranoside (45) were the newly found phenolic compounds.

Likewise, Bhardwaj et al. (2018a, b) carried out the micropropagation of *R. imbricata* and analyzed the secondary metabolites present in the micropropagated sample and compared the bioactive constituents present in naturally grown and in in vitro produced species. The chief components identified by HPLC analysis of the methanolic extract of the roots and shoots of *R. imbricata* were the polyphenolic compounds including gallic acid (46), chlorogenic acid (47), 4-hydroxybenzoic acid

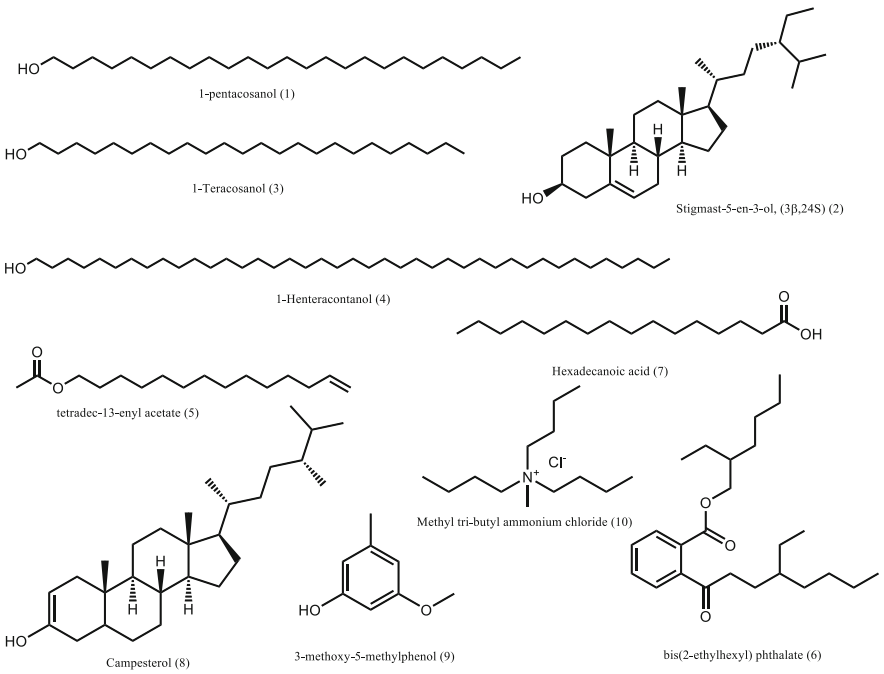


Fig. 17.2 Structures of important phytoconstituents present in *Rhodiola imbricata*

(48), ferulic acid (49), *p*-coumaric acid (50); caffeic acid (51), and phenylpropanoid compounds including rosavin (52). The earlier reports suggested the extraction of the significant bioactive phytoconstituent rosavin, from the rhizome of *R. imbricata* but a large amount of rosavin was extracted from the shoots of the plant by the same research group. Furthermore, it was investigated that gallic acid (46), chlorogenic acid (47), and 4-hydroxybenzoic acid (48) were present in larger amounts in the naturally grown plant. Rattan et al. (2020) developed four callus color variant cell lines each from root and leaf calli (friable white, friable green, friable cream, and compact green) of *R. imbricata* for the qualitative quantification of phenylpropanoid, phenylethanoid, and phenolic metabolites. In addition to gallic acid (46), tyrosol (30), *p*-coumaric acid (50), and rosavin (52) as discovered by other research groups, salidroside (53), cinnamic acid (54), and rosarin (55) were detected (Fig. 17.2).

17.6.2 Phytochemistry of Rhodiola heterodonta

Rhizome of *Rhodiola heterodonta* contains large number of secondary metabolites containing proanthocyanidin compounds which together with phenylethanoids in the ethanolic extract make quantitative and qualitative investigation of these components problematic due to the similarity in the UV profile of these two compounds

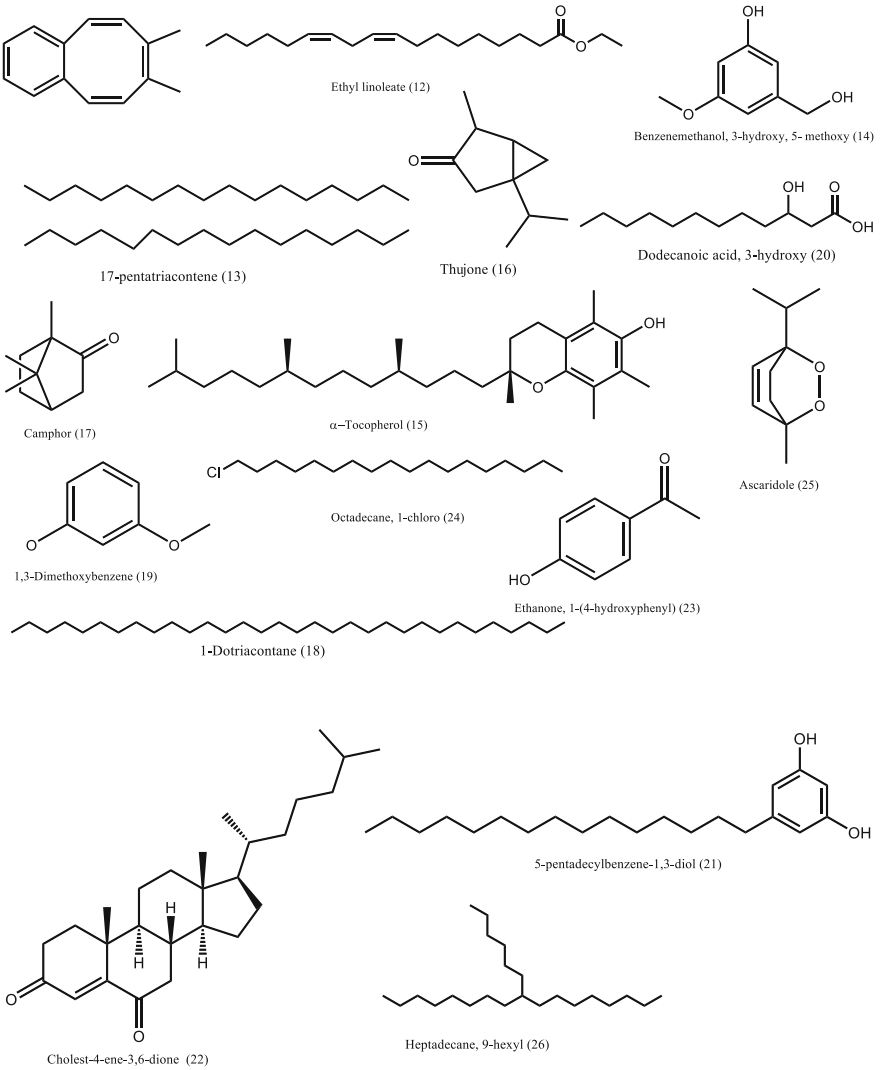


Fig. 17.2 (continued)

and secondly due to appearance of a hump of polymeric proanthocyanidin in the recorded spectra. Therefore, Grace et al. (2009) carried out the fractional process of the ethanolic extract (80%) of *R. heterodonta* and obtained two different fractions: fraction-A containing phenylethanoids and fraction-B comprising proanthocyanidins. The quantification of individual phytoconstituents in the two fractions was performed with the help of HPLC together with LC-MS (Liquid Chromatography-Mass Spectrometry) analysis. Seven prominent components present in the phenylethanoid fraction comprised 17.4% of the ethanolic extract and

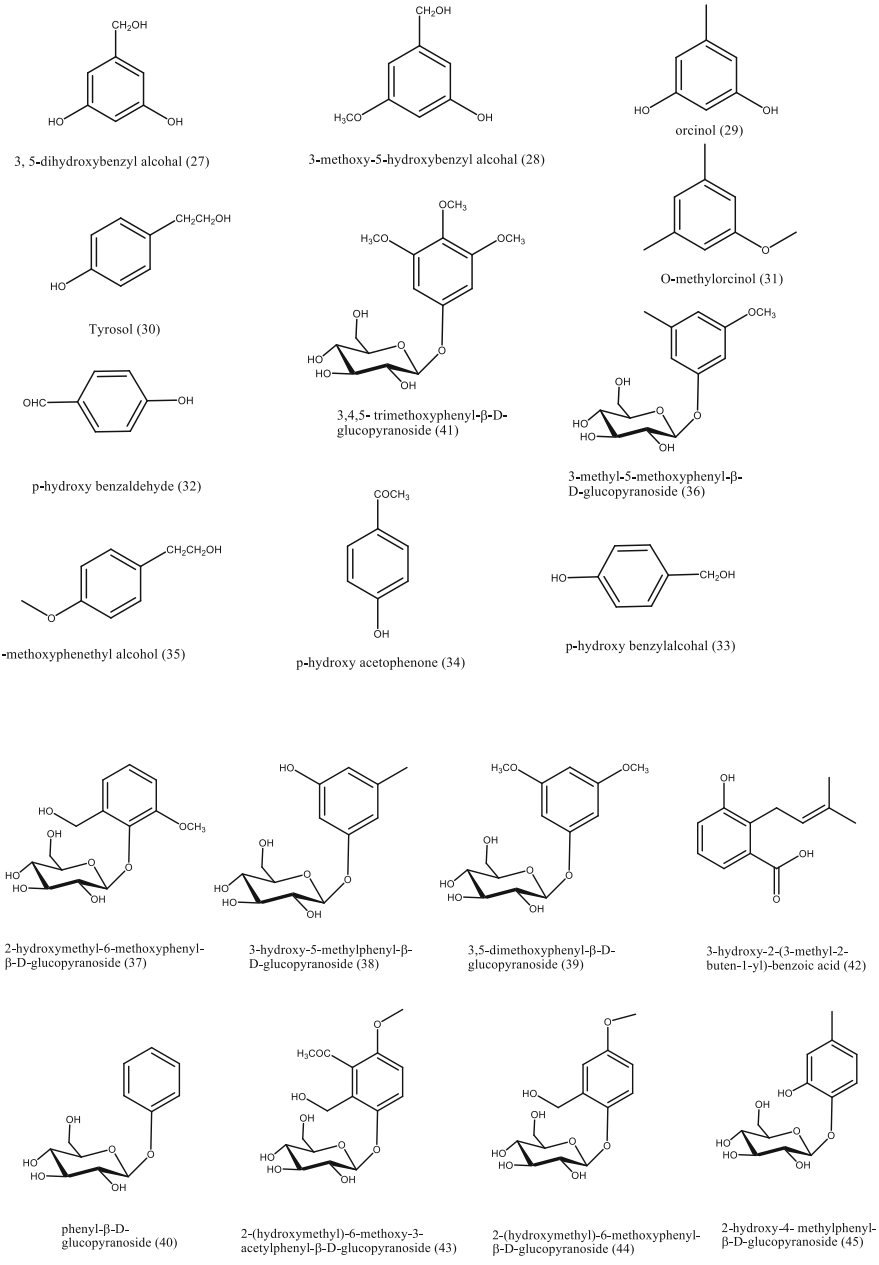


Fig. 17.2 (continued)

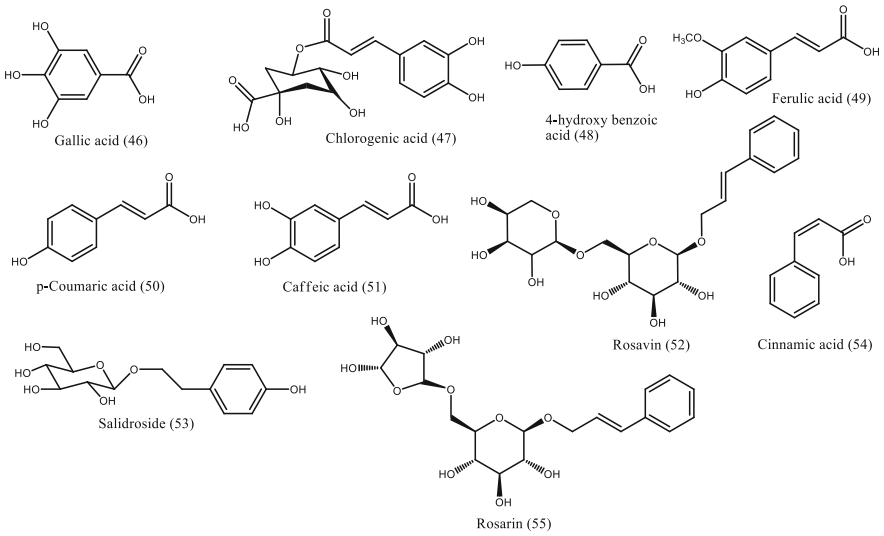


Fig. 17.2 (continued)

included tyrosol (30); heterodontoside (56); salidroside (53); tyrosol methyl ether (57); viridoside (58); mogroside (59); and cyanogenic glucoside rhodiocyanoside A (60). Out of these seven components, mogroside (59) and tyrosol methyl ether (57) were characterized by the research group for the first time. Moreover, heterodontoside (56) was found to be the most abundant component present in the ethanolic extract. The semi-preparative HPLC and LC-ESI-MS analysis of the proanthocyanidin fraction of the ethanolic extract (80%) yielded oligomeric and polymeric compounds and comprised derivatives of epigallocatechin (61–63). Earlier, Yousef et al. (2006) compared the phytochemical composition of *R. heterodonta* and *R. semenovii* in comparison to the phytochemical profile of *R. rosea*. Two groups of compounds namely phenolic and/or cyanogenic glycosides and proanthocyanidins were identified in all the three species but the type of phytoconstituents contained in them were different. The phenolic and/or cyanogenic glycosidic fraction of aq. acetonic extract of *R. heterodonta* on HPLC analysis afforded same components as reported by Grace et al. (2009) including tyrosol (30); viridoside (58); salidroside or rhodioloside (53); rhodiocyanoside A (60); and heterodontoside (56). The HPLC-ESI-MS analysis of proanthocyanidin fraction of *R. heterodonta* afforded (-)-epigallocatechin-3-O-gallate (63).

Yunuskhodjaev et al. (2015) identified different phenylethanoid components present in dried rhizome extract of *R. heterodonta* and studied its pharmacological potential for anti-hypoxic and analgesic activity and acute toxicity too. The nine important phenylpropanoids present in the dry extract were verified with the help of LC-MS analysis. In addition to the seven already discovered components including rhodiocyanoside A (60); dimer of epigallocatechin gallate (EGCG) (61); salidroside

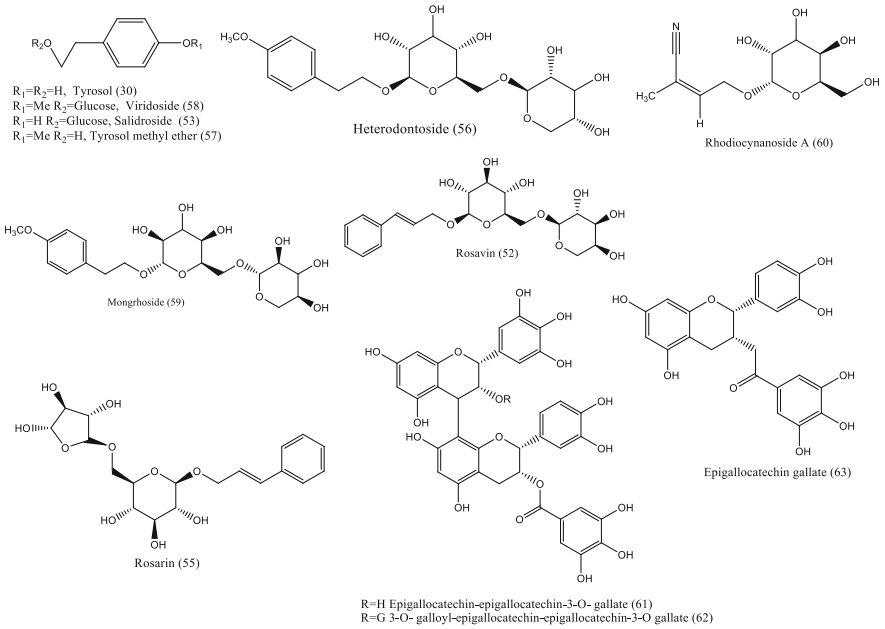


Fig. 17.3 Phytoconstituents present in *R. heterodonta*

(53); tyrosol (30); heterodontoside (56); viridoside (58;) and mogroside (59), two more phenylpropanoid compounds including rosavin (52) and rosarin (55) were also reported to be present in *R. heterodonta* (Fig. 17.3). The RP-HPLC (Reverse phase HPLC) method used for quantitative estimation of phytoconstituents present indicated that the dry extract of the rhizome of *Rhodiola heterodonta* contained 14.50% of total rhodiocyanoside and phenylethanoids whereas the amount of proantocyanidins was found to be negligible (0.75%).

17.6.3 Phytochemistry of R. crenulata

Various categories of phytoconstituents have been isolated and elucidated from *Rhodiola crenulata* which can be categorized into classes including phenols, phenylethanoids, phenylpropanoids, monoterpenoids, flavonoids, cyanogens, lignans, and the corresponding glycosides (Du and Xie 1995; Peng et al. 1995; Nakamura et al. 2008; Wu et al. 2008; Yang et al. 2012; Lee et al. 2013; Jia et al. 2015; Han et al. 2016; Chen et al. 2021). In *R. crenulata*, 29 lignans are found in comparison to other *Rhodiola* species where maximum number of lignans found are only seven. Nakamura et al. (2008) isolated five novel alcoholic glycosides namely creoside I (64), creoside II (65), creoside III (66), creoside IV (67), and creoside V (68) from the methanolic extract of the dried roots of *R. crenulata* whose structures

were elucidated by the combined use of IR (Infra-red) spectroscopy, ^{1}H and ^{13}C NMR spectroscopies and Fast Atom Bombardment (FAB) MS analysis. Han et al. (2016) employed an extremely sensitive technique using HPLC together with Diode-Array Detection and Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (HPLC-FT-ICR MS) along with chromatographic techniques to identify the phytoconstituents (both major and minor) and reported the isolation of 48 components including flavonoids and their glycosides, alcohols and their glycosides, flavanols and gallic acid derivatives, cyanogenic glycoside and organic acids from the aqueous methanolic (50%) of *R. crenulata*. Twenty-six alcohols and their corresponding glycosides, five flavanols, twelve flavonoids and their corresponding glycosides, gallic acid derivatives, one cyanogenic glycoside, and gallic acid derivatives were isolated and characterized from the aqueous methanolic extract of *R. crenulata*. In addition to creosides I, II, and IV, other major components present were identified as salidroside (53); tyrosol (30); kenposide A (69); rhodioloside E (70); rhodiooctanoside (71); n-hexyl-β-D-glucopyranoside (72); 4-hydroxybenzyl-β-D-glucopyranoside (73); quercitrin (74), rhodiosin (75), luteolin (76), catechin (77), epigallocatechin gallate (63), epicatechin (78), ethyl gallate (79), gallic acid (46), caffeic acid (51), and *p*-coumaric acid (50). Zhou et al. (2015) reported isolation of 17 different constituents from the ethanolic extract of the roots of *R. crenulata* and the structures of the compounds were verified by chemical analysis and spectroscopic techniques including Ultraviolet (UV), IR, NMR spectroscopy and Circular dichroism (CD) and HRESIMS analysis. 15 already discovered phenolic compounds along with two novel phenolic compounds: rhodiolate (80) and (+)-syringaresinol A (81) were reported and the absolute configurations of two 2 lignans (82) and (83) was assigned by using CD analysis by the research group. Moreover, ^{13}C NMR data of herbacetin 7-methyl ether (84) was corrected. Twelve already known phenolic compounds 5,7,3′,5′-tetrahydroxy-dihydroflavone (85) (Wang et al. 2010), (+)-dihydrodehydrodiconiferyl alcohol (86) (Fu et al. 2008), kaempferol (87) (Lee et al. 2000), kaempferol-7-O-α-L-rhamnoside (88) (Lee et al. 2000), rhodionin (89) (Du and Xie 1995), ternatumoside II (90) (Warashina et al. 2012), crenuloside (91) (Du and Xie 1995), 2-(4-hydroxyphenyl) ethyl 3,4,5-trihydroxybenzoate (92) (Chu et al. 2014), methyl gallate (93) (Fu et al. 2008), and (+)-isolariciresinol (94) (Jutiviboonsuk et al. 2005) in addition to rhodiosin (75) (Du and Xie 1995) and luteolin (76) (Zheng et al. 2013) were identified.

Yang et al. (2012) reported the isolation of 11 novel lignans (95–105) and one benzonitrile compound; crenulatanoside A (106) from the 80% ethanolic extract of the air-dried roots of *R. crenulata*. The lignan compounds 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, and 105 were the newly discovered compounds. Compounds (100–103) belonged to aryl tetralin lignans whereas 104 and 105 were dihydrobenzofuran neolignan compounds. Additionally, compounds (95–99) and earlier discovered 107, 108 were the isomers of two 8-O-4′ neolignan glycosides. Yang et al. (2012) also identified (7R,8R)-threo-4,7,9,9′-tetrahydroxy-3,3′--dimethoxy-8-O-4′-neolignan-4-O-β-D glucopyranoside (109), (+)-cycloolivil-4′-O-β-D-glucopyranoside (111), (+)-isolariciresinol (112), (+)-isolariciresinol-4′-O-β-D-glucopyranoside (110), (+)-isolariciresinol-4-O-β-D-glucopyranoside

(113), (+)-isolariciresinol-9-O-β-D-xylopyranoside (114), (7S, 8R)-dihydrodehydrodiconiferyl alcohol-3′-O-β-D-glucopyranoside (115), 7S, 8R)-dihydrode-hydrodiconiferyl alcohol-9-O-α-L-rhamnopyranoside (116), (7R, 8S)-dihydrodehydrodiconiferyl alcohol-4-O-β-D-glucopyranoside (117), and olivil-4-O-β-D-glucopyranoside (118). Huang et al. (2008) investigated and quantified ten flavonoid compounds from *Rhodiola crenulata* by employing a simple but efficient HPLC method. In addition to rhodioloside (70), rhodiosin (75), kaempferol (87), and rhodionin (89), new flavonoids comprising kaempferol-3-sophoroside (120); kaempferol-3-rungioside (121); kaempferol-3-O-L-rhamnopyranoside (122); kaempferol-3-O-α-rhamnopyranosyl(1 → 4)-β-D-glucopyranoside (123); kaempferol-4′-O-α-L-rhamnopyranoside (124); and rhodiolin (125) were identified from the ethanolic extract of *R. crenulata*. Recently, Chen et al. (2021) identified 104 compounds from *Rhodiola crenulata* which were further categorized into five subclasses: alcohols and their glycosides, organic acids, flavanols, and derivatives of gallic acid and flavonoids and their corresponding glycosides by employing Ultra-High-Performance Liquid Chromatography (UHPLC)-Quadrupole Time-of-Flight (QTOF) MS as well as UHPLC-LTQ-Orbitrap MS (Ultra-High-Performance Liquid Chromatography coupled with Linear Ion Trap-Orbitrap Mass Spectrometry) technique for the fast detection of the chemical constituents present in *R. crenulata*. Three potential compounds were newly discovered ones, and 59 compounds were notified for the first time. 18 different organic acids present in *R. crenulata* were subdivided into five categories; Type (1, II, III, IV, and V): seven compounds including caffeic acid (51) and compounds 126, 127, 128, 129, 130, and 131 belonged to Type I category (caffeic acid), compounds including *p*-coumaric acid (50), 132 (4-O-glucosylcoumaric acid), and 133 (*p*-coumaroyl-O-hexoside) fall in Type-II (*p*-coumaric acid), Type-III category (vanillic acid) contained vanillic acid (134), compound 135 called iso-vanillic acid (an isomer of 133) and compound 136. Whereas Type-IV (trimethoxy cinnamic acid) category of organic acids comprised compounds 137 and 138 (3-(3,4,5-trimethoxyphenyl) propenoic acid-β-D-glucopyranosyl-β-D-glucopyranosyl ester). Three compounds: 139 (4-hydroxybenzoic acid), 140 (4-hydroxybenzoic acid 4-β-D-glucoside), and 119 belonged to Type-V (4-hydroxybenzoic acids) category of organic acids. Flavanol and gallic acid derivatives were also categorized into three classes including flavanols and gallic acids both, only flavanols and only gallic acids (Fig. 17.4). Lignans were categorized as Type-I containing neolignan glycosides and Type-II comprising aryl tetralin lignans. Flavonoids and their corresponding glycosides were categorized as kaempferol based, herbacetin only position-7 replaced and herbacetin not only position-7 replaced categories. In a similar manner, alcohols and their corresponding glycosides were placed in terpene alcohols (creoside V) and hydroxyl phenylethyl alcohols (2-(4-hydroxyphenyl) ethyl 6-O-β-D-glucopyranosyl-β-D-glucopyranoside and salidroside).

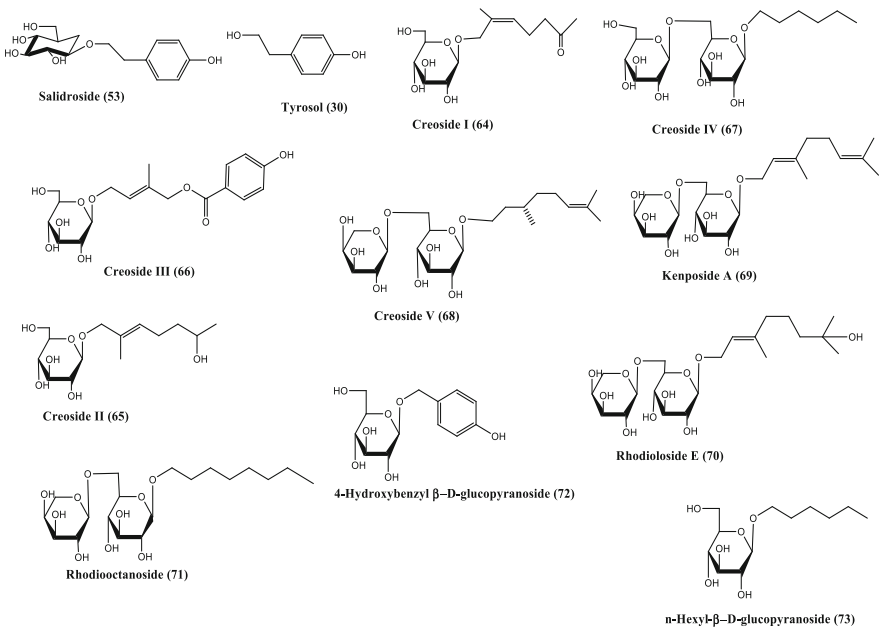
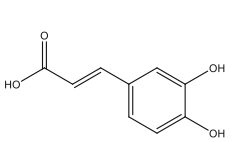


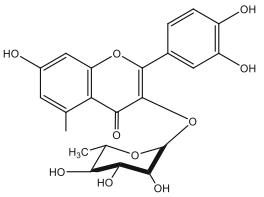
Fig. 17.4 Important phytoconstituents present in *Rhodiola crenulata*

17.6.4 Phytochemistry of R. tibetica

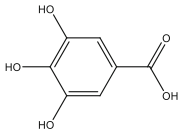
Out of the different *Rhodiola* species being investigated, *R. tibetica* is the least explored one. Fewer reports are available in literature on the phytochemical profiling of *R. tibetica*. Lu et al. (2021) reported thirteen polyketonic metabolites (141–153) from endophytic fungus Alternaria sp. of *R. tibetica*. The separation and purification of the polyketonic compounds was performed with column and preparative HPLC technique from the methanolic extract of the fermentation product of *R. tibetica.* Additionally, *p*-tyrosol and salidroside were the major active constituents present in *R. tibetica*. Shufeng et al. (2014) investigated the partition behavior of these two ingredients in different solvent systems. In case of liquid-liquid solvent extraction in tributyl phosphate solvent at pH below 9.0, salidroside and tyrosol were present in 60% and 90% amounts (Fig. 17.5).



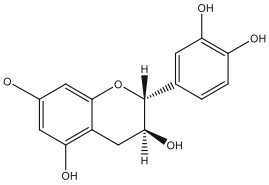
Caffeic acid (51)



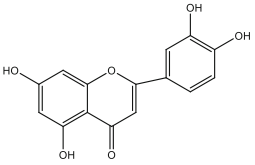
Quercitrin (74)



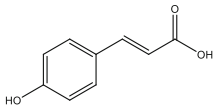
Gallic acid (46)



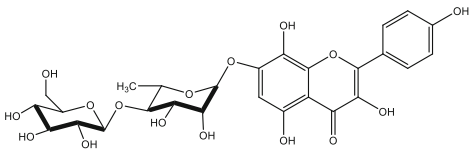
Catechin (77)



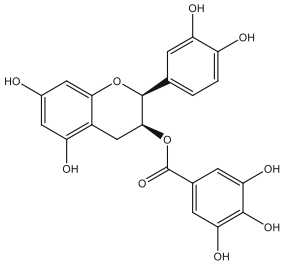
Luteolin (76)



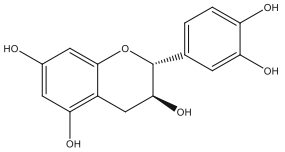
p- Coumaric acid (50)



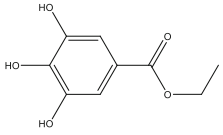
Rhodiosin (75)



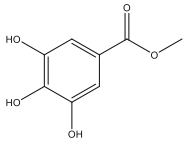
Epigallocatechin gallate (63)



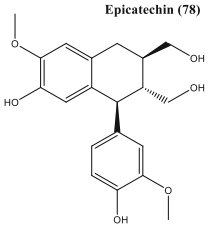
Epicatechin (78)



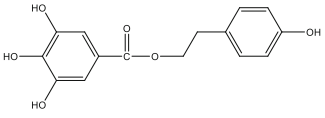
Ethyl gallate (79)



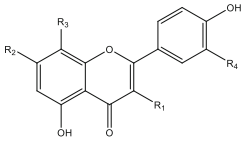
Methyl gallate (93)



(+)-isolarisiresinol (94)



2-(4-hydroxyphenyl) ethyl 3,4,5-trihydroxybenzoate (92)



Kaempferol (87) R_1=R_2=OH, R_3=R_4=H
Kaempferol-7-O-α-L-rhamnoside (88) R_1=OH, R_2=Rha, R_3=R_4=H
Rhodionin (89) R_1=OH, R_2=Rha, R_3=OH, R_4=H
Ternatumoside II (90) R_1=Rha^3-Glc, R_2=OH, R_3=H, R_4=H
Crenuloside (91) R_1=OH, R_2=Rha^3-Glc, R_3=R_4=H

Fig. 17.4 (continued)

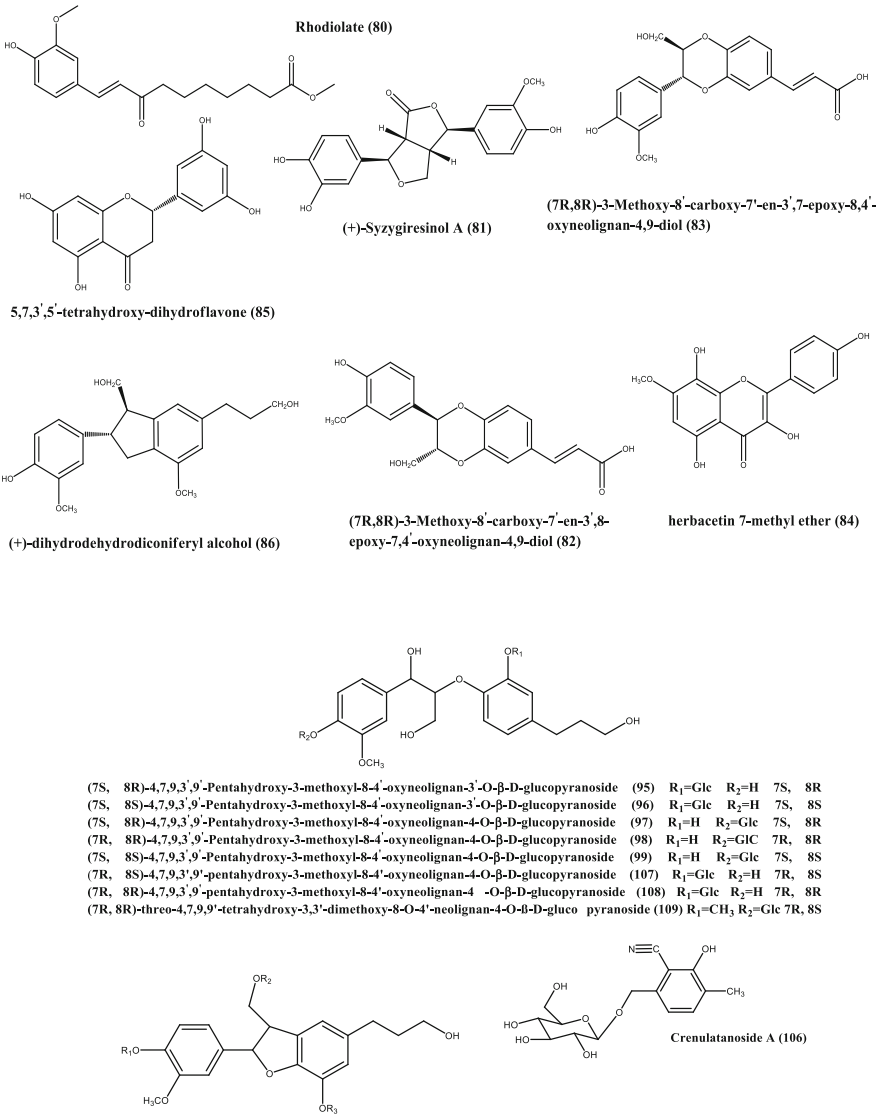


Fig. 17.4 (continued)

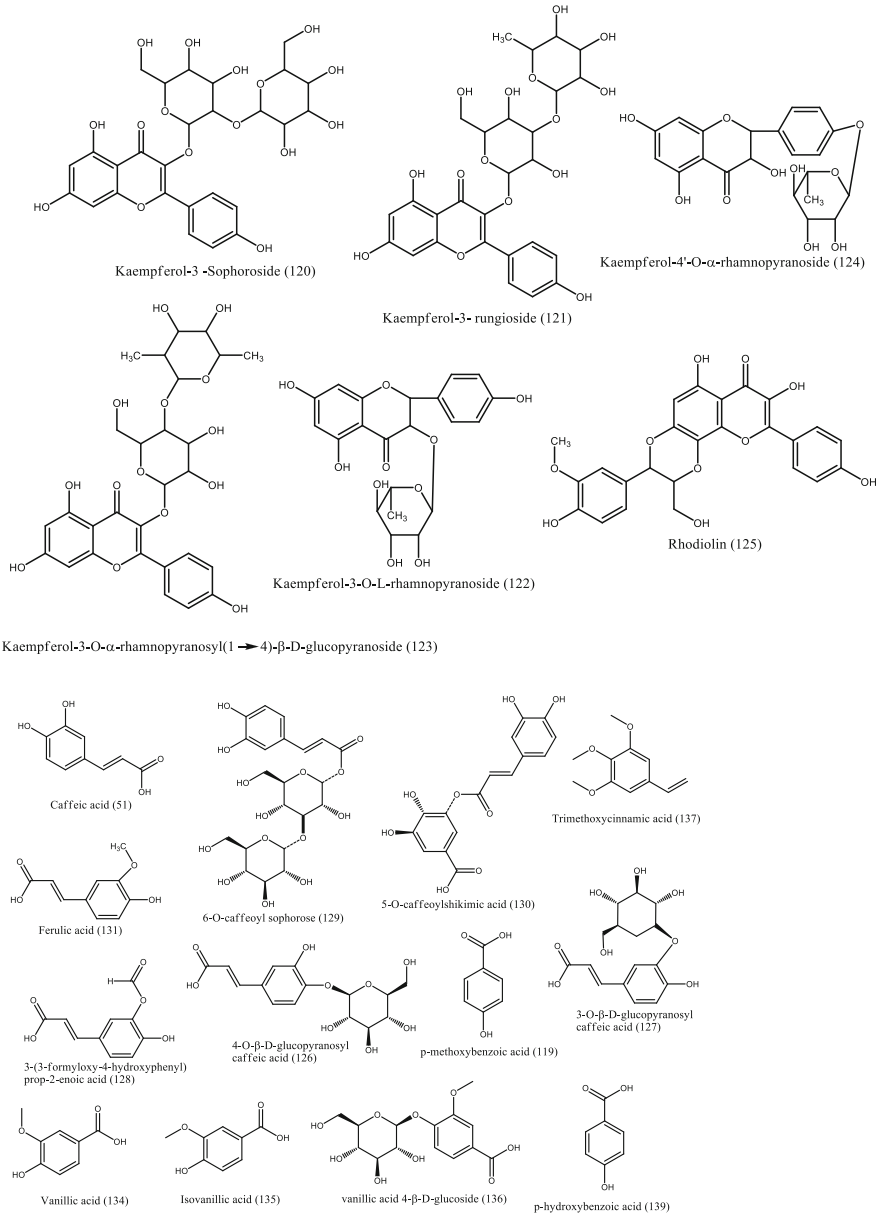


Fig. 17.4 (continued)

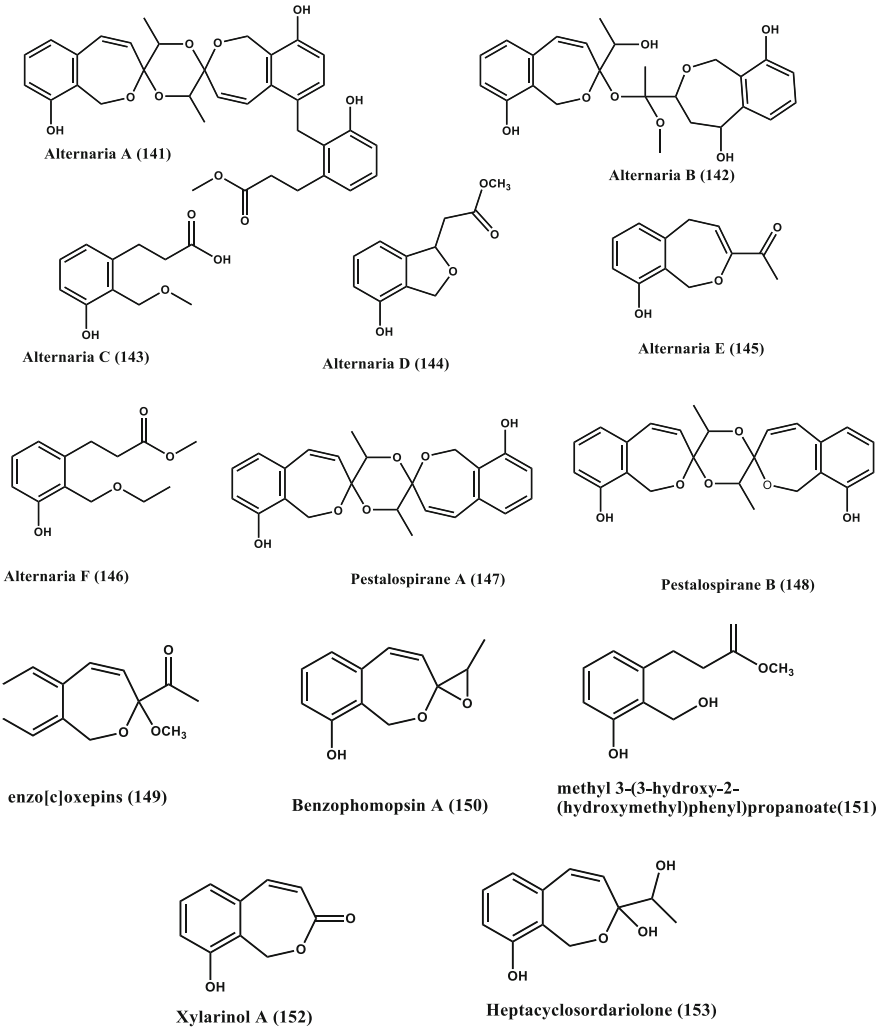


Fig. 17.5 Flavonoids and gallic acid derivatives in *Rhodiola tibetica*

17.7 Pharmacology

17.7.1 Rhodiola imbricata

R. imbricata is a very imperative food crop and has a wide range of medicinal properties. More than 200 species of genus *Rhodiola* occur in nature, of which approximately twenty possess vital medicinal applications (Bawa and Khanum 2009). This plant is exploited as an herbal remedy for treating the limb edema,

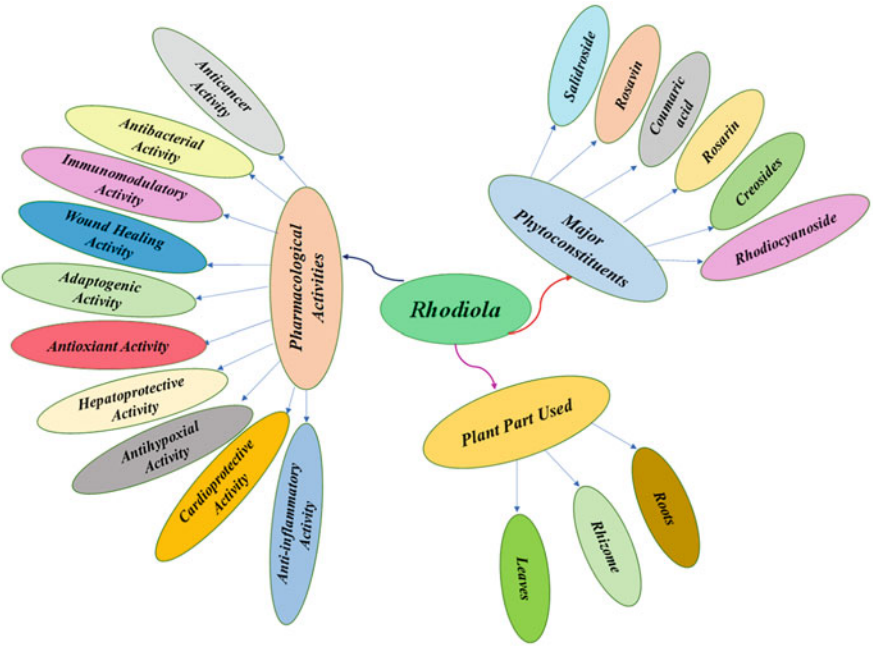


Fig. 17.6 Pharmacological potential of *Rhodiola* sp.

sores and other injuries, asthma, detoxification of the body, fatigue and to increase the physical strength. It is also a main constituent in the herbal tea due to its extensive pharmaceutical value (Kelly 2001; Chen et al. 2008). This plant is being valued for its extensive number of pharmaceutical applications (Fig. 17.6) like cardioprotective, adaptogenic, anti-stress, and anti-cancer activities (Singamaneni et al. 2020).

17.7.1.1 Anti-cancer Activity

Mishra et al. (2008) advised the in vitro protective efficacy of rhizomes of *R. imbricata* against leukemic human cell line. After incubation of the cells with plant extract, the growth of cells was observed to reduce as quantified by the MTT technique. However, no cytotoxic effect was observed in normal lymphocytes of human and RAW-264.7 cell line of mice. After 72 h, death of leukemia cells was noticed due to the production of ROS by *Rhodiola* as detected by PI and Annexin V-FITC staining. Moreover, the extract was observed to arrest the cell cycle at G_2/M phase. In another in vitro study conducted by Senthil Kumar et al. (2013), the antitumor activity of the acetone and alcoholic extract of *R. imbricata* (200 µg/mL) against the growth of human cancer cell line of colon HT-29 was investigated. It was observed that both the extracts prohibited the proliferation of cells by 84% in

dosage and time-dependent manner. This activity was supposed to be due to the more concentration of active constituents like polyphenols in the extracts.

17.7.1.2 Immunomodulatory Activity

One of the approaches to prevent and lessen several fatal infections is the stimulation of immune system. Various herbal extracts have been monitored for the same and are employed as immunomodulators. A huge number of studies have been carried out with *R. imbricata* which depicts that it stimulates the immune system and therefore can be used among immunocompromised patients. Mishra et al. (2009) examine the protective efficacy of water extract of *R. imbricata* on the toll-like receptors-4 (TLR4) and on the levels of granzyme-B in spleen cells of mice. Further the Th1/Th2 cytokine profile was also determined by monitoring the concentration of cytokines in the human mononuclear cells. The amplified levels of TLR4 and Th1 cytokines was observed along with high levels of granzyme-B in splenocytes. In another study of Mishra et al. (2006), the in vitro activity of *R. imbricata* (250 μg/mL) was demonstrated using the human PBMCs and murine macrophage cell line. It was observed to enhance the TNF-α concentration in cell lines through phosphorylation of inhibitory kappa B and NF-kB. Additionally, it was detected to enhance the nitric oxide concentration in RAW cells. *R. imbricata* extract enhanced the IL-2 production in chickens and most pronounced effect was observed at 400 mg/kg supplemented group birds. It advocates that *R. imbricata* supplementation employs immunostimulatory activity in broilers by mediating both cellular and humoral immune response (Kalia et al. 2017).

17.7.1.3 Hepatoprotective Property

Liver is a vital organ which controls the typical internal atmosphere and also plays a significant part in metabolic and inflammatory activities, and detoxification of poisons (Tacke et al. 2009). There are several researches validating the hepatoprotective characteristics of *R. imbricata*. In a study conducted out by Senthilkumar et al. (2014), it was observed that *R. imbricata* has the potential to cure the paracetamol intoxicated rats. It was detected that paracetamol caused the hepatic damage as it enhanced the levels of several hepatic enzymes SGOT, SGPT, and ALP in the serum. The acetone extract of *R. imbricata* ameliorated the liver damage. The oral administration of *R. imbricata* extract brought the levels of SGPT (88 ± 0.3 U/L), SGOT (79.6 ± 0.3 U/L), and ALP (193 ± 0.3 U/L) within the normal range. Moreover, paracetamol-induced histopathological necrosis was found to be repaired after the treatment of plant extract.

17.7.1.4 Wound Healing and Adaptogenic Activity

The wound healing potential of *R. imbricata* has been tested in the murine model. It was observed that the cover of epithelial cells could be formed at a faster rate (7 days earlier) after the treatment of wounds with the ethanolic extract of rhizome of *R. imbricata* as compared to the untreated animals. The extract enhanced the growth of cells and assisted in the formation of collagen proteins at the place of wound as indicated by the concentration of proteins like hydroxyproline and hexosamine. The concentration of these collagen synthesizing proteins was detected to be more in those animals which were treated with the extract in comparison to the povidone-iodine ointment treated positive control animals. Moreover, the histological investigations further validated the healing characteristics of this plant (Gupta et al. 2007). Supplementation of 100 mg/kg of water extract of *Rhodiola imbricata* showed highest resistance to cold-hypoxia restraint stress-stimulated hypothermia and accelerated retrieval from the stressor (Gupta et al. 2008). Moreover, the water extract of *Rhodiola imbricata* displayed concentration-dependent adaptogenic activity (Gupta et al. 2010).

17.7.1.5 Antioxidant Activity

The intake of *R. imbricata* extract in a single dose can cause remarkable reduction in MDA in blood, lower LDH in blood, liver, and muscles and also increase the GSH and SOD levels in blood. Moreover, multiple doses of the *R. imbricata* extract results in the further rise of GSH, GST, and CAT levels in blood, liver, and muscle and limit an upsurge in LDH production. It is also worth adding that the five-fold rise in the dose of the extract enhance the level of CAT, verifying the cumulative antioxidant potential of *Rhodiola imbricata* (Jamiol et al. 2021). Antioxidant and anti-proliferative potential of *R. imbricata* extracts were noticed in the colon cancer cells. The occurrence of phenols in *Rhodiola* rhizome showed anti-proliferative and anti-oxidative activity of these extracts is due to their metal chelating ability (Senthilkumar et al. 2013). The cyto-protective action of water and alcoholic extracts of *Rhodiola imbricata* was determined in human macrophages treated with tert-BHP oxidant. Both extracts (250 μg/mL) hampered the production of free radicals and programmed cell death (Kanupriya et al. 2005).

17.7.1.6 Other Therapeutic Activities

Several *Rhodiola* spp. have been observed to be the portion of Amchi medicine system. It is common among the conventional therapy for the cure of cold, cough, and fever among the ethnic people of Leh-Ladakh region of India (Ballabh and Chaurasia 2007). *The methanolic extract of R. imbricata* has been found to be a capable prophylactic agent in the crises of nuclear and radiological radiations

(Chawla et al. 2010). Gupta et al. (2010) prepared the water extract of roots of *R. imbricata,* and its different doses were fed to the rats through oral route. After 30 min of administration, rats were exposed to cold, about 5 °C and hypoxic conditions, 428 mm Hg and restraint conditions. Various parameters such as lipid peroxidation, levels of antioxidant, malondialdehyde (MDA), and lactate dehydrogenase (LDH) were done to check the adaptogenic activity of this plant. The levels of glutathione and superoxide dismutase (SOD) were observed to be in the normal range after the treatment of the plant. This illustrates that the plant has beneficial adaptogenic capacity as it alleviated the stress-stimulated alterations by improving the normal magnitudes of the numerous markers and enzymes in the rats.

17.7.2 Rhodiola crenulata

Rhodiola crenulata is a renowned traditional Chinese medicine described to increase hypoxia durability. This plant extract has been recommended for the treatment of high-altitude illness and mountain sickness (Chen et al. 2014). *R. crenulata* displayed an extensive number of biological properties like anti-inflammatory, anti-hypoxia, antioxidant, anti-cancer, radioprotective, and neuroprotective (Arora et al. 2005, 2008; Qu et al. 2009; Chawla et al. 2010; Zhang et al. 2019). Ingestion of *R. crenulata* extracts cause remarkable improvement in the cerebral functions (Qu et al. 2009), decrease mental fatigue (Zhang et al. 2009), and oxidative insults (Kanupriya et al. 2005). *Rhodiola crenulata* has a wide range of active components like salidroside, rosavins, tyrosol, and crenulatanoside which might be responsible for robust biological potential of this plant. It was observed that *R. crenulata* has good wound healing property, and it caused fastened recovery of injured spinal cord tissue (Zhao-Bo et al. 2017).

17.7.2.1 Antibacterial and Antioxidant Activity

The root extract of *R. crenulata* has been revealed to be a strong antioxidant in in vitro and in animal studies. The crude ethanolic and butanol extract of *R. crenulata* were found to be rich in flavonoids and phenolic acids. Both crude ethanolic and ethyl acetate *R. crenulata* extracts displayed marked antibacterial and antioxidant activities. The antibacterial and antioxidant potential of ethyl acetate extract of this plant was due to the presence of active phytoconstituents. Among them, rosavin showed outstanding antioxidant action as demonstrated by radical scavenging assays. Herbacetin, gallic acid, and ethyl gallate can hinder the multiplication of pathogenic bacteria which play a crucial role in the antibacterial potential of the extract (Zhong et al. 2020). Intake of RCE modulated the neurogenesis by shielding the neurons from oxidative free radicals, thus thwarting the apoptosis of neural stem cells. Prolonged administration of RCE caused marked decline in the oxidative stress in brain and has reduced memory debilities induced through

streptozotocin in rats (Qu et al. 2009). The protective effect of RCE against ionizing radiation-stimulated skin injury was investigated. Cancer cell lines (HepG2, Caco2, A549, and OECM) as well as non-cancerous skin cell line HaCaT cell lines were pretreated with RCE for 1 day and then exposed these cell lines to the ionizing radiation. The cell viability, apoptosis, matrix metalloproteinases (MMPs), oxidative stress markers, and pro-inflammatory cytokines were investigated. RCE treatment markedly protected HaCaT cells; however, this plant did not save cancer cells from the apoptosis caused due to the exposure of ionizing radiation. Supplementation of RCE weakened the radiation stimulated oxidative stress markers, apoptosis, levels of MMP and cytokines. Moreover, RCE restricted the levels of p53 and p21 induced due to radiation exposure. This study depicts that RCE exhibit protective effect on the skin cells from radiation without changing its potential to harm cancer cells. Thus, RCE may serve as an ideal candidate for radioprotective therapy (Lin et al. 2018).

17.7.2.2 Neuroprotective Effect

There are many studies which demonstrates the neuroprotective characteristic of *Rhodiola crenulata*. Sun et al. (2020) reported that supplementation of *Rhodiola crenulata* extract brought the concentration of 19 lipids in the normal range. Mechanistic studies depicted that the protective action of RCE might be linked with the sphingolipid and linoleic metabolism. Therefore, this investigation depicts a novel viewpoint on the use of RCE in the cure of Alzheimer's ailment. Li et al. (2020) proposed that the neuroprotective efficacy of RCE against Alzheimer's disease might be regulated through the glutathione and arachidonic acid metabolism. In another study, pre-administration of *Rhodiola crenulata* in animals with Alzheimer's disease triggered a substantial enhancement in the concentration of ATP and cytochrome c oxidase. Moreover, the apoptotic neurons and mitochondrial injury were observed to be decreased in the animals treated with RCE in comparison to the untreated control. Moreover, RCE administration averts dysfunctioning of mitochondria and defends hippocampal neurons from programmed cell death in animals with Alzheimer's disease (Wang et al. 2017). RCE restored the weakened neurogenesis in the hippocampus of animals with Alzheimer's disease. This protective effect of RCE is attributed to the presence of active secondary metabolite salidroside in the extract which reduced the levels of intracellular ROS (Qu et al. 2012). In another study, it has been seen that the active phytoconstituent, salidroside of RCE exhibited recovery in cognitive deficits by controlling the levels of oxidative radicals and inflammatory mediators in the hippocampus of rat (Zhang et al. 2013), upgrading cell death like the reduction of the pro-apoptotic gene and the enhancement of the levels of anti-apoptotic gene Bcl-2 (Yu et al. 2008; Yang et al. 2013).

Chen et al. (2020) demonstrated that RCE can be an ideal agent against the neurodegeneration induced by D-galactose in animals. The intake of D-galactose can cause the brain aging identical to human brain aging in several facets such as cell death, cognitive deficit, neural deterioration, amplified oxidative stress, and

mitochondrial DNA mutation, reduced ATP production in the brain. Treatment of RCE showed the neuroprotective effect through the inhibition of apoptosis by blocking the Fas/FasL and mitochondrial-mediated signaling in neurons. Administration of RCE augmented the PI3K/Akt signaling in cortical as well as hippocampal neurons (Shwe et al. 2018).

17.7.2.3 Anti-hypoxial Activity

Traditionally, *R. crenulata* is employed as an herbal medicine for the cure of high-altitude illness and pulmonary injury stimulated by hypoxia. Supplementation of *R. crenulata* extract in zebrafish after exposure of 72-h hypoxia modulated the levels of citrate synthase, lactate dehydrogenase, and hypoxia-induced factor-1α (HIF) in brains. This plant revealed anti-hypoxic activity in zebrafish by altering the sphingolipid metabolism, HIF- and AMPK-related signaling (Ma et al. 2019). Hsu et al. (2017) demonstrated that the administration of RCE showed reduction in the cardiac apoptosis induced due to hypoxia. Exposure of hypoxia to the animals showed remarkable increment in the lung's edema and elevated levels of ROS, MDA, and MPO. Pre-administration of RCE displayed reduction in hypoxia induced lung edema by decreasing all the markers of pulmonary injury associated with hypoxia (Lee et al. 2013). Supplementation of RCE to broiler chickens caused remarkable enhancement in the levels of red blood cells and hemoglobin. It was observed that addition of RCE diminished the impact of hypoxia on broiler chickens and lower the mortality rate (Li et al. 2014).

17.7.2.4 Anti-diabetic

Oral supplementation of RCE displayed significant improvement in the sensitivity of insulin in diabetic rats and control carbohydrate and lipid metabolism (Wang et al. 2012). Treatment with RCE in HepG2 cells reduced the production of glucose and expression of gluconeogenic genes. Additionally, RCE supplementation boosted the concentration of phosphorylated AMPK and repressed the amount of gluconeogenic gene in the rat liver. Therefore, in vitro and in vivo examination of *Rhodiola crenulata* displayed glucose-lowering effect by hindering the liver gluconeogenesis and stimulating the AMPK signaling. RCE regulate the nitric oxide (NO) defects, apoptosis, and oxidative radicals in high glucose environment. Moreover, RCE administration caused markedly reduced high glucose-stimulated vasoactive markers such as fibronectin, endothelin-1, and vascular endothelial growth factor. However, the supplementation of RCE recovered the AMPK-eNOS signaling and shielded endothelial cells from hyperglycemic insult. Thus, *R. crenulata* can be used as an ideal agent for the cure of vascular diseases related to diabetes mellitus (Huang et al. 2017, 2020).

17.7.2.5 Cardioprotective

Rhodiola crenulata administration demonstrated protective efficacy against cardiomyocyte cell death caused due to chronic sporadic hypoxia through Fas-based and mitochondrial-based apoptotic signaling (Lai et al. 2015). Treatment with RCE repressed the concentration of IL-17 and its related markers which were facilitated by the stimulation of numerous MAPKs that ultimately reduced the stages of fibrosis and apoptosis (Hsiao et al. 2021). Huan-Huan et al. (2021) demonstrated that RCE administration assisted in the control of pulmonary arterial hypertension through the hinderance of oxidation of fatty acids and autophagy.

17.7.2.6 Anti-inflammatory

Administration of RCE as a complementary therapy caused a significant decline in the levels of IFN-γ and T cells in chronic obstructive pulmonary disease (COPD) patients. However, treatment with RCE augmented the levels of $CD4^+$ $CD25^+$ $FOXP3^+$ and $CD4^+$ $CD25^+$ $CD45^+$ $FOXP3^+$ in the blood in COPD patients (Chen et al. 2015). Wang et al. (2021a) reported that RCE modulated the signaling of immune response particularly IL-17 and TNF signaling. This plant extract can play significant role in the control of cytokine storm of COVID-19 through regulating the IL-1, TNF-α, and IL-6. Intake of RCE boosted the expression of antimicrobial peptide genes in response to the ingestion of toxic compound and enhanced the viability of adult flies. Thus, *Rhodiola crenulata* extract can be used for the control of inflammatory intestinal infections (Zhu et al. 2014). Water extract of RC showed anti-inflammatory action on macrophages cell line RAW 264.7 cell and murine peritoneal macrophage cells. The expression of iNOS, MAPK, and NF-κB proteins were found to be reduced after the treatment of RC, and it activated autophagy-associated markers. RC supplementation caused a reduction in the nitric oxide, IL-6, and IL-1 in both lipopolysaccharide-induced macrophages (Lee et al. 2020).

17.7.2.7 Hepatoprotective

Root extract of *R. crenulata* showed protective efficacy against liver gluconeogenesis and lipid metabolism in human HepG2 hepatoma cells. Administration of RCE in high glucose atmosphere for 6 h enhanced the synthesis of liver glycogen and inhibited the lipogenesis through AMPK signaling, and it might be active in the cure of non-alcoholic fatty liver infection (Lin et al. 2016). In another investigation, RCE at the concentration of 50 mg/kg decreased the hepatic triglyceride content in fructose-fed rats. In addition to it, RCE enhanced the levels of autophagosome indicators and reduced the autophagolysosome marker p62 in the liver of fructose-fed rats (Yuan et al. 2020).

17.7.2.8 Anti-cancer and Anti-fatigue Effect

R. crenulata extract displayed antitumor activity against brain tumor glioblastoma. RCE showed decline in the proliferation of cells, enhance cell differentiation, and remove formation of tumorsphere. These effects are linked with the inhibitory effect of RCE on Wnt/b-catenin signaling (Mora et al. 2015). In another study, pre-administration of RCE exhibited anti-fatigue activity and caused improvement in the exercise performance in mice by regulating the skeletal muscle markers (Hou et al. 2020).

17.7.2.9 Other Therapeutic Effects

Supplementation of *Rhodiola crenulata* extract weakened the hypoxia-stimulated brain injury as demonstrated through reduced concentration of MDA, GSSG, and LDH. RCE amplified the levels of GSH and SOD as well as upgrading the histological alterations in hippocampus and boosted the cell vitality through reduction in the cell apoptosis. Moreover, RCE treatment caused increment in the COX10, HIF-1α, and Bcl-2 expression, however intensely hindering levels of Cyto-c, Apaf-1, Caspase-3, and Bax. Thus, RCE diminished hypoxia-stimulated brain injury by modulating the cell death and mitochondrial energy metabolism through the COX10 signaling pathway (Wang et al. 2019). Moreover, RCE supplementation can improve the insulin resistance by controlling sarcolemmal and intracellular CD36 reorganization in muscle (Pearson et al. 2010; Chen et al. 2016). Moreover, RCE demonstrated protective efficacy against dextran sulfate sodium (DSS)-stimulated colitis in mice. This plant extract remarkably lessened the pathological aberrations in colitis mice such as colonic damage and decreased pro-inflammatory markers. The DSS-stimulated epithelial apoptosis and sustained colonic blockade function were diminished by RCE by the enhancement in the concentration of tight junction proteins like occludin. Moreover, RCE restored the microbial richness and diversity by prohibiting gut dysbiosis in colitis mice and declining the plenty of pathogenic *Parasutterella* and *Staphylococcus* and enhancing the number of valuable microbes in *Bifidobacterium* and, *Lactobacillus* which were associated with its shielding action against colitis (Wang et al. 2021b).

17.7.3 Rhodiola heterodonta

Rhodiola heterodonta root extract contained the remarkable number of total phenols (79.21 mg GAE/g) and flavonoids (269.3 QE/g) (Kumar et al. 2010). Ethanolic extract of *R. heterodonta* triggered improvement in the survival rate of the mice under hypoxic environment than the untreated control. It was found that rate of survival of mice under hypoxia conditions enhanced by 192% after the

administration of *R. heterodonta* extract which serve as an adaptogenic agent (Grace et al. 2009). In another study, it was found that *Rhodiola heterodonta* extract displayed average adaptogenic activity and can be employed as an adaptogen. Moreover, negligible toxic effects were found after the treatment of *Rhodiola heterodonta* extract as confirmed by histological studies (Yunuskhodjaev et al. 2014).

17.7.4 Rhodiola tibetica

The medicinal value of *Rhodiola tibetica* is documented in the Tibetan medicine book (Tan and Zou 2001). *R. tibetica* is known as anti-stress agent and it reinstates memory as health stimulant (Bhadrecha et al. 2017). In addition to it, this plant contained polyketone metabolites which displayed significant inhibitory activity against the SARS-CoV-2 virus ($EC_{50} = 0.02–0.3$ mM) (Lu et al. 2021). In another study, treatment of *Rhodiola tibetica* showed reduction in the high-altitude pulmonary edema in rats in contrast to the untreated control group (Li 2013).

17.8 Toxicity Studies

Tulsawani et al. (2013) monitored the safety index of water extract of root of *R. imbricata* by orally administering it at various dosages of 100–500 mg/kg body weight for 3 months. At the concentration of 100 mg/kg, no alteration in the body weight, histology, hematological and biochemical markers were noticed. In another study, oral supplementation of water root extract of *R. imbricata* was observed to be non-toxic in murine models for acute and sub-acute studies. Moreover, long-term administration of *Rhodiola heterodonta* did not reveal any toxicity and alteration in the body weight of animals. The LD_{50} of *R. imbricata* extract was detected to be more than 10 g/kg body weight, which confirmed negligible toxicity of this extract (Gupta et al. 2008; Yunuskhodjaev et al. 2014).

17.9 Tissue Culture

The medicinal plants are haphazardly harvested in the name of exploration well before the seed setting for raw materials, and this unorganized exploration in addition to lack of cultivation policy/strategies has put an extra burden on natural resources. To overcome this situation, these wild populations need to be saved naturally as well as in artificial conditions, which will reduce the pressure on biodiversity and also meet the increasing demand. One of the most important artificial methods of conservation is micropropagation/tissue culture techniques

which may also serve as a substitute for plantlets production on the large scale or rapid cloning of desired genotypes. Tissue culture techniques are very basic and practical aspects that have evolved over time. In *Rhodiola* spp. in vitro study was performed to develop the plant in laboratory conditions to use for various medicinal purposes. Since it is evident that selection of explant is very crucial for the success of morphogenesis in isolated cells. These explants may be used to determine organogenic diversity and also maintain genetic stability after cloning (Rai et al. 2000). Chang-zhong et al. (2005), studied the possibilities of induction or maintenance of callus cultures in *R. quadrifida*, a highly medicinal herb used in the Chinese medicine system. They revealed that MS medium (2,4-D (1.0 mg/L + NAA (2.0 mg/L) + BA (0.5 mg/L) + kinetin (0.1 mg/L) maintained the callus induction in the species and also revealed that the compound salidroside was formed in callus (1 month old). Similar observations were made in *R. kirilowii* by Li et al. (2005) where importance of explant and temperature conditions were discussed for the production of salidroside in callus. Hong et al. (2008) revealed that MS medium (BA (0.5 mg/L) + IAA (2.0 mg/L) induces callus induction in *R. coccinea*. Further, Jianfeng et al. (1995) obtained callus from different explants of *R. sachalinensis* and observed that treatments with BA3 mg/L + NAA 0.3 mg/L, at temperature of 21–25 °C, were suitable conditions for the callus induction. The same species was studied by Jianfeng et al. (2007a), and the results revealed that application of BA (2.0 mg/L) + NAA (0.5 mg/L) induces callus formation in leaf explants (83.33%) with green, yellow, and red colors, of which application of BA (1.0 mg/L) + NAA (0.1 mg/L) only induces green color in explants. Jianfeng et al. (2007b) reported that application of phyto-growth regulators BA 2.0 mg/L + NAA 0.5 mg/L induces green callus in *R. sachalinensis*. Similarly, mesophyll protoplasts obtained from leaves of *R. sachalinensis* induces callus in 40 days treated in MS medium (2,4-D—1.0 mg/L + zeatin—0.5 mg/L + 0.5 M/L mannitol + casein hydrolysate 500 mg/L), phytohormones (BA 1.0 mg/L + NAA 0.1 mg/L), and organogenesis were also observed in the form of buds after *p*-calli (Jianfeng et al. 2009). Similarly, Hai-jun et al. (2006) studied in vitro propagation in four species of *Rhodiola*, viz. *R. crenulata, R. fastigata, R. yunnanensis,* and *R. sachalinensis* using two different forms of explants. The results further revealed that bud induction was best in stem explant, while organogenesis was best in leaf explants of *R. crenulata*. Further, *R. crenulata* and *R. yunnanensis* combination phytoregulators (2.5 mg/L BA+0.1 mg/L NAA) was best for organogenesis and induces bud formation in *R. cranulata* (71%) and *R. yunnanensis* (84%). Similarly, bud formation in *R. fastigata* and *R. sachalinensis* was induced by application of higher concentrations of phytoregulators (NAA-0.5 mg/L + BA 2.5 mg/L), which also show a high percentage (66%) of adaptability in soil. Wu et al. (2003) performed in vitro culture in *R. crenulata* revealed variation in in vitro multiplication by applying different concentration of plant growth regulators in different explant(s), viz. flower buds (2.0 mg/L kinetin), leaf discs (BA 2.0 mg/L + IAA 0.2 mg/L), stem explants (2.0 mg/L kinetin+1.0 mg/L IAA), and stem nodes (2.0 mg/L BA+1.0 mg/L IAA). In the result, leaf explants showed best results for in vitro development as it revealed 100% shoot induction in MS medium and PGR (0.2 mg/L NAA), while other explants failed to do so.

17.10 Synthesis of Secondary Metabolites

All the species of the genus are reported with salidroside, which constitute the basic component of a biologically active complex. For example, cinnamyl alcohol glycosides (CAGs) are one of the important constituents of *R. rosea* extracts (Zapesochnaya and Kurkin 1982). However, roseroot cultivation does not provide standard quality of raw materials, which may be due to variable content of the active metabolites in various morpho/ecotypes under different cultivation conditions which needs a long-term monitoring and investigation (Linh et al. 2000; Peschel et al. 2012). Such variability in chemical constituents of different populations of *R. rosea* has been studied in different domestication efforts (Aiello et al. 2013; Adamczak et al. 2014). A number of in vitro studies had been conducted to enhance and stimulate the production of active metabolites in *R. rosea* where the precursor feeding turns out to be the most efficient approach (Grech-Baran et al. 2015). In another species, *R. imbricata*, blue light exposure of callus culture, enhances the amount of Salidroside in 21 days when compared to the other light conditions (Kapoor et al. 2018). Further, UV–Vis spectrophotometric analysis in the same study also revealed the increased amount of total phenolics content in callus cultures exposed to blue light conditions compared to the others. Thus, the study revealed that abiotic stress factors, viz. drought, salinity, light, and temperatures, etc. influence growth and secondary metabolite production in higher plants.

17.11 Clinical Trials

There are several clinical studies of *Rhodiola* have been conducted since the 1960s. Moreover, a huge number of papers on *Rhodiola*'s therapeutic effectiveness (of variable scientific rigor) have been reported in journals of India, Russia, China, and other countries. Various researches have monitored the efficiency of *Rhodiola* on depression, fatigue, mountain sickness, and heart-related diseases. *R. crenulata* has been shown in several studies to improve physical performance in healthy people, suggesting that it has an ergogenic action (Chen et al. 2014; Hovhannisyan et al. 2015). Preliminary clinical trials of *Rhodiola* demonstrated potential in monitoring the concentration of glucose in blood in diabetic patients (Fan et al. 2007). *R. crenulata* displayed anti-inflammatory activity and it caused the increment in exercise durability, thus possessing the efficacy to cure chronic obstructive pulmonary disease (COPD). A 12-week, randomized, double-blind clinical trial was carried on 57 COPD patients (age 70 years) with administration of RCE at the concentration of 250 mg two times/day ($n = 38$) or a placebo at the similar concentration ($n = 19$). There were no remarkable alterations detected in pulmonary functioning, ability of 6-min walk, superiority of life, and exercises among the 2 clusters at registration. After the completion of 12 weeks, it was observed that patients remarkably tolerated RC and showed significant decline in thickness of

triceps skin. Patients revealed better capability to do work and marked enhancement in tidal breathing and ventilation output ratio. It was observed that treatment with RC resulted in protection against acute aggravation of COPD patients. However, more studies with large population size are required to validate the effect of RC on COPD patients (Chuang et al. 2015).

17.12 Patents

Many species of *Rhodiola* find great importance in the field of nutritional medicine due to differences in efficacy and their chemical composition. *Rhodiola crenulata* is associated with a characteristic bitter and sweet taste and affects the heart and lung meridian and improves qi by triggering blood circulation and curing asthma. Its anti-fatigue effect is many folds superior to acanthopanax and ginseng and its stimulating intellectual activity is better than acanthopanax root, it is used as a nutritional medicine as an anti-fatigue and enduring coldness. The major components responsible for its effectiveness is a flavonoid namely herbacetin and its glycosides. Herbacetin functions by scavenging DPPH and hydroxyl radicals and preventing oxidation of proteins. It has been investigated that free herbacetin content in *Rhodiola* L. species is very small and is as low as 0.0022% to 0.061%. Therefore, the separation of herbacetin is a very difficult process. Taizhou Danding (Patent No. US 10,435,384 B2) proposed an efficient method for extraction of herbacetin from *Rhodiola* L. by carrying out the extraction from pulverized *Rhodiola* L. using methanol-aqueous solution or ethanol-aqueous solution as the extracting solvent and then condensing the solution to attain an extract and the aqueous layer so obtained after leaching with petroleum ether is then acid hydrolyzed. Leaching is carried out on the acid hydrolyzed solution using an organic solvent. Herbacetin extract is obtained by concentrating the organic layer. Crude herbacetin was obtained by passing the above collected herbacetin through a column containing polyamide, eluting it with petroleum ether and ethyl acetate (1:1, v/v) and collecting the fraction containing herbacetin and condensing it. The crude product is subjected to reverse-phase column chromatography (silica based) and the herbacetin containing outflow on drying by condensation method and recrystallization by a methanol solution yields pure herbacetin. In another invention, *Rhodiola crenulata* extract finds application in boosting the metabolism of cutaneous tissue of mammals, when applied topically. The extract was first prepared by the extraction of *Rhodiola crenulata* roots, and/or low stem by using a polar/non-polar solvent particularly water-glycolic or water-alcohol mixture followed by the isolation of the active fraction via a fractioning step. The extract containing crenulatine or at least one of its ester/acid derivatives or no less than one of its salts is then purified. The topical application of 0.01–10% (w/w) composition of *Rhodiola crenulata* extract increased energy metabolism of the cutaneous tissue human beings and delayed the loosening and intensified the plasticity of the cutaneous tissue. It also caused an anti-wrinkle effect,

delayed the appearance of wrinkles and acne, and exerted a depigmenting effect on the skin by faster multiplication of cells of the cutaneous tissue.

0008 U.S. Pat. No. 6,399,116, to R. Xui (the 116 patent) is directed to *Rhodiola crenulata* extract for curing many diseases and conditions in mammals, particularly to increase blood oxygen levels in conditions of muscle fatigue. *Rhodiola crenulata* extract administered in any form by using any effective way is associated with many valuable properties such as increasing oxygen levels, endurance and working capacity, increasing concentration and sharpening of memory, to improve cardiovascular and cardiac functions and as antioxidant. It is also beneficial in decreasing stress, helps in DNA repairing, reduces levels of glucagon, decreases allergic reactions by reducing histamine release, functions as sleep and testosterone modulator, acts as a radiation shield and lowers cholesterol levels, promotes weight loss, and improves sexual performance and treating impotency.

17.13 Herbal Formulations

The herbal adaptogenic appetizer for appetite modulation using *Rhodiola* extract, in particular *Rhodiola imbricata* mixed with pulp of apricot and dry powder of sea buckthorn has been formulated to overcome weight loss problems at high-altitude regions due to reduced energy consumption. The formulation is rich source of polyphenols, vitamin C, flavonoids, sterols, vitamin E, niacin, rosavin, isorhamnetin, and carotene. It aids in improving appetite and digestion and enhances serum antioxidants too. The major ingredients present in the formulation are *Prunus armeniaca*, *Hippophae rhamnoides,* and *Rhodiola imbricata*. Moreover, a herbal preventative formulation is prepared from various parts including leaves, fruits, and roots of high-altitude exotic and medicinal plants to improve mental and physical performance. The major constituents of the formulation are *Codonopsis pilosula*, *Rhodiola imbricata*, *Zingiber officinale*, *Bacopa monnieri*, *Tribulus terrestris*, *Ginkgo biloba*, and *Withania somnifera*. The formulation acts as a very good source of withanine, astragalosides, aloin, triterpenes, shogaol, anthraquinones, gingerol, bacosides, and vitamins. In addition to it, an antioxidant enriched herbal tea has been formulated by Defence Institute of High Altitude Research (DIHAR) in Leh. The major ingredients present in herbal tea are *Mentha longifolia*, *Hippophae*, *Bidens, Dactylorhiza*, *Achillea, Bunium persicum*, *Origanum*, and *Rhodiola* sp. It is used as a remedy for cough, cold, body pain, headache, fever, high altitude-related sickness, memory loss, high blood pressure, weakness, and memory loss.

17.14 Conclusion and Future Perspectives

Rhodiola, the golden root as immunomodulator enhances functioning of immune response in different ways. It is known by the name of golden root just as gold possess remarkable useful properties, *Rhodiola* has immense value in the

improvement of mucosal immunity and can be used for the treatment of cancer and tumor patients. It is employed as strong anti-viral and anti-oxidative agent. This plant is exploited in the cure of depression, anxiety, stress, and other lifestyle diseases. Thus, *Rhodiola* can be measured as a wonderful drug, which can be useful for all the parts of body. The chief active phytoconstituents which are responsible for the biological potential of *Rhodiola* are rosin, salidroside, tyrosol, rosarin, rhodionin, and catechin. However, the mode of action behind the activity of *Rhodiola* is still not investigated and has to be explored further so that it can be used for the cure of fatal infections effectively. *Rhodiola* species have proved their innumerable pharmacological potential for about 2000 years. Further clinical studies are required to be conducted to form adaptogens and preparations from *Rhodiola* against fatal infections. Equivalently, more attention must be needed for the preservation of these golden root species as they are very susceptible for environmental variations.

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Chapter 18
Saussurea Lappa C.B. Clarke: Kushta/Kut

Urvashi and Ramandeep Kaur

18.1 Introduction

Natural medicines obtained from medicinal plants have become an integral part of human lives with each civilization having its own understanding of concepts and thus giving various medicine theories such as Ayurveda (Indian subcontinent), Unani medicine (Iran), and traditional Chinese medicine (Vitalini et al. 2013). World Health Organization (WHO) reported that approximately three-fourth of the global population practice medicinal plants as basic healthcare therapies (Pan et al. 2014) and make up a significant percentage of approved drugs worldwide. Over the last few years, many scientists have focused on medicinal plants because of their widespread pharmacological properties (Newman and Cragg 2016). Since time immemorial, Himalaya's natural flora is the source of medicinal herbs to be used traditionally to cure different diseases.

S. lappa commonly referred to as costus is one of the prestigious plants of the Indian Himalayan region having therapeutic properties. It is a dominant medicine in the retail market worldwide. *S. lappa* is widely used in Ayurveda and other traditional medicinal systems for the treatment of various diseases related to skin and digestion (Lin et al. 2015). The pharmacological properties exhibited by *S. lappa* are well-documented in the literature (Trinh et al. 2020). The major constituents of *S. lappa* (Costunolides and dehydrocostus lactone) are also known to possess medicinal properties (Hassan and Masoodi 2020). However, it is now reported as a critically endangered species due to its overexploitation for various medicinal tasks (Kuniyal et al. 2005). This chapter is an effort to provide comprehensive information related to chemistry and pharmacological studies related to *S. lappa*.

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18.2 Taxonomic Classification of *S. Lappa* (Gautam and Asrani 2018)

Division	Tracheophyta
Subdivision	Spermatophytina
Infradivision	Angiospermae
Kingdom	Plantae
Subkingdom	Viridaeplantae
Infrakingdom	Streptophyta
Subphylum	Euphyllophytina
Infraphylum	Radiatopses
Class	Magnoliopsida
Subclass	Asteridae
Superorder	Asteranae
Order	Asterales
Family	Asteraceae
Tribe	Cynareae
Genus	*Saussurea* DC.
Species	*Lappa*

18.3 Common Names: Kur, Kusthah, and Costus

S. lappa is mentioned differently in various parts of the world such as Costus (English), Kostwurz, Practige (Germany), Mu Xiang (Chinese), Costus Eligant (French), Kust-a-behri, Kush, Quist (Arab), and Qust (Persian). In India, *S. lappa* is called by different vernacular names as mentioned below (Prasad and Subhaktha 2002; Khan et al. 2013).

Punjab	Kuth, Kust, Kot
Urdu	Kut
Kashmir	Post-Khai, Chob-i-kud, Kuth
Gujart	CuplateKut, Upalet/Upaleta, Kath, Kur
Sanskrit	Kushta, Kashmiraja, AgadaKashtam, Utpalam, Amayam, Pushkara
Assam	Kud, kur
Bengal	Kudo, Pachak, Kur, Kut
Hindi	Kot, Kur, Kut, Kust, Pachak
Karnataka	ChangalKushtha, koshta
Kerala	Kottam, sepuddy
Maharashtra	Upleta, Kushtha
Orissa	Kudha
Tamil Nadu	Goshtam, Kostam, Kottam
Andhra Pradesh	Changala, Kustam

18.4 Flowering/Fruiting Season

S. lappa is cultivated in the sandy loamy soil. The plant grows in the temperate and sub-alpine region. The seeds are sown in April or May in nursery and transplanted when seedling are 15 cm long. The crop is harvested after 3 years to obtain a maximum yield of root tubers in the month of September/October/early spring.

18.5 Distribution

S. lappa is widely distributed in the moist slopes of the Himalayas in India, Pakistan, and China at an altitude of 2600–4000 m (Zahara et al. 2014). Worldwide, it is cultivated in the cold arctic and temperate areas of Asia, North America, and Europe continents (Shah 2019). In India, the species are mostly available in the Himalayas and Western Ghats region (Gautam and Asrani 2018). Its wild varieties are reported at high altitudes of Jammu and Kashmir, Sikkim, and Arunachal Pradesh. To conserve the plant species, the commercial cultivation of *S. lappa* has been started in Himachal Pradesh, Uttaranchal, and Uttar Pradesh (Butola and Samant 2010; Kuniyal et al. 2005).

18.6 Morphology

S. lappa, also known as costus, is a massive, perennial aromatic plant which grows upto 1–2 m in height. Its roots are dark brown to grey colored, long (about 60 cm), stout, and have a specific aroma. The roots taste bitter when dried. The leaves are membranous, irregularly toothed, and lobate; radical leaves are large with long lobately winged trunk while upper leaves are small. The stem is stout, erect, and fibrous. The flowers are blue-purplish to blackish, sessile, and have auxiliary and terminal heads arrangement. The fruits are hairy, curved, and cupped (Pandey et al. 2007).

18.7 Traditional Uses

Since times immemorial *S. lappa* is used as a potent medicinal herb in traditional Chinese, Tibetan, and Indian (Ayurvedic) medicinal systems. In Atharvaveda, its medicinal properties are documented under name “kustha.” It is of two types, i.e., Sweetish and bitter but the latter is real “kustha” (Prasad and Subhaktha 2002). In the

Unani system, it is used for brain stimulation, digestive, excretory, and blood problems. It is also helpful in the treatment of eye, ear, and nose-related problems (Madhuri et al. 2012).

Since time immemorial, costus stem, roots, and flowers are being used for its medicinal purposes to cure stomachache, dysmenorrheal, altitude sickness rheumatoid arthritis, and cough with cold in China, Japan, and Nepal (Pandey et al. 2007; Madhuri et al. 2012; Choi et al. 2013; Lin et al. 2015). It was a well-known plant for various health hazards such as fever, skin diseases, and headache. Contemporary research lead to the belief that can be used for blood purification and disinfection (Waly 2009). *S. lappa* is traditionally used as aromatic, thermogenic, deodorant, digestive, carminative, anodyne, aphrodisiac, and skin-related issues. The roots of costus are also found effective for the treatment of bronchitis and vagotonic-type asthma (Madhuri et al. 2012; Ansari 2019).

In North India, the dried roots of costus are placed in shawls and woolen fabrics for their protection against insect pests. The dried leaves and the aerial parts of costus are also used for smoking, fuel, and fodder (Butola and Samant 2010) in Northern most state of India. Costus oil is highly valued for its specific aroma and is used in perfumes and hair oils. The oil is also used for curing leprosy.

18.8 Chemical Constituents

Phytochemical analysis revealed that the chemical constituents that belong to diverse classes such as glycosides, steroids, terpenoids, tannins, flavonoids, and saponins (Singh et al. 2017; Madhuri et al. 2012; Hassan and Masoodi 2020; Singh 2015) were present in *S. lappa* roots.

The rhizome consisted of an alkaloid, dual lipid resins, valeric acid salt, a solid resin, an astringent principal, and ash having manganese. Approximate proportion of other compounds in the costus oil were aplotaxene (20.0%), dihydrocostus lactone (15.0%), costic acid (14.0%), costus lactone (10.0%), costol (7.0%), costen (6.0%), phellandrene (0.4%), terpene alcohol (0.2%), and camphene (0.04%). Mobile primes of the radicle were (i) an essential oil of a powerful aromatic perforating and aroma odor 1.5%, (ii) a glucoside, and (iii) an alkaloid saussurine 0.05% (Khan et al. 2013). Kustha radicle is composed of resinoids (6%), essential oil (1.5%), alkaloid (0.05%), inulin (18%), saussurea lactone (20–25%), a fixed oil and minor compounds such as sugars and tannins (Prasad and Subhaktha 2002; Khan et al. 2013).

18.8.1 Essential Oil

The percent yield of costus essential oil obtained ranged from 0.8 to 6 using various extraction methods. Costus oil is a viscous liquid with a pale yellowish to brownish color. It has a typical soft but persistent odor. Liu et al. (2012) documented that EO extracted from roots of costus were enriched in sesquiterpenoids. They also reported the presence of steroids, lignans, triterpenes, sesquiterpenoids, monoterpenes, flavonoids, and glycosides in the chemical investigation of *S. lappa* roots. The higher percentage of sesquiterpenes in *S. lappa* root essential oil was also confirmed by Zahara et al. (2014) who reported dehydrocostus lactone (1,46.75%), costunolide (2, 9.26%), 8-cedren-13-ol (3, 5.06%), and α-curcumene (4, 4.33%) as major constituents among the total 39 compounds detected. On the contrary, another study found that the essential oil was mainly composed of monoterpenes such as anethole (5), phellandrene (6), camphene (7), thymol (8), α-pinene(9), β-pinene(10), camphor (11), limonene (12), cryptone(13), ocimene(14), citronellal (15), and menthone(16) (Chang and Kim 2008; Gwari et al. 2013). Figure 18.1 presents the structures of key volatile constituents present in *S. lappa.*

18.8.2 Sesquiterpenoids

Sesquiterpenes are major chemical components found in *S. lappa*. Based upon carbocyclic skeleton, sesquiterpenoids isolated from roots of *S. lappa* can be classified into three groups namely, Guaiane (50%), eudesmane (40%), and germacrane (10%) (Govindan and Bhattacharaya 1977).

18.8.2.1 Guanines Type Sesquiterpenes

Guanine type contributed a major part to sesquiterpenoids. The major lactone isolated was Dehydrocostuslactone (Govindan and Bhattacharaya 1977) (1). The other lactones reported include Isodehydrocostus lactone (17) (Kalsi et al. 1983); 11β, 13-epoxyisozaluzanin C (Chhabra et al. 1998; Kalsi et al. 1983) (18); 11, 13-epoxydehydrocostus lactone (19); isozaluzanin C (20); zaluzanin C (21); 11β,13-dihydroepizaluzanin C (Chhabra et al. 1997) (22), 11, 13-epoxy-3-ketodehydrocostus lactone (23) (Chhabra et al. 1998), and cynaropicrin (24) (Cho et al. 1998).

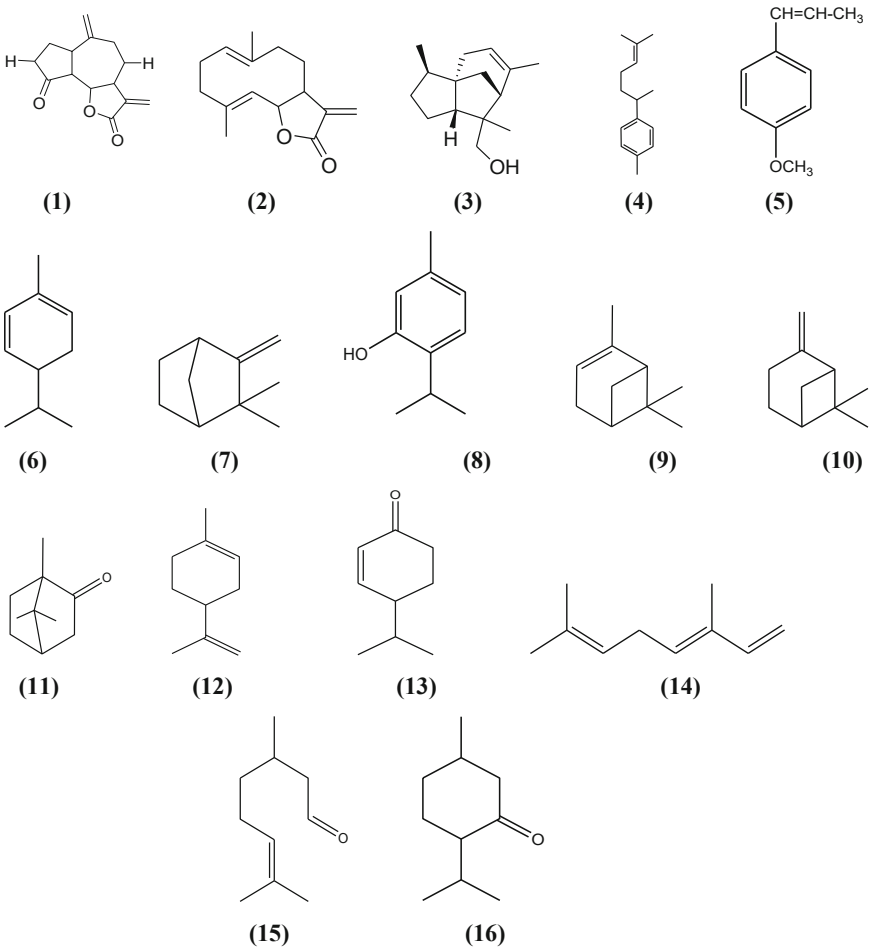
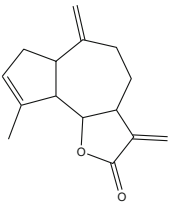
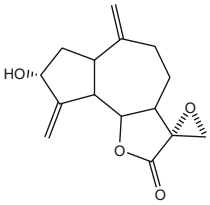


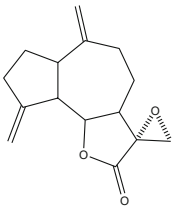
Fig. 18.1 Structures of key volatile constituents present in *S. lappa*



(17)



(18)



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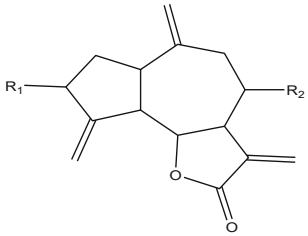
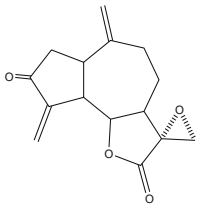


20 R_1 = ···OH R_2 =
21 R_1 = ◀OH R_2 = –H
22 R_1 = ◀OH R_2 =



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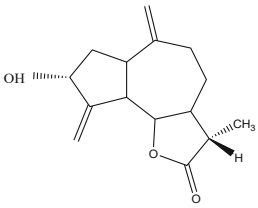
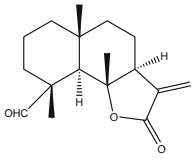


(24)

18.8.2.2 Eudesmanes Type Sesquiterpene

Talwar et al. (1992) isolated a eudesmanes type sesquiterpenoid possessing plant growth regulatory activity from the costus roots and named as saussureal (25). It was a new type of modified eudesmane skeleton.



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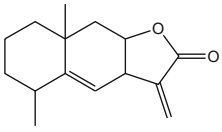

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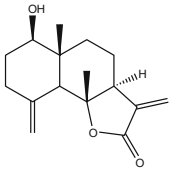
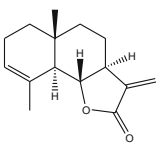


(27)

Alantolactone (26), α-cyclocostunolide (27), β-cyclocostunolide (28), isoalantolactone (29) (Govindan and Bhattacharaya 1977), reynosin (30), 11β, 13-dihydroreyosin (31), magnolialide (32), 4α-hydroxy-4β-methyldihydrocostol

(33), 4β-hydroxyendesin-11 (13)-en-12-al (34), α-costol(35), santamarine (36), isocostic acid (37) (Cho et al. 1998), and 13-sulfodihydrosantamarine (38) (Yin et al. 2005) were reported as eudesmanes type sesquiterpenoids.



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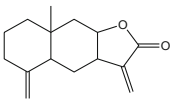

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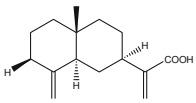

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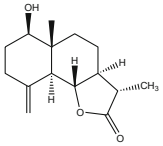

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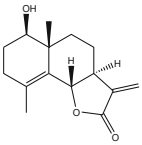

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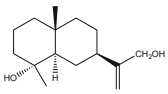

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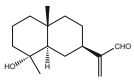

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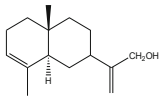

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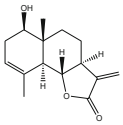

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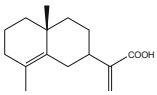

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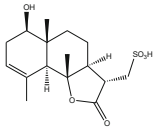
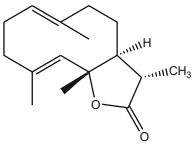


(38)

18.8.2.3 Germacranes Type Sesquiterpene

Kang et al. (1999) reported sesquiterpene lactones having germacranes type skeleton, i.e., costunolide, dihydrocostunolide (39), costunolide 15-o-β-d-glucopyranoside (40), 12-methoxy dihydrocostunolide (41), etc.



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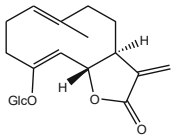

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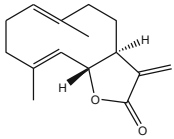
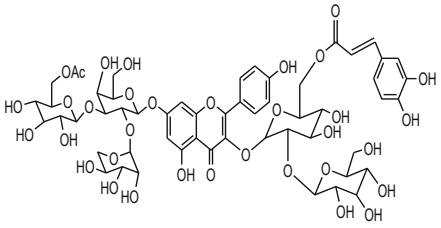


(41)

18.8.3 Flavonoids

Flavonoids having one gylocoside such as rutin, apigenin-7-O-β-D-glucoside, and luteolin-7-O-β-D-glucoside were mainly isolated from the *S. lappa* roots (Alaagib

and Ayoub 2015). Acylated flavonoid glucosides, *viz.,* Kaempferol 3-O-β-D-glucopyranosyl-(1 → 2)-β-D-(6 α'-O-caffeoyl) galactopyranoside 7-O-(β-D-6″'-O-acetyl-β-D-glucopyranosyl-(1 → 3)-[β-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside(42), 3′ [(3R)-3-acetoxy-5, 5-dimethylcyclopent-1-en-1-yl]-4'-O-methylscutellarein 7-O-(β-O-6″'-O-acetylglucopyranosyl-(1 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside(43), kaempferol 3-O-α-L-(2α', 3α'-(E)-di-p-coumaroyl) rhamnoside 7-O-(6″'-O- acetyl-β-D glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (44), and kaempferol 3- O-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside 7- O- (6″'-O- acetyl-β-D-glucopyranosyl- (1 → 3)- [α- L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (45) were isolated from *S. lappa* roots Rao et al. (2007).



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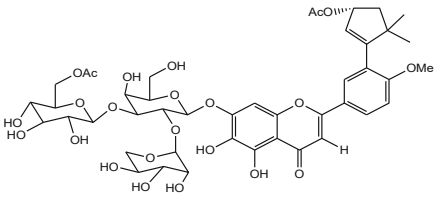

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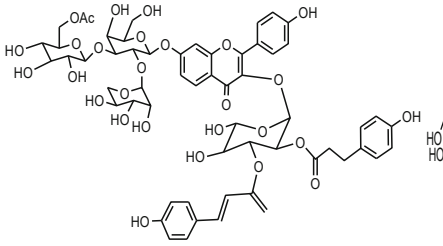

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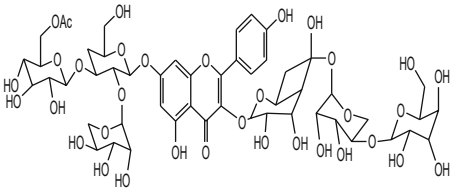


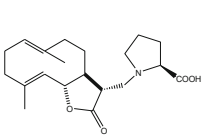
(45)

18.8.4 Other Constituents

Five amino acid-sesquiterpene conjugates, Saussureamine A (46), Saussureamine B (47), Saussureamine C (48), Saussureamine D (49), and Saussureamine E (50) were separated together with a lignin glycoside, (−)-massoniresinol 4ϩ-O-β-D-glucopyranoside from costus roots. (Matsuda et al. 2000).

A new sesquiterpenoid, namely 10α-methoxy-artemisinic acid (51) was separated from the costus roots (Cao et al. 2016). The separation was carried out with 85% purity by the high-speed counter-current chromatography method. Hanh et al. (2021)

identified two new sesquiterpenoids, namely, saussucostusosides A (52) and saussucostusosides B (53).



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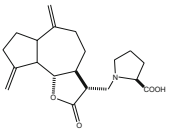

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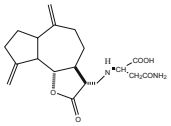

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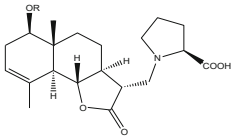

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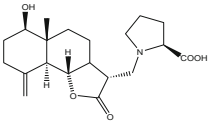

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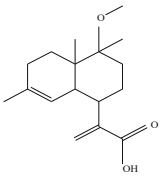

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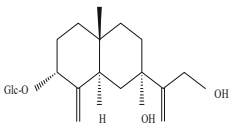

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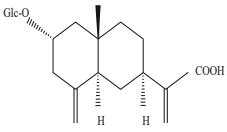
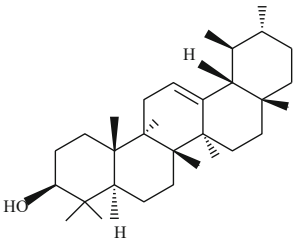


(53)

Three anthraquinone derivatives separated from the costus roots were rhein-8-O-β-D-glucopyranoside, chrysophanol, and aloe emodin-8-O-β-d-glucopyranoside (Hua et al. 2014). These components hindered the action of protein tyrosine phosphatase (PTP-1B). α-amyrin (54) (Yang et al. 1997a, 1997b), 3-β-Acetoxy-9(11)-baccharene (Li et al. 2006), and α-amyrineicosanoate (Robinson et al. 2010) were triterpenes isolated from *S. lappa* roots.



(54)

18.9 Pharmacology

The therapeutic properties of *S. lappa* is a well-documented. The major part explored for the medicinal properties of this plant is root which constitutes mainly two sesquiterpenes lactones in major quantity, viz., dehydrocostus lactone and costunolide. The number of immune-enhancing and pharmacological properties of

this plant and its constituents were reported in the past years. The details are discussed in the next subsections:

18.9.1 Antioxidant

Antioxidants are compounds that are useful to eradicate the influence of free radicals which are produced in the human body during various metabolic processes. These free radicals cause damaging effects on the DNA, protein functioning and thereby causing oxidative stress in the body. Nowadays increasing pollution and exposure to certain chemicals are also responsible for the generation of free radicals which led to several health issues and add to the decrease in immunity of a normal human beings. Therefore, the search for antioxidants in natural sources is gaining interest these days. *S. lappa* due to its wide range of medicinal properties has been explored for its antioxidant potential. Pandey et al. (2005) reported the antioxidant potential of ethanolic-aqueous extract (50%) of *S. lappa* roots via five different assays (DDPH radical scavenging, lipid peroxidation, reduced glutathione, nitric oxide, superoxide) and found that 1000 ppm extract was able to inhibit 85.2% of DDPH radicals and 72.2% of lipid peroxides. Overall, the activity of the extract was correlated to chlorogenic acid content in the extract. The antioxidant potent of chloroform extract was better (IC_{50} value DPPH activity = 78.3 mg/mL and reducing power = 11.5 mg/mL) as compared to methanol extracts (IC_{50} value DPPH activity = 120.5 mg/mL and reducing power = 12.4 mg/mL) when carried out using DPPH assay and reducing power method (Thara and Zuhra 2012). Further in similar study Chang et al. (2012) evaluated the antioxidative potential of ethanol root extract and its sequential fractions (hexane, chloroform, and n-butanol) and reported that the maximum activity was shown by butanol fraction at 1000 ppm concentration (DPPH activity = 92.98% and reducing power = 0.38). Both reports showed a linear relationship between antioxidant activity and the concentration of the extracts. The antioxidant activity was found to depend on the total phenolic content in the extracts (Iskandar et al. 2018). Alnahdi et al. (2019) found that the antioxidant potential of aqueous extracts of *S. lappa* roots was dependent on the phenolic content of the extract in a linear manner. Singh and Chahal (2018) compared the antioxidant potential of different root extracts prepared in three solvents-hexane, dichloromethane, and methanol via the soxhlet extraction method. The methanol extract was best in its antioxidant potential. Benedetto et al. (2019) studied the influence of plant harvesting period on chemical composition of *S. lappa* root essential oil as well as its antioxidant capacity. The summer collected roots were showing higher potential as compared to winters. Ethyl acetate extract of costus roots was evaluated for their antioxidant property via ABTS, lipid peroxidation, and nitric oxide scavenging assays and showed EC50 values of 51.23, 47.35, 42.67, and 38.97, respectively (Premalatha and Lakshmi 2020). The costus roots were investigated mostly for their antioxidant potential in various studies reported till date but recently Lee and Kang (2020) investigated the ethyl acetate fraction of *S. lappa* fruit

extract (ethanolic extract) which showed more than 80% DPPH radical scavenging activity at 100 ppm concentration. Although the plant is showing high potential to be used as an antioxidant, the seeds of this plant possessed very low antioxidant potential (Ijaz et al. 2021). Other plant parts like flowers and leaves are still unexplored for their antioxidant potential.

Several in vitro studies were reported on the antioxidant potential of *S. lappa* root extracts, essential oil, as well as fruit extracts, and are discussed above. The oxidative stress is also caused by various pollutants as well as microorganisms therefore recent reports on the antioxidant potential of *S. lappa* extracts against these oxidative stresses were also published.

18.9.2 Anticancer/Antitumor

The root extracts of *S. lappa* have been very well-documented for their anticancer potential against several cancerous cell lines as well as different types of cancers (breast, prostate, oral, and gastric cancer). Apart from the extracts only the major compounds isolated from this plant were also explored for their anticancer/antitumor properties. Costus roots hexane extract and its bioactive compound (dehydrocostus lactone) were investigated against DU145 and TRAMP-C2 prostate cancer cells. The hexane root extract inhibited the movement of both these cancer cells by inhibiting the regulation of TIMP-1 (tissue inhibitor of metalloprotein) and MMP-9 (metalloproteinase) as well as increased secretion of TIMP-2. This potential was thought to be due to the presence of dehydrocostus lactone which influences the results in a concentration-dependent manner (Kim et al. 2012). The methanolic extract of dried roots of *S. lappa* exhibited anticancer potential against KB human oral cancer cells with IC_{50} value of 30 ppm. It was observed that *S. lappa* methanolic root extract caused inhibition of cell proliferation via the apoptosis pathway in oral cancer cells (Moon et al. 2013). On a similar line, Ko et al. (2004) documented the anticancer properties of roots ethanolic extract against AGS gastric cancer cell lines and the mode of action was proposed to be similar as reported earlier, i.e., by up- and downregulation of apoptotic and tumor suppressor genes. Apart from extract, the bioactive compounds isolated from the roots of this plant have shown huge anticancer potential. The major bioactive compounds present in the roots are costunolide and dehydrocostus lactone. The hexane extract along with its isolated sesquiterpenes lactones were assessed for their anticancer potential against different cell lines of cancer (colon, lung, skin, and breast cancer). Further, derivatization of these compounds was also commenced which showed that α-methylene-γ-lactone moiety is crucial for their anticancer potential (Robinson et al. 2008). Hexane extract of *S. lappa* is the major source of bioactive lactones which are responsible for its anticancer properties. Lin et al. (2016) stated the therapeutic properties of hexane root extract of *S. lappa* against hepatocellular carcinoma cells. The main mechanism of action was cell apoptosis and arresting of cell proliferation. The ethanolic extracts of *S. lappa* roots are effective against prostate cancer cell lines and the mechanism

remains the same as cell apoptosis (Tian et al. 2017). Rasul et al. (2012) have covered the anticancer properties of costunolide, one of the major constituents of *S. lappa* roots reported till 2012. The main mechanism of action of these molecules lies in it to initiate apoptosis in various cancer cell lines along with that it also inhibited cell proliferation, angiogenesis, and metastasis. Further Choi et al. (2013) reported the potency of costunolide to suppress metastatic breast cancer growth via inhibition of TNF-α-induced NFκB activation. Dehydrocostus lactone, another major bioactive compound from the root of *S. lappa*, has shown anticancer properties against human breast and ovarian cancer cell lines via apoptosis (Choi and Kim 2010). Dehydrocostus lactone and costunolide, major components isolated, induced apoptosis of neuroblastoma cells. These two molecules are also able to suppress migration as well as invasion of neuroblastoma cells (Tabata et al. 2015). Further Lin et al. (2015) have compiled the information about anticancer properties of these two major bioactive compounds from *S. lappa* roots and discussed the anticancer mechanism of both these compounds. Both these compounds acted by blocking different stages of carcinogenesis. Still, clinical trials are required to confirm the proteins associated with the anticancer properties of these two molecules. Owing to the potential of costunolide and dehydrocostus lactone as potential candidates for cancer treatment. Peng et al. (2017) investigated the combined effect of two bioactive molecules against breast cancer cell lines. These molecules were able to arrest cancer cell growth via Myc/p53 and AKT/14–3-3 signaling pathways. Apart from the roots, leaf extracts of *S. lappa* also possessed anticancer effects against different cancer cells. Shati et al. (2020) investigated the leaf extracts prepared in six solvents for their anticancer potential against breast, liver, and colon cancer cells. Out of this hexane, chloroform, methanol, and ethylacetate extracts showed remarkable activity against these cancer cell lines. They act by apoptosis in these cancer cell lines. The fruits of costus were also able to regulate cell apoptosis in various human cancer cell lines. The aqueous extract of fruits of this plant was able to control lung, dermal, colon, kidney, and breast cancer with IC_{50} values in the range between 2.5 and 0.85 mg/mL. Out of all these, lung cancer cells were most susceptible to aqueous fruit extract of *S. lappa* (Patel et al. 2020). The anticancer potential of aqueous, ethanolic, and hydroalcoholic root extracts of costus showed a potential against HepG2 cancer cells (Ansari et al. 2021).

The most predictable tumor in adults is Glioblastoma multiforme. The glioblastoma (U118, U251, or U87) cells were treated with dehydrocostus lactone (concentrations 0–100 μM and incubation time 12, 24, 36, or 48 h) showed remarkable inhibition in the viability, proliferation, and migration. Dehydrocostus lactone was found to induce mitochondria-mediated apoptosis by promoting the release of cytochrome c into cytosol, which activated the caspase signaling pathway. These conclusions gave the pharmacological information for the progress of dehydrocostus lactone as a potential agent against glioma (Wang et al. 2017a, b).

18.9.3 Immunomodulators

The hydroalcoholic extract of *S. lappa* showed no immunomodulatory activity at lower doses (<100 mg/kg) but at higher doses (> 200 mg/kg) it exhibited immunomodulatory power in the immune system of humoral and biological arms of the immune system (Pandey 2012; Choudhary 2015). The combined formulation prepared from *S. lappa* along with the other two plants was able to enhance immune response. The extract was able to excite the immune cytokines (interferon -α,-β,-IL-1β,-6,-IL-10-IFN) expression, COX-2 macrophages (Trinh et al. 2020). Kretschmer et al. (2012) investigated the effects of isolates from costus on three soft tissues of sarcoma cell lines of different origins via activity-guided isolation. The effects on cell cycle distribution, cell proliferation, ATP binding cassette transporter protein, and apoptosis induction were examined. Dehydrocostus lactone inhibited cell viability dose and time dependently whereas there was no effect on cell cycle, little in caspase3/7 activity, and low levels of cleaved caspase-3 after 24 and 48 h. IC_{50} values for dehydrocostus lactone ranged from 26.8 to 36.1 μM and for costunolide it was from 41.3 μM to 42.0 μM. Dehydrocostus lactone significantly reduced cells in the G1 phase and increased cells in the S and G2/M phase and it also improved caspase 3/7 activity, cleaved caspase-3, and cleaved PARP indicating apoptosis induction. In addition to this, the impact of dehydrocostus lactone on the expression of ATP-binding cassette transporters related to multidrug resistance (ABCB1/MDR1, ABCC1/MRP1, and ABCG2/BCRP1) was analyzed using real-time RT-PCR. The expressions of ABCB1/MDR1 and ABCG2/BCRP1 in liposarcoma and synovial sarcoma cells were significantly downregulated by dehydrocostus lactone. Therefore, dehydrocostus lactone may be considered as therapeutic agent against drug-resistant tumors. Dehydrocostus lactone was examined to evaluate its inhibitory behavior towards the killing function of cytotoxic T lymphocytes (CTL) with structural variations and the induction of intercellular adhesion molecule-1 (ICAM1). Research revealed that inhibitory potential of dehydrocosus lactone towards CTL killing function and the stimulation of ICAM-1 was mainly attributed to α-methylene γ-lactone moiety (Yuuya et al. 1999).

18.9.4 Hepatoprotective Activity

The anti-hepatotoxic activity of water-methanol costus roots extract was examined against lipopolysaccharide (LPS)-induced hepatitis and D-galactosamine (D-GalN) in mice. Pretreatment of mice with various dosages of *S. lappa* significantly inhibited the D-GalN and LPS-induced rise in plasma transaminase levels in a dose-dependent manner. The posttreatment was carried out with 600 ppm dose which considerably constrained the movement of hepatic damage induced by D-GalN and LPS. These findings suggested that *S. lappa* showed a hepatoprotective effect in mice (Yaeesh et al. 2010). Both water and methanolic extracts of *S. lappa* showed hepaprotective

activity against D-galactosamine (D-GalN) and lipopolysaccharide (LPS)-induced hepatitis in mice (Khan et al. 2013; Choudhary 2015; Singh et al. 2017). Hepatitis B surface antigen (HBsAg) in human hepatoma Hep 3B cells had toughly suppressed by two bioactive constituents costunolide and dehydrocostus lactone but the viability of the cells was slightly affected (Pandey et al. 2007; Gautam and Asrani 2018).

18.9.5 Anti-Convulsant Activity

Ambavade et al. (2009) observed the anti-convulsant potential of different *S. lappa* root extracts in mice. The water, alcoholic, and petroleum ether extracts were tested for picrotoxin and pentylenetetrazole-induced convulsions, and maximum electroshock test in mice. Alcoholic extract (300 ppm i.p.) and petroleum ether extract (100 ppm i.p.) decreased the mortality in picrotoxin and pentylenetetrazole-treated mice and increased latency to clonic convulsions. The water extract (300 mg/kg i.p.) significantly decreased mortality and convulsion episodes. In the electroshock test, water extract (100 and 1000 ppm i.p.), alcoholic extract (100 and 1000 ppm i.p.), and petroleum ether extract (100 and 300 ppm i.p.) decreased mice's mortality. Thus, results revealed that petroleum ether extract had the potential as an anti-convulsant agent against picrotoxin and pentylenetetrazole-induced convulsions in mice, by elevation of the seizure threshold via GABAergic mechanism.

18.9.6 Cardioprotective Effect

Costus root water extract was examined for cardioprotective action against isoproterenol-induced myocardial injury in rats. Pretreatment of rats was done with oral administration at different concentrations ranging from 100 to 300 ppm, p.o. Extract remarkably restored the level of creatinine kinase, lactate dehydrogenase, aspartate transaminase, decreased myocardial glutathione level, and increased myocardial thiobarbituric acid reactive substances level. The results obtained in the study were comparable with the α-tocopherol standard used. The dose of 200 ppm exhibited the most effective response in rats against isoproterenol-induced myocardial injury (Saleem et al. 2013).

18.9.7 Anti-Psychotic

The different solvent (water, benzene, chloroform, and methanol) extracts of *S. lappa* was assayed to study their effect on the mice's central nervous system (CNS) via intraperitoneal administration, i.e., antinociceptive effects, body temperature alterations, potentiation of hexobarbital sleeping time, and impulsive

locomotor activity changes. Benzene solvent extract (100 mg/kg, i.p.) was found to be more active which was then separated by column chromatography into five different fractions. Isolated costunolide and dehydrocostus lactone were considered as the active CNS constituent. Both compounds were activated by the intragastric, intra cerebroventricular routes of administration, and intraperitoneal and reduced motility prompted by both methamphetamine and apomorphine. After their administration, the amount of monoamines and other metabolites remained the same whereas there increase in the amount of homovanillic acid in the brain. Similar observations were recorded in chlorpromazine-treated mice. This outcome showed that both constituents can be used to cure psychiatric disorders (Okugawa et al. 1996).

18.9.8 Anti-Inflammatory

The anti-inflammatory potential of costus root extracts and its key sesquiterpenes lactones have been reported by many scientists since 1990s. Pandey et al. (2007) have compiled the anti-inflammatory potential of *S. lappa* extracts as well as its bioactive compounds reported in the literature till 2007. Sunkara et al. (2010) studied in vitro anti-inflammatory activity against TNF-α levels and nitric oxide (NO) levels in mouse macrophage cells. The results showed that the test compound significantly affected TNF-αl levels as 33.76% inhibition was recorded with the test component. The extract roots were examined for its potential for adjuvant-induced monoarthritis in rats. The ethanolic extract of *S. lappa* was found effective as anti-inflammatory agent on adjuvant-induced monoarthritis in a concentration-dependent manner and has the ability to decrease infiltration of inflammatory cells and synovial hyperplasia thereby protecting the joint deterioration in rats (Tag et al. 2016). The *S. lappa* ethanolic extract in combination with *Dryobalanops aromatic* was evaluated for its anti-inflammatory property via the determination of the production of NO by macrophages in response to antigens. The AT000 extract showed NO production with IC_{50} value of less than 20 μM (Iskandar et al. 2018). The anti-inflammatory effect of ethanolic extract was evaluated on Triamcinolone acetonide (TA)-induced oxidative stress in spleen and lungs of rats. Various combinations of *S. lappa* extract alone or with TA were fed to rat. The *S. lappa* extract was able to cancel out the oxidative and apoptotic effect induced by TA. The maximum-effect was observed for the prophylactic group (El-Rahman et al. 2020). Further carrageenan-induced paw edema in Wilson albino rats was also controlled by *S. lappa* root ethanolic extract @ 13.73% at 200 mg/kg concentration although it was not as effective as ibuprofen which showed a 22.79% decrease in inflammation (El-Marghani et al. 2020). The fruit pulp extract of *S. lappa* @ 100 mg per mL was also capable of inhibiting cytokines pro-inflammatory response in BV-2 microglia induced by lipopolysaccharides. This fact was due to the presence of some compounds in *S. lappa* extract which regulates the inflammatory signaling pathways (Lee and Kang 2020).

18.9.9 Antimicrobial Activity

Costus oil, its fractions, and bioactive compounds (costunolide and dehydrocostuslactone) responded significantly against Mycobacterium tuberculosis H37Rv. The mixture of lactones showed a better response as compared to pure compounds. This confirmed that lactones worked in a synergetic manner (Luna-Herrera et al. 2007).

Hydro-distilled essential oil of *S. lappa* showed concentration-dependent antibacterial potential against both Gram (+ve) and Gram (−ve) bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli,* and *Candida albican*s. However, no cytotoxic influence was observed when different concentrations of Costus oil were tested against *Artemia salina* (brine shrimp) (Abdelwahab et al. 2019).

Chang et al. (2011) explored the antibacterial property of ethanol and *n*-hexane of *S. lappa* root extracts against food-borne bacteria and observed that extracts showed strong inhibition against *Vibrio parahaemolyticus* and *B. cereus* in comparison to standard ampicillin. In a similar study, ethanol extracts of *S. lappa* displayed inhibition to the growth of bacteria resistant to standard methicillin, viz., *P. aeruginosa, Klebsiella pneumonia, Extended Spectrum β-Lactemase, E. coli Acinetobacter baumannii,* and *S. aureus* with MIC value ranging between 2 and 12 μg/μL (Hasson et al. 2013).

Negi et al. (2014) studied the antibacterial property of methanol extracts of *S. lappa* against *Citrobacter freundii*, *Enterococcus faecalis, E. coli,* and *S. aureus* with MIC values ranged from 3 to 50 μg/μL. The authors correlated the antibacterial activity with the amount of sesquiterpene lactones in the extract. Chloroform extract of cotsus roots showed more promising results as compared to petroleum ether, methanol, and aqueous extracts against both categories of bacteria (Alaagib and Ayoub 2015).

Similarly, different solvent (water, CH_3OH, and $CHCl_3$) extracts of *S. lappa* root were found to be effective against *S. aureus*, *B. cereus,* and *C. albicans* but ineffective against *E. coli* and *P. aureus*. Among all extracts, methanol extract displayed maximum inhibition against *S. aureus* and *B. subtilis* (Mohamed et al. 2017). Recently, Omer et al. (2019) observed that ethanol extract of costus roots possessed significant antimicrobial action in comparison to aqueous extract. Extracts were more effective against Gram-positive bacteria (*S. aureus*) whereas ineffective against Gram-negative bacteria (*Salmonella* sp.).

18.9.10 Spasmolytic Activity

Gilani et al. (2007) reported that aqueous-methanolic crude extracts of the costus roots contained active constituents responsible for relieving constipation and spasms in guinea pig ileum. Increase in the concentration resulted in the extracts causing the

blockage of Calcium channels and spasmolytic activity increased with an increase in concentration. The results indicated that gut-simulating cholinergic-type components were mainly responsible for constipation. *S. lappa* extracts were found to have antiperoxidative effects and significantly relaxed the contraction caused by carbachol (30 μmol/ L) in guniea pig aorta. This effect is primarily attributed to the sesquiterpene lactones. Sesquiterpenes are recognized to stimulate the sGC which stimulates the extrusion of K+ ions and thereby reduces intrinsic Ca++ ions through activation of cGMP and PKG pathway, leading to relaxation of smooth muscles (Hung et al. 2010). Alcoholic extracts of *S.lappa* has the ability to relax the precontraction in tracheas caused by carbachol and can be used for the treatment of asthmatic patients (Nagar et al. 2013).

18.9.11 Antiparasitic Activity

Oral administration of S. lappa is found to display a slight antiparasitic effect against *Clonorchis sinensis*, a nematode causing infection in rabbits (Rhee et al. 1985). The methanolic extracts at a concentration of 50 ppm significantly reduced the percentage of fecal eggs of nematodes per gram in children having worm infections. The antiparasitic effect of the extract was comparable to standard pyrantel pamoate (Akhtar and Riffat 1991).

18.9.12 Hypolipidemic Activity

Aqueous extract of costus was reported to show an antihyperlipidemic effect in rabbits when administered orally. Cholesterol and triglyceride levels were also reduced significantly in blood serum (Upadhyay et al. 1996). The rats treated with costus root ethanolic extract showed a significant reduction in triglyceride levels and weight gain (Anbu et al. 2011).

18.9.13 Angiogenic Activity

Costunolide, a major component of *S. lappa* might act as an angiogenesis inhibitor as it was found to suppress the proliferation of endothelial cell and vascular endothelial growth factor-induced chemotaxis. It might block the angiogenic factor signaling pathway to exhibit angiogenic activity (Jeong et al. 2002).

18.9.14 Antidiarrheal Activity

Hemamalini et al. (2011) stated that costus roots methanol extract showed significant inhibition to diarrhea at a concentration of 500 ppm body weight and the result was in comparison to the loperamide, the standard drug used to reduce diarrheal stools in Wistar rats. Negi et al. (2013) also observed the effectiveness of methanol extract on diarrhea caused by castor oil.

18.10 Clinical Studies

Trivedi and Dixit (2015) studied and compared the effect of ethanolic root extract of *S. lappa* (SLR) on periodontitis (chronic inflammation of tooth-supporting tissues) with chlorhexidine gluconate (CHG) and scaling and root planning (SRP). The patients with periodontitis were divided into three groups. Group 1 was treated with SRP; group 2 with SRP + SLR; group 3 with CHG + SRP. Overall SLR proved to be a potential adjunct to conventional treatment options for periodontal infections.

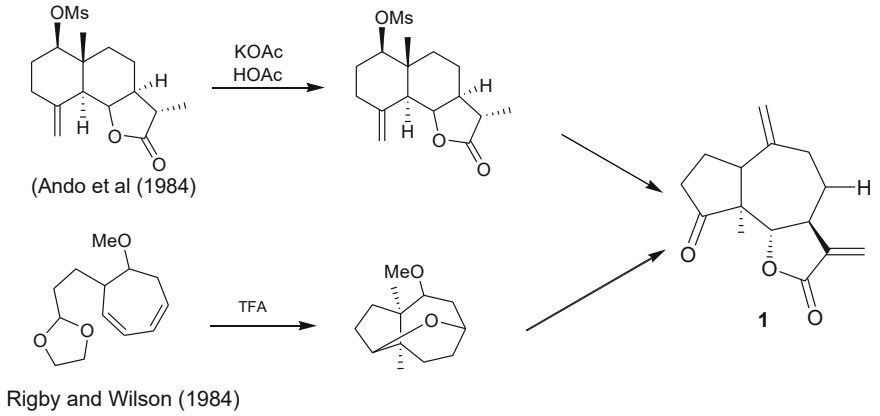
18.11 Toxicology

According to Food and Drug Administration, the consumption of *S. lappa* as a dietary supplement is considered safe. However, a few side effects, viz., nausea, dizziness, and certain kind of allergy can happen. Its use is not recommended for pregnant women and for those who are following any medication. *S. lappa* is usually substituted with *Aristolochia*, which contained aristolochic acid that is cancerous in nature and results in kidney damage.

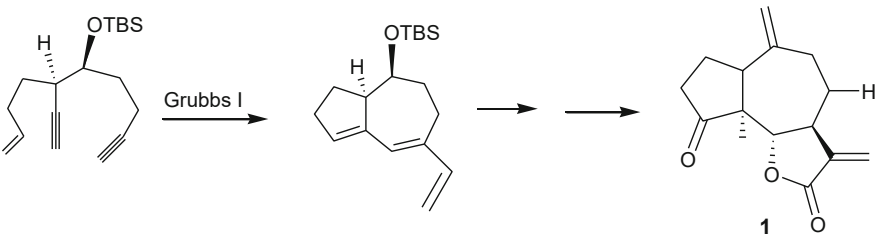
18.12 Synthetic Strategies for Key Secondary Metabolities

Dehydrocostus is the major sesquiterpenoid isolated from *S. lappa*. Rigby and Wilson (1984) reported the first synthesis of dehydrocostus lactone. Afterward, the first ex-chiral synthesis was carried out using the solvolytic approach by Ando et al. (1984). However, the overall yield of the dehydrocostus lactone was less than 3% (Scheme 18.1).

Recently, Kaden and Metz (2021) reported the efficient asymmetrical generation of (−)-dehydrocostus lactone by domino enediyne metathesis with an increase in the overall yield of 12%.



Scheme 18.1 Synthesis of Dehydrocostuslactone



Kaden and his coworkers further extended their work for the improved asymmetrical synthesis of (−)-dehydrocostus lactone via using a domino dienyne metathesis strategy (Kaden et al. 2021).

18.13 Cultivation and Harvesting

S. lappa is a plant that grows in hilly regions. In India, it is growing as a wild plant along the borders of Pakistan and India in the state of Jammu and Kashmir (Kuniyal et al. 2015). Due to its high demand in the pharmaceuticals and Ayurvedic medicines quick techniques are being developed such as propagation (Johnson et al. 1997). In India, the plant is cultivated in Himachal Pradesh and Uttarakhand to serve the dual purpose of germplasm conservation and medicinal use. The cultivation of this herb began in Himachal Pradesh during the year 1920 and in Uttarakhand in 1929 (Kuniyal et al. 2005, 2015), and by the year 1950 the area under the cultivation of Costus plant was about 600 hectares. This was the major source of supply of *S. lappa* to both inland trades in India as well as export to China (Kuniyal et al. 2015). Due to high demand, the annual production was around 300–400 MT (metric tons) in Himachal Pradesh. Nowadays it is considered as critically endangered species due

to its excessive demand and haphazard collection thereby decreasing its natural occurrence (Zahara et al. 2014). Due to the Indo-China conflict, export to China was affected during 1962 and area under the costus cultivation was reduced. *S. lappa* is best grown in sandy-loam soil with high organic carbon and moisture content. The best climatic conditions are temperate and alpine regions at an altitude of 1200–1800 m. All these conditions are best suitable for the better emergence of *S. lappa* seedlings, their survival, and productivity. The sowing of *S. lappa* seeds starts during April and May in the nursery. Once the seedling reached a height of about 15 cm these are transplanted in the field. Irrigation is required when the seeds start sprouting. On the whole, the crop requires 5–6 irrigations and that too between the months of May and September. As this is a medicinal plant no chemical fertilizer and pesticide are recommended to be sprayed on this plant however organic manure like FYM (farm yard manure), green manure, or vermicompost are added as per the requirement of the crop. The bio-pesticide prepared from the neem (seed, leave pr kernel), Dhatura, Chitrakmool or cow urine, etc., can be used from time to time if required. The roots of this plant are of main medicinal value. These plants are grown for the procurement of roots. These are harvested during the months of September or October or early spring. Generally, it took 2–3 years for the roots to get mature enough for their medicinal use.

Still, it is advised to wait for at least 3 years to get a proper yield of this crop. After harvesting, the roots are washed with water to get rid of dirt and soil particles adhered to them, if any. After cleaning these are dried and stored for further use. Approximately 200–300 kg of tubers (roots) are harvested per hectare after 2–3 years of plantation. The market price of these tubers in India is around Rs. 40–50/kg (Vashisht 2021).

18.14 Patents

Several patents mentioning the use of *S. lappa* have been filed under different agencies as given below.

Sr. No.	Title	Inventors	Issuing agency	National/ International	Patent Number	Year of publication
1.	Composition comprising herbal extracts or fermented products thereof having lactic acid bacteria for preventing or treating respiratory diseases	MA JinYeul, KIM dong Seon, and Yang min Cheol	European patent specification	International	EP 2716295 B1	Feb 2, 2018

(continued)

Sr. No.	Title	Inventors	Issuing agency	National/ International	Patent Number	Year of publication
2.	High protein supplement	George Scheele	United States patent	International	US 8,779,189 B2	Jul 15, 2014
3.	Treatment of virus using chelator and antiviral agent	Bruce Halstead	United States patent	International	US 2002/ 0182227 A1	Dec 5, 2002
4.	Therapeutic composition for treating gangrene	Sidgi Syed Anwer Abdo Hasson and Ali A. H. Al—Jabri	United States patent	International	US 9, 999, 643 B2	Jun 19, 2018

18.15 Formulations

Nanoparticles prepared from *S. lappa* CB. Clarke. (SL) roots essential oil displayed an anti-inflammatory (reduction in metalloprotease MMP-9 enzyme activity and RNA expression of inflammatory cytokines: TNF-α, GM-CSF, and IL1β), a high anti-Alzheimer's effect ($IC_{50} = 25.0$ and 14.9 μgmL^{-1} against acetylcholinesterase and butyrylcholinesterase, respectively), and a strong antidiabetic effect ($IC_{50} = 22.9$ and 75.8 μgmL^{-1} against α-amylase and α-glucosidase, respectively). Moreover, these nanocapsules were not cytotoxic at a concentration of 25 μgmL^{-1}. (Lammari et al. 2020).

The wound healing property of emulgel and *in situ* gel formulations of methanol extract of roots of *S. lappa* (SLRE) were tested at various concentrations. These formulations remained stable for up to 1 month when stability was checked at 40 °C for emulgels and at 25 °C for in situ gels. The wound healing property of in situ gel (100 ± 0.0028) of SLRE was higher than emulgel (F6, 99 ± 0.004), SLRE, and standard pyodine gel (91 ± 0.014, $p < 0.05$) in albino rats (Ahsan et al. 2020).

MgO nanoparticles are synthesized using two biomasses (Qustalhindi and Qustalbahri) of costus roots and tested for their antimicrobial activity. The zone of inhibition of Qustalbahri biomass nanoparticles were 15, 16, 18, 17, 14, and 10 mm against *E. coli*, *P. aeruginosa*, *C. tropicalis* and *C. glabrata*, *S. aureus,* and *Bacillus subtilis* while inhibition zone diameter of 12, 8, and 17 mm was observed in case of nanoparticles of Qustalhindi biomass, respectively. Qustalbahri also exhibited high cytotoxicity against MCF-7 cancer cell lines as well as degradation of methylene blue on ultraviolet radiation exposure as compared to Qustalhindi in the cellular studies (Amina et al. 2020).

Silver nanoparticles (AgNPs) of aqueous extract *S. costus* root were found to be effective upto 84.6% in the degradation of color of saffron dye using UV/VIS spectrophotometers for 72 h of exposure time (Abd El-Aziz et al. 2020) and thus provide a green alternative for dye degradation.

18.16 Conservation

S. lappa is reported as critically endangered species due to its overexploitation for various medicinal tasks by Appendix 1 of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Pandey et al. 2007). In spite of adoption of various conservation strategies for wild species of *S. lappa*, there is a decrease in the availability of some wild species day by day. The possible reason might be the lack of awareness among people who extensively harvested the plants for local uses. Uncontrolled grazing in the wild areas also leads to the destruction of natural habitat of species. In addition, *Arcitum lappa* L., a local weed closely resembled *S. lappa* and can be distinguished only at flowering stages that take more than 2 years. This also led to a loss in interest in its cultivation among farmers (Chauhan et al. 2018).

In a survey conducted in Chamoli District, Uttarakhand, it has been reported that during the year 2014–15, the area under Costus cultivation per farm was about 0.5 or 0.6 hectares and the roots production per farm ranged from 128 to 156 kg per year. There is a continuous decrease in market prices for dried roots/kg over time. Lack of awareness about the legal formalities related to export policies is the major problem faced by farmers. Improvement in state and national policies can improve this situation (Kuniyal et al. 2019).

To meet the requirements of International and National markets, the commercial cultivation of *S. lappa* is urgently needed. The commercial cultivation of costus has been started extensively in forests of the Himalayas, where it grows naturally.

A multidimensional approach is required for the preservation and conservation of costus species from being extinct. Various techniques like micropropagation, in vitro (Arora and Bhojwani 1989) and ex situ strategies have been adopted for the rapid propagation of *S. lappa* (Johnson et al. 1997; Arora and Bhojwani 1989; Sher and Hussain 2010). A few suggestions for the conservation of this plant species are as follows:

(i) Detailed knowledge about medicinal properties and its preservation has to be provided.
(ii) Simplification of rules and regulations related to export.
(iii) Increase in the market price of cultivated products.
(iv) Development of socio-entrepreneurial approaches.
(v) Intervention of more governmental or non-governmental entities to develop costus-based small-scale industries.
(vi) Permanent monitoring programs to check overexploitation of wild habitat.

(vii) Legal amendments in conservation acts and promotion of forest management practices that help in the conservation of biodiversity.
(viii) Proving medical facilities to local people.
(ix) Receptiveness programs at local low level should be started.

18.17 Trade

The international demand of *S. lappa* is huge due to its application in the medicinal industry. The estimated trade of *S. lappa* is approximately 100–200 MT per year. This species has been enlisted as endangered medicinal plant in Appendix I of CITES (Convention on International Trade in Endangered Species). As per Foreign Trade Development Act (1992), the trade of *S. lappa* is illegal Internationally. Import and export permit can be granted in only those cases in which it is used for research purposes (Zahara et al. 2014). According to Illicit Trade Report published in 2014 by the World Customs Organization (WCO), costus root was ranked at second number in illegally traded endangered plants (Anonymous 2014).

18.18 Conclusion

S. lappa is an extremely valued medicinal herb used primarily in countries like India, China, and Korea in the local medicinal system. Traditionally, the costus roots are recommended for the treatment of skin, stomach, and throat ailments. Experimental evidence confirmed the medicinal properties of *S. lappa* and its bioactive components. Higher National and International demand for pharmaceutical purposes led to the inclusion of *S. lappa* in the category of endangered plant species. A broad and multidimensional approach is required for the conservation of this plant. Various strategies have been adopted to preserve the species in its natural habitat. In the end, we conclude that there is a great scope for utilization of *S. lappa* plant for the extraction of the component to be used in pharmaceutical research for the new chemical of medicinal significance.

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Chapter 19
Hippophae Rhamnoides L.: Sea Buckthorn

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19.1 Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is a robust deciduous plant that is inhabitant (native) to northwest Europe to central Asia, including the Altai Mountains, the northern Himalayas, as well as northern and western China. Hippophae is a Latin word that combines the words hippo means horse, and phaos means flare or gloss. As a result, it is referred in the literature as the "shining horse." Dr. L. Skinner of Agriculture and Agri-Food Canada, Morden, Manitoba, introduced this plant from Russia to the Canadian prairies in the 1930s (Li and Beveridge 2003). It is indigenous to India, Sweden, Switzerland, Hungary, France, and extends north to Afghanistan, Pakistan, Bhutan, Nepal, China, Russia, Kazakhstan, Finland, Mongolia, the United Kingdom, Germany, and Norway (Pundir et al. 2020). It is presently grown in many places and territories of the world especially for its

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nutritional and therapeutic belongings. Because of its robust vegetative propagation system and powerful complex root structure with nitrogen-fixing nodules, this plant is drought and winter tolerant. This plant is also beneficial in terms of land reclamation and farmstead protection (Suryakumar and Gupta 2011). Sea buckthorn (SB) is also well known for its multipurpose capabilities, which include the development of culinary products such as jam, jelly, juice, smoothie, sauce, oil, and so on, as well as herbal teas with fine amount of flavonoids and detoxifying constituents. Sea buckthorn by-products can also be used to make fodder supplements. It is also used in cosmetics, medicines, and as a fuel source for firewood (Tkacz et al. 2021). It has low growing conditions requirements and acts invasively if grown in low humidity, sedimentary gravel, wet landslips, and riverside.

SB fruits, leaves, branches, and oils are very popular due to their extensive nutritional, therapeutic, and health-promoting potentials, which are well known in traditional medicine. It is regarded as a multipurpose plant for a large number of bioactive constituents, with berries being the most important and well-known component, as they have several times high vitamin C content than many popular fruits like lemons, strawberries, and blackberries. They are well known for their high oil content (Tkacz et al. 2021). For almost 1000 years, dating back to the Tang Dynasty, SB has been administered in traditional Asian medicine. In oriental traditional medicine, SB has an extensive and vast application in the treatment of asthmatic problems, skin infections, gastric ulcers, and several respiratory complications. Sea buckthorn has been shown to retain a wide range of therapeutic and therapeutic potentials, including antioxidant, anti-atherogenic, immunomodulatory, anti-stress, radioprotective, hepatoprotective, and tissue renovation properties (Suryakumar and Gupta 2011).

19.2 Taxonomy

Sea buckthorn (Fig. 19.1) is a member and belongs to the Elaeagnaceae family, which means “pasted all over” and indicates to its plentiful fruits, which are indistinctly seated on little pedicels. Sea buckthorn, also familiar as seaberry, sandthorn, sallowthorn, or Siberian pineapple, can be found throughout Europe, Canada, and Asia, ranging from dry, sandy places to hilly areas to seashores and river valleys. Originally, it was just single species named, *H. rhamnoides*, with three subspecies: *rhamnoides*, *tibetana, salicifolia* (Li and Beveridge 2003). Rousi (1971) re-categorized *Hippophae*, recognizing three species depending on morphological differences: *Hippophae rhamnoides* L., *Hippophae salicifolia* D. Don, *Hippophae tibetana Schlect.*

Sea buckthorn is a deciduous shrub, or a small plant having branched thorns. Scale-hairs or stellate hairs typically cover all organs. The flower bud shape is noticeable and stable. Petioles are 13 mm long and have simple alternate, opposite, or three leaves in whorled leaves. The chromosome number is $2^n = 24$. It thrives in locations with far more over 350 mm of annual rainfall, as well as territories with less

Fig. 19.1 Sea buckthorn fruits

than 350 mm of annual rainfall but a high level of ground water or surface runoff (Yongshan et al. 2003). Most recent classification of the genus *Hippophae* was reported by Lian and Chen (1997), Lian (1988), and Lian et al. (2000). The taxonomic outline of sea buckthorn is as follows. There are 6 species and 12 subspecies in total.

Section A. Hippophae

1. *Hippophae salicifolia* D. Don.
2. *Hippophae rhamnoides* L.
   Subspecies:
   a. *carpatica* Rousi
   b. *caucasica* Rousi
   c. *fluviatilis* van Soest
   d. *mongolica* Rousi
   e. *rhamnoides*
   f. *sinensis* Rousi
   g. *turkestanica* Rousi
   h. *yunnanensis* Rousi
3. *H. goniocarpa* (Lian) X.L. Chen and K. Sun
   Subspecies:
   a. *litangensis* Lian and X.L. Chen
   b. *goniocarpa* Lian

Section B. Gyantsensis Lian

4. *H. gyantsensis* (Rousi) Lian
5. *H. neurocarpa* S.W. Liu and T.N. He
   Subspecies:
   a. *stellatopilosa* Lian and X.L. Chen
   b. *neurocarpa* S.W. Liu and T.N. He
6. *H. tibetana* Schlecht

Bartish et al. (2002) used chloroplast DNA (cpDNA) and a collective set of data of morphological characteristics and cpDNA to validate this most recent classification. They proposed that *H. goniocarpa subsp. litangensis* be recognized as a species because *H. goniocarpa* and *H. litangensis* are evidently not monophyletic, but rather sister to two separate species in the assessment.

19.3 Common Names

The generic Latin name "Hippophae" translates literally as "shiny horse." Seaberry, Siberian pineapple, Sandthorn, or Swallowthorn are the common names of sea buckthorn (www.omafra.gov. . .).

19.4 Flowering/Fruiting Season

Flowering occurs when the plants are completely leafless, during the mid-April and the first week of May. Anthesis begins in the second to last week of April. Male plant anthesis occurs 24–48 h before female plant anthesis, ensuring that airborne pollen grains are accessible when female flowers are receptive. Pollen can travel to a distance of 15 m, so it is recommended that the distance between male and female plants be kept between 10 m and 15 m for proper and adequate pollination (Stobdan et al. 2017).

19.5 Distribution

The sea buckthorn plant is native and endemic to Asia and Europe. An illustration of the natural distribution of this plant within Asia and Europe can be seen from Fig. 19.2.

In Russia, China, and Mongolia, the total cultivable area for sea buckthorn is only around 3,00,000–5,00,000 ha and natural growths are 8,10,000 ha in those countries (Li and Schroeder 1996). Nonetheless, sea buckthorn shrubs are also widespread

Fig. 19.2 Natural distribution of Sea buckthorn in the Europe and Asiatic region (Yang and Kallio 2002)

naturally in other European countries too such as Germany, Finland, and Poland, on the banks of the rivers and coastal sandbanks or dunes along the Baltic beach (Biswas and Biswas 1980; Kluczynski 1989) and on the western coast and Gulf of Bothnia in Sweden. However, in the Asia, it is extensively spread all over the Himalayan region including India, Nepal, and Bhutan along with northerly portions of Pakistan and Afghanistan (Lu 1992). Overall, SB is indigenous to India, Switzerland, Hungary, Sweden, France, and extends north to Finland, Russia, Nepal, China, Mongolia, Afghanistan, Pakistan, Bhutan, Kazakhstan, the United Kingdom, Germany, and Norway (Pundir et al. 2020).

19.6 Morphology

Sea buckthorn female and male plants exist separately because it is a dioecious plant. Male plant can produce pollen, and they also feature flowers that lack petals, as well as four stamens on each flower. Females produce fruits and seeds, as well as flowers with no petals. They are deciduous shrubs that usually grow to be 0.5 to 6.0 m tall within comparable spread but can grow to be up to 18 m tall in the central Asiatic

regions. The plant is distinguished by its bright orange berries that protrude densely from the shoots (Pundir et al. 2020). The staminate plants stand taller than the pistillate ones, which spread to an extent. The branches are vigorous, rigid, and thorny, having spines on both terminal and axillary twigs (www.omafra.gov. . .). The glabrous, linear, or lanceolate-shaped leaves range in length up to 8 cm and are not more than 7 mm in width. They are arranged in an alternating pattern and possess a deep grey-green top surface and a distinguishing pale, silvery-grey bottom surface (Wu et al. 2007). Flowers come out before the leaves do. They only grow on wood that is 2 years old and come out in tiny racemes along the length of the branch in the spaces between the leaves. Male flower buds are two to three times larger than female flower buds. Female flower pollination takes place in the mid of May and is completely reliant on airflow to disperse pollen from the male flowers. The fruit of this plant ripens approximately after 100 days of pollination (www.omafra.gov. . .). The fruits may vary in size, shape, and color, but they are usually oval-shaped berries, varying from yellow to bright orange color, with juicy and fleshy pulp, and 6–9 mm long (Pundir et al. 2020). It has a tart taste with a strong lemon flavor. It is composed of 60–80% juice. The juice consists considerable amount of sugar, organic acids, amino acids, tannin, and vitamins. Besides, the fruit-pulp contains up to 5% pulp oil and approximately 20% seed oil. It also has a distinct aroma that is similar of pineapple. Sea buckthorn is a fruit with a single seed. The seed is ovate-oblong in shape, measuring length 4–7 mm, width about 2.5–3.5 mm, and approximately 1.6–2.2 mm in thickness. SB seed's skin is leathery and glossy, and it is greyish-brown or dark brown in color. A parchment-like ovarian wall surrounds the seed. The seed contributes only 10% of the overall weight of the fruit sample. Depending on the plant species, the seed contains 10–20% oil. The leaves, on the other hand, are small having a length of usually 3–8 cm and width of around 1.0 cm, alternating, linear, lanceolate, and having a silvery coating on the backside that reflect sunlight and decrease moisture loss. It has a massive as well as a very well tap root system, with primary, secondary, and tertiary roots protected in root hairs, which are more prominent in the apical portion. The root system of this plant species can be so enormous that the roots seem to be branched multiple times during the growing season, generating a complex system of roots. It is approximated that the roots have twice the nitrogen-fixing capacity of soybean roots (Rajchal 2009). Table 19.1 shows the plant and fruit morphology of sea buckthorn.

19.7 Traditional Uses

As early as 900 AD, people of Tibet used sea buckthorn as a medicinal plant for their daily needs. The ancient Tibetan medicinal texts contain references to the medicinal use of this plant. It has also been used as a traditional medicine agent to prevent numerous diseases by the people of Central and Southeast Asia for centuries. The plant and its fruits, leaves, roots, branches, and bark have long been recognized as a valuable bio-resource, having been used for hundreds of years as fuel, food,

Table 19.1 Morphology of plants and fruits

Active period of growth	Status in the summer and spring
Bloat	Negative
Coppice potential	Positive
Fall conspicuous	Positive
Resistant to fire	Negative
Flower	Yellow colored
Flower noticeable	Positive
Foliage	White-grey colored
Texture of foliage	Coarse
Fruits/seeds	Orange
Fruits/seeds visible	Positive
Growth	Rapid growth
Height	18.0 feet approximately

medicine, and nutritional supplement (Suryakumar and Gupta 2011). Berry oil is used to treat gastritis, stomach ulcers, uterine erosion, and genital organ inflammation (Li 1999). Sea buckthorn branch and bark extracts have traditionally been used internally to treat colitis and enter colitis, as well as externally in topical compresses for dermatological disorders, wounds, and rheumatoid arthritis.

In the traditional medicinal history of Tibet and Mongolia, berries of this plant were used to treat sputum and cough, as well as to improve blood circulation and digestion. In Russia and the Himalayan section of India, sea buckthorn was used to treat skin diseases, liver disease, i.e., jaundice, gastrointestinal problems, asthma, as a laxative, and to treat rheumatism (Singh 2005). Local individuals of Afghanistan and Tajikistan are using these berries to treat hypertension, digestive system disorders, and skin problems. It has long been used in Germany to ecologically rehabilitate damaged lands, mainly for a forestation of different industrial, and coal mining sites, as well as to avoid soil erosion (Singh and Moersel 2005). In Greece, leaves and branches of sea buckthorn were given to the animals as a feed, resulting in weight increase and a gleaming coat, particularly in horses (Suryakumar and Gupta 2011).

19.8 Phytochemistry and Nutritional Composition

The fruits and leaves of sea buckthorn contain a variety of bioactive components that can be used for unique applications, and the plant components are being evaluated for specific compounds. Research revealed a wide range of phytoconstituents in several parts of *H. rhamnoides* that can diverge depending on environmental conditions, sources, and extraction methods (Zeb 2004). This plant has been linked to more than 190 bioactive compounds (Maertz 2006). Polyphenols, carotenoids (-β-carotene, lycopene, lutein, and zeaxanthin), flavonoids (isorhamnetin, isorhamnetin-3-β-D-glucoside, quercetin, isorhamnetin-3-D-glucosaminide;

Table 19.2 Nutritional composition of berries[a] (Ciesarová et al. 2020)

Attributes	Average values		Attributes	Amount (μg/mL)
	g/kg fresh weight	g/kg dry weight		
Moisture	53–86		Sodium	48.5–76.9
Total carbohydrate	291	368–420	Potassium	497
Total oil	15–105	365–443	Calcium	113–143
Crude protein	11–31	86–100	Magnesium	70.4–88.9
Crude fiber	47.1	62–73	Phosphorous	131
Ash	3–18	40–41	Chromium	0.178–0.699
Vitamin C	0.25–30	–	Copper	0.384
Vitamin E	0.03–0.21	–	Manganese	1.27–1.67
Vitamin K	1.10–2.30	–	Nickel	0.189–0.237
Flavonoids	1.68–10.0	–	Iron	7.58–28.2
Phytosterols	13–20	–	Zinc	0.763–3.29
Total carotenoids	0.01–0.40	0.12–1.42	Boron	1.06
			Arsenic	<0.5
			Lead	0.01–0.551

[a]The values presented here are averages derived from various studies. The attributed values of various studies fall within the range shown in this table

kaempferol, etc.), organic acids, fatty acids (saturated 13.7% and 86.3% unsaturated), amino acids, sugar alcohols, macro- and micronutrients have all been found in extracts from various parts of *H. rhamnoides* (Suryakumar and Gupta 2011). Sea buckthorn's phytochemical characteristics can be observed independently through the phytochemistry of its fruits, oils, and leaves. Berries are high in vitamins, particularly in vitamin C (ascorbic acid), which presents in an amount more than any citrus fruits like oranges and lemons, and as a result, such type of berries are said to be the most healthy and nutritious of all plant part(s). Vitamins B_1, B_2, B_9, E, and vitamin K are also present in sea buckthorn (Bal et al. 2011). However, these components may vary according to fruit maturity and sizes, species, geographical region, environment, and extraction methods (Zeb 2004). Berry pulps of sea buckthorn is superior in essential amino acids, proteins, different macronutrients and micronutrients, along with a number of minerals like K, Mg, Cd, Zn, Cu, Fe, and few organic acids such as quinic acids, oxalic acids, tartaric acids, and maleic acids (Bal et al. 2011). Table 19.2 shows the nutritional composition of sea buckthorn. In addition, the berries are high in antioxidants. In several antioxidant assays, isorhamnetin isolated from sea buckthorn marc demonstrated significant antioxidant activity (Pengfei et al. 2009). Besides, chromatographic analysis of SB berries revealed zeaxanthin and β cryptoxanthin esters (Andersson et al. 2009).

The plant's leaves contain nutrients and phytochemicals, the majority of which are phenolics. Other active components include gallic acid, procyanidins such as leuco-anthocyanidins, gallocatechin, epicatechin, and epigallocatechin. The other constituents present include amino acids such as methionine (0.13%), lysine(0.73%), cysteine (0.13%), proteins (20.7%), folic acids, ellagic acid, ferulic acid, isoprenols,

Table 19.3 Antioxidant compounds and antioxidant properties of different parts of SB (Wani et al. 2016)

Plant part	Antioxidant compounds	Extraction technique	Phenolic content	Antioxidant potential
Leaves	Kaempferol-3-O-β-D-glycoside, isorhamnetin-3-O-glucoside, quercetin-3-O-β-D-glucopyranosyl-7-O-α-L-rhamnopyranoside, quercetin-3-O-β-D-glucopyranoside, isorhamnetin-3-O-rutinoside, 1-feruloyl-β-D-glucopyranoside	Aqueous extract	67.91–88.69 GAE/g	76.44–88.82%
		Butanol fraction	477 GAE/g	–
		Maceration extraction	28.35 mg/g	86.35 TE/g
		Soxhlet extraction	43.77–77.85 mg/g	133.31–255.87 TE/g
		Subcritical water extraction	60.22–86.70 mg/g	164.03–343.86 TE/g
		Phenolic rich fraction	319.33 GAE/g	–
Fruits	Flavonoids, carotenoids, organic acids	Phenolic rich fraction	21.31–55.38 GAE/g	80.38–93.54%
Seeds	Catechin, catechin (4α-8), epicatechin, gallocatechin, epigallocatechin			
Oil	Carotenoids, tocopherols, β-sitosterol, tocotrienols			

GAE gallic acid equivalent, *TE* trolox equivalents

esterified sterols, ellagic acid, ferulic acid, β-carotene, triterpenols, catechins, tocopherol, and few minerals such as potassium (K), calcium (Ca), and magnesium (Mg) (Pundir et al. 2020; Suryakumar and Gupta 2011).

H. rhamnoides' oil is the most well-known product. The reported yield of oil from ripe seeds, dried fruit pulps, and berries is about 7.3%, 1.7%, and 2.1% of oil on dry basis, respectively (Bal et al. 2011). The morphological features of the berries, such as size and color, as well as the cultivation practices, influence the oil content. Carotenoids are found in the seeds in amounts ranging from one-tenth to one-fifth of that found in the fruits (Yang and Kallio 2002). However, the pulp and seed oils show variation in their fatty acid structures and composition. The pulp of SB oil contains saturated and mono-saturated fatty acids, whereas the seed oil contains linoleic acid and linolenic acid (Bal et al. 2011; Yang and Kallio 2002). The commercial value of SB oil, on the other hand, is generally based on the amounts of carotenoids and tocols. The branches and bark are also nutritious. They contain tannins, tocopherols, phytosterols, and other bioactive compounds such as hippophan (5-hydroxytryptamine). Table 19.3 shows the antioxidant compounds and antioxidant properties of this plants focusing its various parts.

The berries and leaves of sea buckthorn possess a wide range and good amounts of bioactive compounds, which can further be divided into phenolic and

non-phenolic compounds. The complete distribution and chemistry of bioactive compounds are illustrated in Fig. 19.3. Among the available bioactive constituents, the most abundant phenolic compound group in the berries is flavanol glycosides (FGs). The total FG content varied from 23 to 400 mg/100 g FW, which is up to 150 times richer than tomatoes (2 mg/100 g FW) and 10 times richer than chokeberry (*Aronia melanocarpa*, 27 mg/100 g FW) and American cranberry (Ma et al. 2020). The FG of sea buckthorn leaves has been studied as to be comparable to that of the berries on a dry weight basis (Pop et al. 2013). Among the tannins, condensed tannins, also familiar as proanthocyanidins (PAs) are oligomers and polymers of flavan-3-ol units synthesized via the biosynthetic flavonoid pathway, which is found throughout the plant kingdom. Both the leaves and fruits are high in phenolic acids such as gallic acids, salicylic acids, and caffeic acids. The seeds contain the majority (approximately 70%) of the phenolic acids.

Furthermore, the seed kernel has a higher total phenolic acid content (5700 mg/kg DW) than the berry pulp and seed coat (Ma et al. 2020; Arimboor et al. 2008). On the contrary, the most abundant organic acid in sea buckthorn berries is malic acid, that is followed by quinic acids and ascorbic acids. Ascorbic acid is one of the most abundant antioxidants, and its concentration correlates positively with the corresponding fraction's free-radical scavenging capacity (Gao et al. 2000).

Sea buckthorn characteristically possesses a low level of sweetness. The sweetness is caused by the two major sugars: glucose and fructose; aside from the ration of sugar-acid. The berries of sea buckthorn berries have a much lower sugar-acid ratio than other types of berries. Astringency and bitterness, on the other hand, are commonly considered negative factors that limit SB berry consumption (Laaksonen et al. 2015). These sensory characteristics may pose a challenge for the use of SB berries and berry-based products as food ingredients in the food industries. Figure 19.4 shows the sensory profile of SB berries and the contribution of its bioactive components to their taste properties. The sweetness of the taste may be associated with the presence of glucose, fructose, inositol, alkylated sugars, and ethyl—D-glucopyranose.

19.9 Pharmacology and Clinical Studies

Due to its significant medicinal value, sea buckthorn is considered as the next generation of novel botanicals. The pharmacological effects of this plant are well recognized in Asia and Europe. During the 1950s, clinical studies on therapeutic uses were introduced in Russia. For centuries, the Chinese have used the SB for medicinal purposes. Sea buckthorn was formally recognized in the Chinese Pharmacopoeia by the Ministry of Public Health (MPH) in 1977, cementing its repute as a therapeutic herb. More than ten drugs derived from this plant are available in various forms and can be used for the treatment of several diseases (Li and Beveridge 2003). SB has also become popular for its wide range of antioxidant, cardioprotective, anti-carcinogenic, anti-atherogenic, anti-diabetic,

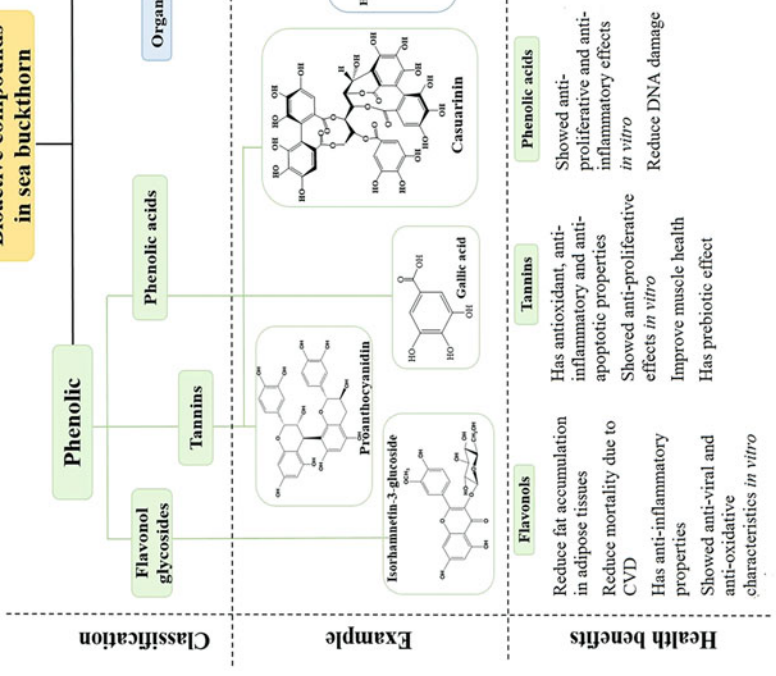

Fig. 19.3 The classification, example structures, and potential health benefits of bioactive compounds of sea buckthorn berries and its leaves (Ma et al. 2020)

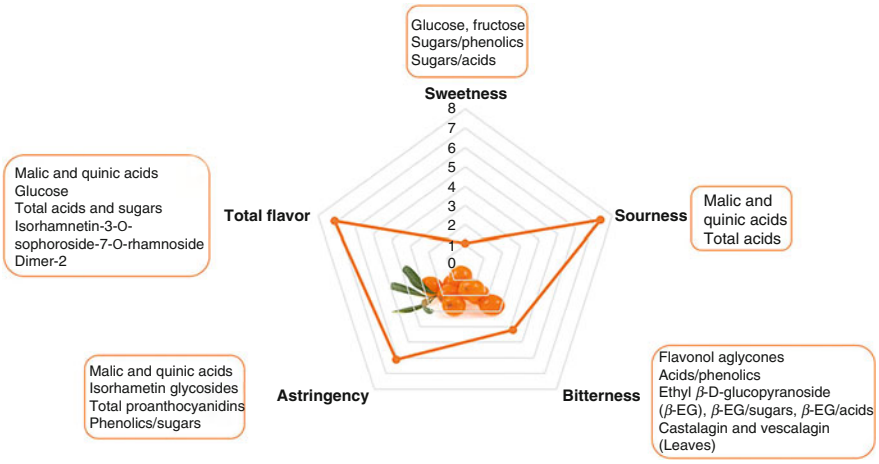

Fig. 19.4 Sensory profile and compounds contributing to taste properties of sea buckthorn berries. The data of sensory profile is based on the mean of 4 European cultivars (Tytti, Terhi, Hergo, and Leikora) (Ma et al. 2020)

hepatoprotective, immunomodulating, antiviral, antibacterial, and anti-inflammatory effects. It also removes the problem of stomach ulcers, promotes wound healing, hastens the therapies of skin disorders, and alleviates pain (Suryakumar and Gupta 2011). Some therapeutic effects of SB and its phytoconstituents are shown in Table 19.4.

The branches have (−)-epigallocatechin and ursolic acid, which possess anti-inflammatory properties. The leaves of this plant are prescribed for managing diarrhea, gastrointestinal, and dermatologic disorders. The leaf extracts are good enough for the treatment purpose of enterocolitis and colitis in humans and animals. In children with functional dyspepsia, sea buckthorn is reported to enhance the amount of plasma leptin and neuropeptide Y. It improves stomach secretion, gastric ulcers, digestive gastrointestinal function, and promotes growth and development of infants. The oil derived from fruit extracts has been linked to the treatment of thrombosis and atopic dermatitis (Wani et al. 2016).

Hiporamin, a new phytochemical drug discovered from the leaves of SB, possesses a broad range of antiviral and anti-microbial activities. It is very effective against the influenza and Herpes virus. Hiporamin is a polyphenol fraction that has been purified and contains monomeric hydrolyzable gallo-ellagi-tannins, most preferably casuarinin, strictinin, iso-strictinin, casuarictin, pedunculagin, etc. (Shipulina et al. 2006). The leaf extracts (aqueous and hydrophilic) inhibit growth of *Bacillus cereus*, *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterococcus faecalis* (Upadhyay et al. 2010). Table 19.5 shows different clinical and experimental studies that was carried out on sea buckthorn.

Table 19.4 Major phytochemicals and their therapeutic effects (Suryakumar and Gupta 2011)

Phytoconstituents	Therapeutic effects
Tocopherols	Antioxidant activity; lipid oxidation minimization; role in relieving pain
Carotenoids	Act as antioxidant; play a role in collagen synthesis; help in epithelial growth
Vitamin K	Helps to stop bleeding; stimulates wound healing; anti-ulcer potential
Vitamin C/ascorbic acid	Antioxidant activity; maintains integrity of cell membrane; helps to facilitate collagen synthesis
Vitamin B complex	Stimulation of cell repairment; regeneration of nerve tissues
Polyphenolic compounds	Antioxidant activity; cytoprotective and cardioprotective potential; support wound healing
Polyunsaturated fatty acids	Immunomodulatory characteristics, neuroprotective benefits, and anti-tumor potential
Organic acids	Reduce the threat of cardiovascular diseases and stroke; also manage the anti-ulcer effects, facilitate wound healing, and have anti-arthritic properties
Coumarins, triterpenes	Help in maintaining normal appetite and sleep; improve memory and learning capabilities
Zinc	Improves blood circulation; has anti-tumor potential; induces cell proliferation; functions as enzymes co-factor; enhances the utilization of vitamin A in body
Phytosterols	Improve skin microcirculation; possess anti-cancer, anti-ulcer, anti-atherogenic properties; help regulate the inflammatory process
Omega 3,6,7, and 9	Maintain cardiovascular health; help to function brain and nervous system; keep the digestive system healthy; stimulate healthy urogenital lining; maintain prostrate and colon health

19.10 Toxicology

Sea buckthorn extract significantly provides protection against arsenic-induced oxidative damage. However, it was found not so much effective in chelating and removing arsenic from the binding sites. This suggests that administration of fruit extracts along with a chelating agent might be an effective therapy in the treatment of arsenic poisoning (Gupta and Flora 2006).

Studies were conducted by Saggu et al. (2007) to ascertain the ability of SB leaves in improving the stress tolerance and adaptogenic ability of subjects. Treatment for 30 days with the aqueous SB leaf extracts, either acute or sub-acute, had no significant effect on liver, kidney, or renal function, fuel metabolism, and other hematological parameters. No deleterious effects were observed in experimental rat models fed with SB leaf aqueous extracts for 90 days at a regular dose of 100 mg/kg-body weight (BW) (Tulsawani 2010). Another study focused on the safety and toxicity of CO_2-supercritical fluid extraction (SFE) extracted seed oil. In chronic, acute, and sub-acute oral toxicity testing, no detrimental effects were found in any of the SB SFE seed oil groups (Upadhyay et al. 2009).

Table 19.5 Different experimental and clinical studies conducted with sea buckthorn

Effects	Study model and sea buckthorn parts used	Observations
Wound healing	Rat model; fruit pulp (flavonoid): 1% (w/v) flavonoid in propylene glycol	Improved wound contraction, accelerated epithelialization, increased hydro-xyproline and hexosamine levels, and reduced oxidative stress (Gupta et al. 2006)
	Dogs and calves; seed oil	Dry wounds and less inflammation (Mahajan et al. 2002; Kumar et al. 2003)
Healing of burns and scalds	Rat model; seed oil (CO_2 extract): 2.5 mL/kg of body weight; 200 μl (topical)	The oil of this plant has remarkable wound healing potential, and it is also free of toxicity or any mentionable side effects (Upadhyay et al. 2009)
Anti-inflammatory	Adjuvant-induced arthritis rat model; leaf extract: intraperitoneally	This is suitable for the treatment of arthritis; showed significant anti-inflammatory activity (Ganju et al. 2005)
Anti-cancer	Mice model; juice	Decreased carcinogen-induced forestomach and skin tumorigenesis (Padmavathi et al. 2005)
Anti-aging and photo-aging effects	Ultraviolet-B (UVB)-irradiated human fibroblasts; seed extract (alcoholic)	Increased procollagen synthesis, decreased MMP-1 expression, and inhibition of IL-1, IL-6, and COX-2 production (Kim et al. 2012)
	Clinical study; SB extract: 1% w/o hydro-alcoholic extract of SB	Improved the skin mechanical parameters (elasticity maintained) and skin hydration (Khan et al. 2012)
Dengue virus	Human blood-derived macrophages; leaf extract	Maintaining the cell viability of Dengue-infected cells as well as decreasing and increasing the TNF-α, and IFN-γ, respectively (Jain et al. 2008)
Antibacterial effects against skin bacteria	Microbial isolates from animals; leaf extracts: Different concentrations inoculated on Mueller- Hinton agar	Positive inhibitory effect (Verma et al. 2011).
Antioxidant, cytoprotective and antibacterial effects	Various in vitro systems; leaf extracts: Different concentrations of hydro-alcoholic extracts	Potent antioxidant activity, hypoxanthine-xanthine oxidase induced damage to BHK-21 cell line, cytoprotective activity against hydrogen peroxide, growth inhibition of *Bacillus cereus*, *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Enterococcus faecalis* (Upadhyay et al., 2010)

(continued)

Table 19.5 (continued)

Effects	Study model and sea buckthorn parts used	Observations
Anti-tumor activity	Human hepatocellular carcinoma cells (BEL-7402); Isorhamnetin isolated from SB: 50 g mL^{-1} isorhamnetin for 48 h	The treatment and management of isorhamnetin caused in the appearance of a hypodiploid peak (sub-G0/G1 peak) (Teng et al. 2006)
Nicotine-induced oxidative stress	Blood samples from rat model; SB extract: Nicotine (0.5 mg/kg/d, intraperitoneal, i.p.); nicotine1vitamin E (75 mg/kg/d, intragastric, i.g.); nicotine1HRe-1 (1 mL/kg/d, i.g.); and control group	Prevention of nicotine-induced oxidative stress (Suleyman et al. 2002)
Adaptogenic activity	Rat model; leaf extracts: Different concentrations administered orally for 14 days (1 g/kg and 2 g/kg daily) and for 30 days (100 mg/kg once daily)	Potent adaptogenic activity without toxicity (Saggu et al. 2006)
Skin diseases	Human patients; seed and pulp oils: 5 g oil for 4 months	Increased the quality of docosapentaenoic acid and reduced the proportion of palmitic acid in the skin glycerophospholipids (Yang et al. 2000)
Cardiovascular disease (CVD)	Human patients; SB juice: Different doses	Positive effects on the cardiovascular disease risk factors (low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), platelet aggregation and plasma soluble cell adhesion protein concentration) (Eccleston et al. 2002)
Antioxidant and immunomodulatory properties	In vitro (lymphocytes); plant extract: Chromium (VI) as potassium dichromate (10 μg/mL)	Cytoprotective properties (Geetha et al. 2002)
Cerebral vascular injury	Rat; leaves	Provide protection in hypobaric hypoxia, also modulate transvascular fluid leakage in lungs (Purushothaman et al. 2008)
Anti-inflammatory activity	In vitro murine macrophages; leaf extract	Anti-inflammatory activity and potential against inflammatory diseases (Padwad et al. 2006)
Anti-atherogenic effects	Rabbit; supercritical CO_2 extracted SB seed oil	Anti-atherogenic and cardioprotective activity (Basu et al. 2007)

19.11 Synthetic Strategies for Key Secondary Metabolites

The secondary metabolite spectra of Hippophae species differ, resulting in disease management and health care competency differences. Furthermore, various species and subspecies possess completely unique and own remarkable characteristics, such as fruit and seed qualities, ease of harvesting and processing, and drought- and cold-tolerant characteristics, which are particularly important in the management of different ailments (Liu et al. 2017). Plant metabolomics has already attracted a lot of attention since it focuses on the holistic properties of plants. This new branch of study has been recognized as an essential strategy for modern medicinal plant research. In metabolomics, the most commonly utilized analytical methods are LC-Ms, GC-MS, and H-NMR. Among two chromatographic methods, H-NMR-based metabolomics is regarded as quite effective and promising analytical tool than rest of the available techniques because it enables for the instantaneous revealing of primary and secondary metabolites in one run and yields a non-biased copious metabolic profile (Liu et al. 2017). Furthermore, because of its easy sample preparation stages and high reproducibility, this H-NMR method may be utilized to investigate numerous classes of chemical components both qualitatively, and quantitatively. Thus, H-NMR-based metabolomics in combination with multivariate data processing has proved its appropriateness for metabolomics research for food, agriculture, and herbal medicine categorization, identification, and quality evaluation (Fan et al. 2012).

19.12 Cultivation, Harvesting, and Processing

Seeds, cuttings (hardwood, softwood, layering), and suckers have traditionally been used to propagate SB. Although seed propagation is a straightforward and affordable approach, distinguishing between male and female plants is difficult until they begin to blossom (Kalia et al. 2011). Land preparation is essential prior to planting because the SB orchard should be planted with the goal of surviving for 10 to 15 years, the crop's estimated life span with maximum productivity. Typically, fertilizer application and land preparation should begin at least a year before planting. Land preparation procedures vary according to soil characteristics, types, and existing condition, previous crop status, vegetation cover, and regional climate. SB is a sun-loving plant, which means it thrives and develops best in bright sunlight. As a result, when choosing a location, the availability of sunlight must be considered (Li and Beveridge 2003). The land preparation techniques include the following:

(a) Sub-soiling—This entails breaking up a hardpan layer by loosening a tiny section of soil 50–75 cm deep.
(b) Summer fallow—Cultivating the site 1 year before planting to conserve soil moisture and control weeds.

Table 19.6 Adverse symptoms caused by physiological factors

Symptoms	Factors	Solving the problems
(i) Smaller crowns (ii) Lower yields	Shading effect	Planting in open and well-lit areas
(iii) Poor plant growth (iv) Decay/rot of root zone	Water logging and/or water level is too high.	Planting on gentle slopes and/or in well-drained sandy loam soil
(v) Smaller leaf area (vi) Low fruit set	Inadequate soil moisture	Regular irrigation, especially in the summer
(vii) Die back of branches (viii) Smaller leaves	Damaged by winter, frost, or heavy snowfall	Shedding during heavy snowfall
(ix) Breakage of branches	Heavy snowfall	Shedding during heavy snowfall
(x) Yellowish leaves (xi) Wilting (xii) Dropping of leaves	Lack of moisture	Regular irrigation, especially in the summer

(c) Cover cropping—It is beneficial to use a cover crop preceding planting to improve organic matter in the soil. Oats, barley, fall rye, and other cereal grains can be planted in the spring at a rate of 80–150 kg per hectare and plowed beneath before seed set to allow for decomposition. The following spring, sea buckthorn is planted. This method is especially beneficial on sandy, erodible land.
(d) Discing—This may loosen the soil, allowing for better moisture reception and retention, as well as improved soil tilth for spring planting.

Sea buckthorn species can withstand summer temperatures of up to 40 °C. In British Columbia, it displayed temporary stress symptoms such as wilting and impeded growth over a period of time throughout the peak of summer when temperatures exceeded 35 °C and 25 °C in day and night, respectively. Extreme heat, drought, or flood, hailstorms, and strong winds are the main climatic constraints. Snowfall can damage or break branches during the winter. The optimum soil for growing sea buckthorn is well-drained sandy clay loam soil, while the clay and heavy loam are unsuitable without organic improvements. As a general principle, if the land is saline but produces a good crop of barley, SB will thrive and grow as well, although the growth and fruit production will be constrained (Li and Beveridge 2003). The seeds must be soaked in water for long 2 days (48 h) before planting, and the floating ones must be discarded. There may be a number of symptoms that indicate a variety of relevant causal factors (Li and McLoughlin 1997). Table 19.6

shows the different adverse signs that may be occurred by a number of physiological factors.

SB seeds may be planted directly outdoors or transplanted indoors. In the case of outdoor planting, one seed per planting site at a distance of 1 m within the row and 4 m between rows is suggested. It can also be planted inside the house in sterilized soil in pots in January and at the starting days of February, with just a seedling per pot expected to grow for 3 months prior to transplanting in the early May. They should be irrigated or watered once in every week after planting. For orchard gardening, a distance of 1 m within the row and 4 m between rows is also suggested, while high density planting of 1 × 1 m is being investigated in Europe. SB can be propagated through hardwood or softwood cuttings, layering, or suckers (Li and McLoughlin 1997).

The optimal harvesting period is among the most important factors effecting the quality of the fruits. A commonly practiced commercial harvesting procedure is to eradicate an entire branch. Harvesting of mature SB is the most challenging and time-consuming procedure due to the plant's thorniness and the fruit's softness, smallness, delicateness, and perishability, as well as its strong attachment to the plant. In developed countries, a variety of fruit harvesting techniques are used, including hand picking, branch chopping, branch beating with sticks, machine harvesting, fork and mineral water bottle use, and so on (Li and McLoughlin 1997). After harvesting, the fruits should be stored in flats no more than 6 inches deep in a shaded area. If there are high temperatures (20 °C) during the harvesting time, pre-cooling for storage is recommended, especially if breakage happens during harvest and even before the cleanup. The fruits or berries can indeed be frozen to − 18 °C for long-term storage (up to 1 year) without losing any of its components.

19.13 Tissue Culture

SB tissue culture comprises callus and organ culture. Since 1988, there was a significant progress was found in several areas SB tissue culture, including explants selection and sterilization, media selection to generate callus, buds, and roots. Nowadays, research also focused prevention of browning and transplant tube seedlings. A large-scale manufacturing framework, on the other hand, has yet to be constructed, and micro-propagation techniques are currently being researched. There has been no study on somatic embryogenesis, cryopreservation or long-term storage of germplasm, or synthetic seed production (Ruan et al. 2007). Table 19.7 shows the optimal elements and concentration levels for *H. rhamnoides* organ culture commencement, multiplication, and rooting media for various explants and varieties, as well as different lines within the same variety. Callus culture is another type of tissue culture that is important for sea buckthorn. It is critical due to callus differentiated seedlings utilized for quick replication and screening of valuable mutations. Large-scale callus culture also used to extract valuable, vigorous components, and secondary metabolites makes it more critical for callus culture (Ruan et al. 2007). in vitro

Table 19.7 Tissue culture and organ culture of sea buckthorn

Varieties	Explants	Optimal initiation medium	Optimal multiplication medium	Optimal rooting medium
Kaniya, 90-3-2, 90-2-14 (*H. rhamnoides ssp. mongolica*)	Apical meristem	MS + NAA 0.03 mg/L ~ 0.05 mg/L + KT 0.3 mg/L ~ 0.5 mg/L	–	MS + NAA 0.002 mg/L + KT 1.0 mg/L (Sun 2005)
92-1-18, 92-5-15, 92-3-1, 92-7-8 (*H. rhamnoides ssp. mongolica*)	Meristem	MS + IBA 0.01 mg/L + BA 0.2 mg/L	–	Guo and Xu 2000
H. rhamnoides ssp. sinensis	Stem segments, lateral bud	½B5 + IBA 0.4 mg/L + sucrose 1.5% + agar0.46%	–	½B5 + IBA 0.2 mg/L + IAA 0.2 mg/L (Guolin et al. 1989)
Yousheng (*H. rhamnoides ssp. mongolica*)	Stem segments	½MS + 6-BA 1.0 mg/L + IAA 0.5 mg/L	1/4MS + 6-BA 0.5 mg/L + NAA 0.2 mg/L	¼MS + NAA 0.03 mg/L + IBA 0.1 mg/L (Kang et al. 2002)
H. rhamnoides ssp. sinensis, hybrids of Qiuyisikke (*H. rhamnoides ssp. mongolica*) and *H. rhamnoides ssp. sinensis*)	Stem segments	½B5 + 6-BA 0.5 mg/L + IAA 0.5 mg/L	½B5 + 6-BA 0.5 mg/L + IAA0.5 mg/L	½B5 + IAA (0.3–0.5 mg/L) (Yang et al. 2004)
Shiyou 1 (*H. rhamnoides ssp. mongolica*)	Meristem	½MS + 6-BA 1.0 mg/L + IAA 0.5 mg/L	½MS + 6-BA 2.5 mg/L + IAA 0.2 mg/L	Zhou et al. 2006
	Hydroponic leaves	½MS + 6-BA 0.5 mg/L + KT 0.2 mg/L + NAA 0.02 mg/L	½MS + 6-BA 0.5 mg/L+ KT0.2 mg/L + NAA0.02 mg/L	½B5 + 6-BA 0.3 mg/L + IBA 0.4 mg/L (Zhou et al. 2006)

Media: *MS* Murashige and Skoog (1962); *B5* B5 basic media (Gamborg 1968). Plant growth regulators: *6-BA* 6-benzyladenine, *IAA* indole-3-acetic acid; *IBA* indole-3-butyric acid; *KT* kinetin, *NAA* naphthalene acetic acid.

studies suggest that axillary buds are used to cultivate tissue from several species of SB. However, there are numerous constraints during tissue culture, such as poor cellular proliferation, cryogenic preservation, moderate browning, weak bases and roots, as well as genotype differences among the varieties (Montpetit and Lalonde

1988; Nilov and Tretyakova 1993). Direct somatic embryogenesis was induced from SB leaves, cotyledons, and hypocotyls of sea buckthorn (Liu et al. 2007).

However, *Hippophae salicifolia* were propagated by Saikia and Handique (2014) using cotyledonary explant. Proper tissue culture-raised plantlets produced new leaves with a well-developed cuticle, capable of producing organic food via photosynthesis. High mortality was identified upon transfer of plantlets to ex vitro conditions as the cultivated plants show non-functional stomata, a fragile root system, and a poorly developed cuticle.

19.14 Formulation and Market Products

Sea buckthorn is increasingly utilized in the manufacturing of juice, chocolates, jam, jelly, alcoholic and non-alcoholic beverages, and also used as a flavoring agent in dairy products due to its beneficial characteristics and distinct taste and flavor. The most valuable product with medicinal properties is essential oil extracted from seeds and berry pulp (Bal et al. 2011). On the other hand, there is currently inadequate research on prescribing the berries for animal nutrition. Nonetheless, the fruits, seeds, and leaves have been shown to be suitable for feeding animals (Christaki 2012). Sea buckthorn pigments are widely used as food additives. Flavors, carotene, and vitamin E are all found in this plant. Its physico-chemical characteristics, i.e., texture, solubility, color parameters, appearance, heat or light stability, and pH make it a very valuable food additive. It can also be used to make tea, squash, jam, juice powder, and fenugreek pickles, among other things (Chandra et al. 2018).

SB juice, according to recent research, encourages the growth of a variety of good and presence of beneficial bacteria in the gut, most likely as a result of its pre-biotic qualities (Attri et al. 2018). Selvamuthukumaran and Khanum (2015) demonstrated that this plant may can stimulate the growth of various lactic acid bacteria. Their studies focused on the effect of variation of SB extracts and milk powder on the sensory quality, physiological, and functional characteristics of yoghurt. Other researchers investigated the use of SB as an active component in the cheese. Terpou et al. (2017) inspected the symbiotic effect of SB berries with a pro-biotic strain of *Lactobacillus casei* (ATCC 393) included in the feta type cheese. These researchers used dry SB berries as a pro-biotic strain immobilization transporter. Nonetheless, SB has been added to other fermented food products to achieve a change in most contexts an increase in performance—in their structure, flavor, antioxidant capacity, shelf life among other attributes.

Many countries are developing brands with therapeutic and cosmetic properties based on SB, such as creams, lotions, shampoos, pills, pastes, liniment, serums, soaps, and different suppositories. Dermal-cosmetics and other generic cosmetics of SB are well known and marketed vastly for their wonderful benefits in smoothening and rejuvenation. They are used because of anti-dandruff potential, eczema alleviation, scalp nourishment, pigmentation, and conditioning of the scalp and advancement of growth, and health of hairs (Table 19.8).

Table 19.8 Commercially available cosmetics of *Hippophae rhamnoides* (Pundir et al. 2020)

Products	Formulation and ingredients	Function	Manufacturer
Oil	Cold-pressed oil	Relieves irritation from affected skin, restores skin from damaged condition, hydrates dry skin, relieves sunburn, cuts, heals wounds, and acts as an anti-aging agent	Devinez
Suppositoria Hippopheas	Rectal suppository: Seed oil	Improves suppleness and tone, and aids in the prevention of hemorrhoids	Monpharm
Bio skincare oil	Sea buckthorn oil added with different oils (almond, green tea, passion fruit, lilac, jojoba, olive), extracts (saffron and mulberry), and vitamin E	Dry skin management, anti-aging function, stretch marks, pigmentation, sunburn, chickenpox scar, burn marks, appendectomy scar, and insect bite signs	Pavitra
Oil	Seed oil	Nourishes skin and hair	Ryaal
Face oil	Sea buckthorn oil added with different oils (tamanu and jojoba)	Rejuvenates skin, cures and heals skin from damaged and broken condition, imparts a natural glow to the face, slows the aging process, and prevents acne scars and blemishes	Mirah Belle
Bath soap	Oil	Revitalizes, softens, and moisturizes the skin	Divinectar
Berry seed oil	Seed oil	Rosacea, eczema, beauty therapy, anti-aging, small burns and wounds, and other skin conditions	Sibu
Hair oil	Sea buckthorn oil added with different oils (Indian gooseberry, spearmint, rosemary, soybean, jojoba, olive, almond, sunflower, fenugreek), and murumuru butter	Anti-dandruff, scalp nourishing and conditioning, encourages hair development, healthy, shiny, lustrous, and black hairs	Greenberry Organics
Night miracle serum	Sea buckthorn extract added with different oils (kumkumadi, sesame, and chamomile)	Anti-aging function, anti-wrinkles, pigmentation, skin whitening, and age spots reduction	Alanna Naturally Beautiful
Vitamin C serum	Sea buckthorn oil added with different oils (argon, carrot seed), vitamin E, acids (hyaluronic and ferulic), and pomegranate extract	Reduces facial aging blemishes, anti-wrinkle, anti-aging	Insta Natural
Hair oil	Sea buckthorn oil added with jojoba and apricot oil	It is suitable for oily, dry, damaged, frizzy, dull, rough, and thin hair.	Betty's Skin Care

(continued)

Table 19.8 (continued)

Products	Formulation and ingredients	Function	Manufacturer
		Prevents hair loss, repairs split ends, receding hairlines, promotes hair regrowth, removes dandruff, treats itchy scalp, greying, and serves as a volume-boosting agent	
Sulfate-free shampoo	Sea buckthorn oil added with other oils (Cedrus atlantica), different extracts (Centella asiatica, reetha, ashwagandha), sodium hydroxide (50%), fragrance	Anti-dandruff, antimicrobial, anti-fungal, antioxidant, anti-inflammatory, treats split ends, and controls frizzy hair	Aaranyaa
Face toner	Sea buckthorn extract added with other extracts (jasmine, ashwagandha, green tea, aloe-vera, licorice, and rose), sodium benzoate, potassium sorbate, and hyaluronic acid	Tones the face, has anti-aging and skin brightening qualities, decreases facial blemishes, deeply hydrates the skin, has anti-inflammatory characteristics, and calms the skin, making it ideal for dry or older skin	Timeless Beauty Secrets
Peel off mask	Sea buckthorn oil	Removes blackheads, whiteheads, acne, pollutants, and blemishes for toned, radiant skin; maintains smooth and delicate face; and shrinks pores	Seabuck Essence
Gel	Aloe vera gel and sea buckthorn gel	Psoriasis, eczema, skin infections, anti-septic, sunburn, anti-tan, heals acne, pimples, pigmentation, and rejuvenates skin after sea and pool baths	Ida's Essence
Fruit pack	Fruit juice	Visibly fairer, smoother, healthier, more even-toned and younger-looking skin	Seabuck Essence
Bleach cream	Fruit extract	Makes skin appear fairer	Seabuck Essence
Face wash	Sea buckthorn fruit juice and activated charcoal	Removes dirt and impurities from deep into the skin, resulting in a fresh face and acne control	Seabuck Essence
Monkey balm sticks	Fruit extract	Eczema treatment, dry skin, reduce the severity of sunburns, windburns, small scratches, and rashes	Balmers

(continued)

Table 19.8 (continued)

Products	Formulation and ingredients	Function	Manufacturer
Deodorant	Sea buckthorn added with different plant extracts, chemical preparation, and other oils	Adequate protection against unwanted body odors	Weleda
Body lotion	Sea buckthorn oil added with different oils (macadamia ternifolia seed), water (aqua), Butyrospermum parkii (shea) butter, glyceryl stearate SE, glycerin, Malva sylvestris (mallow) extract, xanthan gum, tapioca starch, cetearyl alcohol, limonene, linalool, citronellol	Intensive body care for dry skin	Weleda
Anti- redness+ rosacea cream	Sea buckthorn oil added with different oils (argan, Bulgarian rosewater, black seed, rosehip), wild chrysanthemum flowers, rooibos extract	Reduces irritation, provides relaxation, softens and smoothens skin; improves skin health, rosacea and redness relief, anti-aging	Argan Organics
Organic serum	Sea buckthorn seed oil added with different oils (sweet almond, green tea, Matricaria flower, orange peel), rosemary leaf extracts, and tocopherol (vitamin E)	Anti-aging	Dr. Mercola
Foot scrub	Sea buckthorn leaf extract, grapefruit seed extract	Clean and rejuvenate softens feet and cracked heels	Taashi
Retention hydrating gel	Sea buckthorn extract, sodium PCA, glycerin, sodium benzoate, potassium sorbate, and perfume	Rejuvenating benefits on the skin, increases skin's water retention capacity, providing smooth, nourished skin, healing therapy for dry and injured skin	Organic Harvest
Under eye gel	Sea buckthorn leaf extract and berry oil, xanthan gum, different extracts (aloe vera and daruhaldi), glycerin, triethanolamine, potassium sorbate, phospholipid, purified water	Reduces puffiness, revitalizes tired-looking eyes, lightens dark circles, and helps to prevent wrinkles	Taashi
Eczema and dermatitis soothing salve (cream)	Sea buckthorn seed oil added with different oils (rosehip, coconut, pomegranate, helichrysum, chamomile), candelilla wax, and cocoa butter	Anti-microbial, analgesic, and anti-inflammatory properties help to alleviate the severe itching and inflammation associated with eczema and dermatitis, as well as heal and repair dry, damaged, cracked, chapped, irritated, and inflamed skin	SB Sea Buckthorn
Lip balm	Oil	Nourishing treatment for dry, chapped lips	The Healing Arc

(continued)

Table 19.8 (continued)

Products	Formulation and ingredients	Function	Manufacturer
Healing salve (cream)	Sea buckthorn oil added with different oils (rosehip and olive), beehive wax, vitamin E, and seed extracts from grape	Heals wounds, eczema, psoriasis, and dermatitis	Mirah Belle
Sanitizing cream	Sea buckthorn oil and different vitamins	Dry, cracked, flaky, irritated, and itchy skin is instantly and permanently relieved. It kills 99.99% of the germs that cause inflammatory skin problems, making it an effective moisturizer and sanitizing cream for eczema, dermatitis, rosacea, seborrheic dermatitis, and shingles	Coresatin

19.15 Conservation

Sea buckthorn is extremely beneficial to wildlife, particularly in its native habitat. Many animals use it as a source of food and shelter. The shrubs provide valuable habitat for the native sharp-tailed grouse in the prairies. SB is not a native species in many countries; however, there are a few nature reserves created specifically for wildlife, such as birds and animals, and its spread occurred only within the habitat. There are a few methods for controlling the natural spread of sea buckthorn. Herbicide implementation within the row, regular machine mowing and plowing between rows to prevent sucker development, and selection of cultivars with few or no suckers produced are all practices used in orchards. Sea buckthorn is a dioecious plant that produces seeds after female flowers are pollinated by wind-dispersed pollen. Strong winds may transport pollen over long distances, and the resulting hybridization may tend to blur the boundaries between populations and taxa (Li and McLoughlin 1997).

Sea buckthorn has significant nitrogen-fixing capacity. It has a crucial relationship with the bacterium *Frankia,* a genus that is usually found in the root nodules. The improved root system benefits the soil ecosystem by providing more organic matter, more oxygen, and more soil organisms. It is evident that older plants fix more nitrogen than younger plants. As a result, plantation of SB makes significant contribution in enrichment of nitrogen and organic matter in the soil. Mountain ecosystems, on the other hand, require conservation and improvement in order to maintain their impact on downstream ecosystems, freshwater resources, and social conditions. It demonstrates a strong tolerability against different toxic pollutants in soil and air. For this reason, it may be appropriate for use in revegetating heavily industrialized areas and reclaiming mining sites for this purpose (Singh 2004). Because of its soil-binding ability, its plantation near the river's edge prevents landslides and reduces siltation of major river dams. This plant would be ideal for

planting near the entire major trans-Himalayan rivers to protect the Himalayan ecosystem. Furthermore, sea buckthorn has the ability to alter runoff and sediment transport characteristics. The plantation and cultivation of this plant has enormous environmental and economic potential for the trans-Himalayan cold deserts. The oil and berries have the potential to generate income for the locals (Acharya et al. 2010).

19.16 Conclusion and Future Perspective

The study of medicinal plants has seen an exponential growth in the recent years. Despite the fact that all medicinal plants have a long history of usage in the orthodox medicine system and a high degree of appreciation, current science is looking for remedies from these natural systems, with a more definitive and fool-proof approach. A lot of studies have shown that natural therapeutic herbs have no or little negative effects when administered to the human body. *Hippophae rhamnoides* is a magical plant because it contains a diverse range of nutritional and medicinal components. As this plant is a common and widespread species, sea buckthorn deserves to be protected. Its distribution has been described as highly fragmented, with isolated populations frequently genetically distinct. There are numerous opportunities to work on genetic diversity, propagation, root nodulation, environmental conservation, and medicinal properties. Because the phytoconstituent status of different plants and their relevant products vary considerably as a consequence of the wide varieties, global climatic conditions, maturity, post-processing storage, storage, and consistency, extensive research on the structure, composition, and physiological value of medicinal plants, as well as standardization of formulations based on components, is critical.

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Chapter 20
Species of the Genus *Selinum* Found in the Western Himalayas

Apurba Gohain, Shahnaz Alom, Dimpee Choudhury, and Ashoke Kumar Das

20.1 Introduction

From ancient time, traditional medicine is widely practised in various parts of the world such as China, India, Japan, Pakistan, Sri Lanka, and Thailand. China attributes around 40% of overall medicinal usage to traditional tribal remedies alone. In this section, we will discuss the valuable medicinal and aromatic herb Selinum, which is a member of the Apiaceae family. This family consists mostly of herbs with hollow stems. They are seldom woody at the base. The family has 250–440 genera and 3300–3700 species that are found across the temperate zones of both hemispheres. There are various species of Selinum; however, only *S. vaginatum* C. B. Clarke, *S. wallichianum* (DC.) Raizada & H.O. (synonyms *S. candollei* Edgew, *Selinum tenuifolium* Salisb.), and *S. carvifolia* (L.) L. are recognised names, while the status of other species remains unknown (The Plant List 2013). In India, the genus Bhutkeshi is well-known for its medicinal and fragrant properties (Pandey et al. 2013). It is an indigenous, alpine perennial plant that is hairy, rhizomatous, endangered, and primitive. However, the IUCN Red List 2001 listed the species *Selinum carvifolia* as endangered in the United Kingdom (IUCN Red List of Threatened Species 2005). Additionally, the IUCN's 2009 Red List classified *Selinum vaginatum* as an endangered species and placed it in the low-risk category (Chawla et al. 2008). The taxon has not been evaluated by the IUCN Red List since that time. Other Selinum species, however, are not included on

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the IUCN list owing to a lack of study and knowledge gaps. Selinum species are now included in the Catalogue of Life via Catalogue of Life Indexing ("IUCN Red List of Threatened Species", 2005). In India, Khare (2007) recognised two Selinum species, *S. tenuifolium* and *S. vaginatum*. The plant's components, mostly the root and leaves, are used to cure a variety of conditions, including diarrhoea, cuts, wounds, swelling, and vomiting (Mohan et al. 2013). Selinum spp. has a long history of usage in Unani and Ayurvedic medicine as an insecticide, nervine sedative with antispasmodic and stimulating qualities, and for constipation, menstruation, and digestion. The purpose of this research is to determine the long-term viability of Selinum spp. for medicinal and industrial purposes not just in India, but around the globe. Despite the fact that we now have a broad range of contemporary medications at our disposal, the need to identify and develop novel therapeutic agents remains critical. The increasing frequencies and evidence of microbial resistance to a wide variety of antibiotics reaffirm this necessity. According to some assessments, hardly more than one-third of known human illnesses have an appropriate treatment. As a result, the struggle against sickness must continue unabated. Traditional plant remedies continue to have a key role in contemporary pharmaceutical businesses owing to their low toxicity and synergistic activity when combined with other substances. Selinum has the potential to become a sustainable and appropriate resource in the pharmaceutical industry in this situation. This review aims to address the same since there is a dearth of research on Selinum's anatomical, morphological, pharmacological, and phytochemical features.

20.2 Botany

According to "The Plant List", the species, *Selinum wallichianum* (DC.) Raizada & H.O. Saxena plants have been employed for their therapeutic properties for a long length of time prior to prehistoric times, particularly in Ayurveda. Several pharmaceutical enterprises have employed the seeds, roots, and leaves of Selinum spp. in the creation of Ayurvedic remedies. The oil extracted from the roots is leucodermal, hypotensive, sedative, and analgesic (Saraswat et al. 2020a). Selinum rhizome preparations are used in Ayurvedic medicine as a stimulant, bitter tonic, and antispasmodic to treat epilepsy and hysteria (Pandey et al. 2013) (Figs. 20.1 and 20.2). The image of *S. vaginatum* and *S. tenuifolium* are given in Figs. 20.1 and 20.2.

Selinum comprises roughly 35 species dispersed across the globe, only a handful of which have been resolved. Selinum rhizome extracts are employed as a stimulant, a bitter tonic in humus-rich mountain slopes (Chauhan et al. 2012a). *Selinum vaginatum* is a perennial plant with upright, tall, glabrous and hairy stems, and biennial tubers. It is readily recognised by clusters of tiny, white flowers on long, thin stalks. Selinum spp. are hollow and coarsely grooved, with lanceolate, serrate, lobed, or pinnatifid leaf segments (Hutt 2019). Rhizomes are stunted and coated in a thick tuft of bristly fibres. They measure around 4 cm in length and 2 cm in diameter. The roots have a filthy brown colour and may reach a length of 15 cm or more and a

Fig. 20.1 *S. vaginatum* and *S. tenuifolium*

Fig. 20.2 *S. vaginatum* (A) and *S. tenuifolium* in its natural habitat

diameter of 1 cm. Selinum spp. roots and rhizomes are very fragrant and harsh in flavour. Transverse cut of the root of *S. vaginatum* reveals an outermost cork formed of thin-walled, tangentially elongated cells, phellogen, primary cortical secretory

canals, parenchyma cells, secondary phloem, and xylem. The rhizome is characterised by a limited cork zone, a thick primary cortex and phloem and secondary phloem with phloem rays, phloem parenchyma, xylem, and medullary rays. A mesophyll-containing leaf transection reveals a row of vascular bundles flanked by a centrifugal area of sclerenchyma. In the phloem, epidermis, hypodermis, radial wall, and vast intercellular gaps of *S. vaginatum*, the stem contains chlorenchyma, collateral vascular bundles, and tiny secretory canals. Rhizome exhibits cambial activity, resulting in a continuous ring of xylem and phloem (Mehra and Jolly 1963). *S. vaginatum* has a large cortex with around 14–21 layers of cells, many schizogenous canals, and no pith. He examined the microscopic properties of *S. vaginatum* and discovered that its powder is brownish in colour, bitter in flavour, and has a distinct odour. The rhizome's surface view reveals schizogenous channels, fibre segments, medullary rays, and starch granules (Vineet et al. 2011).

20.3 Distribution

Selinum sp. is a significant medicinal and aromatic plant (MAPs) that grows between 6000 and 14,000 feet above sea level in temperate parts of the Western Himalayas, China, South Africa, and Andean highlands (Chauhan et al. 2014). *Selinum tenuifolium* (synonym *S. candollei*) and *Selinum vaginatum* have been documented from the Kumaun and Garhwal regions of Uttarakhand, India (Kumar Joshi et al. 2016). It grows wild in the Rohtang pass (Himachal Pradesh) and in Uttarakhand's Tungnath, Milam glacier, and Niti (Chamoli) (Chauhan et al. 2012c). These plants thrive in the temperate and alpine zones of South African and Andean Mountain ranges. Plants of the genus Selinum are also found in Nepal, Bhutan, and western Pakistan (Hutt 2019). These plants are abundant in the alpine, subalpine, and temperate Himalayas and occur at elevations ranging from 2000 to 4000 m. They are particularly abundant in the western and central Himalayas of India, specifically in Himachal Pradesh, Kashmir, Uttarakhand, and Sikkim. They are also found in Kangra, the Holi range of Chamba, the Parvati valley, the Rohtang area of Kullu, and the Rampur and Rohru divisions of Shimla district (Fig. 20.3).

20.4 Cultivation and Soil Condition

S. vaginatum leaves at two different altitudes in natural habitat (3600 m) and artificial habitat (550 m), at lower altitudes exhibited some cuticular elevation on the upper surface and finer cuticular cristae on the lower surface, as compared to leaves from higher altitudes (natural habitat). They measured the absorptance, reflectance, and transmittance of upper and lower leaf surfaces at two altitudes and discovered that plants cultivated at a higher altitude (natural habitat) had more

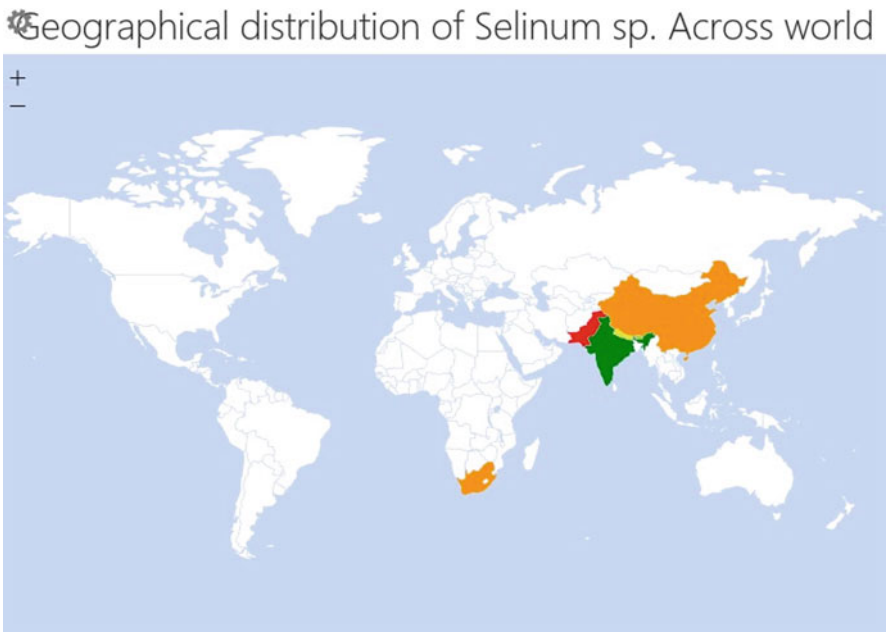


Fig. 20.3 Geographical distribution of selinum sp. across world

absorptivity at all wavelengths than those produced at a lower height (Purohit et al. 1988). *S. vaginatum* rhizome was identified based on macroscopic characteristics such as organoleptic character, odour, and taste, all of which are bitter and extremely distinctive. Multiple environmental cues are necessary for the embryonic development and germination of *S. carvifolia* (L) L seeds. When incubated at varied temperatures of 20°/10 °C and 15°/6 °C in both light and darkness, the seeds of *S. carvifolia* germinated to a high percentage. After 36 weeks of incubation at 5 °C, 10 °C, 25 °C, 15°/6 °C, and 20°/10 °C, seeds germinated. When seeds were transported to a higher temperature after cold stratification at 5 °C, an increase in germination percentage was seen. After 8–12 weeks of cold stratification, the embryo of *S. carvifolia* extended fast when seeds were moved to warmer temperature settings. The experiment indicated that the seeds of this species are physiologically dormant and must be disrupted in order for the embryo to extend (Vandelook et al. 2007). *S. candollei* cell and tissue cultures were cultured with and without mineral oil overlay on MS medium supplied with suitable growth regulators, and it was observed that mineral oil-covered cultures grew and morphogenesis at a much slower pace than controls. This increased the duration of the subculture from 35–45 days to 150–240 days, resulting in considerable labour, material, and time savings. Following that, the shelf life of encapsulated *S. candollei* propagules was increased from 25–30 days to 150–240 days (Mathur et al. 1991). Under a mineral oil overlay, somatic embryogenesis was enhanced in *S. candollei* DC. Cell suspensions obtained on liquid Murashige and Skoog's medium supplemented with 4.52 M

2,4 D and 1.16 M kinetin proliferated into a callus and subsequently produced 15–20 somatic embryos within 60 days when plated on solid medium devoid of 2,4-D. After 30–45 days, however, when plated cells were coated with mineral oil, a reduction in callus development was seen, along with a fourfold increase in the number of somatic embryos per gramme fresh weight of the cells (Mathur 1991). The optimal preparation for *S. wallichianum* seeds from alpine and subalpine provenances was established. For the first time, somatic embryogenesis and synthetic seed production were observed in *S. tenuifolium* Wall. using a mature leaf explant in Murashige and Skoog (MS) medium supplemented with 3 M 2,4 dichlorophenoxyacetic acid (2,4 D) and containing 3% (w/v) sucrose and 0.7% (w/v) agar, which resulted in a significantly higher frequency of callus induction (67%) after 4 weeks of experimentation. The thermostability of acid phosphatase in *S. vaginatum* was determined at two different altitudes (low and high) and revealed that tropical plants (lower altitude) showed more thermostability than temperate and arctic zone species (Bhadula et al. 1986). The comparative morphology and anatomy of leaves, stems, and roots of *S. vaginatum* and *S. tenuifolium* revealed a broad variety of anatomical and morphological changes across altitudinal zones. This information may be used for identification purposes and may also be used to help produce regulatory documents for this medicinally significant species (Srivastava et al. 2018).

20.5 Phytochemical Investigations

20.5.1 Volatile Compounds

Selinum's whole plant is a source of essential oil. The essential oil extracted from the aerial portions of *S. tenuifolium* has the following components:—bisabolol (55.55%), p-cymene (3.50%), ocimene (E) (2.70%), −copaene (2.40%), 2-methylbutyl isovalerate (2.30%), and p-cymene-8-ol (2.23%) (Kumar Joshi et al. 2016). The essential oil composition of *S. vaginatum* was determined using GC-FID and GC-MS analysis from two distant locales, Rohtang (Himachal Pradesh) and Tungnath (Uttarakhand). Only 12 compounds were found to be similar to both populations. Due to environmental factors and genetic makeup, the variety in essential oil content from a remote site is also noticeable in qualitative and quantitative metrics. These variations may be used to evaluate genetic diversity and its relevance to crop development programmes (Chauhan et al. 2014). Several chemical compounds of *S. candollei* DC. have been found, which aided in the chemotaxonomic identification of taxa in the genus Selinum (Chauhan et al. 2012b). The volatile oil extracted from *S. tenuifolium* aerial parts was examined using GC and GC-MS, and 31 components were identified, accounting for 95.16% of the oil. The chemical composition of essential oil extracted from the underground part of *S. tenuifolium* Wall. was determined using a Clevenger type apparatus and GC/FID and GC/MS. Nine constituents were identified, five of which were

polyacetylenes, and four compounds, nona-3,5-diyn-2-one, nona-4,6-dine-3-one, nona-3,5-diyn-2-ol, and nona-4,6-d. Twenty components were identified in the essential oil of *S. candollei*, accounting for 64.32% of the total essential oil. *S. tenuifolium* aerial portions included –bisabolol, a significant component that may be used in the pharmaceutical and cosmetic sectors. The essential oil extracted from air-dried *S. tenuifolium* aerial parts included 37 components that were identified using GC-FID and GC-MS analyses. Bisabolol (71.80%), fernesol (3.50%), germacrene D (2.39%), citronellyl propanoate (2.35%), -bisabolol oxide B (2.26%), sabinene (2.00%), -fernesene (1.53%), and limonene (1.21%) were discovered as major components of essential oil (Mohan et al. 2013). According to GC/FID and GC/MS analysis, the volatile oil composition of *S. vaginatum* from their subsurface section had 37 ingredients that accounted for 96.4% of the total volatile part (Chauhan et al. 2012a). The essential oil composition of *S. wallichianum* aerial and root parts from Munsiyari and Nainital region revealed the presence of sabinene (31%), −phellandrene (18.2%), and –bisabolol (16%) as major compounds in the aerial parts, and –phellandrene (34.5%), −phellandrene (11.2%), and sabinene (11.5%) as major compounds in the root. The oil (aerial component) from the Nainital collection included 53.8% 3, 5-nonadiyne, 31.4% sesquiterpene hydrocarbons, and 12.2% monoterpene hydrocarbons, whereas the root oil had a high concentration of the acetylene hydrocarbon 3, 5-nonadiyne (90.5%). The Munsiyari area plant population may represent a novel chemotype of *S. wallichianum* (Joshi, Melkani, Nailwal, Prasad, et al., 2018). The essential oil of *S. tenuifolium* was found to have a significant amount of 3, 5-nonadiyene (94.32%), which may be used in the pharmaceutical business (Rajendra S. Chauhan et al. 2017) (Fig. 20.4).

20.5.2 Terpene Compounds

From the chemical makeup of the oil, a novel sesquiterpene called vaginatin ($C_{20}H_{30}O_4$) was isolated from the roots of *S. vaginatum* (Mesta et al. 1968). Additionally, a novel ester terpene I was isolated from *S. carvifolium* root (Joshi, Melkani, Nailwal, Prasad, et al., 2018) (Srivastava et al. 2019) (Fig. 20.5).

20.5.3 Coumarin Compounds

Selinidin (I), a novel coumarin having diuretic activity, was isolated from *S. vaginatum*. Its chemical formula is $C_{19}H_{20}O_5$. When selinidin was treated with aqueous alkali (10%) media, it produced selinidin (II) and tiglic acid in low yields, but when treated with alcoholic alkali medium, it completely hydrolysed to produce selenitin and tiglic acid in equimolar amounts (Seshadri et al. 1964). Numerous coumarin compounds, including oroselol, angelicin, and selinitin, were identified from *S. vaginatum*. Selinidin's hydrolysis product was selinitin (Seshadri and Sood

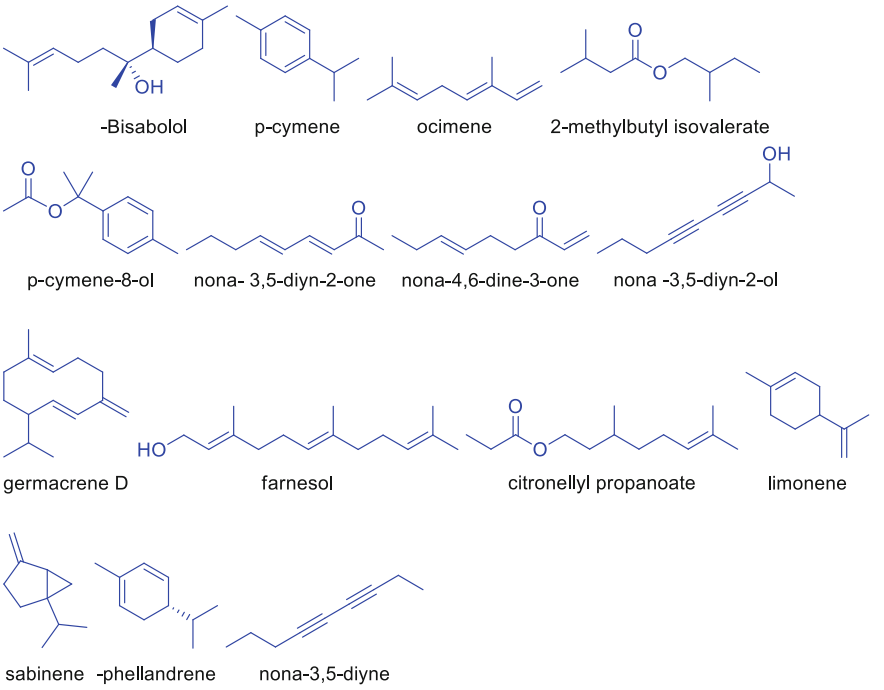
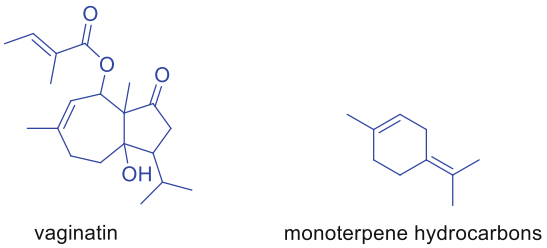


Fig. 20.4 Chemical structure of different volatile compound present in various *Selinum* sp.

Fig. 20.5 Chemical structure of different terpene compound present in various *Selinum* sp.



1967). Two novel coumarin components, selinidin and vaginidin, were identified from *S. vaginatum*, as well as three recognised coumarin compounds, angelicin, oroselol, and lomatin. Selinidin and vaginitin have the chemical formulas C19H20O5 and C19H22O6, respectively (Rao et al. 2006). Three furanocoumarins, bergapten, heraclenin, and heraclenol, were identified from the roots of *S. tenuifolium*, as well as another coumarin from the same source, ST-1, which was shown to be a combination of imperatorin and 8-geranyl oxypsoralen by examination of several reaction products (Adityachaudhury et al. 1974) (Fig. 20.6).

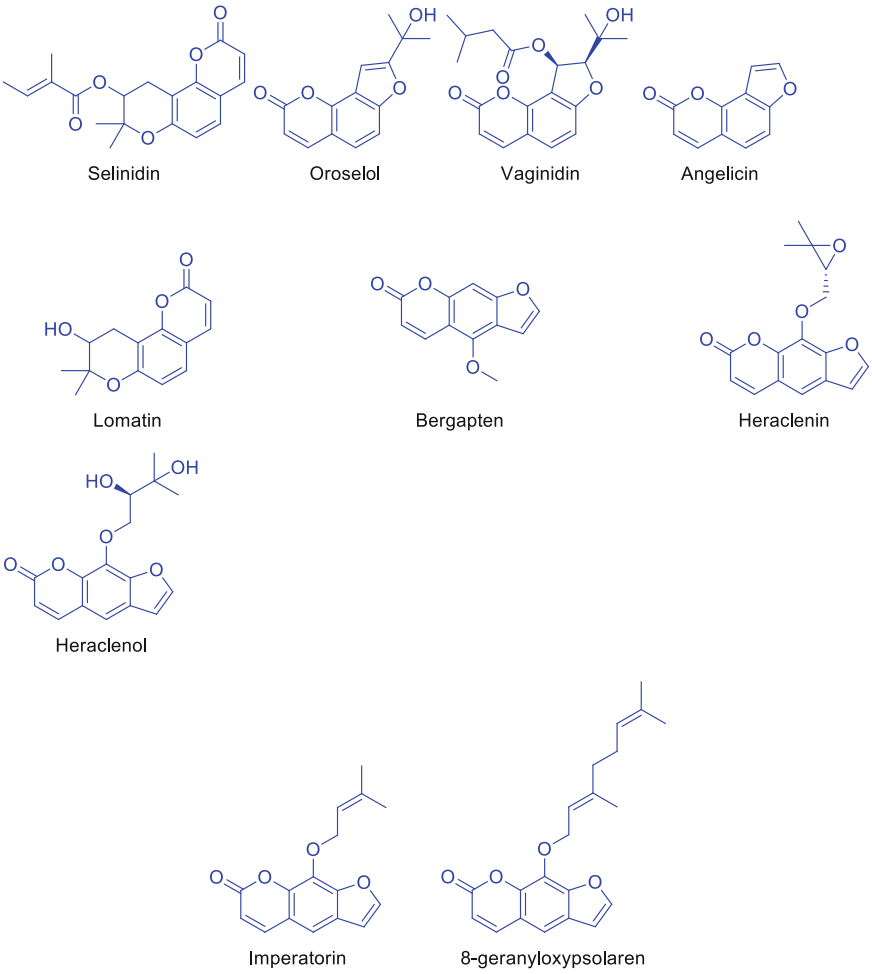


Fig. 20.6 Chemical structure of different coumarin compound present in various *Selinum* sp.

20.5.4 Phenolic Compounds

The phenolic profile and antioxidant activity of *S. vaginatum* showed the presence of antioxidant polyphenolics termed chlorogenic acid and ferulic acid in the methanolic extract, as well as a hydroxyl-cinnamic acid derivative (Pandey et al. 2013). *Selinum vaginatum* rhizome had a significant amount of valerenic acid (Vandelook et al. 2007) (Fig. 20.7).

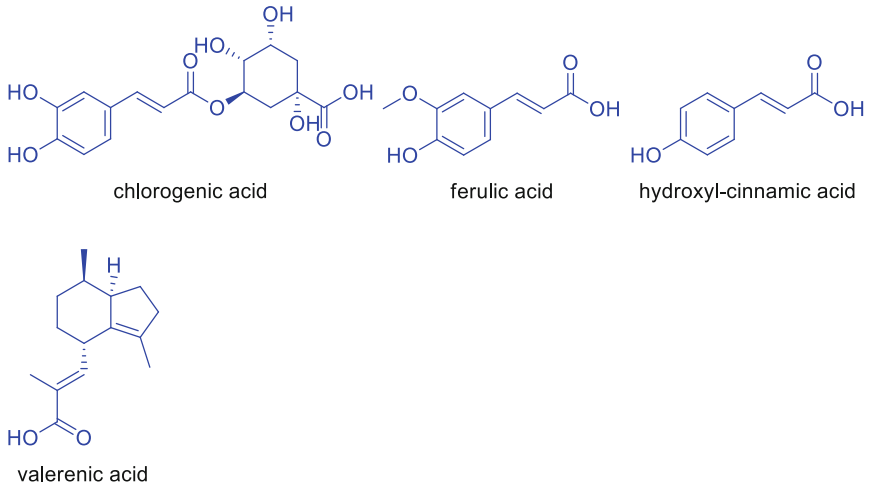


Fig. 20.7 Chemical structure of different phenolic compound present in various *Selinum* sp.

20.5.5 Other Compounds

Two new diacetylene glycosides, bhutkesoside A and bhutkesoside B, are described, along with ten previously described compounds, falcarindiol, chlorogenic acid, 5-O-p-coumaroylquinic acid, 3,5-di-O-caffeoyl-quinic acid, 4-hydroxy-7-methoxy-phenylethanol, ferulic acid, dehydro 3,5-nonadiyne was discovered in the roots of *S. tenuifolium* Wall. (Dev et al. 1984). For the first time, the chemical constituents of *S. cryptotaenium and S. vaginatum* were isolated, including umbelliferone, osthole, coumarin, (+)- heraclenol, longshengensin A, anomalin, ferulic acid, galactitol, stearic acid, melissic acid, lingoceric acid, valerenic acid by using precise high efficiency thin layer chromatography (TLC) (Vineet et al. 2011). The non-volatile compounds cnidioside A, a benzofuran derivative; quercetin 3-O-D-glucopyranoside, rutin, and 4′-O-methylquercetin 3-O-D-glucopyranoside; two coumarin derivatives scopoletin and umbelliferone; a phenylpropene derivative eugenol 4-O-D-glucopyranoside (Srivastava et al. 2010) (Fig. 20.8).

20.6 Traditional Uses

Essential oils derived from fresh leaves and blossoms of *S. tenuifolium* have been utilised as scent additions in culinary, medicinal, and cosmetic products (Kumar Joshi et al. 2016). The genus is antispasmodic and diuretic. *S. tenuifolium's* smoke has been used to kill or repel insects. *S. tenuifolium* aerial portions were shown to be stimulating and carminative, while the roots' essential oil had antimicrobial and antibacterial effects (Chauhan et al. 2012b). Various traditional herbal treatments

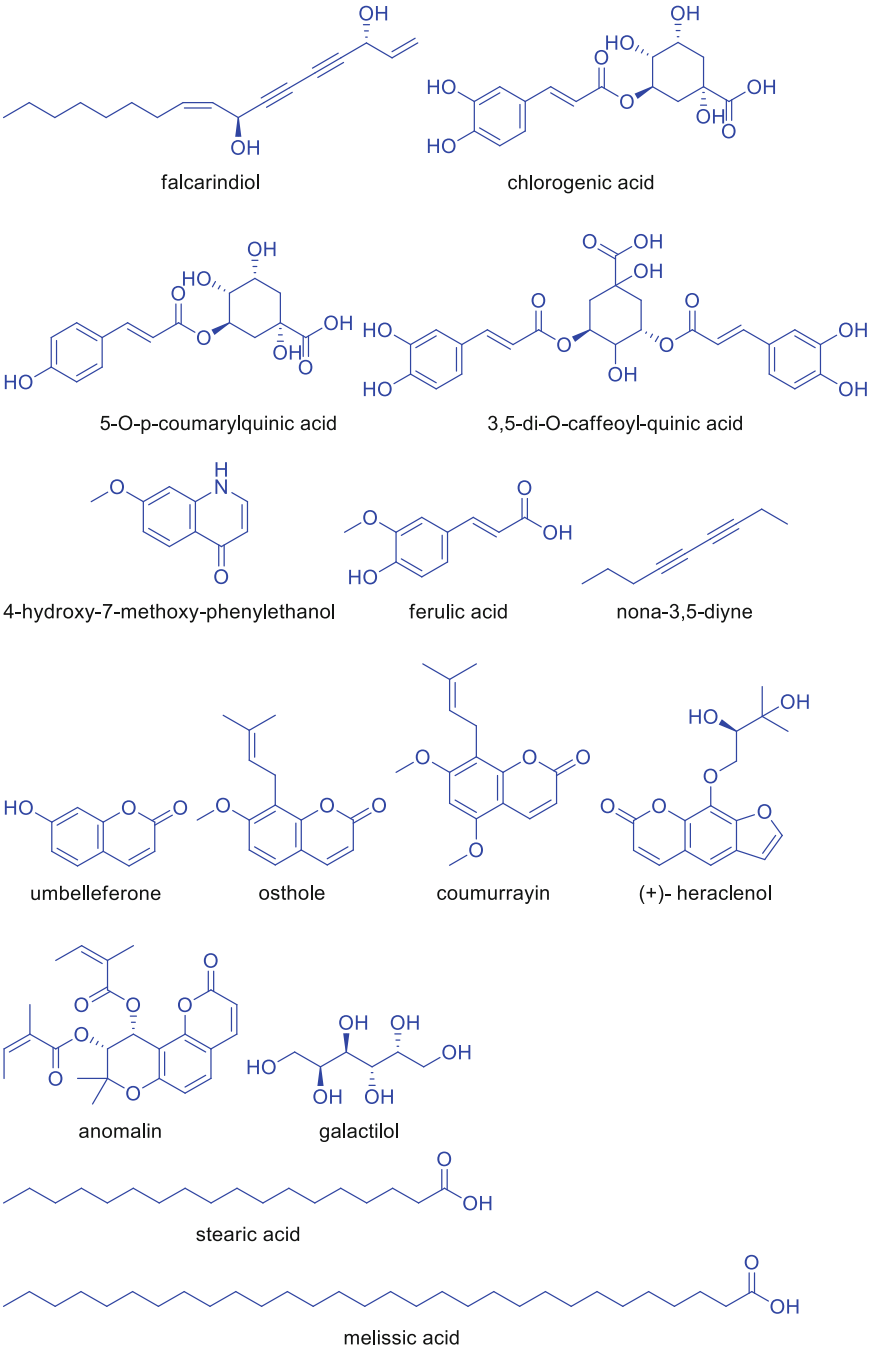


Fig. 20.8 Chemical structure of other compounds present in various *Selinum* sp.

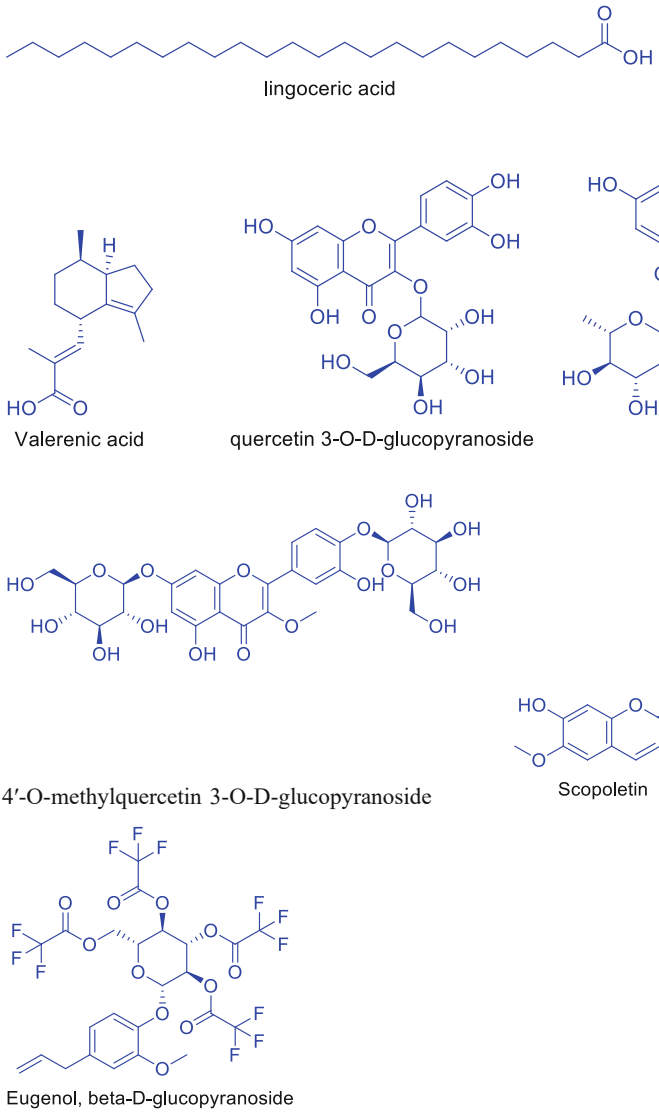


Fig. 20.8 (continued)

have employed *S. vaginatum* to treat neurological problems such as epilepsy, hysteria, syncope, convulsions, and mental weakness (Pandey et al. 2013). Other Selinum species have been used to treat a variety of ailments, including root decoctions for diarrhoea, cuts and wounds, fever, stomach-ache, and vomiting. A blend of root powder and mustard oil has been used to treat postpartum oedema in women (Mohan et al. 2013). *S. wallichianum* roots were traditionally used to cure bodily discomfort, fever, cough, and cold, while the flower and leaves were used to

heal stomach aches, cuts, and wounds. *S. wallichianum* has historically been used to make incense and as a remedy for common maladies such as coughs, colds, fevers, wounds, stomach-ache, and toothache among others (Srivastava et al. 2018; Singh and Toppo 2012). The root oil of *S. tenuifolium* was hypotensive, fragrant, sedative, and analgesic, and the entire plant or root was used as a nervine sedative and given as an alternative for *Nardostachys jatamansi* DC (Vineet et al. 2011). *S. wallichianum* seeds, leaves, and roots were employed in the creation of Ayurvedic medicine by pharmaceutical companies and as a spice and fodder by rural residents (Saraswat et al. 2020a). The roots of *S. cryptotaenium* have been used to make the Chinese traditional medicine "Qian-Hu", which is said to cure a variety of diseases including coughs, bronchitis, and asthma (Rao et al. 2006). The roots of *S. vaginatum* were used to make an aromatic medicine that was used to fumigate dwellings (Srivastava et al. 2019; Pandey et al. 2013).

20.7 Pharmacological Properties

Species of Selinum belonging to the family of Apiaceae is an important aromatic plant from its medicinal point of view (Srivastava et al. 2018, 2019). Different parts of the plants mainly leave and roots are used in different diseases such as inflammation, diarrhoea, vomiting, cuts, and wounds (Mohan et al. 2013). In Ayurvedic and Unani system of medicine, the different species of Selinum has been used as sedative, antispasmodic, bitter tonic, antiepileptic, in menstrual and digestion-related problem and as an insecticide from ancient time (Pandey et al. 2013). The essential oil derived from this plant possesses analgesic, hypotensive, leucodermal, and sedative properties (Saraswat et al. 2020a).

Antimicrobial activity: Bhoj Raj Singh et al. in their studies determined the antimicrobial activity of essential oil of *S. wallichianum*. In their studies, they determined the minimum inhibitory concentration of essential oil of *S. wallichianum*, which was extracted from both leaf and tender stem extracts on two reference strain of E. coli and 94 other bacterial strains which were isolated from food items, environment, and clinical cases. From their experiment they found that the essential oil of *S. wallichianum* possess some potent antimicrobial properties, also *S. wallichianum* can be used in the treatment of food poisoning, gastrointestinal infection, wound infection, and pyrexia (Singh and Toppo 2012). Shandesh et al. from their studies reported that the root extract of *S. wallichianum* can inhibit the growth of *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis,* and *Staphylococcus aureus*. The antibacterial property was determined by using disc diffusion method (Bhattarai et al. 1970).

S. vaginatum also possesses some potent antibacterial properties (Saraswat et al. 2020a). Joshi et al. in their experiment examined the antibacterial properties of essential oils of *S. vaginatum* by determining minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) in disc diffusion method against different strains of bacteria. From their experiment they found that, the essential oil

collected from the aerial parts and roots of *S. vaginatum* possesses significant antibacterial properties. Joshi et al. further stated that, the antibacterial property was mainly due the presence of γ-terpinene, β-pinene, (2E, 6E)-farnesol, elemol, and (2E, 6Z)-farnesol, which had the ability to inhibits *Erwinia chrysanthemi*, *Bacillus subtilis*, *Enterococcus faecalis*, *Klebsiella pneumonia*, and *Salmonella enterica* (Joshi et al. 2018a).

Antioxidant activity: S. vaginatum possesses some potent antioxidant activity (Saraswat et al. 2020a). Pandey et al. stated that the methanolic extract of roots of *S. vaginatum* contained a significant amount of phenolic content due to which it showed good antioxidant properties. Because of this antioxidant properties, *S. vaginatum* have been useful in the treatment of neurological disorders such as epilepsy, syncope, and hysteria (Pandey et al. 2013). *S. tenuifolium* (synonym: *Ligusticopsis wallichiana* (DC.)) shows significant antioxidant properties. Due to the presence of chlorogenic acid and 3,5-di-O-caffeoyl-quinic acid *S. tenuifolium* shows good antioxidant activity against DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical (Adhikari et al. 2016). Also, due the presence of rutin and quercetin 3-O-β-D-glucopyranoside in leaves of *S. tenuifolium*, it shows significant free radical scavenging properties (Devkota et al. 2018).

Antifungal activity: Along with other activities *S. vaginatum* also possesses significant antifungal activities (Saraswat et al. 2020a). Kumar et al. determined the antifungal activity of essential oil from roots of *S. vaginatum*. By using agar dilution method, the minimum inhibitory concentration (MIC) of essential oil of *S. vaginatum* was determined against *R. solani*, *S. sclerotiorum, A. tenuis*, *F. oxysporum*, and *C. graminicola*. From their findings they found that essential oil of *S. vaginatum* inhibit the growth of fungi in a dose-dependent manner. *C. graminicola*, *S. sclerotiorum*, and *A. tenuis* were completely inhibited by the essential oil of *S. vaginatum*, while *R. solani* and *F. oxysporum* inhibited up to some extent by this essential oil (Kumar et al. 2019).

Neuroprotective activity: Essential oil extracted from *S. vaginatum* possesses some potent neuroprotective activities (Srivastava et al. 2018). Thiagarajan et al. from their experiment stated that the essential oil of *S. vaginatum* have the ability to improve the neural damage caused by the methylmercury. In their research, they used rat brain to study the fraction of mitochondria. Coadministration of essential oil, restored the low level of glutathione caused by methylmercury. Due to its chelating effect against oxidative stress, essential oil of *S. vaginatum* have the ability to protect the neuronal damage caused by methylmercury. The catalase activity and the restoration of the low GSH levels was determined by MTT assay (Thiagarajan et al. 2018).

CNS protective activity: Due to the presence of significant amount of phenolic content, *S. vaginatum* shows non-selective mono-amine oxidase activity. Anuj Kumar et al. from their experiment stated that the ethanolic extract of rhizome of *S. vaginatum* showed good CNS activity in experimental animal models. From their findings they revealed that the administration of ethanolic extract significantly reduced the anxiety in animal models, as well as it possesses potent CNS depressant, muscle relaxant, sedative and hypnotic properties. Ethanolic extract of rhizome of

S. vaginatum shows significant inhibition of the central and peripheral activities and also reduces the behavioural activities in experimental animal models (Kumar and Khan 2020).

Antidiabetic activity: Due to the presence of chlorogenic acid in *S. vaginatum*, it shows potent antidiabetic and neuroprotective activity. Chlorogenic acid possesses strong antioxidant properties; hence, it has a strong ameliorative action on diabetes as well as diabetic neuropathy. Nikita Saraswat et al. from their studies stated that chlorogenic acid improved lipid profile, locomotor changes, and liver damage caused by diabetic neuropathy. Chlorogenic acid restored the pain caused by neuropathy, by decreasing the oxidative stress, release of inflammatory cytokine, TNF-α (tumor necrosis factor-α), nitric oxide levels, and conditions caused by apoptosis. Chlorogenic acid also restored the levels of Na^+K^+ATPase pump, which was impaired due to diabetic neuropathy. Thus, *S. vaginatum* shows potent antioxidant and antidiabetic properties (Saraswat et al. 2020b).

Other activities: Due to the presence of alpha-pinene, beta-pinene, and bornyl acetate, *S. vaginatum* shows good anticonvulsant activities, insecticidal and inhibition seed germination activities (Pandey et al. 2013). Srivastava et al. stated that the roots of *S. vaginatum* showed good anti-inflammatory properties (Srivastava et al. 2010). Also, the roots of *S. vaginatum* possesses significant hypotensive, sedative aphrodisiac and analgesic effects (Sharma et al. 2004). The whole plant of *S. vaginatum* possesses good diuretic, antispasmodic activities and are used in different neurological disorders (Pandey et al. 2013, Saraswat et al. 2020a).The whole plant and roots of *S. wallichianum* possesses leucodermal, sedative, hypotensive, and analgesic properties. Also, it is useful in the treatment of fever, cough, stomach pain, and common cold (Joshi et al. 2018b; Vineet et al. 2011).

20.8 Conclusion

Numerous medicinally active Selinum species are accessible worldwide. Phytochemicals derived from these plants are being described and investigated for their biological activity in order to see if they may be beneficially employed to cure a variety of diseases. These plants may include a variety of antibacterial, antifungal, insecticidal, antioxidant, repellent, antifeeding, and anti-inflammatory activities. This review article discusses the botany, pharmacology, pharmacognostic and medicinal qualities, and applications of natural chemicals identified in Selinum spp. in India. Additionally, it emphasises the plant's significance in the Indian medicinal system for its potential for exploitation as a potent drug, as well as the current status of Selinum spp. through a comprehensive summary of various natural compounds, uses, and recent findings from research into its processing, phytochemistry, and pharmacology. The plant is being utilized as a substitute for *Nardostachys jatamansi*, a member of the Caprifoliaceae family (Pandey et al. 2013). While several papers on the anatomy, morphology, and chemistry of *S. vaginatum* exist, *S. wallichianum* seems to be less studied. However, the recent report on a new

species of *S. wallichianum* expands the possibilities for species identification, opening the way for the resolution of the status of other unsettled Selinum species. Previous phytochemical and pharmacological research demonstrate that the numerous chemicals extracted from different portions of the Selinum plant maintain a variety of pharmacological properties, including antibacterial, antioxidant, antidiabetic, and anti-inflammatory properties. The presence of numerous alkaloids, coumarins, essential oils, flavonoids, and polyacetylenes, among other constituents, indicated that additional research is necessary to gain a better understanding of the detailed biochemical and physiological mechanisms underlying Selinum spp. therapeutic effect. Although more than 20 compounds have been separated and identified utilising various separation methods and procedures, these compounds have not been subjected to substantial or in-depth pharmacological investigations. The majority of pharmacological research have employed crude extracts of the plant produced using organic solvents. Due to the unknown chemical ingredients employed in this research, it is impossible to repeat the findings. As a result, bioassay-guided identification is required to localise bioactive chemicals. More advanced study is required on their chemistry and pharmacological effects in vivo and in vitro. Additionally, additional research is necessary to provide more effective and compelling evidence for the species' other traditional usage in the treatment of a variety of illnesses and disorders, including dysentery, diarrhoea, and irregular menstruation. The provided data, together with the phytochemistry and pharmacology potential of this plant, elucidates the therapeutic potential of Selinum spp., which may be employed as an alternative medicine for a variety of serious human disorders. There is relatively little information available on the pharmacological activity associated with or allocated to a specific chemical component. It should be noted at this point that no clinical studies have been done so far, and hence no dosage administration has been established. Simultaneously, no toxicity or adverse impact has been recorded as yet. The current research provides an overview of the applications and pharmacological activities of Selinum spp. in India, and although these activities may support the plant's medicinal potential, clinical studies are required to demonstrate that potential.

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Chapter 21
Swertia chirayita (*Roxb. ex Flem.*): Chirayata/Chiretta

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21.1 Introduction

Indigenous herbal plants have been utilized for millennia around the world and have a significant contribution in the prevention, management, cure, and providing relief from several ailments in humans. They can be used as potential and prospective grounds to cure various diseases, either as traditional arrangements, practices, or as pure active principles, and for the vast majority of individuals in the developing and industrialized world, medicinal plants and traditional herbal medication systems are the only way of disease prevention and healing. The availability and usage of appropriate pharmaceuticals is one of the prerequisites for the advancement of the primary healthcare system. In the primary healthcare system, traditional medicine remains the most affordable and accessible source of treatment. Because of their safety, efficacy, ease of availability, and lack of side effects, medicinal plants, and herbal supplements have grown in popularity throughout the world (Sewell and Rafieian-Kopaei 2014). *Swertia chirayita* is a type of herb that has been employed as a hepatoprotective in traditional medicine. The utilization of these plants in the form

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of crude medications is much sought after throughout the subcontinent (nearly 400 tons per year), and this demand is expected to grow by more than 10% per year (Dey et al. 2020).

Swertia is a genus in the Gentianaceae family which contains approximately 140 species and all of them are annual and perennial herbs. They are commonly used in a variety of herbal preparation. In the subcontinent, there are nearly 40 species of Swertia that have been identified, with *Swertia chirayita* being the most valuable for its therapeutic potential. *S. Chirayita* was first characterized by Roxburgh in 1814 as *Gentiana chyrayta*. *S. chirayita*, also known as "Chiretta," threatened with extinction, is a medicinal plant that usually has its habitat at high elevations from Kashmir to Bhutan in the Himalayan sub-temperate sections (between 1200 m and 2100 m) (Bentley and Trimen 1880; Clarke 1885). This indigenous *S. chirayita* is mostly better known for its bitter aftertaste, which can be attributed to the occurrence of numerous chemical elements including swerchirin, swertiamarin, amarogentin, (most bitter substance identified to date), and other biologically active chemicals that are unswervingly related to the health benefits of people (Joshi and Dhawan 2005). It is an antimicrobial agent that is effective against both Gram-positive and Gram-negattive bacteria. The whole plant can be utilized as an astringent, also acts as a heart tonic, liver tonic, and used for the treatment of cough, sore throat, scanty-urine, dropsy, melancholia, sciatica, and skin disease in Unani literature. *S. chirayita* is sometimes applied as a tonic having a bitter taste in gastrointestinal (GI) diseases such as anorexia/dyspepsia. This plant is said to be a digestive, laxative, and malaria preventative, which is especially effective in fever. In addition, the plant is helpful for intestinal parasites, body scorching, bronchial asthma, and bowel regulation. As a result, *S. chirayita* has attracted the attention of a growing number of researchers. Despite its numerous medical benefits, particularly as a potent and powerful anti-herb, this plant has not received the attention it deserves in the commercial arena. It could be because of a shortage of some well-established standards or doses of medications developed from it, accompanied by trust issues in such medication over commercially recognized brands (Dey et al. 2020).

21.2 Taxonomy

Swertia L. (Gentianaceae) is a taxonomically distinct but morphologically diverse genus. *Swertia chirayita* (Roxb. Ex Fleming) H. Karst is similarly known as *Swertia chirayita* Buch.-Ham., *Ophelia chirayita* Grisebach, *Agathotes chirayita* Don., and *Gentiana chirayita* Roxburgh (Sharma et al. 2011). The taxonomic classification is as follows (Aleem and Kabir 2018).

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida

(continued)

Order	Gentianales
Family	Gentianaceae
Genus	Swertia
Spicies	Chirayita
Bionomial name	*Swertia chirayita*

21.3 Common Names

Chirata, having the trade name Chiretta, is an ancient Ayurvedic drug that is also known as "Nepali neem" due to its prevalence in Nepalese forests (Patil et al. 2013). It goes by a variety of names, indicating its widespread use. However, the common names of this plant are as follows (Sharma et al. 2011).

1. English: Chirata (Indian Gentian).
2. Hindi: Chirayata, Charaita, Chirata.
3. Arabic and Farsi: Qasabuzzarirah.
4. Marathi: Charayatah, Chirayita.
5. Tamil: Anariyatittam, Nilavembu, Shirattakuchi.
6. Malayalam: Kiriyatta, Kiriyattu, Nilaveppa, Uttarakiriyattu.
7. Telegu: Nelavemu.
8. Kannada: Nelabevu.
9. Urdu: Chiraita shireen.
10. Deccan: Charayatah.
11. Sanskrit: Anaryatika, Ardhatika, Bhunimba, Chiratika, Haima, Jvarantaka, Kairata, Kirata, Naditika, Naipala, Neplanimba, Nidrari, Ramasenka, Sannipatha, Sutiktaka, Tikta, Trinanimba, Viktaka, etc.
12. Bengali: Chireta.

21.4 Flowering/Fruiting Season

Chirayita has several miniatures, opposing, axillary, lax cymes placed as little branches, and the complete inflorescence is 2 ft. long. While the plant blooms during July and September, it is harvested and processed for the pharmaceutical industries. Seed set occurs between October and November. The seeds grow quickly after dropping and after 15 days of freezing treatment, around 91% of the seeds are fertilized, although another study found a maximum of 81% fertilization. Chirayita's post-germination growth phase is slow (Nadeem et al. 2020). It is noteworthy to add that, if seeds are collected before November, their viability is very low. Seeds collected after October are reported to have low viability. Flowering typically occurs between July and October, with fruiting occurring between August and December (Sharma et al. 2011).

21.5 Distribution

Swertia chirayita is native and indigenous to North India, commonly found in the hilly and rocky locations, and the Indians value it highly as a medicine and healing element. However, the literature that were discovered citing the *Swertia chirata*'s unique traits are not very consistent. Chirata has been described as an annual by a number of researchers and a biennial or pluri-annual by others (Patil et al. 2013). The plant thrives in open, wet areas, and forest openings. It can also be found on open land and in recently burned woodlands. The abundance of this plant species is not uniform; rather it is influenced by elevation and inclination. On the south-facing slopes, it can grow up to a height of 3000 m, and on the north-facing slopes, it grows under 1500 m. *S. chirayita* grows best in acidic soil at a pH range between 4.7 and 5.5, and it has been found growing alongside other species. In general, a range of 2000 m altitude is the most preferable. The plant is indigenous to the temperate Himalayas, where it can be found at elevations ranging from 4000 to 10,000 ft. (1200–3000 m) distributed from Kashmir to Bhutan, as well as in Khasi hills at an elevation of 4000–5000 ft. (1200–1500 m). This plant may also be found in sub-temperatue climates at elevations ranging from 1500 to 2100 m (Sharma et al. 2011). Figure 21.1 illustrates the *Swertia chirayita*'s regional distribution in relation to medicinal plant markets in India.

21.6 Morphology

The morphological characteristics of *Swertia chirayita* have both qualitative and quantitative features (Tables 21.1, 21.2, and 21.3). *S. chirayita* is an annual/biennial herb with a cylindrical intermediate part, and a quadrangular upper end with a prevalent decurrent line at each angular position, as well as a stiff stem (Kumar and Van Staden 2016). It has a luminous tap root scheme, along with the key root narrowing and mounting to a limited remoteness, and countless secondary and tertiary roots developing across the anterior end. Lots of small flowers are produced in leafy panicles that abide the axillary or terminal bunches. The calyx is composed of four equal-sized everlasting sepals that unite at the base and thus are green, trapezoidal with a sharp tip, shorter than the corolla but extending as the capsule expands. This plant has a solitary upright stem that is spherical in the lower portion and quadrangular in the top serving, especially in the branches, with a noticeable recurrent line at each angular position and a color that varies from dark green with a purple hue in the lower section to light green with and without a purple hue in the upper section, with densely light yellow pith (Raina et al. 2013). Transecting the stem reveals a single-layered epidermis that is outwardly coated by a thick-lined cuticle which is evident in the stem at an early phase and stays intact and unchanged in the adult epidermis. When cells develop, they flatten and laterally elongate. Four ribs too have parenchymatous cortical cells and epidermis, and also an endodermis

Fig. 21.1 *Swertia chirayita*'s regional distribution in relation to medicinal plant markets in India (Cunningham et al. 2018)

with anti-clinal or peri-clinal walls. Cells are cortical, and resin containing microscopic droplets of oil is present as a deep brown aggregate in particular cortical cells. There are a lot of little blooms with green-yellow panicles that are colored purple and have green or white hairs (Joshi and Dhawan 2005). The plant calyx comprises four lobes, and the corolla contains four twisted, and overlaid lobes connected at the bottom by a couple of nectaries covered in long hairs. The ovary is unilocular, with laminal placentation, parietal ovules, and a couple of stigmas. Capsules are oval in shape with two valves and a yellowish pericarp. The seeds of *Swertia chirayita* are many, small in size, and dark brownish in hue (Chandra et al. 2012). Figure 21.2 illustrates the whole plant, leaves, flowers, and seeds of *Swertia chirayita.*

Table 21.1 Qualitative morphological features of *Swertia chirayita* (Raina et al. 2013)

Qualitative characteristics	
Plant part	Features
Plant characteristics	Annual, biannual, or pluri-annual, erect herb
Root	Simple, yellowish, tapering, with an oblique or geniculate shape. Fracture is short, complete, and splinter. It is uneven because of the irregularly broken surface and roughness.
Stem	Erect, with a spherical outline below, highly branched, and quadrangular upwards. The lower region is rusty brown or purple brown, while the upper section is greenish yellow or dark green. The texture is uneven and rough.
Leaf	Lanceolate, alternate, without stalks, acuminate, chordate at the lower region, sessile, and 5–7-nerved. The texture is smooth.
Inflorescence	Flowers are produced in axillary or terminal clusters of 3–5 flowers in a leafy panicle.
Flower	Bracteates, pediculate, actinomorphic, bisexual, tetramerous, bright green on the outside and purple on the inside.
Fruit	Capsule egg-shaped and green colored, with yellowish pericarp and compacted by a single side containing frequent seeds.
Taste	All parts of the plant (from root to tip) are extremely bitter.

Table 21.2 Morphological features (quantitative) of *S. chirayita* plant (Raina et al. 2013)

Quantitative characteristics		
Plant part	Value	
Height in cm	93.4 ± 0.9 (64.6–142.3)	
Root length in cm	18.5 ± 0.2 (14.0–23.4)	
Leaf	Length in cm	Breadth in cm
Morphotype I	11.5 ± 0.3 (9–14 approx.)	4.5 ± 0.1 (3–5 approx.)
Morphotype II	13.2 ± 0.3 (11–14 approx.)	6.0 ± 0.2 (5–6 approx.)
Morphotype III	10.6 ± 0.3 (9–13 approx.)	4.5 ± 0.1 (3–5 approx.)
Morphotype IV	14.4 ± 0.4 (10–17 approx.)	5.9 ± 0.1 (5–6 approx.)

Table 21.3 Morphological features (quantitative) of *S. chirayita* flower (Raina et al. 2013)

Flower	
Diameter (mm)	9.5 ± 0.2 (8.5–10.0)
Length (mm)	6.3 ± 0.2s (6.0–7.0)
Sepal length (mm)	4.0–5.2
Sepal breadth (mm)	1.0–2.0
Petal length (mm)	5.2–6.4
Petal width (mm)	2.7–3.2
Capsule length (mm)	4.5 ± 0.2 (3.8–5.1)
Capsule width (mm)	1.3 ± 0.1 (1.0–2.0)

Fig. 21.2 (**a**) Plant, (**b**) leaf, (**c**) flower, (**d**) seeds

21.7 Traditional Uses

Swertia chirayita is well-recognized for its bitter taste and has diversity in traditional medicinal purposes. Various medical uses of this plant are documented in Indian, British, and American pharmacopoeias. It is a beneficial plant in Ayurvedic medicines and is used as a blood cleanser, febrifuge, anthelmintic, carminative, digestive, expectorant, antidiarrhoeic, antiperiodic, and laxative, as well as for flatulence, skin infections, malarial fever, gout, bronchial asthma, and other ailments (Raina et al. 2013). This plant act as an antimicrobial agent which is very effective against both Gram-positive and Gram-negative bacteria. The herb has an extensive list of advantages in Unani literature as well. It has been described as a liver and heart tonic, astringent, bronchial asthma reliever, and skin disease healer. The herb is frequently applied as a bitter tonic, particularly for the management of fevers and gastrointestinal diseases such as dyspepsia or anorexia; it is also thought to be quite useful in the

Table 21.4 Traditional uses of *S. chirayita*

Plant part	Traditional uses
Whole plant	Employed in a variety of old-style medicinal treatments, i.e., Ayurveda and Unani system of medicine (Joshi and Dhawan 2005).
Whole plant	The leaves along with the sliced stems are supposed to be soaked in water overnight for healing headaches and high blood pressure. One glass of water is used to make a paste, which is then filtered. The preparation should be drunk once in a day for up to 3 days (de Rus Jacquet et al. 2014; Malla et al. 2015).
Whole plant	*S. chirayita* is divided into small pieces and cooked in half liter of water. Once the volume becomes less than half a glass, filtrates are shifted into a glass jar, and provided to the patient (children) once a day for 2 days. In case of adults, one spoon daily for at least 2 days, then 3–4 times a day until they are cured (de Rus Jacquet et al. 2014).
Whole plant	One cup of decoction is boiled in water and administered orally for Malaria treatment (Shah et al. 2014).
Whole plant	Paste of Chirayita can be used to heal skin disorders like eczema and acne (Malla et al. 2015).
Plant root	It is taken as a medicine and tonic for the treatment of fever, cough, cold, joint pain, weakness, and asthma (Joshi and Dhawan 2005).

treatment of malaria (Aleem and Kabir 2018). Table 21.4 shows the traditional uses of *S. chirayita.*

21.8 Phytochemistry and Nutritional Composition

S. chirayita is well-known for its strong medicinal and pharmaceutical properties. It contains a high concentration of alkaloids and flavonoids, many of which have wide-spectrum actions. During the past two decades, several investigations on the isolation, characterization, identification, and structural identification of active compounds of *Swertia chirayita*, as well as their biological and pharmacological interactions, have been performed (Sharma et al. 2011). *S. chirayita*s are biologically active due to the presence of a wide spectrum of pharmacological bioactive substances from various classes. The active biological compounds present in the *S. Chirayita are* xanthones and its derivatives*,* flavonoids, alkaloids, terpenoids, secoiridoids, and many other substances including chiratin, palmitic acid, oleic acid, and stearic acids. The first identified and isolated dimeric xanthone of this medicinal herb was chiratanin, which was found in various parts of the plant. The bitter elements in this plant are chiratin and ophelic acid. Chiratanin has a higher fraction, and when steamed with hydrochloric acid (HCl). Chiratin and ophelic acid have never been found in crystalline form. Ash of *Swertia chirayita* contains potassium, magnesium, calcium phosphates, and carbonates. Tannin is almost absent in these plant parts. The yield of essential oil from this plant is approximately 0.240 g/kg. Because of the presence of different chemical compounds (swerchirin, swertiamarin, and amarogentin), the bitter taste of this medicinal plant is predominant that makes it

more popular (Kumar and Van Staden 2016). The leaves of *Swertia chirayita* are reported to contain a vast amount of microelements such as Zn (about 7%), Cu (about 2.0%), Mn (about 5%), Fe (about 85%), and Co (about 9%). On the other hand, the presence of the macromolecules in the leaves of *Swertia chirayita* are also abundant as reported as Na (about 30.0%), K (about 92%), Ca (about 20%), and Li (about 4.0%) (Negi et al. 2010a). There are approximately 77 constituents of this plant's essential oil that are related to the categories of aldehyde, alcohol, acid, ester, and ketone as well as various hydrocarbons. Of all these phytoconstituents, ketones are recognized as a major element nearly 30%. Furthermore, camphor, buten, heptadecanone, ethenylcyclohexenone, and geranylacetone act as the main ketone compounds in this plant. The alcoholic groups of compounds are the second most important chemical group in this plant, accounting for 27.45% of the total. This plant contains more than 20 xanthones. Cedrol is by far the most prevalent alcoholic component, but terpene alcohols such as β-eudesmol, patchoulol, p-cymen-3-ol, farnesol, and linalool have also been found in *Swertia chirayita* (Seher et al. 2020). Figure 21.3 presents the structures of key secondary metabolites present in *S chirayita.*

21.9 Pharmacology and Clinical Studies

Pharmacological properties of *Gentianacece* plants are well-known.The most valuable, important, and significant Swertia species is *Swertia chirayita* (Brahmachari et al. 2004). It is an important and traditional plant that has been noted in pharmacological codexes, traditional medical arrangements, and numerous pharmacopoeia (Arya et al. 2011). The numerous ethnobotanical applications of this plant have prompted a number of pharmacological studies. Earlier studies have shown that *Swertia chirayita* extracts have a broad range of biological effects, including antibacterial, antifungal, antiviral, anticancer, anti-inflammatory, antidiabetic, and antioxidant properties (Arya et al. 2011; Chen et al. 2011; Alam et al. 2009; Verma et al. 2008). Running at the same time, the pharmacological effects of this medicinal plant have long been evaluated using a variety of in vivo and in vitro studies. *Swertia chirayita* aqueous, alcoholic, and methanolic extracts offer a variety of outstanding pharmacological effects, according to laboratory investigations. Table 21.5 shows the biological activities of *Swertia chirayita.*

The therapeutic properties and pharmacological properties are attributed to bioactive compounds. Pant et al. (2000) identified 43 constituents, whereas Brahmachari et al. (2004) identified 48 constituents, which include tetraoxygenated xanthones, xanthone glycosides, terpenoids, secoiridoid glycosides, and alkaloids. However, Joshi and Dhawan 2005 identified 40 constituents. Among these phytochemicals, xanthones and its derivatives, as well as secoiridoid glycosides, have been discovered significant pharmacological properties, including antidiabetic properties, antimalarial, hepatoprotective, anti-leishmanial, anti-carcinogenic, antioxidant, anthelmintic, antimicrobial, anti-pyretic, and immune-modulatory activities

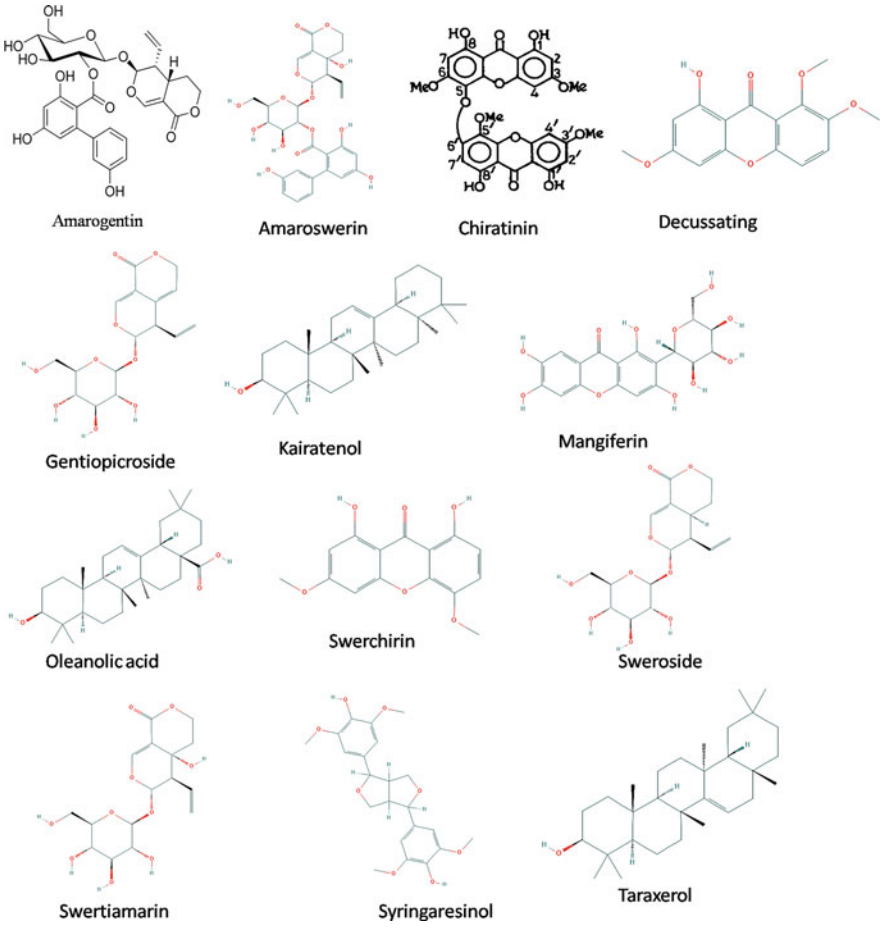

Fig. 21.3 Bioactive constituents of *Swertia chirayita*

(Nagalekshmi et al. 2011; Chen et al. 2011; Kweera et al. 2011; Balaraju et al. 2009; Saha et al. 2004, 2006; Iqbal et al. 2006; Brahmachari et al. 2004; Kumar et al. 2003; Medda et al. 1999; Ray et al. 1996; Saxena et al. 1993). Amarogentin, a secoiridoid glycoside found in the plant, has been reported to have anti-leishmanial and anti-carcingenic properties (Saha et al. 2006). Table 21.6 shows the antioxidant potential of *S. chirayita* after extraction by different solvent extracts. Because of its high content of iridoid glycosides, xanthones, and flavonoids, the plant is well-known for its medicinal and pharmacological effects. Research revealed that swertiamarin is the most bounteous bioactive compound among all other components (Wang et al. 2008). Amarogentin is the most bitter of these compounds, as stated earlier, with anti-proliferative, and proapoptotic characteristics (Saha et al. 2006). These therapeutic herbs' metabolites vary widely depending on geographical, seasonal, environmental, and other conditions. Swertia herbs become more widespread, and as a

Table 21.5 Biological activity of *Swertia chirayita*

Biological activity	Plant part (s)	System and toxicity test	Test organism	Test models	References
Antibacterial	Whole plant	In vitro; no toxicity test	*Klebsiella pneumonia; Pseudomonas aeruginosa; Proteus vulgaris; Escherichia coli*	ATCC 15380 ATCC 25619 ATCC 6380 ATCC 26922	Rehman et al. (2011)
Antibacterial	Whole plant	In vitro; no toxicity test	*Bacillus polymyxa; Staphylococcus aureu; Vibria cholera; Escherichia coli; Salmonella typhi; Bacillus subtilis; Streptococcus pyogenes; Proteus mirabilis; Providentia alkalifaciens; Pseudomonas aeruginosa*	MTCC 3160 MTCC 1927 MTCC 723 MTCC 3216 MTCC 736 MTCC 3906 MTCC 1429 MTCC 783	Laxmi et al. (2011)
Antibacterial	Whole plant	In vitro; no toxicity test	*Staphylococcus aureus*	–	Alam et al. (2009)
Antibacterial	Stem	In vitro; no toxicity test	*Enterococcus faecalis; Staphylococcus aureus; Pseudomonas aeruginosa; Salmonella typhi*	ATCC 14506 ATCC 6538 ATCC	Khalid et al. (2011)

(continued)

Table 21.5 (continued)

Biological activity	Plant part (s)	System and toxicity test	Test organism	Test models	References
				27853 ATCC 14028	
Antibacterial	Stem	In vitro; brine shrimp assay–positive	*Bacillus subtilis; Sarcina lutea; Bacillus megaterium; Staphylococcus aureus; Shigella flexeneriae; Salmonella typhi*	–	Sultana et al. (2007)
Antifungal	Whole plant	In vitro; no toxicity test	*Aspergillus flavus; Cladosporium oxysporum; Aspegillus niger*	MTCC1883 MTCC 1777 MTCC 1881	Laxmi et al. (2011)
Antiviral	Leaves or stem	In vitro; cytotoxicity test negative	*Herpes simplex* virus type-1	–	Verma et al. (2008)
Anti-hepatitis B virus	Whole plant	In vitro; no toxicity test	HepG cells line	HepG 2.2.15	Zhou et al. (2015)
Antimalarial	Leaves or Stem	In vitro; no toxicity test	*Plasmodium falciparum*	FCK 2	Bhat and Surolia (2001),
Anti-leishmanial	Aerial part	In vitro; no toxicity test	*Leishmania donovani*	UR6	Ray et al. (1996)
Anti-leishmanial	Whole plant	In vitro; cytotoxicity test negative	*Leishmania donovani*	AG83	Medda et al. (1999)
Anti-inflammatory	Aerial part	In vivo; no toxicity test			Banerjee et al. (2000)
Anti-inflammatory	Root	In vivo; no toxicity test			Das et al. (2012)
Hypoglycemic	Whole plant	In vivo; no toxicity test			Kar et al. (2003)
Hypoglycemic		In vivo; no toxicity test			

	Whole plant				Alam et al. (2011)
Hypoglycemic	Leaves	In vivo; no toxicity test			Verma et al. (2013)
Antidiabetic	Whole plant	In vitro; no toxicity test	Streptozotocinnicotinamide (STZ-NAD)-induced diabetic albino mice		Grover et al. (2002)
Antidiabetic	Whole plant	In vitro; no toxicity test	Streptozotocinnicotinamide (STZ-NAD)-induced diabetic albino mice		Arya et al. (2011)
Anti-carcinogenic	Whole plant	In vivo; no toxicity test			Saha et al. (2004)
Hepatoprotective	Aerial parts	In vivo; no toxicity test			Nagalekshmi et al. (2011)
Analgesic	Root	In vivo; no toxicity test			Das et al. (2012)
Analgesic	Leaves/ stem	In vivo; no toxicity test			Alam et al. (2010)

Table 21.6 Antioxidant potential of *S. chirayita*in different solvent extracts

Plant part/ parts tested	Test systems: Toxicity test	Models/Test organisms
Whole plant	In vitro; no toxicity test	IC_{50} = 267.8 μg/mL (DPPH); IC_{50} = 1.5 ± 0.2 μg/mL (β-carotene); IC_{50} = 6.5 μg/mL (ABTS); Butylated hydroxy anisole (BHT), and vitamin C (Chen et al. 2011)
Whole plant	In vitro; no toxicity test	IC_{50} = 27.7 μg/mL (DPPH); BHT (Sharma et al. 2013b)
Whole plant	In vitro; no toxicity test	IC_{50} = 222.7 μg/mL (DPPH); BHT (Ahirwal et al. 2014)
Whole plant	In vitro; no toxicity test	IC_{50} = 315.8 μg/mL (DPPH); Gallic acid (Kumar et al. 2003)
Whole plant	In vitro; no toxicity test	IC_{50} = 156.6 μg/mL (DPPH); L-Ascorbic acid (Phoboo et al. 2013)
Whole plant	In vitro; no toxicity test	IC_{50} = 551.3 μg/mL (DPPH); IC_{50} = 557.6 μg/mL (DPPH); IC_{50} = 551.9 μg/mL (DPPH); IC_{50} = 559.1 μg/mL (DPPH); Gallic acid (Kshirsagar et al. 2015)
Leaves	Aqueous	IC_{50} = 86.0 μg/mL (DPPH); butylated hydroxyanisole (BHA); BHT
Whole plant	In vivo; cytotoxicity test negative (Chen et al. 2011)	

DPPH 2,2-diphenyl-1-picrylhydrazyl; *ABTS* 2,2-azino-bis (3-ethylebenzthiazoline-6-sulfonicacid)

result, an excellent standard is required to identify the raw resources for trading and pharmaceutical uses. Table 21.7 shows phytochemical and biological activity of *S. Chirayita*.

An intriguing study found that *S. chirayita* plant extract, which includes various essential phytochemicals, is helpful in controlling insulin release from pancreatic cells in preclinical animals such as mice (Fig. 21.4). In an experimental trial with albino Wistar rats, researchers evaluated the antidiabetic effectiveness of *S. chirayita* plant extract to the usual oral medication glibenclamide (Kavitha and Dattatri 2013). It was determined that the presence of mangiferin in plant stem parts is primarily responsible for the plant's antidiabetic activity.

21.10 Toxicology

Concerns about the safety and efficacy of conventional medicines are critical disputes for the pharmaceutical industries. Some frequently used medicinal plants have been shown in research findings to be mutagenic or cytotoxic, especially when used for an extended period of time. There are mounting evidence on the toxicity effect of crude extracts, and related isolated compound(s) from a number of plant species. Despite a long culture of usage in traditional system of medicine, scientific research on the safety and toxicity of *S. chirayita* is still lacking. However, Chirayita is probably safe in medicinal doses when taken orally. Although, there is no reliable scientific data on the use of Chirayita during pregnancy, breastfeeding, diabetes, or

Table 21.7 Bioactive compounds of *S. chirayita* and their biological activity

Phytoconstituents	Biological activities
Mangiferin	Anti- (viral, inflammatory, diabetic, tumor, HIV, atherosclerotic, Parkinson), immune-modulatory, chemopreventive, and hypoglycemic (Kavitha et al. 2013; Pardo-Andreu et al. 2008; Muruganandan et al. 2005; Kumar et al. 2003; Sánchez et al. 2000)
Swertiamarin	CNS depressant, anti- (cholinergic, bacterial, cancer, hepatitis, atherosclerotic, diabetic, arthritic), cardioprotective (Saravanan et al. 2014; Vaidya et al. 2009; Kumarasamy et al. 2003; Wang et al. 2001; Suparna et al. 1998)
Amaroswerin	Gastroprotective (Niiho et al. 2006)
Amarogentin	Anti- (leishmanial, cancer, diabetic, anthelmintic), topoisomerase inhibitor, gastroprotective (Ray et al. 1996; Medda et al. 1999; Saha et al. 2006; Pal et al. 2012; Phoboo et al. 2013; Niiho et al. 2006)
Swerchirin	Hepatoprotective, hypoglycemic, pro-hematopoietic, chemopreventive, antidiabetic (Hirakawa et al. 2005; Ya et al. 1999; Saxena et al. 1996; Sekar et al. 1987)
Sweroside	Hepatoprotective, hyperpigmentation, osteoporosis, antibacterial, and anthelmintic (Liu et al. 1994; Luo et al. 2009; Jeong et al. 2015; Sun et al. 2013)
Oleanolic acid	Anti- (microbial, tumor, inflammatory) (Jesus et al. 2015; Soica et al. 2014; Liu 1995)
Syringaresinol	Hepatoprotective (Chakravarty et al. 1994)
1-hydroxy-3,5,8-trimethoxyxanthone	Antimalarial (Mandal and Chatterjee 1994)
1,5,8-trihydroxy-3 1 methoxyxanthone	Improves insulin resistance (Ghosal et al. 1973)
Chiratol	Anti-inflammatory (Banerjee et al. 2000)
1-hydroxy-3,5,8-trimethoxyxanthone	Spasmogenic agent, antiulcerogenic (Ateufack et al. 2007, 2014)
Bellidifolin	Hypoglycemic (Basnet et al. 1995)

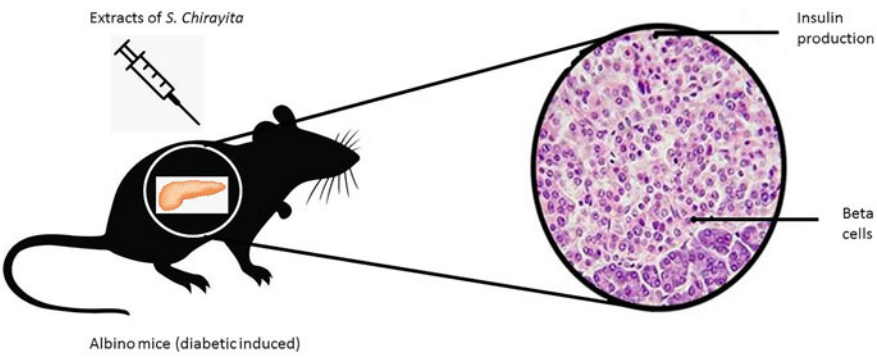

Fig. 21.4 Schematic presentation of regulated insulin production from pancreatic β-cells of *S. chirayita* extract injected mice

stomach ulcers. Medda et al. (1999) conducted a clinical study and as consequence he was not able to find any evidence of toxicity in liposomal aspects of *S. chirayita*. Furthermore, in order to examine the safety of this plant, extensive research are required to characterize the toxicological effects including toxicity and mutagenesis. Even so, laborious drug trials comprising diverse appliances are still required to verify and ensure the safety standards of *S. chirayita* in traditional medicine for its effective and safe applications (Kumar and Van Staden 2016). Regardless of the fact that the benefits of medicinal herbs are generally acknowledged, a greater understanding of safety evaluation is required to discern between harmful effects and pharmacological importance of the extracts (Aremu and Van Staden 2013).

21.11 Synthetic Strategies for Key Secondary Metabolites

Pal et al. (2018) conducted an experiment to compare the greenhouse phenomenon with *S. Chirayita* plants grown by tissue culture (Fig. 21.5). They discovered that the production of *S. Chirayita* plants was poor when compared to field-grown crops as a result of the physiological, functional, and biochemical discrepancies among the plants produced in photoautotrophic versus photoheterotrophic forms of nutrition. To identify the critical molecular elements involved in secondary metabolite formation, comparative transcriptomes of this plant were generated. On the other hand, correlational transcriptomes of chiretta disclosed differentially regulated transcription factors and ABC-type transporters reported to be involved in secondary metabolism. Furthermore, for functional identification and classification, the NCBI

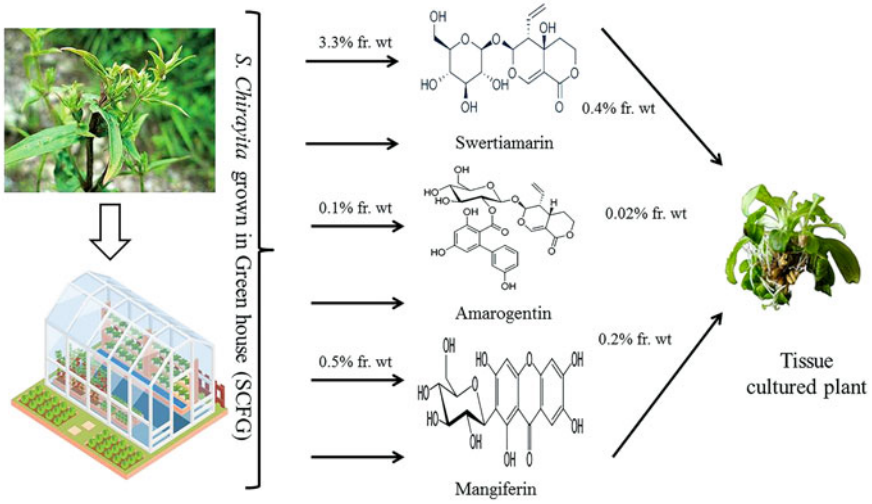

Fig. 21.5 Major secondary metabolites content in *S. chirayita* plants grown in the greenhouse (photoautotrophic, SCFG) and tissue culture (photoheterotrophic)

Biosystems database was used accordingly. They postulated that the structural elements involved in *S. chirayita* secondary metabolite synthesis are involved in various modes of nutrition (photoautotrophic vs photoheterotrophic).

Kumar et al. (2014) established a well-organized procedure for plant regeneration. In *Swertia chirayita*, he attempted a process involving direct and indirect organogenesis. Specimens cultivated on Murashige and Skoog (MS) plant growth medium with 0.5 mg/L 2,4-D and 0.5 mg/L Kinetin exhibited 84% of callusing, and 1.0 mg/L 6-benzyladenine (BA) in combined effect with 100 mg/L Adenine sulfate +0.1 mg/L indole acetic acid (IAA) was outstanding for an optimum vegetative growth (12.69 ± 1.30) formation within 4 weeks. Direct organogenesis produced the greatest number of shoots (7.14 ± 0.99) per leaf explant. The MS media added with 0.1 mg/L IAA, gave a maximum frequency of rooting (11.46 ± 1.56) per leaf explant. The shoots that were rooted finely were further transplanted to plastic containers filled with sand, gravel, and soil rite before being shifted to the greenhouse for additional and much-improved care, growth, and development. Using high performance liquid chromatography (HPLC), four main secondary metabolites were examined, characterized, and quantified. Secondary metabolite levels were observed to be substantially greater in the in vitro explants than in vivo explants and callus grown from *Swertia chirayita*.

The initially found secondary metabolites of Swertia species are xanthones. The characteristics, structure, and formation of xanthones are very much comparable to the flavonoids, as well as to the chromatographic properties. Even though flavonoids are very common in nature, xanthones are discovered in a few families. Gentianaceae and Guttiferae are the only families that have them. Xanthones are occasionally seen as the mother polyhydroxylated components; however, the majority of xanthones are either mono methyl ethers or poly methyl ethers, or glycosides (Hostettmann and Miura 1977). Unlike iridoids, xanthones do not really appear to exist in all Gentianaceae plant species. Hostettmann-Kaldas et al. (1981) conducted a thorough research to support this. Organic xanthones were largely been obtained from 150 plants of four families: Moraceae, Polygalaceae, Guttiferae, and Gentianaceae. Vieira and Kijjoa (2005) reported almost 300 natural xanthones out of a total of 515 xanthones. Xanthones recovered from other larger plants seem to be connected mostly with Clusiaceae family (consists of 55 species; 12 genera) and Gentianaceae family throughout this time period (comprising 28 species; 8 genera). Table 21.1 shows the isolated chemicals and biological activities of Swertia species. The six major categories of xanthones isolated from nature are: (a) simple xanthones, (b) xanthone glycosides, (c) xanthone olignoids, (d) prenylated xanthones, (e) bis xanthones, and (f) miscellaneous xanthones. They have been also classified as per the extent of oxygenation as non-, mono-, di-, tri-, tetra-, penta-, and hexa-oxygenated (Demirkiran, 2007). Swertia species have also yielded xanthones and their glycosides.

In *S. macrosperma, S. chirayita, S. mussotti, S. connata*, and S. *cordata,* mangiferin is the most frequent C-glycoside. Swertianolin (xanthone-o-glycosides) obtained from *S. japonica* and *S. ciliata* is also described accordingly (Plouvier et al., 1967). Norswertianolin-1-o-glucosyl-o-glycoside was the first xanthone

O-glycoside which was isolated from *S. perennis* (Hostettmann and Wagner 1977). Brahmachari et al. (2004) gathered the separated chemical ingredients, ethnopharmacology, biological activity, and pharmaceutical uses of Swertia species from the literature review. Xanthones in *Swertia chirayita*, *Swertia speciosa, and Swertia paniculata* were examined by High performance liquid chromatography (HPLC) (Negi et al. 2010a). Minerals may play a variety of functions in plant and human health depending on their concentrations. Nine (9) minerals such as zinc, copper, manganese, iron, cobalt, sodium, potassium, calcium, and lithium in the *Swertia chirayita and Swertia speciosa* was determined by atomic absorption spectrometry (Negi et al., 2009, 2010b). *Swietenia macrophylla, Rhus coriaria, and Rhus typhina* also included kaempferol, catechin, epicatechin, and polyphenols, which were separated and identified. (Kossah et al. 2010; Falah et al., 2008). *G. senegalensis* extracts are high in flavonoids and have anti-inflammatory properties (Sombie et al. 2011). *Swertia chirata's* stem and leaves demonstrated high antibacterial activity against Gram-negative and Gram-positive bacteria (Alam et al., 2009).

This plant's roots have been discovered to accumulate a variety of secondary metabolites; however, extracting these tissues and plant parts is harmful. As a result, the hairy root technology caused by *Agrobacterium rhizogenes* has lately gained interest and has occupied a powerful opportunity for practical research in producing biopharmaceutical lead compounds. For different biotechnological applications, the hairy root cultures are required to expand on a much larger scale (Guillon et al. 2006). Initiatives are undertaken to standardize the *A. rhizogenes* altered root cultures for the generation of active secondary metabolites of *S. chirayita* in vitro (Keil et al. 2000). Hairy root technology, as well as numerous parameters impacting the synthesis of root biomass and bioactive chemicals, is essential for the commercialization and market expansion of *S. chirayita* adventitious roots and explaining the potential for profitable deployment. Consequently, micro-propagation in a controlled setting will aid in the prevention of existing plant biodiversity conservation issues caused by the overharvesting of wild populations. This should considerably enhance the quality of bioactive secondary metabolites of *S. chirayita* as an ancient medicinal plant.

21.12 Cultivation, Harvesting, and Processing

The plant can thrive in a wide range of soil types, especially sandy clay that is high in carbon and humus. It can be grown in sub-temperate climates at elevations ranging from above 1500 to 2100 m. Nursery beds are prepared in carefully selected areas with favorable weather conditions. During the months of May and June, seeds are sown. After 3–4 months, seedlings are transplanted in rows 45–60 cm apart in the field. Weeding and hoeing must be done on a regular basis in the gardens and fields. It is crucial for *Swertia chirayita* as a medicinal plant to be cultivated in the natural environment such as manures, nourishments, or pesticides should not be a chemical

source. Organic fertilizers such as farm yard manure, vermicompost, and green manure can be utilized depending on species' needs. Nursery beds and filed after plantation might be irrigated depending on needs (maybe weekly or fortnightly). The plants are watered until they bloom. Sowing for propagation is usually done in the spring when the temperature is not higher than 10 ° C and in the humus-rich soil. When the seedling is large enough to handle, they are separated and planted in different pots or containers. The young plants are replanted outside in the early summer. *Swertia chirayita* is typically harvested just as the seeds start to appear and then sun-dried for later use. If the plant is harvested after the seeds are matured, it can regenerate naturally. Harvesting without regard for plant age and seed maturity reduces regeneration significantly (Patil et al. 2013). Harvesting takes place from November to December, and all harvesting is done by hand, with no instrumentation. The entire plant is removed, washed with running water to remove soil particles followed by sun drying for a few days or oven dried for few hours at 80 °C (Bhatt et al. 2006). Aside from fresh leaves, a few other popular chirayita products including essential oil and dried leaves (freeze-dried, sun-dried, and mechanically dried) might be produced from *Swertia chirayita*. To minimize discoloration, oxidation, and browning, chirayita leaves can be dried over two pieces of paper. Chirayita dried leaves are ground into a fine powdery form using a mortar and pestle or a high-capacity grinder. Cold maceration is used to create a powder extract with 70% ethanol, which is then concentrated in a rotary evaporator at a temperature of 45 °C and elevated pressure. The Soxhlet apparatus and 70% ethanol are also used to create an extract of shaded dried leaves. Ethanol extract of dried leaves is condensed in a water bath followed by lyophilization at 55 °C to get solid extract (crude). This extract needs to be stored in the desiccators for further use. However, the yield of Chirayita is reported to be 1.40% (weight/weight) (Nagalekshmi et al. 2011).

21.13 Tissue Culture

Micro-propagation, cryopreservation, the use of appropriate bioreactor programs that enable advanced plant tissue culture processes, mass production, and the conservation of these vulnerable plants can all help to improve the conservation scenario for *S. chirayita*. In recent years, micro-propagation, an in vitro effective culture method, has attracted considerable attention as one of the most practicable way for a large amount of propagation of such threatened and vulnerable medicinal plants. Quick micro-propagation by incorporating shoot tip explants from in vitro growing seedling was attempted by Balaraju et al. (2009) to facilitate the prolonged production and ex situ conservation of *Swertia chirayita*. Biotechnology opens up new possibilities for promoting biodiversity and biotechnological strategies through micro-propagation approaches to develop genetically unvarying plants for the Swertia industrial sectors (Fig. 21.6). Thus, it is expected that the advancement of well-organized micro-propagation methods and protocols may assure an ample supply of *Swertia chirayita* plants free of environmental restrictions, resulting in a

Fig. 21.6 *Swertia chirayita* (E) shoot reproduction and multiplication in the plant tissue culture systems (Kumar and Van Staden 2016)

reduction of uncontrolled harvesting pressure of *S. chirayita* wild populations. In the current years, in vitro propagation applying somatic embryogenesis has received a great deal of appreciation as the most important system for the rapid growth of uniform plants for exploration and investigation as well as its prolonged production. The procedure is ideal for the creation of artificial seeds of *Swertia chirayita* as a supplement of the natural seeds. Table 21.8 presents the different tissue culture studies and micro-propagation data for *Swertia chirayita*.

21.14 Formulation and Market Products

Currently, the extract from *S. chirayita* has been effectively used during the treatment of diabetes and other associated ailments in the form of diverse formulations. The powdery formulation of the substance is often employed as an ingredient of ayurveda tonic'sudarshan churna,' which is utilized in herbal medicine to treat all types of fevers (Dey et al. 2020). This plant is commonly available in the market throughout the world in the form of dried powder, leaves, and seeds. People frequently buy these products for their health benefits. People in the subcontinent use powdered and dried leaves as a home remedy for a variety of diseases, including fever, gastric disorders, liver function, blood sugar and diabetes, skin problems, and so on. These products are not only sold in stores, but they are also widely available in

Table 21.8 Micro-propagation for *Swertia chirayita*

Tissue culture	Explant type	Optimal concentration	Remarks
Regeneration	Seed(s)	3.0 μMBA	Shoot(s) regenerated from rot explants (Wawrosch et al. 1999)
Regeneration	In vivo stem with node	0.5 μMBA +4.7 μMKN	Regeneration took place from nodal explants (Chaudhuri et al. 2007)
Regeneration	Seeds	2.2 μMBA +2.2 μMKN +0.5 μMNAA	Regeneration was observed from the culture of immature seeds (Chaudhuri et al. 2009)
Micro-propagation	In vitro; axillary bud or shoot apices	0.5 mg/lBA + 1.0 mg/L GA_3	Rapid propagation (Ahuja et al. 2003)
Axillary multiplication	Nodal explants (seedling-derived)	4.0 μMBA +1.5 μM 2iP	Shoot proliferation should be improved (Joshi and Dhawan 2007)
Direct shoot Multiplication	In vitro; *S. chirayita* leaves	2.2 μMBA +11.6 μMKN +0.5 μM NAA	Plant propagation (Chaudhuri et al. 2008)
In vitro regeneration	Node	2.0 mg/L BA	Plant propagation (Koul et al. 2009)
Shoot organogenesis	In vitro; *S. chirayita* root	4.4 μMBA +1.1μMNAA	Plant regeneration (Pant et al. 2010)
Callus culture	In vitro; *S. chirayita root*	13.3 μMBA +0.9 μM 2,4 D	Regeneration of plant (via indirect organogenesis) (Pant et al. 2012)
Efficient regeneration	In vitro; shoot tip	0.5 mg/L BA +1.0 mg/L GA_3	Proliferation of shoot (Kumar and Chandra 2013)
In vitro flower production	In vitro; *axillary* bud	1.0 mg/L BA +70 mg/L adenine sulfate	Flowering for plant regeneration (Sharma et al. 2013a)
Somatic embryogenesis	In vivo; *S. Chirayita* leaves	0.5 mg/L 2,4-D + 0.5 mg/L KN	Regeneration of plant (Kumar and Chandra 2014)
Regeneration (direct & indirect)	In vivo *S. chirayita* leaves	1.0 mg/L BA +100 mg/L adenine sulfate +0.1 mg/L IAA	Plant regeneration took place through organogenesis (direct and indirect) (Kumar et al. 2014)

popular online stores and websites these days. Table 21.9 shows the available formulations of *S. chirayita* in the market.

21.15 Conservation

Plant resource destruction and large-scale deforestation have become a common practice in modern times. Nowadays, the hustle of extinction caused by human intervention is believed to be more than a hundred times quicker than the usual

Table 21.9 Available formulations of *S. chirayita* in the market

Product type	Formulation	Intended use	Country of origin
Powder	Dried powder	Diabetes; liver function	India
Mother tincture Q	Homeopathic medicine	Hepatitis; gastric complaints; skin disorders	India
Luminous facial serum	*Swertia chirayita* extract; Ascorbic acid 2-glucoside; hydrolysed hyaluronic acid (Primalhyal 50); Brightenyl	Ultra-hydrating facial serum	USA
Seed	*Swertia chirayita* seeds	Improves liver function; anti-parasitic; anticancer	India
Leaf	*Swertia chirayita* dried leaves	Detox liver; antioxidant; anti-inflammatory	India
Leaves	Dried leaves	Fever; constipation and stomach upset; loss of appetite; intestinal worms	India

rapidity of extinction (Chapin III et al. 2000). Countless medicinal and herbal plants, including *S. chirayita*, are now on the brink of extermination as a result of urbanization in the Himalayan region. Because of its numerous applications, *S. chirayita* is predominantly marketed and utilized as a traditional medication; nevertheless, demand from both local and global trading is increasing, resulting in significant harvesting and over-collecting of wild habitats. This phenomenon has resulted in a drastic reduction of *S. chirayita* populations in the Himalayan region. However, a serious concern is the absence of detailed data on the annual collection and trade of this plant.

The conservation status of *S. chirayita* has been termed as critically endangered by International Union for Conversation Nature (IUCN) (Joshi and Dhawan 2005). National plant board of India (NPBI) has identified *S. chirayita* as one of the most highly ranked medicinal plants in the subcontinent. The consequences of the extinction of these plants include both the deletion of genes crucial for evolution, growth, and plant development or manufacture and synthesis of novel components, as well as the destruction of potentially novel substances with pharmacological or nutraceutical value. Cultivation must be increased to cope up with the ever-rising demand for raw plants in domestic and foreign markets. It is expected that the introduction and implementation of appropriate micro-propagation procedures will assure enough supply of *S. chirayita* that will be supposed to be free of environmental restrictions, and, as a result, there will be less spontaneous harvesting demand on wild populations. A number of researchers indicated that somatic embryogenesis, micro-propagation, and acclimatization procedures are capable of producing a large number of unvarying *S. chirayita* clones all around the time (Kumar and Chandra 2013, 2014; Kumar et al. 2014). Table 21.7 provides the micro-propagation protocols for *S. chirayita* that have already been planned and carried out utilizing various explanting strategies.

S. chirayita can only be found in a single habitat and at a specific height (up to 3000 m). It is critical to preserve its nesting sites, despite the fact that there have been numerous management (in situ*)* issues, i.e., unauthorized harvesting, grazing, stealing, and fodder collection. As a result, this is critical to educate the local population about the importance of sustainable harvesting of this plant. Establishing strict monitoring and management of native areas with the help of local residents, as well as promoting cultivation on personal land, community forests, and other vacant fields, might also aid in the preservation of this plant. Rest of the factors that help initiatives include the formation of local groups of users to monitor conservation and consciousness-building campaigns to manage and control the collection and conservation of *S. chirayita*, as well as assisting local users and groups in monitoring the native trade network to safeguard efficient and reasonable trade and gathering information about future demand and trade perspective. The establishment of a *S. chirayita* farmers' cooperative will also aid in the promotion of this plant's cultivation by facilitating seed altercation, information and facts sharing, and cooperative bulk trade (Phoboo and Jha 2010).

Many secondary metabolites accumulate in plant roots, but extracting these organs is harmful. As a result, *Agrobacterium rhizogenes*-triggered hairy root technique has lately gained courtesy and has occupied a novel stage in fundamental research in the generation of pharmaceutical lead phytoconstituents. The extensive and significant expansions of root cultures (hairy) are critical for biotechnological processes (Guillon et al. 2006). Efforts were completed to systematize and optimize the *A. rhizogenes*-modified root cultures for the in vitro synthesis of *Swertia chirayita* bioactive secondary metabolites (Keil et al. 2000). Hairy root technique, as well as many other factors influencing the synthesis of root biomass and bioactive chemicals, are necessary for commercialization of *Swertia chirayita* adventitious roots and determining their economic potential. Overall, micro-propagation in a controlled environment will aid in the prevention of present plant biodiversity preservation issues caused by overharvesting of wild habitats and can significantly progress the superiority of this age-old medicinal plant's bioactive secondary metabolites.

21.16 Conclusion

In developing countries such as India and Bangladesh, modern herbal healthcare industries are still working on new inventions and new formulations. The bulk of regularly used medications in contemporary and traditional medicine are obtained from plants, as it is well-established. When compared to conventional medicine, herbal medicines made from various medicinal plants are far safer, with fewer or no adverse effects. Several active and natural components derived from *Swertia chirayita* provide relief from a number of common illnesses such as flu, fever, cough, diarrhea, ache, vomiting, and so forth. *S. chirayita* has a lot of potential in both traditional and modern medicine. It appears to be a promising herbal therapeutic

approach for a variety of ailments. Up to this point, the major toxicity of *S. chirayita* or any side effects of *S. chirayita* have not been documented; nevertheless, additional toxicological investigations are required to validate *S. chirayita's* safety in human subjects. But, still, more investigation is necessary, particularly to assess its pharmacological and in vivo (biological) activities, as well as mutagenic and toxicological qualities, to further corroborate the suitability of numerous plant-derived chemicals. Clinical trials are also required to establish the efficacy as well as to confirm the treatment doses for using *S. chirayita* in medicinal fields. The demand for *S. chirayita* is increasing over time in the domestic and international markets due to its numerous applications in human welfare. As a result, enhanced manufacture of *Swertia chirayita* bioactive components will be accessible for drug manufacture at a lower cost. Over-exploitation and deforestation have led to a substantial decrease in the plant's easy availability. Any research that is intended to carry out the commercialization process and focuses on the conservation and sustainable supply of raw materials for this critically endangered plant must be considered with utmost priority. Strict policies should be put in place to prohibit local collectors, traders, and herbal drug industries from extracting wild plants. Furthermore, in nearby future, hairy root techniques might be employed as a prototype and instruments to enhance the beneficial phytochemicals of *S. chirayita. S. chirayita* will hopefully stimulate their usage in modern medicine in the coming days, while fresh biotechnological techniques are required for further conservation.

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Chapter 22
Taxus wallichiana Zucc.: The Himalayan Yew

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22.1 Introduction

Taxus wallichiana Zucc. (commonly described as the Himalayan Yew) is a gymnosperm, coniferous tree that grows in various conditions including the temperate and tropical submontane of the Himalayas at an altitude of 900 m. Locally in the regions of India, Nepal, and China, the Himalayan Yew is believed to have many health-promoting benefits such as immune boosting, hepatoprotective, anticancer, anti-inflammatory, and antibacterial among others (Sinha 2020). Several parts of the Himalayan Yew tree are therefore used in tea, ointments, or as ingredients in different cuisines for its remedial effects (Juyal et al. 2014), resulting in greater interest in understanding the composition of the plant and the role of the bioactive compounds in fighting diseases.

The Himalayan Yew has been shown to contain hundreds of chemical constituents. Some of those are taxoids, terpenoids, phytosterols, lignans, organic acids, alcohols, and a range of linear and branched alkanes (Khan et al. 2006). Of those, taxol is a cyclodecane shown to have anticancer properties. Taxol, which was

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initially identified in *Taxus brevifolia* (also known as Pacific Yew), is a well-sought-after chemical for the manufacture of chemotherapy drugs. Due to the wide deforestation of the Pacific Yew in Northern America and the slow regeneration of the tree, other sources of taxol are therefore valuable. The Himalayan Yew is an underestimated plant and has a lot of potential to bring to the health, pharmaceutical, and food industry. This chapter discusses the latest discoveries about the Himalayan Yew and its usability in pharmacology and clinical trials.

22.2 Taxonomy, Distribution, and Morphology of Plants

The *Taxus* trees are endemic to the North Hemisphere. Its low rate of growth, shrubby appearance, and bright colored fruits rendered them an ideal candidate for low maintenance and decorative hedges in many parts of North America, Europe, India, China, and Japan (Khan et al. 2006). *Taxus wallichiana Zucc*, specifically, is an Indian species that is found in the Himalayan region. Also described as an evergreen tree, the Himalayan Yew has been discovered in other parts of the Himalayas including the East Himalayas with moist temperate zones, North-East India, Nepal, Pakistan, and Western Bhutan (Purohit et al. 2001; Pant and Samant 2008; Nimasow et al. 2016).

The Himalayan yew has been known for its miraculous properties of medicinal uses from historical time. A major advantage of the early use of the Himalayan Yew as a home remedy is the fact that it is not poisonous. Unlike the European Yew (*Taxus baccata*), the Himalayan Yew does not contain the toxic chemical taxine (Khan et al. 2006). Therefore, the exploration of the use of the Himalayan Yew in Ayurveda and Traditional Chinese Medicine has been comprehensive. For example, a broth of the young stem of the Himalayan Yew is believed to be effective in treating a range of mild discomforts such as headaches, unstable heart rate, cold limbs and extremities, or gastrointestinal aches. The extract from Himalayan Yew leaves is thought to have anti-inflammatory properties and is often used by villagers suffering from mental and psychological challenges. It was recently shown that the water extract from the Himalayan leaves sedates the central nervous system of mice (Malik et al., 2019; Rana et al., 2014). Table 22.1 represents the ethnobotanical and traditional uses of *Taxus wallichiana* Zucc.

The genus *Taxus* has gained significant attention because it produces taxol, an alkaloid diterpene metabolite, which is also known as paclitaxel. Originally discovered in the Pacific Yew, paclitaxel has been proven to be a successful candidate in fighting various cancers but is mostly combatting breast, ovarian, and lung tumors (Khan et al., 2006). While Wall (1995) reported several taxoids of diverse structural types showing promise as an anticancer remedy, they also revealed that Taxol is an alkaloid which is used in combination or monotherapy (Baloglu and Kingston 1999). In other words, up to a thousand kilograms of taxol would be needed annually and this would equate to between three and four million kilograms of trees being cut down for pharmaceutical purposes. Evidently, the large-scale deforestation of Yew

Table 22.1 Ethnobotanical and traditional uses of *Taxus wallichiana* Zucc. Himalayan yew

Region	Local name	Ethnobotanical uses	Reference
Asi ganga sub basin, Uttarakhand, India	Thuner	Bark and seed extract with warm water is given orally for treatment of internal wound	Nand and Naithani (2018)
Urgam valley, Chamoli Garhwal, Uttarakhand,	Thuner	Bark extract is used as tea for treatment of high blood pressure.	Singh et al. (2019)
Nanda Devi biosphere reserve, Uttarakhand	Thuner	Bark is used as a substitute of tea. The powdered bark is used for the treatment of cold.	Tiwari et al. (2010)
Kedarnath wildlife sanctuary, Garhwal Himalayas, Uttarakhand, (34)		Bark and bark paste used for the treatment of fractured bones, headache, breast piles	Bhat et al. (2013)
Niti Valley, Uttarakhand,	Thuner	Dry powder of bark with salt and ghee is mixed with water to make tea and used for treatment of high blood pressure and cancer. Paste of bark with egg yolk is used as plaster for treatment of fracture	Phondani et al. (2010)
Jakholi block,	Thuner	Juice of leaves are used for the treatment of boils, cuts, and wounds	Tiwari et al. (2010)
Mornaula reserve forest, Kumaon, Uttarakhand, IndiaBark,	Thuner	Oil and leaves are used for treatment of cancer. Bark is also used as fuel	Singh and Thakur (2014)
Shimla Manali wildlife sanctuary, Himachal Pradesh	Thu/Barmi	Tincture from young shoots is used for treatment of headache, giddiness, feeble, and falling pulse, diarrhea, and severe biliousness. Leaves are antispasmodic and used for treatment of nervousness, hysteria, epilepsy, and stones	Singh and Thakur (2014), Tiwari et al. (2010)
Manali wildlife sanctuary, Himachal	Rakhal	Barks and leaves are used for treatment of cancer, swelling, and as a contraceptive	Rana and Samant (2011)
Pabbar Valley, Himachal	Thuna	Tea prepared from needle and bark is used for treatment of congestion and cough	Rana and Samant (2011)
Mandi and Hamirpur district, Himachal	PradeshRakhala/ Talispatra	Tea prepared from barks and leaves is used to treat asthma. Bark is used for the treatment of cancer	Kumari et al. (2018)
Churah subdivision, district Chamba, Himachal Pradesh,	India Nadgaun/ Brahmi	Bark is used as flavoring agent	Kumari et al. (2018)
Shimla water catchment sanctuary, Himachal Pradesh, India	Rakhal	Leaves used to cure cancer. Bark used for preparation of tea	Kumari et al. (2018)

(continued)

Table 22.1 (continued)

Region	Local name	Ethnobotanical uses	Reference
Kathua, Jammu and Kashmir, India	Barmi	Decoction of leaves is used to cure asthma, bronchitis, cough, indigestion, and epilepsy	Singh and Thakur (2014)
Ganderbal, Kashmir, India tea prepared from boiling bark in water is used for cure of asthma, giddiness, arthritis, tumor growths.(47)		Tea prepared from boiling bark in water is used for cure of asthma, giddiness, arthritis, and tumor growths	Phondani et al. (2010)
Bangus valley, Kashmir, India	Postul	Tea made from bark is used to cure sickness in winter	Purohit et al. (2001)
Bandipora, Jammu and Kashmir, India	Postul/Brammi	Bark extract is made into a tea and is used for curing of asthma, headache, giddiness, tumor growths	Purohit et al. (2001)
Galliyat, NWFP, Pakistan	Bermi	Decoction of stem is used for treatment of tuberculosis	Rana et al. (2019)
Neelam valley, Muzaffarabad, Pakistan	Birmi	Tea from leaves is used to cure asthma and high fever	Rahman et al. (2013)
Kel, Pakistan		Decoction of bark is used for treatment of cancer	Rahman et al. (2013)
In India		Use of young shoots in Ayurvedic drug to prepare a curative tint for the cure of biliousness, diarrhea, headache, hysteria, nervousness, and is applied in steam baths to take care of rheumatism	Rahman et al. (2013)
Shogran valley, Pakistan		Plants are used for the treatment of cancer, cardiac disorders, headache, renal disorders, and digestive disorders. The plant is antispasmodic, purgative, and antirheumatic	Kumari et al. (2018)
Manaslu, Sagarmatha, and Kanchenjunga region, Nepal		Used for treatment of cancer and jaundice	Kumari et al. (2018)
In northern India	Unani medicine (Zarnab—a supply of drug)	Extracted from its leaves and bark approved as an aphrodisiac, sedative, and as a treatment for epilepsy, bronchitis, snake bites, asthma, and scorpion stings	

trees became unsustainable and has been enlisted as endangered species (Kumari et al. 2018).

22.3 Pharmacology

Due to its high commercial value and potential medicinal applications, there has been increasing demand for profiling and understanding the bioactive compounds present in the Himalayan Yew. Taxol, which probably is the most valuable biomolecules from a pharmaceutical point of view, has been shown to be mostly present in the leaves and barks of the Himalayan Yew (Kovacs et al. 2007). Additionally, metabolites extracted from the leaf, bark, and heartwood of the Himalayan Yew contain a range of bioactive compounds with antibacterial and antifungal properties (Nisar et al. 2008). Throughout the years, bioactive compounds have been characterized as phenols, polyphenols, tannins, saponins, anthraquinones, alkaloids, and steroids (Prasain et al. 2001).

22.3.1 Drug Composition/Properties

22.3.1.1 Anti-Inflammatory and Analgesic Activities

Inflammation occurs as a response to injury that protects our body from further damage. It involves the accumulation of cells and exudates from the injured tissue ensuring maximum protection (Yuan et al. 2006). Since antiquity, whole plants, parts, or their extracts have been used as anti-inflammatory remedies and the Himalayan Yew is one such plant that possesses numerous bioactivities including anti-inflammatory and immunomodulatory activities. The preceding section contains the previously published reports that provide pieces of evidence on the bioactive potential of the Himalayan Yew.

Qayum et al. (2012) have conducted anti-inflammatory studies against several isolated compounds from the bark extract of the Himalayan Yew by implementing both in vitro (lipoxygenase inhibitory assay) and in vivo approach. The results revealed a significant anti-inflammatory activity from each compound while tasumatrol B revealed an outstanding activity. Besides, while the results showed anti-inflammation and anticancerous potential they did not demonstrate any activity towards the in vitro lipoxygenase inhibitory assay (Qayum et al. 2012).

In another study, Khan et al. (2011) have isolated the compound—taxusabietane A showed considerable anti-inflammatory potential in lipoxygenase (LOX) inhibition assay with an IC_{50} value of 57 ± 0.31 μM. The obtained IC_{50} value was comparable with the standards used (baicalein: 22.1 ± 0.03 μM). Also, taxusabietane A significantly ($p < 0.05$) reduced edema induced by carrageenan at doses of 5 and

10 mg/kg, suggesting promising anti-inflammatory activity and further investigations on the compound (Khan et al. 2011).

Inamullah Khan et al. (2013) have further isolated two abietane diterpenoids and they were evaluated against the lipoxygenase inhibitory assay. Results revealed a potent anti-inflammatory activity with an IC_{50} value of 69.00 ± 0.3 and 73.00 ± 0.14 μmol/L, respectively which were significantly compared to the reference standards of baicalein (22.1 ± 0.03 μM) and tenidap sodium (41.6 ± 0.02 μM) (Khan et al. 2013).

In a study by Chattopadhyay et al. (2006), the authors have evaluated the immunomodulatory activity of the taxoid 1-hydroxy-2-deacetoxy-5-decinnamoyl-taxinine j isolated from the needles of *Taxus wallichiana Zucc.* by using human lymphocytes. The lymphocytes were treated with an immunosuppressant agent, cyclophosphamide, and these treated lymphocytes demonstrated cell proliferation when treated with concanavalin A (5 μg/mL), a well-known immunostimulant. By examining the lymphocyte proliferation in different concentrations of the taxoid, the results revealed that it contains significant immunomodulant properties (Chattopadhyay et al. 2006).

The previous reports suggest potent anti-inflammatory compounds isolated from *Taxus wallichiana Zucc*. (Himalayan yew). Although research including further isolations and investigations of these compounds is complicated, they would still pave the way for novel therapeutic interventions.

Khan et al. (2011) investigated the analgesic characteristics of the bark extracts using the acetic acid-induced abdominal writhing model and showed that tasumatrol B, was one of the major active ingredient responsible for the analgesic activity. Moreover, Qayum et al. (2012) suggested that bioactive compounds in the Himalayan Yew affected the long-chain fatty acids metabolic pathways such that the production of metabolites such as arachidonic acid would be downregulated. As such, tasumatrol B may serve as a potential treatment in the management of pain and inflammation and requires further exploration.

22.3.2 Antioxidant Activity

The role of antioxidants is to defend the body from damage due to oxidation from compounds such as reactive oxygen species and it does so by inhibiting or delaying their onset (Chen et al. 2016). The importance of natural antioxidants in maintaining human health has become a timely topic mainly due to their wide availability and safety concerns of using synthetic antioxidants (Chen et al. 2016). *Taxus wallichiana Zucc*. is a highly valued medicinal plant that is found to contain abundant amounts of secondary metabolites with antioxidant properties. The following reports provide some evidence for the antioxidant activity exerted by *Taxus wallichiana Zucc.*

Ahmad et al. (2015) have evaluated the in vitro antioxidant activity of *T. wallichiana Zucc* rhizome extracted with methanol, ethyl acetate, chloroform, and petroleum ether by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), reducing

power, DNA sugar damage, and lipid peroxidation assays. Further, each extract was evaluated for its total phenolic content. A dose-dependent DPPH radical scavenging activity was reported in each extract. At 100 μg/mL concentration, petroleum ether extract showed the highest scavenging activity (percent inhibition: 25.17%), followed by methanol (19.63%), chloroform (4.63%), and ethyl acetate (1.30%) extracts, respectively. However, at higher concentrations, the maximum percentage inhibition in petroleum ether was 94.81%, while that of methanol, ethyl acetate, and chloroform extracts were 72.78%, 72.45%, and 68.3%, respectively. Likewise, petroleum ether extract showed the highest potential in both DNA sugar damage (percent scavenging: 98.54%) and lipid peroxidation (percent inhibition: 97.63%) assays while methanol (93.05% and 96.56%), chloroform (89.79% and 52.11%), and ethyl acetate (84.51% and 78.94%) extracts showed the corresponding percent scavenging and inhibition values, respectively. Similarly, a dose-dependent antioxidant activity was reported for each extract in the reducing power assay. Also, the highest phenolic content was observed in the petroleum ether extract (106.60 mg%) followed by ethyl acetate (105.80 mg%), chloroform (103 mg%), and methanol (19.15 mg%) extracts, respectively (Ahmad et al. 2015).

Studies by Bhat et al. (2018) assessed the antioxidant capacity of *T. wallichiana Zucc.* leaves extracted with chloroform, hexane, ethyl acetate, methanol, ethanol, and aqueous solvents using 1,1-diphenyl-2-picrylhydrazyl (DPPH), reducing power, microsomal lipid peroxidation (LPO), superoxide and hydroxyl radical scavenging assays. Further, the highest DPPH percent scavenging activity (91.25%) was detected at 700 μg/mL in methanol (with the lowest IC_{50} value of 212.00 μg/mL), (87.64%; IC_{50}: 258.29 μg/mL), and ethanol (85.23%; IC_{50}: 301.80 μg/mL) extracts, respectively. A dose-dependent reducing power (with increasing absorbance values) was observed in each extract.

Similarly, a dose-dependent superoxide radical scavenging activity was observed in methanol (170.30 μg/mL), aqueous (257.00 μg/mL), and ethyl acetate (297.55 μg/mL) extracts, respectively. Further, the highest potential was reported to be 300 μg/mL in methanol extract (89%; IC_{50} value: 82.34 μg/mL) followed by aqueous (78%; IC_{50} value: 175.33 μg/mL) and ethyl acetate (72%; IC_{50} value: 199.05 μg/mL) extracts, respectively. Subba (2018) has also evaluated the in vitro antioxidant activity of leaf and stem methanolic extracts of *Taxus wallichiana Zucc*. and found that the IC_{50} value of leaf methanolic extract was 23.18 ± 6.49 μg/mL whereas the IC_{50} value of stem methanolic extract was 56.75 ± 8.96 μg/mL. In the FRAP assay, the antioxidant power obtained for leaf and stem methanolic extracts were 2.25 ± 0.57 and 2.23 ± 0.29 mM Fe (II)/L, respectively (Subba 2018).

Adhikari et al. (2020) evaluated the influence of altitude on the antioxidant ability of *Taxus wallichiana Zucc*. methanolic needle extract showing a significant increase in DPPH and ABTS radical scavenging activity with increasing altitude suggesting the activity of abundant phenolic compounds present in trees of higher altitudes. However, results obtained from the FRAP assay did not demonstrate a relationship between altitude and antioxidant ability. The IC_{50} values ranged from 185.52 ± 10.98 to 276.88 ± 8.65 μg/mL, 218.56 ± 6.95 to 495.76 ± 9.57 μg/mL,

and from 385.68 ± 3.95 to 487.32 ± 12.05 μg/mL in ABTS, DPPH, and FRAP assays, respectively (Adhikari et al. 2020).

22.3.3 Anticonvulsant and Antipyretic Activity

WHO estimates that developing countries hold approximately 80% of epileptic patients who do not receive adequate treatments and the treatments are known to have adverse effects on the patient and their lifestyles (Gowda et al. 2012). Studies conducted on many medicinal plants have been scientifically proven to have anti-convulsant activities, that suggest developing newer, safer, and more effective neuroprotective agents.

Considering *Taxus wallichiana Zucc.*, Muhammad Nisar (2008) has revealed that *Taxus wallichiana Zucc*. leaf methanolic extract possesses potent anticonvulsant and antipyretic activities by using induced rodents. The methanolic leaf extract with administered doses of 100 and 200 mg/kg, controlled the pentylenetetrazol-induced convulsions by inhibiting myoclonus and clonus while inhibiting tonus and hind limb tonic extension more significantly in mice. The antipyretic activity was also studied in the same study where an administered dose of 200 mg/kg caused a significant inhibition in the yeast-induced pyrexia model compared to the used administered doses of 50 and 100 mg/kg (Nisar et al. 2008).

22.3.4 Analgesic Activity

Further studies carried out by Muhammad Nisar (2008) evaluated the analgesic activity of *Taxus wallichiana Zucc*. leaf methanolic extract in nociceptive mice reporting a significant analgesic effect (67.77 and 74.29%) was observed (Nisar et al. 2008). In another study, Qayum et al. (2012) purified bioactive metabolites from the bark extract of the Himalayan Yew and examined their analgesic activity using acetic acid abdominal writhing and hot plate assays. Compared to other compounds, tasumatrol B, revealed remarkable analgesic activity suggesting it is a potent compound for further investigations (Qayum et al. 2012).

22.3.5 Hepatoprotective Activity

Liver diseases have become one of the global threats caused by excessive doses of drugs and chemicals. These causative agents mainly damage the liver cells by inducing lipid peroxidation (Adewusi and Afolayan 2010). Thus, it is imperative to reveal the hepatoprotective potential of natural products.

Bhat et al. (2018) have reported the hepatoprotective properties of the Himalayan yew by evaluating the liver marker enzyme level in mice. The results demonstrated promising hepatoprotective activity at doses of 100 and 300 mg/kg that resulted in decreased biomarker enzymes. Further hepatic examination of mice revealed almost normal structures without any signs of necrosis (Bhat et al. 2018).

22.3.6 Cytotoxic and Anticancer Activity In Vitro

Cytotoxicity screening is of great importance, as it provides the preliminary idea of selecting a particular natural product with potential cytotoxic activity for future research work. The following section reports the cytotoxic activity of *Taxus wallichiana Zucc* concerning the previously published literature.

Subba (2018) has investigated the toxicity of methanolic extracts of *Taxus wallichiana Zucc.* using brine shrimp lethality assay and revealed that the methanolic leaf extract is pharmacologically active while the stem is inactive (Subba 2018). In Chattopadhyay et al. (2006), the authors isolated a taxoid from the spines of the Himalayan Yew and found that it possesses dose-dependent cytotoxic activity against five human cancer cell lines. The lowest IC_{50} values (MTT: 0.01 ± 0.0025 μg/mL; clonogenic: 0.85 ± 0.0062 μg/mL) were obtained with PA-1 (human ovarian teratocarcinoma) cell line compared to the other cell lines used. Further, the taxoid was nontoxic on normal hepatocytes of mice even at 50 μg/ml concentration (Chattopadhyay et al. 2006).

Cancer-killing chemotherapeutic drugs are often described as cytotoxins but the term can be also used to describe toxins such as venom (Eldrige 2020). The authors have found that it possesses dose-dependent in vitro cytotoxic activity against five human cancer cell lines: MCF-7 (human breast adenocarcinoma), WRL-68 (human hepatic carcinoma), KB (subline of the HeLa tumor cell line), PA-1 (human ovarian teratocarcinoma), and Colo-320DM (human Caucasian colon adenocarcinoma). The highest potential with the lowest IC_{50} values (MTT: 0.01 ± 0.0025 μg/mL; clonogenic: 0.85 ± 0.0062 μg/mL) was obtained with PA-1 cell line compared to the other cell lines used. However, this study also does not provide detailed information about the dosages used (Chattopadhyay et al. 2006). In another study, Qayum et al. (2019) isolated three compounds namely, 4-deacetylbaccatin III, tasumatrol B, and taxawallin J from the bark of the Himalayan Yew and evaluated the in vitro cytotoxic activity against human renal (A498), human hepatoma (HepG2), non-small cell lung (NCI-H226), and human ovarian (MDR 2780 AD) cancer cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The compound concentrations were used as 1.5–100 μM while the vehicle control (DMSO) was maintained at 0.2%. Among the isolated three compounds, tasumatrol B had the highest activity with the lowest observed IC_{50} values per cancer cell line (A498: 147 μM, HepG2: 19.4 μM, NCI-H226: 87 μM, and MDR 2780 AD: 0.82 μM). However, 4-Deacetylbaccatin III and taxawallin J were inactive against MDR 2780 AD (Qayum et al. 2019).

An in vivo study on mice models assessed the impact of Taxotere on transplantable tumors, in which it was observed that nine of 11 tumor models were responsive to the Docetaxel treatment (Bissery et al. 1991). The most sensitive cancer cell line to Taxotere was B16 melanoma, presenting with a more significant tumor growth inhibition of 0% than the application of the precursor taxol, and Taxotere also cured early-stage colon and pancreatic ductal adenocarcinoma. (Bissery et al. 1991).

22.4 Toxicology

Cytotoxicity is the ability of a substance or process to cause damage or death to a cell. Yews are one type of evergreen, gymnospermous shrub that have been reported for their cytotoxic properties. Since antiquity, extracts of yew leaves have been used for homicides as well as suicides (Aslam et al. 2017). Previous studies have reported that the toxicity of *Taxus* species depends on the presence, amount, and stability of alkaloid diterpenes named taxines (Sharma and Garg 2015). Unlike numerous reports on toxicity after consumption of leaves in European yew (*Taxus baccata*) (Tranca and Petrisor 2013; Tiwary et al. 2005) and recent reports on the toxic nature of *Taxus brevifolia* and *Taxus cuspidate* (Wilson and Hooser 2018), Himalayan yew (*Taxus wallichiana*) have not been proven to be toxic. This can be attributed to the lower contents of taxines in Himalayan yew, their instability in alkaline or neutral environment, and the sensitivity of taxines to photodegradation (Aslam et al. 2017). Therefore, Himalayan yew can be quite beneficial in terms of its therapeutic activity compared to other yew species. Cytotoxicity screening is of great importance, as it provides a preliminary idea about the potential side effects of a natural product. The following section reports the cytotoxic potential of *Taxus wallichiana Zucc* based on the current literature.

Subba (2018) revealed that the methanolic leaf extract is pharmacologically active while the stem is inactive. However, this study did not provide detailed information about the dosages used (Subba 2018). Chattopadhyay et al. (2006) isolated a taxoid named 1-hydroxy- 2-deacetoxy-5-decinnamoyl-taxinine J from the needles of *Taxus wallichiana Zucc* and reported in vitro cytotoxicity against five cancer cell lines using the diphenyltetrazolium bromide (MTT) and clonogenic assays. The in vitro cytotoxicity against the cancer cells was on par or even better compared to the standard anticancer drugs vincristine and vinblastine sulfates. Furthermore, the taxoid was found to be nontoxic against normal hepatocytes of mice even at 50 μg/mL concentration. Mudbhari et al. (2019) examined the cytotoxic effect of ethyl acetate and methanol extracts of *T. wallichiana* (prepared with maceration method) using a brine shrimp lethality assay. The macerated methanol extract was more cytotoxic with a lower LC_{50} value of 289.81 ppm compared to the ethyl acetate extract ($LC_{50} = 604.54$ ppm) (Mudbhari et al. 2019).

Overall, reports on in vivo toxicity and clinical side effects of *Taxus wallichiana* are not available in the current literature to the best of our knowledge. Given the potential therapeutic activity of Himalayan yew, future studies should prioritize

understanding the possible toxicity and side effects as well as stability of the taxines found in Himalayan yew and its whole extracts before permitting their use in integrative medicine. Similarly, the potential interactions of extracts or compounds from Himalayan yew with other standard drugs should also be investigated to mitigate any toxicity mediated through herb–drug interactions.

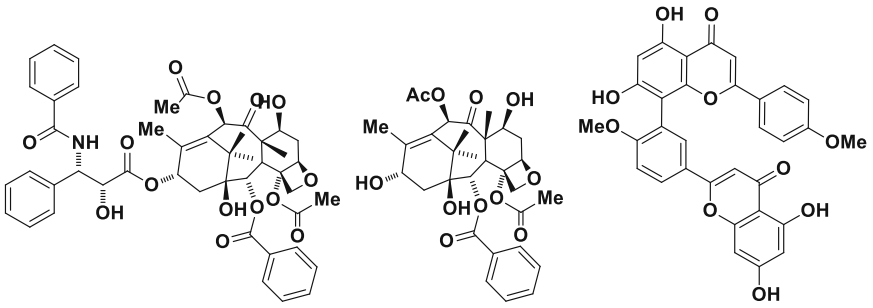
22.5 Clinical Studies

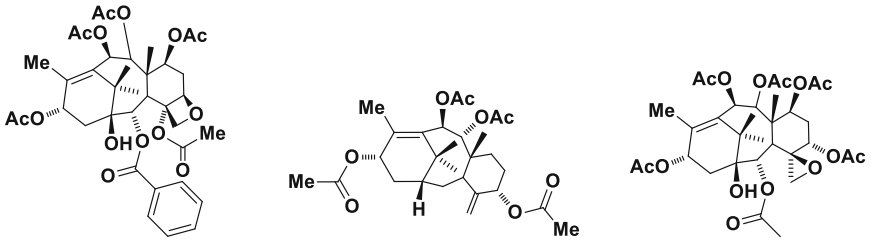
22.5.1 Anticancer Properties

Specific phytochemicals such as diterpenes, lignans, biflavonoids, phytosterols, and phytoecdysteroids extracted from Himalayan Yew have been investigated for their anticancer potential in several studies against various cancer cell lines (Sharma and Garg 2015). The key bioactive phytochemicals present in different parts of the Himalayan Yew are Paclitaxel, Sciadopitysin, Baccatin III, Baccatin IV, 1-β-Hydroxy-baccatin 1, Cephalomannine, Taxusin and their derivatives (Fig. 22.1). These compounds have demonstrated a diverse range of bioactivity; however, further investigation is necessary for their administration in anticancer treatment regimes.

22.5.1.1 Paclitaxel (Taxol)

Paclitaxel, also known as diterpenoid alkaloid taxol, or more commonly taxol, is a notable compound extracted from Himalayan Yew after originally being isolated from the *T. brevifolia Nutt* (Sharma and Garg 2015). This compound has been utilized widely as a standard chemotherapeutic drug in the treatment of several prevalent cancer types, including breast, liver, lung, and blood cancer forms. A phase II clinical trial assessed the efficacy of taxol on metastatic breast cancer and found that the disease progressed in only 8% of the cancer patients after treatment (Holmes et al. 1991). These patients had only undergone one round of chemotherapy, and many of the participants had previously received doxorubicin, another standard chemotherapeutic drug (Holmes et al. 1991). These significant findings led to the conduction of a subsequent clinical trial that aimed to analyze paclitaxel as a first-line treatment method for the metastatic form of breast cancer (Bishop et al. 1997). This was achieved by comparing paclitaxel with standard chemotherapy as front-line treatment in patients who had not previously undergone chemotherapy for metastatic breast cancer. The results depict that the quality-of-life assessments of 100 patients had better results with paclitaxel treatment in comparison to standard chemotherapies (Bishop et al. 1997). Furthermore, it was also observed that the CMFP combination therapy, comprised of cyclophosphamide, methotrexate, 5-fluorouracil, and prednisolone, had lower efficacy than the single-agent paclitaxel treatment against metastatic cancer, and paclitaxel was generally better tolerated as a





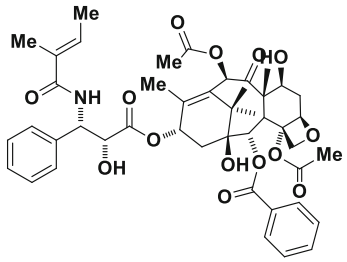


Fig. 22.1 Structure of key secondary metabolites isolated from different parts of Himalayan Yew

front-line treatment (Bishop et al. 1997). A nonrandomized prospective phase II clinical trial aimed to assess the anticancer potential of paclitaxel in ovarian cancer patients with advanced and progressive diagnoses, and a premedication regimen with taxol was implemented to avoid hypersensitivity reactions to the drug, which has been observed with high dosages of taxol (McGuire et al. 1989). This study reported that approximately 30% of the participants were responsive to paclitaxel administration for periods between 3 and 15 months and concluded that it was effective against drug-refractory ovarian cancer and could potentially be used in combination therapy for advanced ovarian cancer (McGuire et al. 1989). A subsequent clinical study also identified that taxol presented with strong anticancer action

in phase I and II clinical trials against four different cancer types; head and neck squamous cell carcinoma (HNSCC), and breast, lung, and ovarian cancers (Srivastava et al. 2005). More recently, a randomized phase III clinical trial assessed the implementation of albumin-bound paclitaxel particles (Nab-P) as a combined therapy with gemcitabine against pancreatic adenocarcinoma, which is an aggressive form of cancer with a poor prognosis (De Vita et al. 2016). This study observed that the combined treatment of Nab-P and gemcitabine had a more effective response and survival rate in cancer patients and presented a strong safety profile for therapeutic administration (De Vita et al. 2016). Another study analyzed the anticancer effect of Nab-P on triple-negative breast cancer (TNBC) cells (an aggressive form of breast cancer with poor long-term prognosis) (Schettini et al. 2016). The authors acknowledged that the strong anticancer activity of Nab-P against aggressive forms of cancer was likely due to greater tolerability and efficacy than other agents, indicating its effectiveness as a neoadjuvant therapy against TNBC (Schettini et al. 2016). However, it is important to note that despite these recent promising clinical findings, the paclitaxel compound is more commonly synthesized chemically in recent years, in comparison to older studies that derived this constituent from natural sources.

The molecular mechanisms of action of paclitaxel have been extensively investigated. It inhibits the proliferation of cancer cells through the stabilization of microtubules in the G_2/M phase of the cell cycle (Juyal et al. 2014). The stabilization occurs as a result of the depolymerization of microtubules to soluble tubulin being blocked by paclitaxel (Juyal et al. 2014). Additionally, the mechanism behind the stabilization of drug-tubulin interactions was attributed to the orientation, structure, and activity relationship of the C-13 side chain of paclitaxel (Srivastava et al. 2005). Despite the clinical benefits associated with paclitaxel, there are several issues associated with its application and distribution as a chemotherapeutic drug for cancer. The primary issue related to obtaining the drug is the slow reproductive and growth rates (low extraction yield of the compound), limiting its bioavailability, as well as problems associated with the infusion of the drug into the body due to poor solubility, which is amplified under circumstances where the dosage must be greater (Srivastava et al. 2005). Additionally, high dosages of paclitaxel can incur high toxicity within the host, limiting its potential utility in a broader range of cancer types, in addition to the breast, lung, and ovarian cancers that have been studied to date (Juyal et al. 2014). The hypersensitivity reactions (HSRs) observed with paclitaxel led to the implementation of premedications to limit these adverse events in patients (Pazdur et al. 1993).

Metabolites/derivatives of existing anticancer drugs have been an evolving and rapidly emerging area of anticancer research in recent years, due to their potential to exhibit more diverse and effective bioactivity than their precursors. A synthetic derivative of paclitaxel, Taxotere (known as docetaxel), was found to be more efficacious in preclinical research, which led to its evaluation in phase I clinical trials (Srivastava et al. 2005). Docetaxel also showed similar action on the microtubules to that of paclitaxel (Pazdur et al. 1993) however with improved water solubility compared to the latter (Srivastava et al. 2005). Similar to paclitaxel, the premedication approach was later adopted with docetaxel treatments due to the

occurrence of HSRs in the clinical investigation (Pazdur et al. 1993). Docetaxel was also found to be effective against both early and advanced-stage colon adenocarcinoma, especially in targeting the poor prognosis evident in advanced-stage diagnoses of colon cancers (Bissery et al. 1991). Due to the observed desirable clinical efficacy, this paclitaxel derivative is currently utilized as a standard chemotherapeutic drug against several cancer types. To date, paclitaxel has been approved for use as a stand-alone treatment and in combination with other anticancer treatments in treating breast, ovarian, and non-small cell lung cancers (Barbuti and Chen 2015). Further investigation is also underway to demonstrate the efficacy in head and neck, bladder, cervical, endometrial, and esophageal cancers (Barbuti and Chen 2015).

22.5.1.2 Lignans

Lignans are a class of phenylpropanoids and are deposited in substantial quantities in the heartwood region of Himalayan Yew (Sharma and Garg 2015). Specific isolated lignans, including taxiresinol, hydroxymatairesinol, isotaxiresinol, and secoisolaricinresinol have been found to possess anticancer activities through experimental studies contributing to a reduction in disease prevalence (Sharma and Garg 2015). The preclinical research into the anticancer potential of taxiresinol has shown that this compound was bioactive against colon, liver, breast, and ovarian tumors (Chattopadhyay et al. 2003). As previously acknowledged, metastatic breast cancer is an aggressive form of breast cancer and a major cause of morbidity (Bishop et al. 1997), and as lignans have demonstrated strong anticancer potential against other invasive breast cancer cell lines, the requirement for clinical research is significant in the treatment of breast and metastatic cancers. However, to date, clinical research has not been conducted on the administration of lignans extracted from Himalayan Yew in the treatment of early or late-stage cancers indicating a substantial gap in the existing literature. However, lignans derived from other natural sources, including fruits and vegetables, have been investigated in clinical trials for their anticancer activity. As lignans are natural products, they have been observed to cause fewer adverse events in cancer patients unlike with chemo- and radiotherapies. The secoisolariciresinol diglucoside (SDG) lignan extracted from flaxseed was assessed in a phase IIB clinical trial of premenopausal women at an increased risk of breast cancer and its application was determined to be tolerable and safe (Fabian et al. 2020). These findings supported an earlier study that identified administration of flaxseed treatment reduced tumor proliferation in breast cancer patients while reducing the risk of breast cancer in premenopausal women (Mason and Thompson 2014). Overall, existing research has demonstrated the anticancer potential of lignans in the treatment and prevention of breast cancer. To support the application of lignans found in Himalayan Yew in cancer treatment regimes, clinical trials must be prioritized to investigate their molecular mechanisms of action against cancer as well as to define effective and safe dosages.

22.5.2 Antimicrobial Activity

Although many studies have indicated the antibacterial and antifungal properties of Himalayan yew in vitro, clinical investigations on its antimicrobial activity are missing from the literature. A review by Amber et al. (2017) discovered that the Himalayan Yew leaf extract is traditionally used to treat bronchitis and other respiratory illness due to viral infections. Further clinical studies are necessary to understand the antimicrobial (antibacterial, antifungal, and antiviral) activity of Himalayan yew, especially against multidrug-resistant pathogenic strains.

22.5.3 Anti-Inflammatory and Immuno-Modulatory Properties

The current literature does not contain any clinical studies on the anti-inflammatory or immunomodulatory activity of Himalayan yew to the best of our knowledge.

22.5.4 Any Other Biological Activity

Although the current literature portrays several preclinical (in vitro and in vivo) studies on the antioxidant, anticonvulsant, antipyretic, analgesic, hepatoprotective, neuropharmacological, and cytotoxic properties of *T. wallichiana Zucc*, there are no clinical reports on these activities to the best of our knowledge.

22.6 Conclusion and Future Perspective

The primary constituent of Himalayan Yew that has been investigated in the clinical setting is paclitaxel. Subsequently, its derivative docetaxel has been widely used as a part of standard chemotherapy for different cancer types. While there have been several phase I and II clinical trials carried out in investigating the anticancer action of these compounds, the clinical studies primarily date back to the late twentieth century and minimal progress has been made in refining the HSR and toxicity issues associated with prolonged exposure to paclitaxel in cancer patients. Future research on paclitaxel must reexamine the most appropriate dosage to improve its efficacy and reduce toxicity and to accommodate for recent advancements made in the understanding of cancer development and drug interactions within the host. Paclitaxel has exclusively been clinically examined against specific breast, lung, skin, liver, and gastrointestinal-based cancers whereas docetaxel has primarily been assessed against melanomas, colon adenocarcinoma, and pancreatic ductal

adenocarcinoma in clinical studies. Therefore, future studies should prioritize repurposing these compounds for other aggressive and rare cancer types as mono or combination therapies with other standard or natural product-based anticancer agents.

In comparison to paclitaxel, lignans found in Himalayan Yew have not been explored adequately, with most research performed in preclinical settings. The experimental studies carried out on lignans such as taxiresinol have demonstrated promising anticancer activity, against multiple cancer types and cell lines highlighting the need for clinical studies to evaluate their efficacy both as stand-alone and combination therapy against cancer. Overall direct clinical studies on the anticancer activity of Himalayan Yew are not available in the current literature. However, given the rich phytochemical content of Himalayan Yew with several anticancer secondary metabolites including paclitaxel, further preclinical and clinical investigations on its extracts and solitary bioactive compounds are necessary for novel anticancer drug discovery.

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Chapter 23
Tribulus terrestris L.: Gokshur/Gokharu

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23.1 Introduction

Humans consume about 30,000 plant origin foods, yet only 30 agricultural plants provide over 90% of their dietary requirements (Tabuti et al. 2004). Persons in underdeveloped nations, notably those in Sub-Saharan Africa, rely on natural leafy green vegetables for crucial nutrients (Shackleton 2003). Green vegetables are nutritionally extremely valuable regardless of the form of eating since they are inexpensive and provide a rich source of fundamental nutrients needed by the human body (Tomori and Obijole 2000). There is little research on the nutritional value of lesser-known green vegetables like *T. terrestris* leaves.

Tribulus cistoides, *Tribulus terrestris*, and *Tribulus alatus* are three species of the genus Tribulus, which related to Zygophyllaceae family. *Tribulus terrestris* is such a well-known therapeutic weed among them, all by Ayurvedic seers and contemporary herbalists. Among them, ancient mystics and contemporary herbalists both recommend *Tribulus terrestris* as a therapeutic plant (Yanala et al. 2016). *The tribulus species has exploited like a solitary medicinal compound as well as a primary/*

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secondary ingredient in a variety of chemical compositions and dietary supplement (Chhatre et al. 2014). It is a Mediterranean, subtropical, and desert environment plant that originates in India, China, the United States, and Bulgaria (Sharma and Kumar 2020). It is a little prostrate hirsute or silky hairy shrub with a height of 10–60 cm. Pinnae from 5–8 pairs, elliptic or oblong lanceolate; leaves are opposite, typically irregular, and paripinnate. The flowers are yellow (Sahani and Hemalatha 2018). Its carpel fruits have a distinctive stellate shape, are relatively round-shaped, compressed, have five corners, and are covered in light-yellow sprinkles. Each crocus contains many seeds separated by transverse walls. The seeds have an oily texture. The root appears brownish and thin, fibrous and spherical, usually branching, yielding bunch of little adventitious roots when mature (Chhatre et al. 2014).

Tribulus includes chemicals which might help to boost hormone production. Furthermore, it would not seem significantly enhance androgens among people (testosterone). (Neychev and Mitev 2016). Tribulus is often referred simply puncture vine since its harsh spikes may collapse cyclist wheels. The whole plant, especially the fruits, was extensively utilized within Indian and Chinese customary remedy to cure variety of illnesses. The identification of some of the plant's helpful pharmacological qualities has resulted from research conducted on it (Hanif et al. 2020).

Throughout conventional healers, *Tribulus terrestris* is being used as a stimulant, erotic, analgesic, abrasive, purgative, vasodilator, and urinal cleanser. (Pokrywka et al. 2017). The herb's dried fruit is particularly beneficial for most genitourinary tract diseases. It is a key component of Gokshuradi Guggul, an Ayurvedic treatment that helps the genitourinary system operate properly and removes urinary stones (Azam et al. 2019). *Tribulus terrestris* was already using in managing hypogonadism, venereal infections, and sexual debility in Ayurveda for ages (Meena et al. 2009). Throughout Bulgaria, the plant is often utilized as a traditional remedy to treat malfunctioning. The Vedic System of medicine of India assigns cardioprotective properties first to rhizome and fruits in addition to all of these uses. The fruits were used to cure eye disorders, puffiness, gastrointestinal pain or swelling, ejection, pathological leukorrhea, as well as loss of libido within Chinese Medicine (Zhu et al. 2017). Within Shern-Nong System of medicine (China's earliest recorded medicinal treatise), *Tribulus terrestris* is stated like an incredibly useful medication for reviving impoverished hepatic, treating heaviness inside the breast, mastitis, flatulence, acute conjunctivitis, headache, and vitiligo. *Tribulus terrestris* was utilized in treating diuretic, mild laxative along with general tonic within Unani remedy (Das et al. 2017).

It is well recognized because of its ability to treat premature ejaculation, libido, and hormonal imbalances in humans and animals. It can also be used as a sexual stimulant. However, recent finding of active compounds that can be used to treat sexual and other diseases, there has been an increase in interest in this plant in the last 30 years (Semerdjieva and Zheljazkov 2019).

23.2 Taxonomic Hierarchy of *Tribulus terrestris L.* (Table 23.1)

Table 23.1 Taxonomic hierarchy of *Tribulus terrestris L*

Domain	Eukaryota—Have nucleus
Kingdom	Plantae—Plants
Subkingdom	Viridiplantae—Plants that are green in color
Infrakingdom	Streptophyta—Plants that grow on soil
Super division	Embryophyta—Plants that produce embryo
Division	Tracheophyta—Plants that have xylem and phloem
Subdivision	Spermatophytina—Plants that produce seeds
Class	Magnoliopsida—Flowering plants
Order	Zygophyllales—Dicotyledonous plants
Family	Zygophyllaceae—Bean caper, caltrops
Genus	*Tribulus L*—Puncture vine
Species	*Tribulus terrestris L*—puncture vine

Sources: National Plant Data Center, NRCS, USDA (2000)
ITIS USDA PLANTS (2007–2010)

23.3 Common Names of *T. terrestris* (Table 23.2 and 23.3)

Recommended name of plant: Puncture vine.

23.4 Flowering/Fruiting Season

The breeding period for *Tribulus terrestris L.* extends around March to October. Shiny yellow flowers with a diameter of almost 1/5–3/5 of an inch (6–15 mm) are developed individually where the stem and leaf stalk intersect. Except perhaps in shaded regions, they only bloom on bright mornings. The taproot of *Tribulus terrestris L.* is deep and woody. The plant creates a thick mat by producing many spreading stalks equal to 3 m lengthy which may heavily divide and emerge out of the highest point of view. The cotyledons of the leaves are rectangular, opposing, short petioled, 2–5 cm long, hairy, along with separated into pinnate ellipse or else rectangular leaflets (4–6 leaflet pairs per leaf); every leaflet is 4–14 mm long. At leaf nodes, the five tiny yellow petals exquisite blooms that grown upon small stems. Fruit is schizocarp, a wooded thorn bears spiky, stiff tentacles that reach a diameter of 1–1.7 cm. Burrs generally have 3–5 seeds per burr; furthermore, the seeds remain contained inside the burrs (Yingxin 1998).

Throughout one growth season, a plant can generate 200–5600 seeds, and a big plant can yield equal to 10,000 seeds (Boydston 1990; CDFA, 2002). Numerous

Table 23.2 Familiar names around the world

English	Bendy eye, caltrop, puncture vine (Tutin et al. 1981)
Spain	Abrojo terrestre (CABI n.d., UK)
France	Croix de Malte (Tutin et al. 1981)
Saudi Arabia	Dacn-ash-sheikh, dreiss, gutiba, kotaba, shiqshiq (Revri et al. 1983)
Portugal	Abrolhos (Tutin et al. 1981)

Table 23.3 Names used in the local communities

Australia	Bindii, yellow vine (Barker 1998)
Ethopia	Akakima, cachito, kurakito, kuremehit, kurumshit, qottbet (Stroud and Parker 1989)
Germany	Erd Buerzeldorn (Tutin et al. 1981)
India	Bakhra, ikshugandha, kokulla, lahangokhru (Nayak and Satapathy 2015; Devi et al. 2015)
Pakistan	Gokhru, Tikandu (Rehmat Ullah et al. 2014)
Punjabi	Bhakhra, Gokhru (Ihsan Ullah et al. 2011)
Iraq	Al-gutub, gotob (Twaij et al. 1989)
Italy	Tribolo (Tutin et al. 1981)
Kenya	Kungu, mbigiri, mbiliwili, okuro, shokolo (Hadidi 1985)
Lebanon	Kutrab (Edgecombe 1970)
Netherland	Aardangel (Tutin et al. 1981)
South Africa	Common dubbeltjie, gewone dubbeltjie (Henderson and Anderson 1966)
Uganda	Esuguru, eziguru (Terry et al. 1987)
USA	Land caltrop; Mexican sandbur; puncture weed (Sampangi et al. 2007)

research have shown that seeds may survive for so many years after being latent inside mud for 3–5 seasons (Whitson et al. 1991). The much more significant methods of seed dispersion are humans and their actions, as well as wildlife (Squires 1979).

Seeds sprout from spring to autumn in wet, moderate circumstances (24 °C–27 °C), and the plant develops quickly, forming a dense root system in a matter of weeks (CDFA, 2002; Scott and Morrison 1996). *T. terrestris L.* sprouting inside the land is capable of beginning subsequent to a rainfall of more than 9 mm, according to a research (Ernst and Tolsma 1988). While conditions are over 20 °C, *T. terrestris L.* can blossom within 3–4 weeks of development, typically from July to August (Parker 1972). When the plant starts to blossom, it will continue to do so for the rest of its life (Reddi et al. 1981). Fruits develop within about 12 to 14 days and then break into pieces shortly later. Till the chilly season arrives, plants continue to multiply and grow fruit (Holm et al. 1977).

During experiments in Washington, Boydston (1990) states that fruit growth ceased in October while usual temperatures fell below 20 °C. This species may

rapidly expand in numbers under optimal circumstances because to its enormous seed output and long-term survival of seeds. After the first cold spells, the plants generally decease (Squires 1979). *T. terrestris L.* bear significant seed dormancy that lasts into the fall as well as cold seasons, a few seeds remaining dormant for extended intervals of life; however, seeds have been recorded germinating in the fall in tropical regions. *Terrestris L.* produces wood roots and grows everlasting in these places when conditions are favorable (Pathak 1971).

Tribulus terrestris can survive in a broad range of climates. It is common in locations with hot weather, such as warm, temperate, and desert climates. *T. terrestris* is sensitive to cold conditions and needs rather high temperatures to develop. It may be found in locations where the yearly lowest rainfall is 270 mm and the maximum annual rainfall is 390 mm (Rice et al. 2002). In shady locations, seedling development was reported to be low. *T. terrestris* tends to produce within extensive diversity of soil kinds, except somehow this thrives in dry, loose, clay soils near sandy beaches and in air loose soil near field edges. It also begins to grow in heavier soils, particularly if they are productive and watery, and on granular soil like those originate next to the elbows of un-surfaced streets or in play areas (El-Ghareeb 1991).

23.5 Cultivation, Harvesting, and Processing

Tribulus terrestris is categorized as an evergreen herb that is found almost all over the world. It is a creeping plant and has a woody taproot that forms thick mats (Raghu et al. 2010). The highest length of its stems is 1.8 m or 6 feet. The spiky hums of this plant are used as medicine (Jamuna et al. 2017). The plant is found to be a cause of puncture tires and its spikes often penetrate in shoe soles, so it is often called "puncturevine." The hums are known to be the seeds or fruits of this plant (Elbalola 2020).

Regions having warm temperature are very effective in *Tribulus* production. High temperature is required for the development and growth of *Tribulus*; moreover, it can habituate to the most range of soils (Zesiger and Ransom 2019). Typically, as it is a weed so it is found in the waste areas, grazing land, cattle farms, dump yards, and disrupted soil. Due to its harmful potential, precautionary measures and strict consideration must be given during the process of farming for the prevention of its escape (Akram et al. 2011). In the countries like United States and Australia, *Tribulus* is considered to be a virulent weed. Your regions may be checked as local regulations disallow farming of *Tribulus* (Semerdjieva and Zheljazkov, 2019).

Seeds are used for the propagation of *Tribulus*. After seeds develop, they germinate immediately. In the months of autumn and winter, the seeds remain inactive and start to germinate in early summer and late spring when the humidity level in the atmosphere is high (Kostova and Dinchev 2005). For the process of germination and growth, high temperature is required. After 3 weeks, yellow flowers start to appear. During the month of autumn and summer, fruits (the burrs) are continuously

produced. 400 fruits are produced by a single plant. The remains of the seeds can be visible for multiple years (Petkov 2010).

Plastic barriers are found to be the best for the growth of this crop as it spreads like vine; moreover, it has invasive herb's potential and continuous fruit production. Plastic barrier is found effective because it prevents the fruit from dropping down into the soil and is also found facilitating in the harvesting process of the fruit (Akram et al. 2011). The amount of fertilizer, the spacing difference between the crops and crop care are still not defined. Deep plunges and pots must be used for the germination of plants so that the plant can be conveyed into rows in the field (Scott 2012).

It is highly recommended that the seed must be sown in the moist sand for the period of one to 3 months for the breaking of seed's dormancy (Stancheva et al. 2011). The seeds are then frequently checked for the signs of sprouting or swelling, as these are the signs of breaking of seed's dormancy. As soon as the signs appear the seeds are removed from sand and sowed in pots or plug trays. *Tribulus* can also be found in the bank of rivers and streams due to the distribution of water (Dinchev et al. 2010).

Like all the crops around the world *Tribulus* is also attacked by the pests. *Microlarinus lareynii*, *M. lypriformis* and two weevils from France, India, and Italy are well-known pests of *Tribulus* (Masheva et al. 2011). These pests attack both the stem and the seed and have been used as bio-control agents in the United States with good success (Miraj 2016).

The objective for the establishment of control measures is to prevent the seed production because they can sustain in soil for the period of 3 to 6 years and fruits that are a cause of injury to livestock and human around (Šalamon et al. 2016). The germination of seeds is irregular during the season and plant could set seeds as well as flowers within very petite period of time so irregular or single cultivations are not enough for the reduction of seeds population.

Tribulus is composed of taproot so the most effective method for cultural control is by shallow cultivation. Cultivation by the method of deep ploughing increases the lives of seed in soil (Tkachenko et al. 2020). Plants must be ripped off just after the ripening of fruits to avoid the ripening of seeds (Fig 23.1). To remove and gather the fruits special rollers "Prickle roller" have been developed. Solarization of soil for the period of three to 6 weeks by the use of transparent polyethylene mulches is found effective in reducing the number of plants and dry weight (Elmore 2012).

The collection of fruits is carried out during the month of May and June. The collection of fruit is generally done by hands, but sometimes the whole plant is plucked and then the fruits are separated afterword. After harvesting, the fruits are then dried in shaded areas and then kept within plastic containers or jars and then preserved within cool and dry places (Boteva et al. 2011). The medicinal efficiency of the fruit is only sustainable for 6 months after harvesting (Fig. 23.2).

Fig 23.1 *Tribulus terrestris L.* Flowers

Fig 23.2 *Tribulus terrestris L.* Fruit

23.6 Distribution

Tribulus terrestris L. is inhabitant herb belongs to Mediterranean areas. It was derived from North Africa mainly from its Saharan areas. At present, it is generally distributed in South Africa, Western Australia, South and Central Europe, Central Mexico, Tropical as well as Temperate Asia, Southern USA as well as in some regions of South America (Frohne 1999; Huffaker et al. 1983; Tutin et al. 1968).

Genus *Tribulus* consists of about 20 different species worldwide, and it belongs to *Zygophyllaceae* family. In India, 3 species out of the 20, i.e., *Tribulus alatus, Tribulus cistoides*, and *Tribulus terrestris* are most commonly found (Evans 2002). Herbalists of modern era and Ayurvedic seers illustrated *Tribulus terrestris* as a significant herb to be used in medicinal purposes (Duke 2002). The herb utilized primarily within diverse formulations as well as dietary supplements for therapeutic purposes. This is yearly plant that is present within desert, and Mediterranean areas all across the globe counting southern USA, India, Bulgaria, Mexico, China, and Spain (Nadkarni 1976). This plant is generally known as *Khar-e-khasak khurd* in Urdu, small (land) caltrops in English, *Nanogokharu* or *Bethagokharu* in Gujrati and *Gokharu* in Hindi. It is widely distributed along geographical perimeters. It is widely present in India up to thousands of feet in Kashmir. It is generally found in common places including dried out, hot and sandy places, i.e., Gujarat along with West Rajasthan (India) (Kokate et al. 2007).

23.7 Morphology

This plant is just 10–60 cm tall, having long silky hairs on its stems. It bears branched stem having flowers on the top (Nukhimovsky et al. 1979; Shreter 1980). Based on environmental circumstances, stem length goes about minimum 15–20 cm to more than 200 cm (Halvorson and Guertin 2003; Semerdjieva 2013). It bears fruits having polymorphic characteristics with medium forms and various epithets (Semerdjieva 2013; Al-Hemaid et al. 1996). This property is also because of harsh climate or hybridization of close species (Al-Hemaid and Thomas 1996). Two cotyledons appear and give rise to several pairs of leaves. Leaves are oval shaped and arranged in opposite direction mostly in five to eight pairs. Flowers are yellowish color and fruits have significant importance having five corners, mostly round in shape, compressed and covered with light yellow colored sprinkles. Each crocus contains partitions that include numerous seeds (of oily nature) among them. Fresh roots of *Tribulus terrestris* are light brown in color, slender, fibrous, branched, and cylindrical and having several rootlets.

Roots are found in pieces, having 7–18 cm height and diameter of about 0.3–0.7 cm. These are woody, tough, and rough apparently because of small nodules on surface. The odor of fruit is aromatic and it tastes acrid or unpleasant. Fruits are greenish yellow in color and bear spines. Fruit is mainly a schizocarp capsule that is

divided into rough, thick, and thorny enclosed parts (nuts) having seeds among them (Artyushenko and Feodorov 1986). Seeds are triangular-oval shaped, flat base (Semerdjieva et al. 2011) and embryo that is round and fully developed (Yankova-Tsvetkova et al. 2011). Fruits are spherical in shape, having five smooth, muriculate, woody wedge-shaped cocci. Each coccus bears two pairs of spines and one pair is always bigger than the other. These spines are arranged in a splendid way that their tips meet together in pairs and form a pentagonal structure. It is also known as "go-ksura" meaning cow-hoofed because its fruit gives an appearance of cloven hoof of cow. In China, it is also known as "Chih-hsing." (Chhatre et al. 2014).

23.8 Usage in the Past

From past several years, *Tribulus terrestris* seems to have a long history of usage within conventional healers, encompassing "southern European herbal medicine," "Indian Culture treatments" (Ayurvedic medicine), and "acupuncture." This utilization depicted its significant importance to be used as medicinal plant (Neychev et al. 2016). In earliest Chinese pharmaceutical monograph "Shen-Nong Ben Cao Jing,", TT was described as top-ranking medicine (Shang 2008). TT is of incredible benefits as erotic, abrasive, edema, renal disinfecting, hypotensive, palliative, lithotriptic, stomachic, and an excellent tonic as well. Its fruit (in dried form) is also used for genitourinary tract diseases. It is an essential component of *Gokshuradi Guggul* that is considered as an effective Ayurvedic medicine, used to regulate body functioning especially genitourinary tract, and also to eradicate urinary stones. It is evaluated that from centuries TT has been applied to treat sexual debility and venereal diseases.

In Bulgaria, it is also extensively used to treat impotence. Its fruits as well broadly utilized within customary Chinese drug in healing edema, sexual dysfunction, abdominal distension, eye trouble, morbid leukorrhea, and emission problems. Chinese Pharmacopeia (Chinese Pharmacopoeia Commission 2015) evaluated that fruits of this plant have been significantly applied not only for kidney tonifying purposes but also for diuretic and cough expectorant for better cure of headache, blockage of mammary duct, skin pruritus, vertigo, and eyesight problems. India also used these fruits for curing impotence, low libido, infertility, and erectile dysfunction in Ayurvedic treatment for many years. Sudan also depicted the use of TT as demulcent for treating nephritis and inflammatory diseases (Mohammed et al. 2016).

Shern-Nong Pharmacopeia depicted that TT can be well used as a significant drug for treating mastitis, acute conjunctivitis, vitiligo, fullness of chest, headache, flatulence, and repairing depressed liver. It is also used to cure general tonic, diuretic, and mild laxative in Unani medicine. It is observed that not only its fruits as well as its roots are used as folk medicine to cure several ailments. Additionally, Ayurvedic Pharmacopeia (India) illustrated cardiotonic attributes of fruits and as roots of TT (Khare 2008). Additionally, fruits and also its roots are estimated to possess cardiotonic characteristics (Mohammed et al. 2016). In Pakistan, TT has also been used for uricosuric and diuretic properties (Akram et al. 2011). Traditional pharmacological

uses of TT are of high importance because of significant chemical composition, i.e., the presence of flavonoids and steroidal saponins which act as better anti-aging as well as anti-inflammatory activities.

23.9 Phytochemistry

Early studies demonstrated that TT comprises various vital chemical constituents like polyphenols, tannins, glycosides, saponins, and alkaloids (Usman et al. 2007). Saponin contents and its composition is evaluated to be varied from region to region. Bioavailability and chemical composition of saponins in TT was observed by Kostova and Dinchev (2005), and they demonstrated the presence of gitogenin, neohecogenin, neotigogenin, chlorogenin, sarsasapogenin, hecogenin, neogitogenin, diosgenin, fructostanol, ruscogenin, and many more vital constituents. Fructostanol glycosides mainly protogracillin and protodioscin are present in this plant. From which protodioscin, a vital saponin is of major importance. Spirostanol glycosides are also present in small amount (Kostova and Dinchev 2005; Xu et al. 2010). Main flavonoids are also observed quantitatively and it was found that these are nearly 1.5 times the amount of main saponins. Thus, it is depicted that further studies on flavonoid contents should be performed for its better understanding and implementations (Wu et al. 1999).

Kaempferol, Kaempferol-3-rutinoside, and Kaempferol-3-glucoside were confined by Bhutani et al. (Bhutani et al. 1969) from fruits and leaves of TT, and these chemical constituents were observed by analysis through spectroscopy. About 18 flavonoids (caffeoyl derivatives and quercetin glycosides such as kaempferol glycosides and rutin) were also observed from leaf extracts of four *Tribulus* species by using HPLC by Louveaux et al. (1998).

In India, Raja and Venkataraman (2011) observed chloroform and ether extracts (of some varieties of) fruits of TT using 1:9 ethyl acetate: benzene solvent system. Alkaloids such as harmane and non-harmane were also observed to be present in this plant. Tribulusterine, β-carboline, and alkaloids are also present in minute amounts in its fruits (Bremner et al. 2005). Methanolic extract of TT plant depicted that a significant constituent, i.e., α-Amyrin is also present, that was observed by gas chromatography-mass spectroscopic analysis (Abirami and Rajendran 2011). Other substances such as amino acids (mainly threonine and alanine (Xuedong et al. 1994)) and organic acids (vanillic, ferulic) (Lv AL, 2007), palmitic, docosanoic acid (Li 2006), Tribulus acid (Chen et al. 2004), and many others) are also constituents of TT. Additionally, uracil nucleic acid (RuYi et al. 2009), emodin, physcion (Jie et al. 2003), and coumarin (Li 2006) are also present in TT (Figs. 23.3 and 23.4).

The level is determined through milligrams per 100 g of dry mass. NS stands for "not specified," which means that the intensity cannot be estimated utilizing data provided within study article.

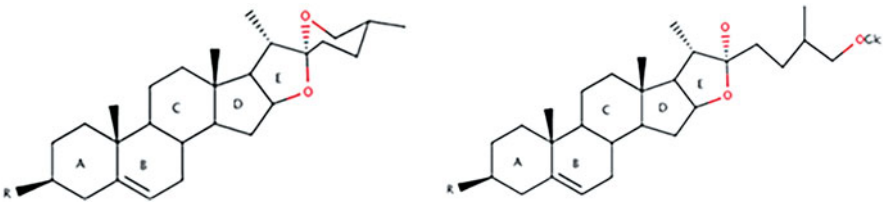


Fig. 23.3 Spirostanol (left) and furostanol (right) saponins

23.10 Pharmacological Properties

23.10.1 Impact as Antioxidants

In vitro tests employing the DPPH, ABTS, and FRAPS techniques have shown that TT extracts exhibit antioxidant property. Phenolics concentrations have been found to range from 0.5% to 3%, whereas flavonoids concentration has been observed to range from 0.05% to 0.6% (Evans 2002). Whenever partitioned extracted have been evaluated for antioxidant properties, it was discovered that the ethanol extract showed the highest DPPH-free radical scavenging action, with the relevant chemicals being 4, 5-di-p-cis- and trans-coumaroylquinic acid (Kokate et al. 2007).

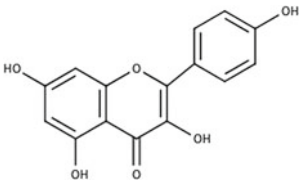
During an in vivo investigation, Semerdjieva et al. (2011) found that administration using TT extracts boosted catalase as well as superoxide dismutase activities while decreasing malondialdehyde (MDA) levels. Para-chlorophenylalanine was used to produce anxiety in diabetic mice and mice having anxiety caused by diabetes (Chhatre et al. 2014). Catalase is an enzyme that disintegrates hydrogen peroxide (H_2O_2) into water and oxygen; moreover, this acting a vital position in defensive cells alongside reactive oxygen species (ROS). Superoxide dismutase is a catalytic enzyme that catalyzes the conversion of superoxide anion-free radical (O_2) to oxygen (O_2) and H_2O_2 (Neychev and Mitev 2016). MDA is measure of oxidative stress plus a few of the conclusion yields of the peroxidation of polyunsaturated fatty acids (PUFAs) (Shang 2008).

STZ-induced diabetes has been demonstrated to enhance oxidative stress, while TT extracts (plant source UAE, 70% ethanolic extract) appear to be able to modulate oxidative stress indicators (Mohammed et al. 2016).

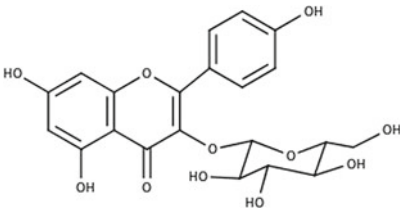
23.10.2 Abnormalities Associated with Sex

Anaerobic muscular strength and circulating testosterone in youngsters rose dramatically following 1 month of administration well with nutritional supplement "*Tribulus*," according to a recent research (Khare 2008).

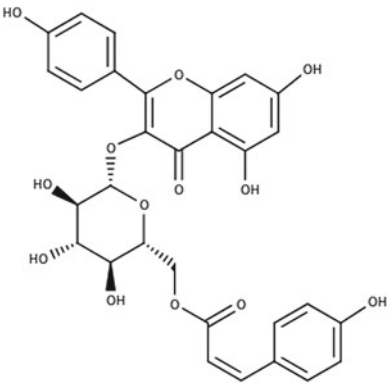
The following is a summary of the available facts on the processes underlying its usage in sexual problems (Fig. 23.5): Others (Akram et al. 2011) deny that steroidal



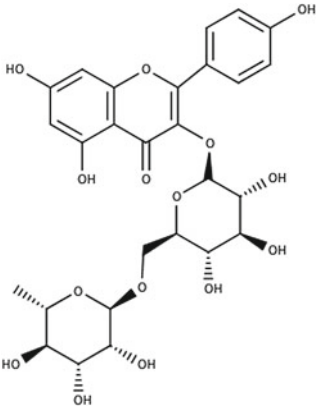
(**a**)Kaempferol



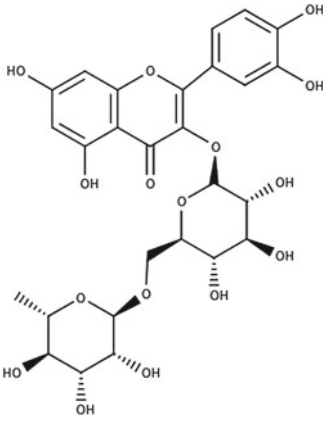
(**b**)Astragalin



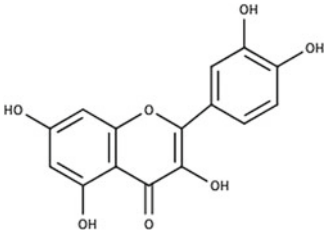
(**c**)Tribuloside



(**d**)Kaempferol3-rutinoside



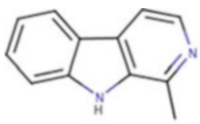
(**e**)Rutin



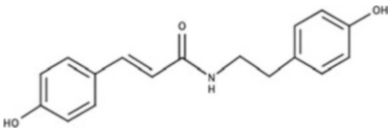
(**f**)Quercetin

Fig. 23.4 Most widespread compounds found in TT extracts

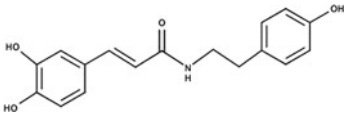
saponins from TT raise endogenous levels of testosterone through an unintended consequence, such as the LH-type effect of the steroidal saponosides or even a mild androgenic antagonist sort activity (Usman et al. 2007). The hormone (LH) controls



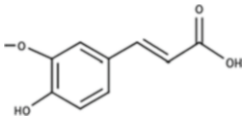
(**g**)Harmane



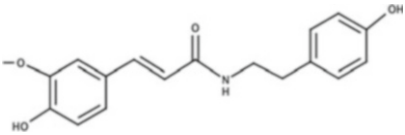
(**h**)Coumaroyltyramine



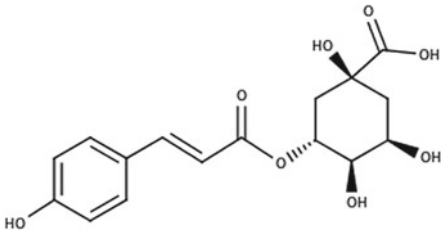
(**i**)Caffeoyltyramine



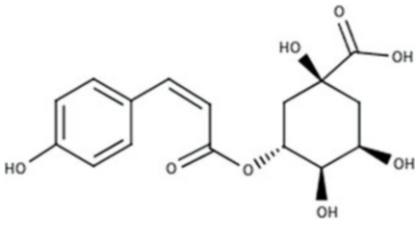
(**j**)Ferulicacid



(**k**)Feruloyloctopamine



(**i**)5-p-*trans*-coumaroylquinicacid



(**m**)5-p-cis-coumaroylquinicacid

Fig. 23.4 (continued)

the enzyme 17-hydroxysteroid dehydrogenase, which converts androstenedione to testosterone (Kostova and Dinchev 2005). Furthermore, because oxidative stress seems connected to arterial stiffness, the antioxidant impact of TT might assist to its stimulant impact. Nitric oxide facilitates the synthesis of cyclic guanosine monophosphate (cGMP); such technique might encourage arousal through dilatation and improved blood flow towards the corpora cavernosa. Reactive oxygen as well as progressive terminal glycation products combines to nitric oxide in the endothelium to create reactive nitrogen species under oxidative stress, which assists in the development of impotence (Xu et al. 2010). Additionally, other research had demonstrated that TT extracts also effective inside females in regard to sexually problems, including positive results in controlled studies on sexually fatigue drive within females and the management of menopausal transition disorders Bhutani et al. (1969).

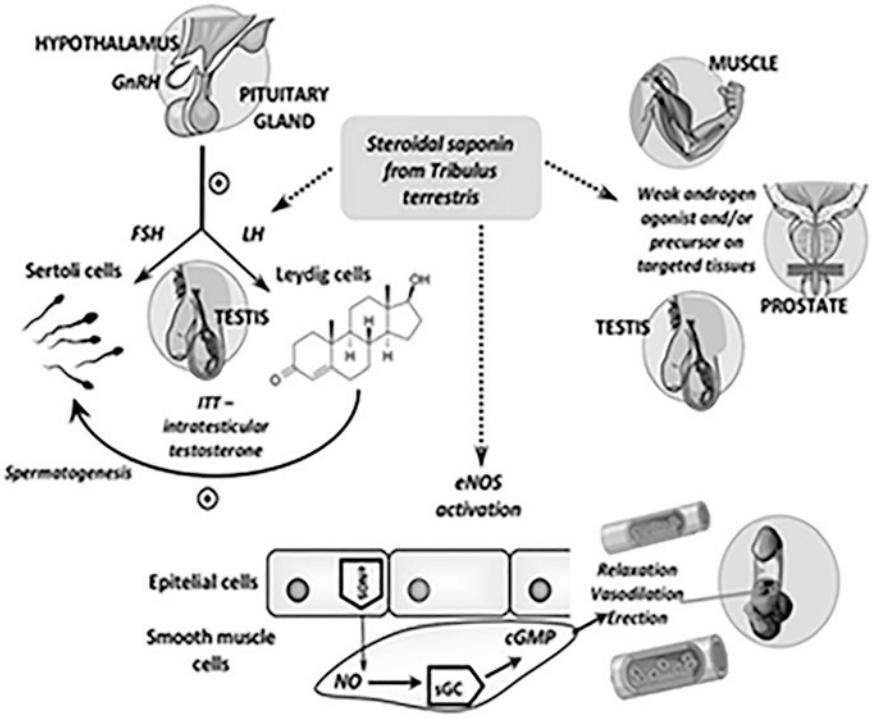


Fig. 23.5 The pathways of administration that are thought to be accountable for the impacts of TT extracts upon fertility issues. GnRH stands for gonadotropin-releasing hormone; FSH stands for follicle-stimulating hormone; LH stands for luteinizing hormone; ITT stands for intratesticular testosterone; eNOS stands for endothelial nitric oxide synthase; NO stands for nitric oxide; sGC stands for soluble guanylate cyclase; and cGMP stands for cyclic guanosine monophosphate

23.10.3 Antihyperglycemic Effect

The saponins of TT fed to mice proved able to postpone postprandial hyperglycemia via blocking alpha-glucosidase, indicating that with in vivo animal research seem to be consistent with in vitro research (Louveaux et al. 1998). TT extracts have been proven to reduce fasting postprandial hyperglycemia mice as well as glucose-loaded rabbits, indicating that the bioactive chemicals possess diverse spectrum of activity (Raja and Venkataraman 2011). Despite the fact that a large number of the clinical studies for TT extracts has been performed on diabetic mice to assess the outcome on comorbidities due to diabetes, the vast bulk of which were associated to sexual dysfunction, most authors confirmed that TT extracts have an anti-hyperglycemic impact (Bremner et al. 2005).

Via PPAR stimulation within fatty tissue as well as oxidative stress regulation, diosgenin was demonstrated to enhance insulin production as well as affect beta cell repair in STZ-induced diabetes in mice (Lv AL, 2007). The insulin-sensitizing

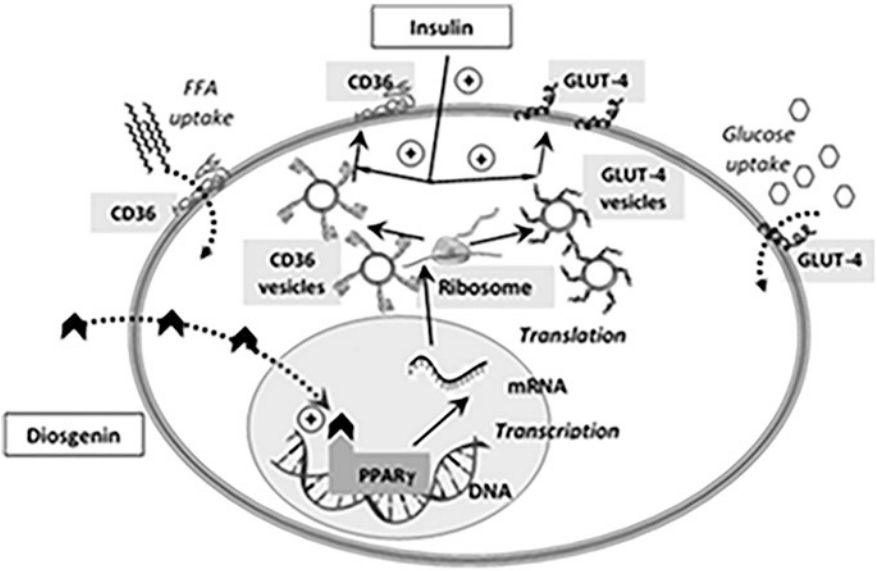


Fig. 23.6 Process through which diosgenin triggers PPAR receptor. CD36, clustering of differentiating 36; PPAR, peroxisome proliferator-activated receptors gamma; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid; FFA, free fatty acids; GLUT-4, glucose transporter 4; and PPAR, peroxisome proliferator-activated receptor gamma

activity of diosgenin might be explained by modifying the free fatty acid/glucose proportion through boosting their cytoplasmic absorption in muscular tone as well as adipocytes via activation of PPAR-specific receptors. The stimulation of the production of GLUT-4 and CD36 like a consequence of PPAR receptor stimulation might lead in cytoplasmic absorption of both sugars and free fatty acids (Fig. 23.6). Imidazolidine derivatives promote insulin release by activating imidazoline receptor type 3 binding affinity by β cells in the pancreas (Jie et al. 2003), suggesting that alkaloids and steroidal saponins might work together.

23.10.4 Antibacterial Activity

TT whole or graded preparations have been shown to display antibacterial activity adjacent to Gram-negative and Gram-positive bacteria inside a number of in vitro experiments. *Staphylococcus aureus, Streptoccocus mutans, Streptococcus sanguinis, Actinomyces viscosus, Enteroccocus faecalis,* and *Bacillus subtilis* were vulnerable between many Gram-positive bacteria, while *Escherichia coli, Salmonella typhi, Proteus mirabilis*, and *Klebsiella pneumoniae* have been vulnerable between Gram-negative bacteria (Faruq et al. 2002). Although it is currently unknown whose constituents seem to be accountable again for antibacterial action,

alkaloids lead to the entire extracts' overall antibacterial impact (Asibey-Berko and Tayie 1999). Saponins have antimicrobial qualities which are well established, & their mechanism of action is predicated just on degradation of the cell surface, which leads to cell death (bactericidal activity), which is likely owing to its amphiphilic nature & surfactant capabilities. Furthermore, saponins have been found to alter ion channels, affecting membrane permeability (Faruq et al. 2002).

The antimicrobial action of extracts from TT roots significantly lesser than that of extracts from the fruits, stems, and leaves, according to (Hassan and Umar 2004). Even though the researchers did not disclose phytochemical constituents of the extracts, we found that furostanol and spirostanol saponins were mostly discovered and measured in the aerial sections of TT instead of the roots (Table 23.4). Alkaloids, on the other hand being found in every tissue. Such findings imply that the saponin concentration of TT is largely responsible for its antibacterial effect. Antimicrobial property of flavonoid extracts from TT leaves and fruits has also been demonstrated towards E. coli, Salmonella, and Streptococcus (Isong et al. 1999).

The quorum dampening outcome of TT (India) root extracts upon *Chromobacterium violaceum, Serratia marcescens*, as well as *Pseudomonas aeruginosa* strains was recently reported in a study. ß-1, 5-O-dibenzoyl ribofuranose (Almasi et al. 2017) found discovered being the primary component.

23.10.5 Anti-inflammatory Properties

TT preparations have been shown to have anti-inflammatory properties in several investigations. Down regulation of the inflammatory cascade protein NFB (Zhao and Chen, 2014) is likely to be one of the key systems occurring. In tribulusterine, the extract was standardized. TT extracts have been demonstrated to encourage apoptotic cell death within human liver tumor cell by means of blocking the NFB signaling corridor, which furthermore a modulator of cell cycle & cell maintenance (Lovkova et al. 1989). The preparations to see an anti-inflammatory effect when applied topically, according to studies, by modulating the calcium channels Orai-1, TRPV3, as well as blocking mast signaling pathways (Gibson et al. 2016). Rutin has been the sole component found in the TT extract. In an in vitro investigation, Gibson et al. (2018) evaluated anti-inflammatory properties of tribulusamide D derived from fruit of TT. Researchers theorized that the impact was caused by enzymes involved in the synthesis of cytokines as well as inflammatory mediators being down regulated. TT fruits extract decreased COX-2 expression, according to (Brima 2017). TT extracts have also been proven to have anti-inflammatory properties in vitro (Almasi et al. 2017).

Table 23.4 *Tribulus terrestris* (TT) contains chemical substances that have been discovered

Compound	Chemical Formula	Plant part	Conc. mg/100 g
Furostanol saponins			
Protodioscin	$C_{51}H_{84}O_{22}$	Aerial parts	420–990
		Fruits	63–89
		Stem	24
Neoprotodioscin	$C_{51}H_{86}O_{22}$	Aerial parts	130–2200
		Fruits	21–28
		Leaves	700
		Stem	40
Prototribestin	C45 H73 $NaO_{20}S$	Fruits	17–65
		Aerial parts	420–990
Neoprototribestin	$C_{45}H_{75}NaO_{20}S$	–	NS
Terestrinin A	$C_{33}H_{48}O_9$	Fruits	–
Terestrinin B	$C_{60}H_{95}O_{30}$	–	–
Terrestrinin D	$C_{33}H_{5}0O_{10}$	–	5.6
Terestrinin J-T		Whole plant	NS
Terestroside A		Roots	–
Terrestrosin K	$C_{51}H_{82}O_{24}$	Fruits	1.27
Terrestrosin I	$C_{51}H_{84}O_{25}$	–	NS
Tribufuroside D	$C_{45}H_{74}O_{21}$	–	–
Tribufuroside E	$C_{45}H_{74}O_{21}$	–	–
Tribulosaponin A	$C_{51}H_{84}O_{21}$	–	–
Polianthoside D	$C_{56}H_{92}O_{29}$	–	–
Spirostanol Saponins			
Dioscin	$C_{45}H_{72}O_{16}$	Aerial parts	6–13
		Fruits	1–2
		Leaves	62
Tribestin	$C_{39}H_{61}NaO_{14}S$	Fruits	0.5–1
		Aerial parts	24
Diosgenin	$C_{27}H_{42}O_3$	Fruits	86
		Aerial parts	0.1–7.7
		Leaves	0.8
		Stem	1.7
Tribulosin	$C_{55}H_{90}O_{25}$	Aerial parts	2.24
		Fruits	420

(continued)

Table 23.4 (continued)

Compound	Chemical Formula	Plant part	Conc. mg/100 g
		Leaves	644
		Stem	185
Tigogenin	$C_{27}H_{44}O_3$	Fruits	0.05
Hecogenin	$C_{27}H_{42}O_4$	Fruits	0.4
Agovoside A		Fruits	NS
Prosapogenin B		Aerial parts	–
25R-5a-Spirost-3,6,12-trione	$C_{27}H_{39}O_5$	NS	–
25R-Spirost-4-ene-3,12-dione	$C_{27}H_{40}O_4$	–	–
25R-Spirost-4-ene-3,6,12-trione	$C_{27}H_{38}O_6$	–	–
Cinnamic acid amides			
Coumaroyltyramine	$C_{17}H_{17}NO_3$	Fruits	NS
Ferulic acid		–	–
Feruloyloctopamine	$C_{18}H_{19}NO_5$	–	–
Quinic acid derivatives			
5-p-cis-coumaroylquinic acid	$C_{16}H_{18}O_8$	Aerial parts	NS
5-p-*trans*-coumaroylquinic acid		–	–
4,5-Di-p-*trans*-coumaroylquinic acid		–	–
4,5-Di-p-*cis*-coumaroylquinic acid		–	–
Flavonoids			
Tribuloside	$C_{30}H_{26}O_{13}$	Leaves, fruits	NS
Kaempferol	$C_{15}H_{10}O_6$	–	18
Astragalin (kaempferol 3-glucoside)	$C_{21}H_{20}O_{11}$	–	NS
Kaempferol 3-rutinoside	$C_{27}H_{30}O_{15}$	–	–
Kaempferol-3- gentiobioside	$C_{27}H_{30}O_{16}$	–	–
Rutin	$C_{27}H_{30}O_{16}$	–	70–250
Quercetin	$C_{15}H_{10}O_7$	–	NS
Quercetin-3-*O*-arabinosyl galactocide	$C_{26}H_{28}O_{16}$	–	–
Quercetin-3-*O*-sophoroside-7-*O*-glucoside	$C_{33}H_{40}O_{21}$	Leaves	–
Quercetin-3- gentiobioside	$C_{27}H_{30}O_{17}$	Fruits, leaves	–
Quercetin 3,7-diglucoside	$C_{27}H_{30}O_{17}$	–	–
Isoquercitrin	$C_{21}H_{20}O_{12}$	–	–
Luteolin-7-*O*-β-D- glucoside	$C_{30}H_{18}O_{11}$	Leaves	–
Isorhamnetin-3-glucoside	$C_{22}H_{22}O_{12}$	Leaves	–
Alkaloids			
Harmine	$C_{13}H_{12}N_2O$	Fruits	14
Harmane	$C_{12}H_{10}N_2$	Leaves, roots	NS
Harmalol	$C_{12}H_{12}N_2O$	Leaves, roots	–

(continued)

Table 23.4 (continued)

Compound	Chemical Formula	Plant part	Conc. mg/100 g
Harmaline	$C_{13}H14N2O$	Roots	–
Norharmane	$C_{11}H8N2$	Aerial parts	–
Tribulusterine	$C_{16}H_{12}N_2O_2$	Fruits	–
n-Caffeoyltyramine		Fruits	–
Perlolyrine	$C_{16}H_{12}N_2O_2$	Not specified	–
Amides and lignanamides			
Terrestribisamide	$C_{13}H_{18}NO_5$	Fruits	NS
Tribulusamide A	$C_{36}H_{36}N2O_8$	–	–
Tribulusamide B	$C_{36}H_{34}N_2O_9$	–	–
Tribulusamide D	$C_{17}H_{15}NO_5$	–	–
Tribulusamide C	$C_{18}H_{15}NO_6$	–	–
Fatty acids and fatty acid esters			
Oleic acid	$C_{18}H_{34}O_2$	Stem	NS
Palmitic acid	$C_{16}H_{32}O_2$	–	–
6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	–	–
Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	–	–
9,12-Octadecadienoic acid, methyl ester (E,E)-	$C_{19}H_{34}O_2$	–	–
Phytosterols			
β-sitosterol-D-glucoside	$C_{35}H_{60}O_6$	Whole plant	NS
Stigmasterol	$C_{29}H_{48}O$	Stem	–
Other compounds			
ß-1, 5-*O*-dibenzoyl ribofuranose	$C_{19}H_{18}O_7$	Roots	NS
1,3-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester	$C_{24}H_{38}O_4$	Stem	–
Apiol	$C_{12}H_{14}O_4$	–	–
Octacosane	$C_{28}H_{58}$	–	–
Heptacosane	$C_{27}H_{56}$	–	–

23.11 Nutritional Composition of *T. terrestris*

23.11.1 Macronutrients of T. terrestris *Plant*

Table 23.5 shows the findings of proximate composition of *T. terrestris* leaves. The relative humidity (64%) was low when compared to the 92% found for cabbage, however greater than the 35% observed for sickle pod leaves. This high moisture level indicates that microbiological activity may be present throughout storing (Hassan, 2004). The ash, which measure of total mineral stuff found, was 5.11%, indicating that the leaves are mineral-rich. The result was lower than the 8.3% recorded by Faruq et al. (2002) in *Senna obtusifolia* leaves, 8.9% in *Corchorus*

Table 23.5 Proximate composition of *T. terrestris* leaves

Parameters	Composition (% Dry matter)[a]
Moisture (%wet weight)	64 ± 0.01
Ash	5.11 ± 0.01
Crude protein	21.29 ± 0.3
Crude lipid	5.03 ± 0.2
Crude fiber	12.99 ± 0.9
Carbohydrate	55.59 ± 2.2
Energy value (kcal/100 g)	291.98

[a]Mean ± standard deviation

tridens, and 14% in *Amaranthus incarvatus*, but greater than the 1.9% observed inside sweet potato leaves (Asibey-Berko and Tayie 1999).

T. terrestris leaves had a significant quantity of crude protein (21.29%). Plant-based foods which yield upwards of 11% of their calorie content from protein, according to Pearson (1976), are significant suppliers of protein. *T. terrestris* fulfills these criteria and more. Likewise, for kids weighing 14, 19, 27, 44 kg, and adults weighing 60 kg or more, the daily recommended protein intakes are 24, 29, 33, 46, and 55 grams, respectively (Faruq et al. 2002). This means that 107, 140, 159, 210, and 260 g of *T. terrestris* per day may meet their respective daily protein requirements.

T. terrestris leaves contain 5.03% crude lipid. Lipids are primary source of energies; however, they must never be consumed in excess of 30 calories per day. A gram of lipid has 9 calories, thus 100 g of *T. terrestris* leaves lipid should offer around 41 calories.

The leaves have a high carbohydrate concentration (55.59%) due to their low lipid concentration. The main sources of energy are carbohydrate and fat; the carbohydrate content of 100 g of *T. terrestris* leaves may supply 199 kcal of energy. It shows that somehow this plant's foliage could provide significantly to an individual's everyday energy needs of 2400 kcal.

Crude fiber is important in human diet because it lowers cholesterol levels in the blood, lowers the hazard of congestive heart failure, colorectal and breast cancer, and lowers blood pressure. Additionally, it improves glucose tolerance and insulin levels (Hassan and Umar 2004). *T. terrestris* leaves have a fiber content of 12.99%.

As per a research, most vegetables have a reduced calorie density (30–50 kcal/100 g), while *T. terrestris* leaves had a significant value of 291 kcal/100 g, which is within the range of 249–308 kcal/100 g found within few lush green veggies (Isong et al. 1999).

23.11.2 Micronutrient Contents of T. Terrestris *Plant*

Herbs are useful in medicine since they possess active ingredients which have a particular pharmacological action within body. Organic and inorganic ingredients are found in medicinal plants. The existence of active elements which are

accountable for significant physiological activities inside living beings has been linked to the pharmacological capabilities of medicinal plants. *Tribulus terrestris L.* steroidal saponins thought to play a role due to its natural commotion of products generated as of this plant. Flavonoids, flavonol glycosides, steroidal saponins, and alkaloids are among chemical ingredients found in various portions of the plant. The amount and content of active saponins determine the activity, which is regulated by the geological distribution of plant substance (Zhao et al. 2014), (Brima 2017), and (Almasi et al. 2017).

Because the levels of Na, Mg, K, Ca, and Zn in the investigated samples of T. terrestris are higher than the values present in a reference plant as shown in table 23.6, it may be concluded that the amount of T. terrestris that contribute to daily human diet is low. The value of *T. terrestris* that contribute to daily human needs might be deemed minimal because the amounts of Na, Mg, K, Ca, and Zn in examined samples of *T. terrestris* are greater compared to the values used as a benchmark. Controlling quality of input material for standardized extracts can be aided by element determination (Gibson et al. 2016). Even though the trialed commodities of *T. terrestris* have high Ca, Fe, and Zn content, they can't be utilized to make up for a lack of food because any plant supplies with elevated substance of 2 and 3-valent ions contain little bioavailability because they are high in phytic acid, polyphenols, as well as tannins, that strictly confines absorption. Phytic acid seems to be a nutritional antagonist in terms of mineral uptake, despite its other benefits (Gibson et al. 2018).

The findings of the Epithermal Neutron Activation Analysis (ENAA) of the *T. terrestris* herbs are shown in Table 23.6. The proportions of major components in *T. terrestris* plants provided in milligrams per kilogram.

Table 23.6 Inorganic component content (mg/kg) with in wild. *Tribulus terrestris L.* based upon their location of the harvest. Remark: Exceeding values are in bold format

Components	Samples of *Tribulus terrestris L.*				Reference plant market (Markert 1992)
	1	2	3	4	
Na	**1030**/3	**643**/3	108/3	146/3	150
Mg	**5190**/2	**5320**/2	4290/2	5410/2	2000
Al	714/4	**805**/4	112/4	41/4	80
Cl	**7350**/8	**8540**/8	4120/8	1240/8	2000
K	**36,400**/9	**30,600**/9	26,000/9	28,500/9	19,000
Ca	**21,400**/7	**21,800**/7	34,600/7	19,600/7	1000
Sc	0.16/6	0.12/6	0.03/18	0.02/18	0.02
V	**1**/12	**1**/12	0.2/12	0.2/12	0.5
Mn	40/5	38/5	19/5	112/5	200
Fe	**613**/10	**482**/10	**246**/10	111/10	150
Zn	**75**/5	35/5	31/5	**140**/5	50
As	0.9/4	0.8/4	0.9/2	1.1/4	0.1
Se	**0.31**/17	**0.22**/19	**0.4**/16	**0.3**/15	0.02
Mo	**1.8**/29	1/29	**2.6**/29	0.3/29	0.6

The values of micronutrients which are more significantly present in T. terrestris as compare to reference plant is in bold.

Samples 1 and 2 taken in the region of Mongolia, near city of Bao Tou; samples 3 were taken from Xian Shan Mountains region, near Beijing. Sample 4 was gathered at the Botanical Institute of the Russian Academy of Sciences (St. Petersburg, Russia). Table 23.6 shows that the concentration of elements such as Na, Mg, Cl, K, Ca, and Fe is higher in wild samples (from China) than in plants produced in the Botanical Garden (Russia). The Russian samples, on the other hand, had a 2–3 times greater magnesium and zinc level than the Chinese samples. The data in Table 23.6 also shows that the hazardous components in the Russian sample are several percent smaller than those from the Chinese sample. The As level, on the other hand, was found to be greater.

The amount of macronutrients such as K (42 mg/g), Ca (37 mg/g), Mg (6 mg/g), and Fe (0.4 mg/g) was previously found in *T. terrestris* herbs (Lovkova et al. 1989). Mn-0.2, Cu-0.6, Zn-3, Mo-0.91, Al-0.21, Ba-11.90, V-0.02, and Se-5.49 are the amounts of trace elements.

A comparison of the collected information with available figures (Selvaraju et al. 2011) reveals that the Chinese *T. terrestris* samples tested accumulate the same chemicals in equal levels. Table 23.6 shows that the amount of Na, K, and Fe in the grass drops with time in the *T. terrestris* grass taken in China throughout the summer months. Under comparison to wild plants, *T. terrestris* cultivated plants collect far less essential elements in the environment of St. Petersburg. The collected findings were compared to the data from the "Reference Plant" (Markert 1992). This comparison reveals that the proportions of components such as Al, Cl, Mo, and Se in all tested plants surpass the "Reference plant" levels.

23.12 Conclusions

It is concluded from the above discussion that TT possess numerous phytochemicals of therapeutic potential. TT is widely used in traditional herbal medicines in Pakistan, Sudan, India, and China as aphrodisiac, energy booster, and one that improves heart health. It possesses a broad spectrum of antibacterial, anti-aging, and anti-inflammatory properties. Recent scientific interventions have explored that steroidal saponins and flavonoids present in this herb showed significant anti-aging and anti-inflammatory properties. Clinical studies on the efficacy of this herb are limited and require for the initiation of randomized placebo-controlled clinical procedures for better understanding about the pharmacological status of this herb. The results and data accessible in this review supply strong technical base for better exploitation of TT and motivating other scientists to further explore this herb for improved innovative therapeutic products.

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Chapter 24
Species of the Genus *Viola* Found in the Western Himalayas

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Abbreviations

C. albicans	*Candida albicans*
$CycO_2$	Cycloviolacin O_2
DTH	Delayed Type Hypersensitivity
F. solani	*Fusarium solani*
M. canis	*Microsporum canis*
MTCC	Microbial type culture collection
NF-κB	Nuclear Factor kappa B
NK	Natural Killer
ROS	Reactive oxygen species
Th1	T helper type 1
V. betonicifolia	*Viola betonicifolia*
V. biflora	*Viola biflora*
V. canescens	*Viola canescens*
V. odorata	*Viola odorata*

24.1 Introduction

Lifestyle is now getting techno-savvy thus hectic day-by-day results in increasing health issues that made people increase the use of allopathic medicine. No doubt allopathy has the potential to heal an array of diseases rapidly but results in various side effects (Ekor 2014). Thus, health practitioners, doctors, dieticians, nutritionists, and researchers are switching from synthetic drugs to traditional or herbal medicines that are free from side effects, comparatively safe, eco-friendly, and locally available

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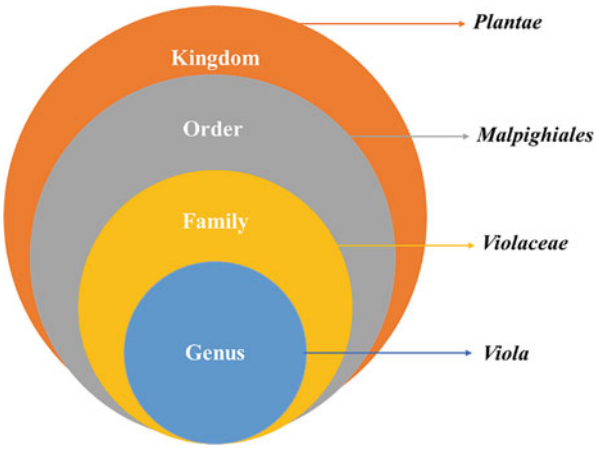

Fig. 24.1 Botanical description of *Viola* genus

in comparison (Kala et al. 2006; Ekor 2014). This is the reason why herbal treatment is growing in popularity across the globe. Also, the limelight for the increasing demand of the usage of herbal medicine attributed to its affordability thus meets the financial status of an individual and allows greater public access.

Herbal medicine (Parle and Bansal 2006) is the use of different parts of the woody and non-woody plants, i.e., leaves, flowers, seeds, stems, and roots that were available within the geographical location to promote the diagnosis, prevention as well as the treatment of physical and mental ailments (Bent and Ko 2004). Herbs are used extensively in a variety of traditional practices such as Ayurveda, Siddha, and Unani to treat a variety of illnesses (Mohammad 2012; Parasuraman et al. 2014).

Traditionally there are a lot of herbs and plants used generally considered as home remedies that are being used by each and every individual in day-to-day-life, viz., cardamom, turmeric (Yadav et al. 2005), tulsi (Borah and Biswas 2018), ginger (Singh and Singh 2019), black pepper (Damanhouri and Ahmad 2014), garlic (Moutia et al. 2018), and onion (Elberry et al. 2014) that either actively or passively acts on the immune system of the body to treat the diseases (Singh 2016). Likewise, various species from the different families of the plant kingdom effects the immunity of the body that does not only resist the foreign substances from entering into the body but also kill them to sustain a healthy life. In this chapter, only the species of the *Violaceae* family and its *Viola* genus have been discussed along with its botanical description in Fig. 24.1.

Violaceae family also recognized as *Retrosepalaceae*, *Leoniaceae*, and *Alsodeiaceae* (Deepak et al. 2017) contains about 20 genera and 800 species that provide a broad spectrum of therapeutic uses (Muhammad et al. 2012a). *Viola* is the biggest genus of the *Violaceae* family consisting of about 500 species, which are found all across the globe; some of which are perennial and annual herbs with short stems and rhizomes.

24.1.1 Geographical Distribution

Species of the *Viola* are found in South Asian countries, viz., India, China, Nepal, Pakistan, Sri Lanka, Malaysia, and Australia. In Indian species, the genus *Viola* is mainly found in the Western Himalayas (Muhammad et al. 2012a) (Table 24.1). *Viola odorata* (Singh and Dhariwal 2018) is well-recognized by the name sweet violet and is native to India and found mainly in hilly areas such as Kashmir and Himachal Pradesh. Also, Other species belonging to the western Himalayas are *Viola biflora* and *Viola canescens* (Mann et al. 2016).

Some other known species, viz., *Viola stocksii*, *Viola falconeri*, and *Viola kunawurensis* (Wang et al. 2017) have been discovered in various parts of India and Pakistan. Meanwhile, *Viola mandshurica* and *Viola arvensis* are native to Nepal and Romania, respectively (Muhammad et al. 2012a).

Violaceae family is not economically productive but the genus *Viola* has been traditionally practiced to prevent different kinds of syndromes, which have also been scientifically validated for their antimicrobial, anti-inflammatory, antioxidant, anti-hypertensive, anthelmintic, antiasthmatic, anti-HIV, antipyretic, antidyslipidemic, anti-HIV, analgesic, and antifebrile properties. Species of this genus are often used in ethnomedicine as detoxicants and to cure severe pyogenic diseases such as carbuncles, furuncles, and boils. The purpose of this chapter is to mark the immune regulation and immune-boosting activities of a few *Viola* species namely *V. odorata* (Datta et al. 2020), *V. betonicifolia* (Rizwan et al. 2019), and *V. canescens* (Masood et al. 2014).

Table 24.1 Geographical distribution of *Viola* species

S. no.	Species	Western Himalayas Region	Species found in other countries
1.	*V. odorata*	Kashmir	Pakistan, Nepal, Iran Afghanistan, Iraq, Mediterranean region (Muhammad et al. 2012a)
2.	*V. biflora*	Jammu and Kashmir, Uttarakhand, and Himachal Pradesh	Europe, Central Asia, and Pakistan (Muhammad et al. 2012a)
3.	*V. canescens*	Jammu and Kashmir, Uttarakhand, and Himachal Pradesh (Mann et al. 2016)	Pakistan, Nepal, and Bhutan (Muhammad et al. 2012a)
4.	*V. pilosa*	Uttarakhand	Pakistan, Ceylon, Nepal, China, and Java (Muhammad et al. 2012a)
5.	*V. serpens*	Uttarakhand, Himachal Pradesh. Other regions are Meghalaya, Nagaland, Manipur, and Orissa (Deepak et al. 2017)	Pakistan, Ceylon, Nepal, China, and Java (Muhammad et al. 2012a)
6.	*V. betonicifolia*	Jammu and Kashmir (Rizwan et al. 2019)	Nepal, Pakistan, Indo-China, Sri Lanka, Japan, Burma, China, Malaysia, and Australia (Muhammad et al. 2012a)

24.2 Immunity and Immune System

Before we go into the immunological aspects of *Viola*, it is necessary to understand what immunity is and how it works. Immunity is described as the capacity of the body to recognize as well as resist a wide variety of contagious and potentially dangerous bacteria, allowing it to avoid or resist infection as well as prevent organ and tissue damage. The immune system is not restricted to a single area of the body. Immune stem cells produced in the bone marrow may remain till it moves to other bodily locations for maturation. As a result, the vast majority of immune cells travel down the bloodstream, exerting specific functions.

A multilayered immune system works on several levels out of which skin is the most apparent and primary defense against infection (Kumar et al. 2012). Anatomic and physiological factors such as body temperature and pH offer unsuitable dwelling circumstances for foreign microbes and augments the innate or natural (Medzhitov and Janeway 1975) as well as acquired or adaptive immune systems thus generating a protective response (Bonilla and Oettgen 2010; Sharma et al. 2017). The functioning of the innate immune system depends upon the hematopoietic cells and the non-hematopoietic cells whereas T and B lymphocytes usually participate in the adaptive immune system (Turvey and Broide 2010). The adaptive immune system resists invading organisms through these two separate but interconnected mechanisms: humoral immunity (the antibody-mediated defense system based on B cells produced in the bone marrow) and cellular immunity (Elberg 1960) (the cell-mediated defense system, i.e., T cells found in thymus) (Maggini et al. 2007). The brief classification of the immune system is discussed in Fig. 24.2 (Turvey and Broide 2010; Sharma et al. 2017).

Acquired or adaptive immune system (Bonilla and Oettgen 2010) is made up of a slew of cells as well as molecules that work together to identify and kill invaders. The complicated signaling system that governs the immune response is ascribed to

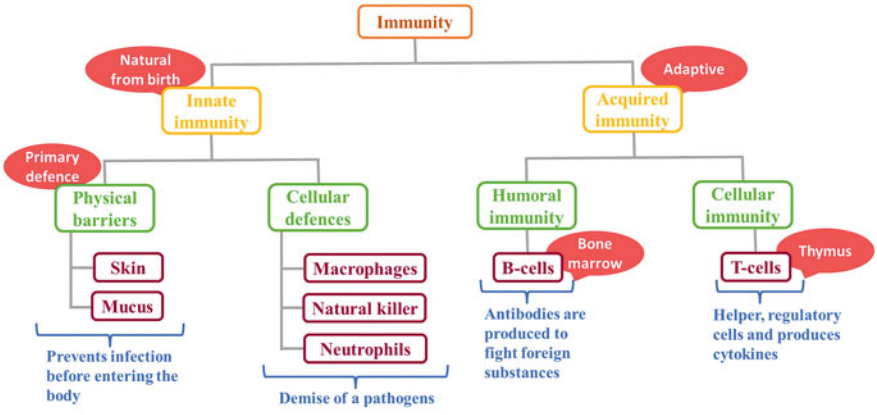

Fig. 24.2 Brief classification of the Immune system

the chemical binding of different receptors to immune cells as well as pathogens, which further determines pathogen identification and elimination. Many plant extracts exercised in the Indian traditional system known as Rasayana (Chulet and Pradhan 2009) (which is aimed at boosting the body's immunity) caught the interest of scientists worldwide (Mukherjee et al. 2014). Some *Viola* species, as listed below, exhibit not only immune-boosting action but also antioxidant, anti-inflammatory, diuretic, anticancer, antiasthmatic, antifungal, pulmonary, and other therapeutic characteristics.

24.3 Viola Odorata

Almost 200 phytochemicals, including terpenoids, essential oils, saccharides, flavonoids, sterols, aromatic compounds, amides, and other derivatives, have been identified from several *Viola* species (Singh and Dhariwal 2018). The various chemical constituents of different species have been discussed below. *V. odorata* (sweet violet) serves *Violaceae* family, is an evergreen perennial herb that can grow upto 10 cm, and blooms in late winters. The flowers are bowing, a rich violet color with a pleasant odor. It is found in Kashmir and the Western Himalayas at elevations ranging from 1500 to 1800 m above sea level. The plant is widely renowned for its therapeutic potential in the Ayurvedic and Unani medical systems (Singh and Dhariwal 2018).

24.3.1 Chemical Constituents

V. odorata contains cyclotides (Slazak et al. 2016; Ajaz et al. 2020), anthocyanins, alkaloid (violin), glycoside (violequercetin), saponins, mucilage, methyl salicylate (1), and macrocyclic peptides (ethyl hexanoate (2) and (2*E*,6*Z*)-nona-2,6-dienol, (*E*, *E*)-hepta-2,4-dienal, hexanoic acid, limonene, tridecane, and eugenol). The 25 compounds in the essential oil composition of *V. odorata* leaves represented 92.77% of the oil, with butyl-2-ethylhexylphthalate (3) (30.10%) and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)- benzofuranone (4) (12.03%) being the two primary components (Mittal et al. 2015) (Fig. 24.3).

The components were 1-phenyl butanone (5) (22.43%), linalool (6) (7.33%), benzyl alcohol (7) (5.65%), α-cadinol (8) (4.91%), globulol (9) (4.32%), and viridiflorol (10) (3.51%) were found to be in major proportions whereas pulegone (11) (3.33%), terpinen-4-ol (12) (2.31%), germacrene A (13) (1.99%), and *p*-methyl anisole (14) (1.09%) found in minor quantities in the *V. odorata* flower oil (Mittal et al. 2015; Feyzabadi et al. 2017) (Fig 24.4).

The plant is also known to exhibit ester of fatty acids, hydrocarbons, alkanols, polysaccharides of glucose, galactose, and galacturonic acids. *V. odorata* is a recognized plant in Ayurvedic, Unani, Iranian, and Persian traditional systems to

Fig. 24.3 Chemical constituents of *V. odorata*

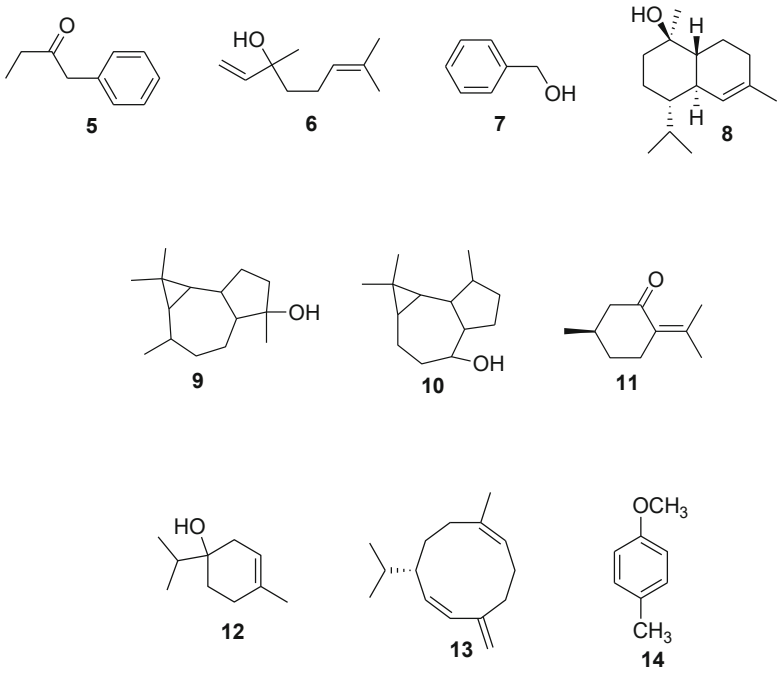

Fig. 24.4 Major and minor constituents of *V. odorata*

treat different ailments. In India, it is used as an antipyretic and to treat common cold, cough, headache, and migraine. In Unani traditional system, the plant is acknowledged for its laxative properties. Furthermore, it can be used to treat palpitations and cardiac disorders (Mahboubi and Kashani 2018). The flowers of the plant can be used in nasal or topical forms in Iranian folk remedies to treat headache, gastric diseases, insomania, etc. Also, some local inhabitants used the plant for treating eczema, jaundice, and febrifuge. In the traditional Persian system, the plant is used for the treatment of inflammations. Additionally, its essential oils can be used in aromatherapy. The syrup of the violet leaves mixed with the sugar softens and manages rectal prolapse in children. The leaves of *V. odorata* have a cooling property that helps to relieve heartburn, eyes as well as genital inflammations, which is why it is also regarded as a cooling herb. (Tobyn et al. 2011).

24.3.2 V. odorata *in Immune Regulation*

The nutritive compositions of the aerial parts of the *V. odorata* indicate the presence of different elements such as calcium, carbon, sodium, magnesium, aluminum, oxygen, silicon, chloride, iron (Datta et al. 2020), vitamin A as well as Vitamin C,

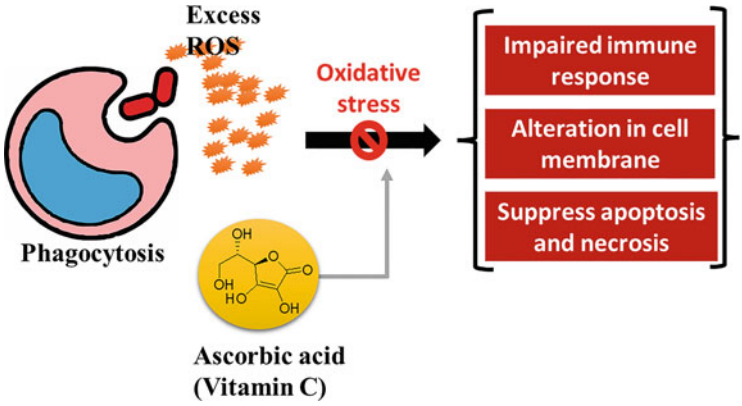

Fig. 24.5 Vitamin C scavenges the production of oxidative stress

which acts as an immunostimulant (Muhammad et al. 2012a). The immunological route used by vitamin C and iron to boost the functioning of the body's immune system is reviewed briefly.

Vitamin C: If reactive oxygen species (ROS) are produced in large concentrations by activated immune cells during the formation of phagosomes, resulting in oxidative stress, impaired immune response, loss of cell membrane integrity, and altered membrane function can be scavenged by vitamin C (Fig. 24.5).

The occurrence of a large amount of leukocytes in vitamin C contributes to its immune-enhancing effect. Indeed, it has been identified that the mechanism of leukocyte activities as seen in Fig. 24.6 found to improve when supplemented with vitamin C in young adults (1–3 g/day) and kids (20 mg/kg/day), especially neutrophil and monocyte mobility has enhanced up to a great extent (Maggini et al. 2007).

Furthermore, vitamin C is also responsible for the bustle of the immune system by increasing T lymphocyte proliferation therefore raising cytokine output and immunoglobulin synthesis. Apart from the aforementioned function's role of vitamin C on various immune cells has been discussed in Table 24.2 (Carr and Maggini 2017).

Vitamin C may potentially have a function in the control of the inflammatory response. Its supplementation improves numerous aspects of the human immune response, including antimicrobial and Natural Killer (NK) cell activity, lymphocyte proliferation, chemotaxis, and Delayed Type Hypersentivity (DTH) response (Maggini et al. 2007).

Iron: Iron is required for transporting electrons, gene control, oxygen binding along with its transport, cell differentiation, and growth regulation. Iron is an essential component for enzymes that produce peroxide as well as nitrous oxide. It is known to regulate cytokine production and for the stimulation of protein kinase C required to intend the phosphorylation of cell proliferation-regulating molecules. Furthermore, iron is required for the action of myeloperoxidase that promotes the death of bacteria by neutrophils via the production of detrimental hydroxyl radicals.

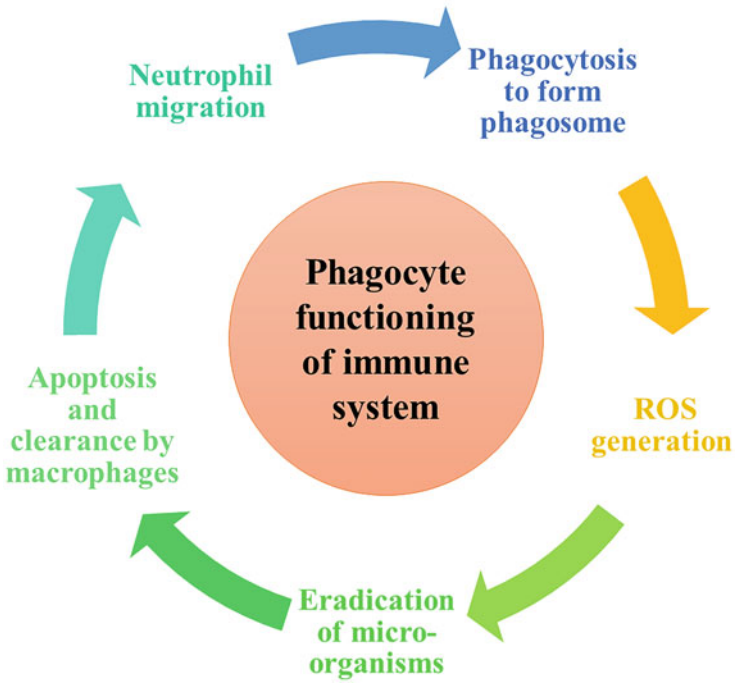

Fig. 24.6 Mechanism of phagocyte function

Table 24.2 Functions of Vitamin C on immune cells

S. no.	Immune cell type	Function of Vitamin C
1.	Epithelial barriers	• Increase production of collagen and stabilization prevents damage caused by ROS and reduces the time for wound healing • Increase keratinocyte differentiation and lipid synthesis, fibroblast proliferation, and migration
2.	Macrophages/ neutrophils	• Electron-donating species • Increases phagocytosis, ROS generation, microbial killing, facilitates apoptosis and clearance, suppresses necrosis, and enhances the monocyte mobility
3.	Inflammatory mediators	• Modulates cytokine production and decreases the concentration of histamine
4.	B and T lymphocytes	• Favors differentiation, proliferation, and antibody levels

As a result, any change in cellular iron equilibrium, whether due to deficiency or excess, results in a negative functional impact on immunity. Pathogens such as infected microorganisms or viruses demand iron as well as other microelements for replication and survival; therefore, it appears critical to limit the infecting microorganism's access to iron while maintaining a suitable concentration of iron

so that the host can mount an optimal immune response while avoiding the possibility of excess iron, which may induce free radical-mediated damage (Maggini et al. 2007).

24.3.3 Other Biological Activities of V. Odorata

The aqueous extract of *V. odorata* inflorescence exhibits anthocyanins which can act as antibacterial, anti-inflammatory, antihypertensive, antidyslipidemic, anticancer, anti-HIV, antioxidant, antipyretic agents, and so on.

24.3.3.1 Antibacterial Effect

A cyclotide named Cycloviolacin O_2 ($CycO_2$), a constituent isolated from sweet violet, has shown significant bactericidal action against various Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumonia,* and *Escherichia coli* (Pränting et al. 2010; Feyzabadi et al. 2017). The aerial portions of *V. odorata* exhibited strong antibacterial action against respiratory bacterial microbes, viz., Influenza Haemophilus *Staphylococcus aureus*, Microbial type culture collection (MTCC) 1144, *Streptococcus pneumonia*, MTCC 655, and *Streptococcus pyogenes*, MTCC 442, *Pseudomonas aeruginosa* (Gautam and Kumar 2012).

24.3.3.2 Anti-Inflammatory Activity

An aqueous extract of violet flowers was tested to treat formalin-induced lung damage in rodents. Pretreatment with the extract was shown to be somewhat helpful in avoiding lung injury and the results were similar to that of hydrocortisone (a medication used to treat inflammation) (Koochek et al. 2003). In a clinical study, a decoction of *V. odorata* was administered enterally accompanied by co-amoxiclav and fexofenadine to mitigate tonsillitis (inflammation of tonsil) and peritonsillar abscess (a bacterial infection in the throat). The analysis revealed that using a decoction on a regular basis can reduce tonsillitis as well as peritonsillar abscess (Feyzabadi et al. 2017).

24.3.3.3 Antihypertensive and Antidyslipidemic Activities

The antihypertensive and vasorelaxant effects of an aqueous-methanol leaf extract of *V. odorata* leaves have been demonstrated in vivo using both anesthetized as well as isolated rat heart tissues (atria and aorta) (Siddiqi et al. 2012). In addition, extract treated with an atherogenic diet when consumed by rodents results in lowering of

total cholesterol level, low-density lipoprotein, and reduction in body weight. As a result, improvements in hypertension, dyslipidemia, and weight reduction demonstrated the potential of the leaf extract of common violet in the treatment of metabolism (Feyzabadi et al. 2017).

24.3.3.4 Pulmonary Diseases

Due to the presence of vitamin C, which creates immunostimulant characteristics, the impact of *V. odorata* flower syrup on cough suppression and asthma relief in infants has recently been explored (Feyzabadi et al. 2017). As a result, the substance was more effective than a placebo and is used as an adjuvant in conjunction with β-agonists (Qasemzadeh et al. 2015).

24.3.3.5 Anticancer Effect

An isolated cyclotide $CycO_2$ of sweet violet exhibits antitumor effects against MCF-7 and MCF-7/ADR breast cancer cell lines (Feyzabadi et al. 2017). The mechanism is mediated by increasing the permeability of the cell membrane and subsequent cellular disruption. Thus, $CycO_2$ is regarded to be an active ingredient to manage drug-resistant breast cancer (Gerlach et al. 2010).

24.3.3.6 Antioxidant Effect

A study reveals that aqueous, dichloromethane, ethanol, and ethyl acetate extract of *V. odorata* scavenges the production of NO radicals thus reducing oxidative stress (Erdogan Orhan et al. 2015). Also, the ethanol extract portrays considerable tyrosinase inhibition with a value of 80.23% $\pm$ 0.87% (Feyzabadi et al. 2017).

24.3.3.7 Laxative Effects

The butanol and aqueous extracts of violet aerial parts reported good laxative effects in rats with 200 and 400 mg/kg dose, respectively. The extract increases gastrointestinal motility and increases the volume of urine with a high concentration of sodium ion (Na+) and potassium ion (K+) (Feyzabadi et al. 2017).

Apart from the biological activities, *V. odorata* has also been studied clinically for the disorders mentioned in Table 24.3 along with its results and effects (Mahboubi and Kashani 2018)

Table 24.3 Clinical studies of *V. odorata*

Disorder	Control group	Patient	Herbal extract	Current treatment	Time period	Results	Effects
Migraine	Placebo control	Adults	Sofoof-E-Banafshe	20 mg Propranolol	30 days	Reduction in migraine headaches and improved quality of sleep	No adverse effects
Cough in intermittent asthma	Placebo control	Children (2–12 years)	Aqueous extract of V. odorata flowers with red sugar	β-blockers	5 days	Suppression of cough and wheezing	No adverse effects
Chronic insomnia	–	Patients between 16–50 age group	Flowers extract in almond or sesame oil	–	30 days	Improved quality of sleep	Cough, post-nasal discharge, and itching in throat

24.4 *Viola betonicifolia*

In Pakistan, the plant *V. betonicifolia* is known by names such as Banafsha or Banfosha. It grows in a wide range of environmental habitats, including woodland, forest, shrubland, and agricultural fields (Rizwan et al. 2019). It is an annual herb 8–20 cm in height. It is long and sleek with arrow-shaped leaves that normally grow from the base, and a stem devoid of V-shaped sinus. The plant is often bright and has a fresh-green hue. Traditionally, the plant was used as a diaphoretic, purgative, astringent, anticancer, antipyretics, as well as to cure a variety of ailments including nerve problems, (Iqbal and Hamayun 2004; Shinwari 2010), cough (Tiwari et al. 2010), skin disorders, sinusitis, blood disorders, pharyngitis (Bhatt and Negi 2006), renal problems, pneumonia, and bronchitis (Bhatt and Negi 2006; Husain et al. 2008).

24.4.1 Chemical Constituents

The plant *V. betonicifolia* forms a prospective and significant source of several phytochemicals including alkaloids, proteins, flavonoids, tannins, phenolic compounds, saponins, sterols, as well as triterpenoids when investigated in methanol solvent (Muhammad and Saeed 2011; Muhammad et al. 2012b, 2013a).

Also, some bioactive constituents have been isolated from *V. betonicifolia* such as 2,4-dihydroxy-5-methoxy-cinnamic acid (15) (Muhammad et al. 2013b), 4-hydroxy coumarin (16) (Muhammad et al. 2014), and 3-methoxydalbergione (17) (Muhammad et al. 2013c) (Fig 24.7).

24.4.2 Viola Betonicifolia for Immunity

V. betonicifolia possesses various macronutrients such as zinc (Zn) and iron (Fe) which act on cellular immunity. Zn accelerates the reproduction of cells found in the immune system and impacts natural as well as acquired immunological activities (Chandra and Dayton 1982; Ibs et al. 2003). It is essential for the production as well as for the functioning of innate immunity, neutrophils, and NK cells

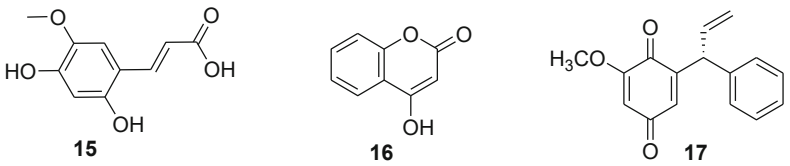

Fig. 24.7 Chemical constituents of *V. betonicifolia*

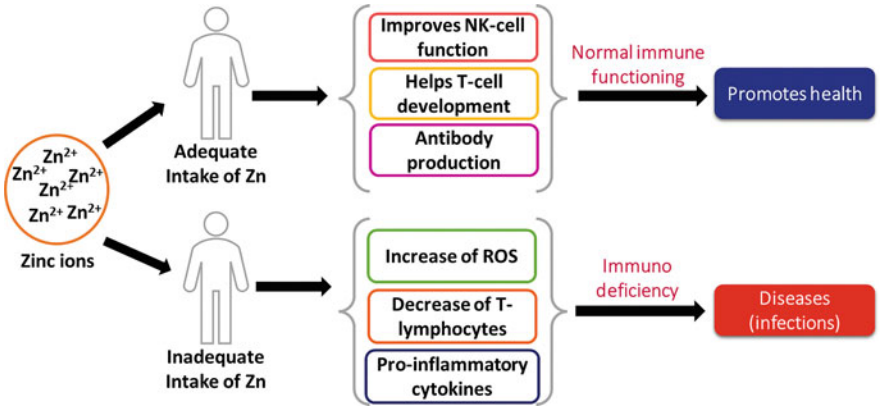

Fig. 24.8 Effects of zinc concentration in the body

(Prasad 2008). Zinc deficiency adversely affects the growth and function of T and B cells (Pamela et al. 1987; Prasad 2000) (Fig. 24.8).

Appropriate zinc consumption promotes a T helper type 1 (Th1) response and aids in the maintenance of skin along with the integrity of mucosal membrane, while free zinc ions explicitly acts on the proliferation of rhinovirus. Zinc intake boosts innate immune cellular components (Prasad 2008) such as phagocytosis by macrophages (Gordon 1998) and neutrophils (Kantari et al. 2008), NK cell activity, oxidative burst production, DTH, antibody responses, and the numbers of cytotoxic CD8βT cells (Th1 response) as mentioned in Table 24.4 (Ibs et al. 2003; Bonaventura et al. 2015). It participates in the cytosolic defense against oxidative stress (superoxide dismutase activity) and is a required cofactor for thymulin, which regulates the secretions of cytokine promoting growth (Maggini et al. 2007; Prasad 2008).

Table 24.4 Effects of Zinc concentration on immunity cells

S. no.	Cell type	Results of Zinc deficiency	High dose of Zinc	Results of high doses of Zinc
1.	NK cells	Decreased cytotoxicity	–	Suppressed killing
2.	Monocytes/ macrophages	Decreased functions	> 30 μmol/L > 100 μmol/L	Normal functioning Direct activation
3.	Neutrophil granulocytes	Suppress phagocytosis	> 100 μmol/L > 100 μmol/L	Normal functioning Direct chemotactic activity
4.	T cells	Effects normal functioning, increased autoreactivity, and alloreactivity	> 30 μmol/L > 100 μmol/L	Functions decreased Functions suppressed
5.	B cells	Apoptosis	–	Apoptosis

Zn deficiency as well as its concentration in excess results in the inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) thus suppressing the production of superoxide anion that participates in the destruction of pathogens (Bonaventura et al. 2015). Deficiency of Zn causes an increased risk of infections and leads to severe diseases such as Alzheimer's, chronic asthma, atherosclerosis, and arthritis.

Besides, *V. betonicifolia* has been investigated for sodium (Na), calcium (Ca), and potassium (K) necessary for different processes occurring in humans, since calcium plays a significant role in bone mineralization, blood clotting, and healthy toning of muscles. Furthermore, calcium governs the activity of skeletal muscle, which is stimulated by exocrine gland secretion, and is engaged in sustaining membrane fluidity as well as permeability and micronutrients such as chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), cadmium (Cd), and lead (Pb) which actively or passively influences the human body's immune system. Also, the herb is a promising source of essential nutrients such as vitamin C, fibers, carbohydrates, proteins, and fats with percentage composition 39.01%, 21.42%, 15.70%, and 18.70%, respectively aids in the development of the body's immunity (Muhammad et al. 2012c).

24.4.3 Other Biological Activities

24.4.3.1 Antioxidant Activity

The crude extract of *V. betonicifolia* in different solvents such as methanol, chloroform, and aqueous was found to be an excellent source of antioxidants due to the presence of polyphenolics (Rizwan et al. 2019). The antioxidant property has been calculated by DPPH free radical scavenging assay at varied concentrations and it was found that chloroform extract has the highest scavenging properties followed by methanol and aqueous owing to the presence of higher concentration of polyphenolics in chloroform extract which displays promising, effective, and imperative antioxidant propensity (Muhammad and Saeed 2011).

24.4.3.2 Antimicrobial Activity

V. betonicifolia n-hexane and chloroform fractions were active against *Candida albicans*, *Fusarium solani*, and *Microsporum canis* (Rizwan et al. 2019). It was observed that the n-hexane fractions were not much effective against *F. solani* in comparison to the *M. canis* with the percentage inhibition found to be 5–10% and 20–40%, respectively. However, the combined fraction of chloroform and ethyl acetate of *V. betonicifolia* exhibits 30% and 40% inhibition against *C. albicans* and *M. canis,* respectively (Muhammad et al. 2013a, e).

24.4.3.3 Neuropharmacological Activities

In Pakistani traditional medicine, *V. betonicifolia* has been used as a sedative to treat different neurological illnesses. To determine its traditional usage, the crude methanolic extract and *n*-hexane fraction obtained from the whole-plant of *V. betonicifolia* were examined to achieve the neuroprotective activities (Muhammad et al. 2013d) say, anxiolytic, muscle relaxant, sleep induction, and hypnotics. The *n*-hexane fraction and crude methanolic extract exhibit efficient dose-dependent anxiolytic as well as muscle relaxant properties (Muhammad et al. 2013c). Also, extracts display sedative effect in BALB/c mice (Rizwan et al. 2019).

24.4.3.4 Analgesic Activity

Pain is an unpleasant feeling as well as an emotional reaction associated with existing tissue damage. Pain often generates a range of discomforts, which can lead to a variety of psychological stress. Analgesics (Twycross 1984) are pain relievers that work upon the central and peripheral nervous system to alleviate pain without altering consciousness (Rizwan et al. 2019). To assess the analgesic potential of *V. betonicifolia,* various studies have been carried out in BALB/c mice via acetic acid-induced writhing test, tail immersion test, and hot plate techniques. In the acetic acid-induced writhing test, the methanolic extract and n-hexane fraction of *V. betonicifolia* showcased analgesic activity with maximal inhibition of 78.9 and 85.2% at 300 mg/kg dosage, respectively (Muhammad et al. 2012b).

24.5 Viola Canescens

V. canescens is common in the Western Himalayan area. It is exclusively found in hilly areas and is primarily found in tropical as well as temperate zones. The plant is mainly found at elevations of 2000 meters or higher across the temperate Himalayas. *V. canescens* is found in the cold desert of the Himalayas in the Pangi valley near chamba district, popularly called as the Trans Himalayan region (Rana et al. 2014). The occurrence of the plant has also been confirmed in the Garhwal area of the Himalayas, which is located in Uttarakhand, India, at an elevation of about 1600 m–2000 m. Also, it is found in the Nanda Devi National Park and the Nainital catchment area in Uttarakhand (Rana et al. 2010; Dua et al. 2011; Agnihotri et al. 2012). *V. canescens* was also discovered in the Dhunkharka locality in Kavrepalanchowk, Nepal (Suyal et al. 2010; Masood et al. 2014).

V. canescens is known as Himalayan White Violet in English due to its association with the Himalayan area. *V. canescens* is a perennial plant that is prostrate, subglabrous, or hairy. It has a long, branching, cylindrical root structure. The leaves

can be seen at the base and the stem is absent. The leaves are oblong, wide, cordate, or obtuse to acute, with serrate-crenate borders.

24.5.1 Chemical Constituents

The phytochemical composition of the leaves of *V. canescens* displays alkaloids, phenolic compounds, tannins, saponins, phytosterols, and flavonoids (Barkatullah et al. 2012). Various phytochemicals are found in *V. canescens* such as glucosides, alkaloids, saponins, and methyl salicylate (18). An alkaloid named violin was isolated from the roots which are less or more similar to emetine (19) but are characterized by different properties (Masood et al. 2014). It has also been suggested that violin is an impure form of emetine but its actual structure is still unknown. It is produced in plants in combination with malic acid which is unexpectedly active and can be toxic. A glycoside called *Viola* quercitrin is also reported which was more or less similar to quercitrin (20) (Masood et al. 2014) (Fig 24.9).

24.5.2 V. Canescens in Immune Regulation

Salicylates derived from *V. canescens* can be utilized to stimulate the immunological system. Salicylic acid and its derivatives have multiple targets, following inflammation, Nuclear Factor kappa B (NF-κB), which upregulates the pro-inflammatory enzymes, chemokines, receptors, cell adhesion molecules, and cytokines thus governing diverse facets of the innate and adaptive immune responses and is regarded as a central mediator of immune response. Thus, the suppression of NF-κB via salicylic acid is among the major anti-inflammatory modes of action for salicylates. Furthermore, quercetin, one of the components of *V. canescens*, has a flavone ring that impacts immunity and inflammation by primarily operating on

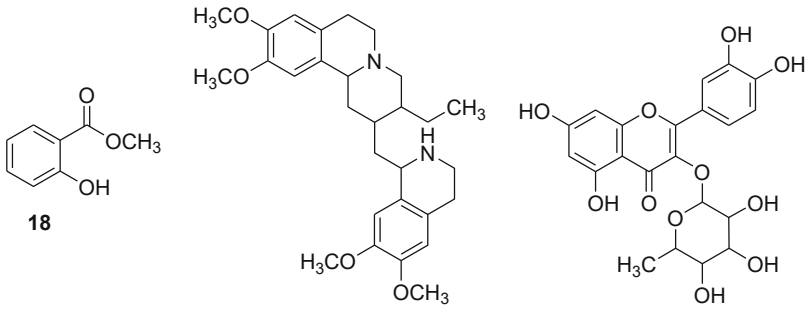

Fig. 24.9 Chemical constituents of *V. canescens*

leukocytes and attacking several intracellular signaling kinases as well as phosphatases, enzymes, and membrane proteins that are mostly required for cellular function.

24.5.3 Other Biological Activities

V. canescens is well-recognized for its antimalarial and antiprotozoal activity. It can be used as antipyretic, demulcent, diphoretic, astringent, carminative, and purgative properties (Ahmad et al. 2012). The plant is also recognized for its anticancerous properties and is used for treating different nervous syndromes (Ali et al. 2011; Hussain et al. 2011).

Ethnobotanically, the plant extract is utilized for stomach, epilepsy, rheumatism, eczema, and gastric problems along with various respiratory disorders (Hamayun et al. 2006). For instance, the pastes of leaves obtained from *V. canescens* when used with brown sugar cures cough. Also, the decoction of its blooms accompanying fennel, cinnamon, and clove is beneficial for respiratory tract problems (Rana et al. 2014). In Himachal Pradesh, the powder of dried flowers and leaves of *V. canescens* is consumed with lukewarm water on empty stomach help in relieving the symptoms of dysentery (Kumar et al. 2013). The infusion of flowers using water is used to cure flu symptoms (Agnihotri et al. 2012; Rani et al. 2013). In Uttarakhand, the plant is used as a remedy for headache, leucorrhoea, menstruation, bronchial asthma, and aphrodisiac. In Nepal, *V. canescens* is used as antipyretic, laxative for boils, and its leaves are used to moisturize the skin (Adhikary et al. 2011). In addition, paste of the plant serves as an antiseptic to heal wounds (Rana et al. 2010). The aerial parts of *V. canescens* are reported to be used against malaria as well (Bahekar and Kale 2013).

24.6 Immunomodulation Effects of Genus *Viola*

The aqueous extracts of some other species of the genus *Viola,* namely, *V. yedoensis*, *V. tricolor*, and *V. diffusa* exhibit extraordinary immunomodulatory properties (Zhu et al. 2015). For instance, *V. yedoensis* had the function of reducing the phagocytic ability of mouse macrophages (macrophages are the cells that interact with foreign or abnormal host cells and their products) and secreting TNF-α (Jeong et al. 2016). In high concentrations, it could pass down the secretion of IL-2 and TNF-α, and suppress the release of inflammatory mediators via macrophages, and, thus, modulating the immunity in mice. *V. yedoensis* also had an inhibitory impact on Hepatitis B virus, particularly resisting intracellular HBsAg, HBeAg, and HBcAg of HepG cells. Furthermore, the aqueous extract of *V. diffusa* might improve immunological function and escalate the formation of phagosomes in macrophages as well as in healthy mice peritoneal macrophages (Zhu et al. 2015).

On the other hand, *V. tricolor* has been known to show immunosuppressive activities (Hellinger et al. 2014). Cyclotides present in *V. tricolor* were recently reported to possess immunosuppressive peptides which inhibit the multiplication of T lymphocytes. An aqueous extract of *V. tricolour* hinders the multiplication of activated lymphocyte by decreasing the ejaculation of IL-2 cytokine without altering the expressions of IL-2 receptors (Lim 2014).

24.7 Conclusion

According to the preceding explanation, it is evident that the species of *Viola* genus found in the Western Himalayan area have traditional and biological significance. Furthermore, the species suggests that it can be employed as both an immunity booster and immunomodulator. However, fewer studies have been conducted on the study of immune-boosting activities, and phytoconstituents, nutritional composition that would be accountable for the same providing a great possibility for future research.

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