Bikarma Singh *Editor*

# Botanical Leads for Drug Discovery

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ISBN 978-981-15-5916-7 ISBN 978-981-15-5917-4 (eBook)
https://doi.org/10.1007/978-981-15-5917-4



This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

I dedicate this book ***Botanical Leads for Drug Discovery*** *to my parents (Shri Jammu Singh and Smt. Sam Devi), for giving me high class education and being an excellent first Guru; my wife Manisha Singh and children (Aryan Singh and Aditi Singh) for being patience at home. I express my deepest gratitude to my mentors Prof. S.K. Borthakur, Dr.(Mrs.) Sandhya Jyoti Phukan and Prof. Saroj Kanta Barik for guiding me with knowledge and to all the authors for their top-class research contributions.*

Dr. Bikarma Singh
Editor and Senior Scientist, CSIR-IIIM

# Preface

Active botanical ingredients, sometimes called phytopharmaceuticals, are prime requirement for herbal formulations and drug preparations. Discovering drugs or developing an ingredient for pharmaceuticals is all about integration of scientific disciplines. Usually, developing a new drug is a serious challenge as it involves time for research trials and the whole processes is very expensive, sometimes huge investments are required, for example, more than 1 billion US$ for one drug. The number of new drugs discovered each year has not kept pace with either the rate of medicine discovery or the money spent in research and development. Curiosity for treating wounds or getting relief from disease by applying local herbs using age-old traditional knowledge and combinatorial chemistry advancement in recent few decades has filled the gap of developing new bioactive chemical ingredients from plants; however, the chances to overcome different hurdles according to FDA guideline and then succeed is very low, as this has to undergo several clinical trials and post-marketing studies. Since ancient times, botanical resources are known to provide many classes of new ingredients for drug discovery. For instance, the bioactive chemical constituents for direct use as medicine like berberine isolated from different species of plants such as *Berberis, Tinospora, Argemone, etc.,* digoxin characterized from *Digitalis purpurea*, identified leads for more active compounds such as paclitaxel from *Taxus baccata* and CBD/THC from *Cannabis*, herbal extracts like green tea, marker compound for standardization of crude extracts and many similar herbal extracts are adding new lead as botanicals. However, in certain cases, drug discovery from plant sources puts extra pressure on natural resources causing unconditional environmental issues for human and animal survival.

In the recent past, refined observations, translational research and hypothesis-driven biological approaches accelerated drug discovery programmes which focused on human and animal biology. The science-driven translational discovery and phytopharmaceutical development have created a new reality and initiated great changes in strategies, technologies and disciplines, and these new changes have embraced the pharmaceutical and biotech industries. Based on the idea of disseminating ongoing research, the goal of this book ***Botanical Leads for Drug Discovery*** is focused and tries to bring together the scientific expertise as well as ideas to discover and develop new formulations and drugs from plants based on

traditional knowledge and carries forward the known scaffolds discovered from active ingredients to clinical trials.

This book contains 20 excellent chapters covering the theme of modern approaches to drug discovery and ethnobotany. I am confident that the research covered herein by all the contributors will provide essential background information on the origin and development of compounds in drug discovery as well as offer a sense of promise of what this field of research may deliver in the upcoming future. I am sure that this particular book will help those aiming to be a part of an exciting and challenging science mission. All chapters have been updated and many have been revised extensively. I would like to acknowledge each author of every chapter for the time and effort spent to write informative articles and reviews on their area of expertise. It is hoped that those seeking a guide to pharma R&D will find this book very helpful and informative.

Jammu, Jammu and Kashmir, India Bikarma Singh

# Acknowledgements

This book ***Botanical Leads for Drug Discovery*** is based on the research conducted on various aspects of plant sciences, biotechnology, chemistry, pharmacology and clinical trials, which provide leads to modern herbal drug and medicine, importantly required for human and animal health care. I deeply indebted this book to the top class researchers and their scientific contributions. My gratitude goes to my worthy Director, Head of Department, colleagues and students as they continuously encourage me for my work and support me whenever required.

As editor of this book, I would like to acknowledge the authors for their valuable contributions. This comprehensive book would not have been possible without their enthusiasm and cooperation throughout the stages of this project. I also would like to express my gratitude to all the anonymous reviewers of sample chapters who improved the quality of this book through their critical comments and constructive suggestions.

I would like to extend my gratitude to Springer Nature Singapore Pte Ltd., Singapore, who agreed to publish this research outcomes. The project and editorial assistance from Gaurav Singh, Ashok Kumar, Suraj Kumar, S. Padmashri and others associated with production and typesetting with english editing is highly appreciated. I personally feel that it takes lots of patience to do all proof reading tasks for such a comprehensive outcome. Special thanks go to the production team for their enthusiastic support.

The presented invaluable contributions come from various themes related to plants and medicine on which project outcomes were invited from various authors. I am sure this publication will greatly help different workers in the task of new research activities and will go beyond expectation of different thematic areas in a new era of society, knowledge and science transfer. I am sure this book will attract academicians, civilians, students, researchers, industrialist and pharma companies willing to understand the value of botanicals. I would like to express my gratitude to many readers who will see me through this book, to all those who provided support, talked things over, offered comments and assistance.

I express my heart-felt gratitude to my family for their patience, especially to my wonderful children, Aryan Singh and Aditi Singh, for always making me smile and for understanding those weekend mornings when I was busy in compiling and

editing this book instead of playing with them and enjoying in the park. Work presented in this book has sometimes been at the expense of family time.

Finally, I would like to beg forgiveness from all those who have been with me over the course of the years whose names I have failed to mention in this book.

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## About the Editor

**Bikarma Singh** is presently working as a Senior Scientist at the Indian Institute of Integrative Medicine (IIIM), Jammu and Kashmir UT, which is one of India's leading pioneer institute of the Council of Scientific and Industrial Research (CSIR) coming under Ministry of Science and Technology, Government of India. He is also serving as a faculty member, Assistant Professor in Academy of Scientific and Innovative Research (AcSIR, Ghaziabad), and Ph.D. Supervisor at the University of Jammu in Botany Department. He is a Post-graduate and Gold Medalist in Botany from the North-Eastern Hill University, Shillong (2005), and completed his Doctorate Degree in Botany from Guwahati University, Assam, and Botanical Survey of India (Shillong), Meghalaya, in 2012. He began his career as Scientist-Ecologist in WAPCOS Limited, Gurgaon, and later joined CSIR-IIIM in the Department of Plant Sciences in 2013. At present, Dr. Singh is actively involved in research with passion and possesses about 15 years' research experience in plant sciences and expertise in systematics and biotechnology, ethnobotany, value addition from essential oils, natural products, ecology, germplasm conservation and EIA. He is serving as a reviewing member of several National and International SCI and UGC listed journals, and also works as lead leader for organizing many seminars and workshops. He was awarded with 'Best Researcher Award-2020 (VDGOOD), Kolkata', 'Outstanding Scientist in Botany in 2019' by Venus International Foundation, Chennai, and two of his Ph.D. students received the best research paper award in 2019. He has

authored/co-authored 9 books and published 88 research papers in peer-reviewed International and National journals. Some of his contributions are *Plants for Human Survival and Medicine*, *Plants of Commercial Value*, *Plant-Human Relations* and *Future Drug Discovery*. He has one published International patents and worked as PI/Co-PI/Team member for more than 40 research projects funded by government and private organizations. He is supervising 5 Ph.D. students in biological science to defend their doctorate degree. He is a member of several research committees and has delivered invited talks as key-note speaker/lecturer under various themes in several conferences, seminars and workshops.

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# Plant-Derived Drug Discovery: Introduction to Recent Approaches

1

Bharat Goel, Bharat Sahu, and Shreyans Kumar Jain

**Abstract**

Drug discovery using plants is an emerging task for leads identification. The drug discovery process involves selection of plant material to phytochemical analysis, characterization, and pharmacological investigation followed by detailed preclinical investigation to clinical trials. Up to 1996, approximately 80% of medicinal products were either directly originated from naturally occurring compounds or motivated by a natural product. 1881 new drugs were approved between the years 1981 and 2019, out of them approximately 23.5% were natural products or semi-synthetic derivatives of natural products, and approximately 25% were either natural product mimic or contained pharmacophore from a natural product. Drug discovery requires the development of efficient and feasible leads, which advance from screening a hit to a drug candidate through structural elucidation identification by GC-MS, NMR, IR, HPLC, and HPTLC. The development of new expertise has modernized the evaluation of natural products in new drug discovery.

**Keywords**

Drug discovery · Isolation · Characterization · Dereplication · Bioassay-guided isolation

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_1

## Abbreviations

| | |
|---|---|
| CE | Capillary electrophoresis |
| CML | Chronic myelogenous leukemia |
| CPT | Camptothecin |
| GBF | German Research Centre for Biotechnology |
| GC | Gas chromatography |
| HKI | Hans Knöll Institute, Jena, Germany |
| HPLC | High performance liquid chromatography |
| HPTLC | High performance thin layer chromatography |
| HTS | High throughput screening |
| IL | Ionic liquid |
| LC | Liquid chromatography |
| MS | Mass spectrometry |
| MS-MS | Tandem mass spectrometry |
| NMR | Nuclear magnetic resonance spectroscopy |
| NP | Natural product |
| PCF | Plant cell fermentation |
| PDA | Photo diode array detector |
| Pdbu | Phorboldibutyrate |
| $R_t$ | Retention time |
| SAR | Structure-activity relationships |
| SFE | Supercritical fluid extraction |
| SPE | Serum protein electrophoresis |
| TLC | Thin layer chromatography |
| UV-Vis | Ultraviolet-visible |

## 1.1 Introduction

Every substance in the universe can be considered as natural products. With respect to pharmaceutical science or medical science, natural product (NP) is a chemical compound or substance produced by a living organism (animals, plants, or microbes), having pharmacological or biological activity that can be clinically useful either as such in crude (traditional medicines) or isolated/modified form even if it can be produced synthetically (modern system) (Cutler and Cutler 1999; Samuelson and Bohlin 1999). For example, the traditional herbs and their preparations were considered as drug in Ayurvedic system of medicine; there are around 700 plants documented in "Sushruta Samhita" (a text of Ayurveda) for the treatment of 1100 diseases. Similarly, other traditional systems (Chinese materia medica, Greek, Arab, Egyptian, and Mesopotamian) and folk system (ethnomedicine) of medicine produced a huge wealth of knowledge.

The modern natural product chemistry started with the work of Serturner (1804) with the isolation of morphine from opium. Many similar developments led

discoveries of bioactive isolated compounds such as quinine (1820) from cinchona bark, strychnine (1818), cocaine (1859), tubocurarine (1935), penicillin, and others. By 1996, approximately 80% of approved medicinal products were either originated from naturally occurring compounds or were motivated by a natural product. The importance of natural products has been extensively reviewed and it was found that out of the new drugs introduced between 1981 and 2019, 33% of the 1394 small-molecule approved drugs were either natural products or derived from natural products and another 35% were created around a pharmacophore from a natural product (Newman et al. 2003; Newman and Cragg 2007, 2020; Cragg et al. 2009). Plants use these secondary metabolites for defense and signaling. Thus, they are important for the survival and reproductive fitness of plants. Secondary metabolites, therefore, represent adaptive characters that have been subjected to natural selection during evolution (Wink 2003). Several specific interactions of secondary metabolites with proteins (enzymes, receptors, ion channels, structural proteins) and other cellular components have been discovered. Structures of these metabolites appear to have been shaped during evolution in such a way that they can mimic the structures of endogenous substrates, hormones, neurotransmitters, or other ligands (Wink 1988; Rosenthal and Berenbaum 1992; Pichersky and Gang 2000; Wink 2010). Hence, these compounds are results of an evolutionary relation, and the internal signaling pathways respond to many of the primordial "exocrine" signaling systems found today in ancient marine animals as well as in the plants (Wink 1988, 2010; Rosenthal and Berenbaum 1992; Robert 1997; Pichersky and Gang 2000).

Parallel discovery of high throughput screening (HTS) and the knowledge of molecular targets of diseases, along with the introduction of combinatorial chemistry-based synthesis, dramatically shifted efforts toward the synthetic medicinal chemistry. The major underlying challenges with the natural product-based drug discovery have been reviewed (Patwardhan et al. 2004; Lam 2007; Cragg et al. 2009) as below:

- An ability is required to build and maintain a high-quality natural product library.
- Natural products are biosynthesized in small amounts and present in extracts as mixtures, isolation of which is an effortful and tedious procedure.
- Obtaining additional quantities for preclinical development requires a large-scale reacquisition or fermentation that would have a significant impact on the growth timeline.
- When screening natural product libraries, rediscovery of known compounds is a major problem. This is due to the lack of active dereplication methodologies.
- Natural products are often complex in terms of structure. It is often difficult to alter the complex natural products using organic chemistry.

To meet the above challenges, various concepts have emerged in the natural product-based drug discovery. Natural product-like compounds can be obtained either by isolation or synthesis based on natural products. These two categories can be classified further according to different concepts involved in methodology, and has been discussed in subsequent subheads.

## 1.2 Plants as Potential Sources for Natural Products

Plants are considered as a classical source of drug discovery; they produce toxic compounds against neighboring species, and the process is known as allelopathy (Bais et al. 2003). Around 300,000–500,000 higher plant species have been estimated, and only 6% of that has been investigated pharmacologically and 15% phytochemically (Cragg et al. 2009). Some of the anticancer natural products are vinblastine and vincristine from *Catharanthus roseus* L. (family Apocynaceae); investigation of *Podophyllum peltatum* L. (family Berberidaceae) led to the discovery of podophyllotoxin, which is the precursor of the semisynthetic anticancer agent, etoposide. *Indigofera* sp. and indigo dye, which have been used for many years to treat chronic myelogenous leukemia (CML) in China, are the sources of indirubin which is under clinical development; another example is flavopiridol, although it is entirely a synthetic molecule, under clinical development as anticancer agent, it was inspired by rohitukine, a chromane alkaloid of *Dysoxylum binectariferum* Hiern., which is related to Ayurvedic plant *Dysoxylum malabaricum* Bedd. ex C.DC. Similarly, camptothecin was discovered from *Camptotheca accuminata* Decne and later from other genus and species; paclitaxel (Taxol), the original source of which was bark of *Taxus brevifolia* Nutt.; another potential example was CA-4-phosphate; combretastatin was isolated from African plant species of *Combretum* genus (Newman et al. 2003; Newman and Cragg 2007; Cragg et al. 2009). The major anticancerous drugs obtained from plant species are shown in Fig. 1.1.

To proceed with the drug discovery and development process, an active compound is required which can interact with specific target. These compounds can be discovered through systematic or random screening of compounds. Synthetic chemistry and natural sources are two major sources for these compounds of interest. Subsequent validation steps required for lead identification, preclinical development, and clinical development at the discovery level of molecules from plants involve several crucial steps such as plant selection, extraction, isolation, and characterization before its biological testing. Figure 1.2 depicts a typical procedure of plant-based drug discovery procedure.

Following are some important aspects of plant-based drug discovery:

- Criteria for the plant selection
- Authentication of plant material
- Extraction methods
- Natural product library
- Bioassay-guided fractionation
- Dereplication and hyphenated techniques

## 1.3 Criteria for the Plant Selection

Following information is helpful to choose the biological material to be investigated for research in drug discovery point of view.

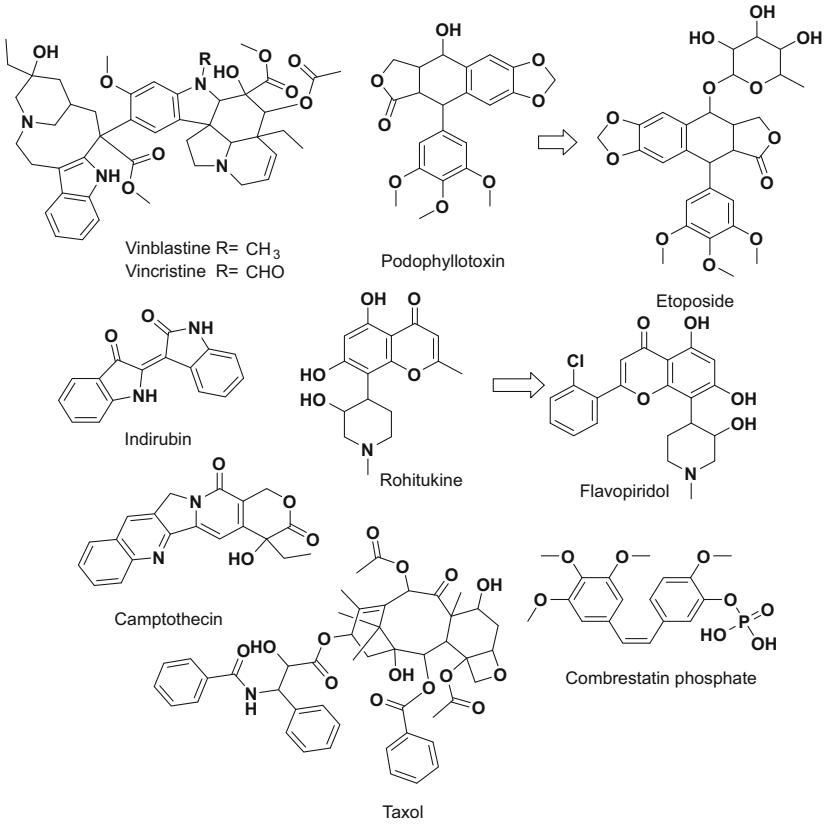


**Fig. 1.1** Anticancerous drugs obtained from plant sources

- Ethnomedicinal uses or information from traditional medicine systems
- Chemosystematic criteria
- Ecological and field observations
- Random collection

### 1.3.1 Ethnomedicinal Uses or Information from Traditional Medicine Systems

Traditional Chinese and Ayurvedic medicines are relied on crude preparations and their complex interactions, for disease prevention and treatment. In these systems, to prevent or control complex diseases, many herbal preparations are considered ideal than a single active ingredient. Studies have shown the ability of plant metabolites such as quercetin, catechins, resveratrol, piperine, and curcumin to potentiate the activity of various anticancer drugs (Schmidt et al. 2007). Various digital platforms, databases, and documents have been compiled to keep this information in a

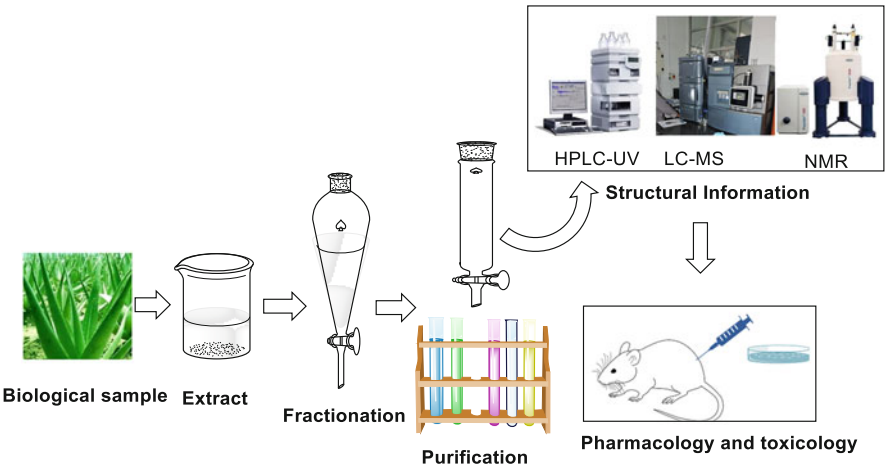


**Fig. 1.2** Typical procedure of drug discovery

user-friendly manner. Various plants have been classified according to their traditional uses; this information can be used to choose plant material to isolate compounds of interest. Many potent immunomodulators with anolides have been discovered from *Withania somnifera* (L.) Dunal based on these criteria.

### 1.3.2 Chemosystematic Criteria

The plant species can be classified based on the presence of some specific class of metabolites. Various genus/species specifically produce some class of compound such as cyanogenic glycosides produced by genera *Beilschmiedia*, *Cardwellia*, *Cleistanthus*, and *Elaeocarpus*; indole-containing alkaloids are mainly synthesized and produced by Rubiaceae, Loganiaceae, and Apocynaceae species.

### 1.3.3 Ecological Approach

Also known as field observations, plants are selected on the basis of relationship between ecological conditions and the secondary metabolites produced in their response leading to potential biological activity. For example, secondary metabolites produced in plants against microbial infections may be used as antimicrobials for humans if they are not too toxic (Briskin 2000).

### 1.3.4 Random Collection

According to this method of plant selection, plants are selected randomly, which is subject to plant availability. This method can be of high importance when the study is carried out in the region with high diversity; therefore, the plant species which have never been investigated can be explored for new chemical constituents.

## 1.4 Authentication of Plant Material

In case of medicinal plants, the awareness of authentication of biological materials has been given a boost by the significantly increasing emergence of herbal drugs from traditional products (Indian and Chinese system) derived thereof in the western world (Bucar et al. 2013; Rivera et al. 2014). Various initiatives have been taken at the international level such as the Chinese Medicinal Plants Authentication Centre at Royal Botanic Gardens, Kew, Great Britain, and their corresponding herbal drugs have been recently illustrated (Bucar et al. 2013).

Primary way of authentication is morphological as well as anatomical analysis, based on texture and organoleptic properties (details of various Ayurvedic herbs are mentioned in Ayurvedic Pharmacopeia of India). The rapid comparison of sample with reference is based on fingerprinting. TLC is the most available and cheapest reliable method; sophisticated version of this, is HPTLC (Reich et al. 2008). HPLC alone (Wolfender 2009; Zhao et al. 2011), and with other techniques (PDA, MS, and NMR) offered a two-way analysis (comparison as well as identification), by these techniques a specific marker can be used for the chemotaxonomic application, such as 3-hydroxy-3-methylglutaric acid acylated flavonol glycosides are the systematic characters of the genus *Rosa* (Porter et al. 2012). GC analysis is a method of choice for volatile oil-containing drugs (Vial et al. 2009). Molecular biology methods are important, particularly with microbial identification, although they are gaining popularity in the identification of plants also; however, these techniques are not utilized regularly, but they are very precise and can differentiate the adulteration within species. DNA barcode database of important biological sources is available freely for comparative analysis at http://www.cuhk.edu.hk/icm/mmdbd.htm (Lou et al. 2010).

## 1.5 Extraction Methods

During extraction, several issues need to be considered, such as polarity and stability of extractives and solvents, toxicity, volatility, viscosity, and purity of the extraction solvents, the probability of artifact formation, and environmental factors. The classical method for extraction often used harsh techniques such as boiling solvents, acid-base extraction, and steam distillation; it suffers high solvent consumption as well as is time-consuming. As isolation technologies have improved, the extraction conditions have become milder by using ultrasound (Roldan-Gutierrez et al. 2008), microwave (Chan et al. 2011), and pressure (accelerated solvent extraction)

(Mottaleb and Sarker 2012); nowadays, extraction with ionic liquids (ILs) is also gaining popularity because it is selective and effective (Tang et al. 2012). The efficiency as well as selectivity of ILs has been demonstrated very well for artemisinin and shikimic acid (Usuki et al. 2011). In comparison to other, accelerated solvent extraction methodology is applied to solid and semisolid samples in 1–100 g scale using common solvents at elevated temperature and pressure (Mottaleb and Sarker 2012). Automation made this easy, and up to 24 samples can be extracted simultaneously. Another popular technique is supercritical fluid extraction (SFE). The high consumption of organic solvent in classical methods caused environmental disturbances. SFE is an attractive alternative to traditional solid-liquid extraction with lower solvent usage and lower working temperature. It is a type of liquid extraction where a super-critical fluid substance that is above its critical point replaces the usual liquid solvent phase. Because of its comparatively low critical temperature (31.1 °C) and pressure (73.8 bar/7.38 MPa), carbon dioxide is basically the only accessible supercritical extraction solvent used. An organic solvent (also called modifier) may be added to the supercritical fluid to enhance its solvating properties (Sticher 2008, Gonzalez-Coloma et al. 2012). This technique was successfully applied for the isolation of fat, terpenoids, alkaloids, phenolics, and other natural products (Bevan and Marshall 1994).

## 1.6 Natural Product Library

Parallel to combinatorial and HTS, the technological advancement is utilized to create extracts, fractions, enriched and semi-pure compounds, and pure compounds-based library. The advancement of new hyphenated technology based on sophisticated detection methods makes rapid online identification of the constituents possible up to a minimum of 100 μg. It has been explained that natural product-based compounds have more diversity with more chance of getting drug-like molecules from natural product-based library; hence, both companies and academia are focusing on creating libraries of natural products (Rolf et al. 2002). One of the first systematic approaches in the direction of pure compound library-based screening was made by the Hans Knöll Institute (HKI), Jena, Germany, in cooperation with partners in academia, the scientific institute GBF and AnalytiCon Discovery. Within about 5 years, the natural product pool comprised more than 6000 compounds derived from more than 70 laboratories in 2001 (Grabley and Sattler 2003). Similarly, Aventis Pharma evaluated a system to generate a library of semi-characterized pure natural compounds with a purity of over 80% and a quantity of over 5 mg (Bindseil et al. 2001). Within a couple of years, around 4000 nonredundant substances were extracted from plants, bacteria, and fungi, 400 of which were selected randomly and categorized by their key structural element. High number of obsolete and omnipresent substances was the biggest problem to be solved. Purified NP libraries can be created by in-house extraction, isolation, and purification or by commercial sources. Meaning of natural product library, in a broad sense, is not only a collection of an isolated compound but a combined collection of

semi-synthetic and totally synthetic NP and NP-like compounds. The different natural product-based libraries have been listed in Table 1.1.

## 1.7 Bioassay-Guided Isolation

Bioassay-guided isolation (Fig. 1.3) represents a typical procedure for the isolation of bioactive compounds from active crude. Developments in method of extraction, fractionation, and isolation and discovery of fast screening methods (HTS) improved the utility of this technique. Many of bioactive compounds have been discovered in the past, but their bioactivity has not been investigated for various reasons. Through modern screening procedures, a number of these compounds are rediscovered, and other activities become apparent (Kinghorn et al. 2003; Balunas and Kinghorn 2005). For example, betulinic acid is present in many genus and species and is frequently isolated; the structure has been known since 1932; and the bioassay-guided fractionation of *Ziziphus* species found the compound as a potential lead for cancer (Su et al. 2002; Arai et al. 2008). Later, the semi-synthetic derivatives of compound were identified as potent anti-HIV agents (Fujioka et al. 1994; Kashiwada et al. 1996). Another similar example is resveratrol (Kinghorn 2001; Savouret and Quesne 2002).

The crude extract may contain some interfering substances, such as tannins, which hamper the biological activity as they have nonselective protein binding affinity and have shown activity against various enzyme targets leading to false results. Similarly, linoleic acid gives false positive test against adenosine receptor assay. Another factor is synergistic effect. Leurosine, a dimeric indole alkaloid from *Vinca*, was found to be inactive in crude, while in pure form it showed potent cytotoxicity (Colegate and Molyneux 2007). This concept is analogous to diversity-oriented synthesis or more accurately, a natural product-like synthesis, hence biology-oriented synthesis. It is biology-oriented isolation that influenced the discovery of new targets and isolated compounds, ultimately leading to a concept of "Bioactivity-Guided Navigation Chemical Space" (Renner et al. 2009; Bon and Waldmann 2010). A database WOMBAT has been developed based on different scaffolds for same targets, which can be a useful tool for drug discovery and virtual screening (Olah et al. 2004; Mitchell 2011).

## 1.8 Dereplication and Hyphenated Techniques

The rapid detection of a known secondary metabolite at early stage of the discovery process (e.g., extract) is known as dereplication (Tawfike et al. 2013). The term "hyphenation" refers to an online coupling of liquid chromatography (LC) or gas chromatography (GC) isolation technique and one or more spectroscopic detection techniques, for example, ultraviolet-visible (UV-Vis), infrared (IR), mass (MS), or nuclear magnetic resonance (NMR) spectroscopy (VanMiddlesworth and Cannell 1998). Nowadays, a number of sophisticated hyphenated techniques, for example,

**Table 1.1** Major natural product–based libraries and their link

| Library name and/or company | Materials available in the library | Website link |
|---|---|---|
| Albany Molecular Research Inc. | Microorganism and plant-derived samples | https://www.amriglobal.com/solutions/discovery/libraries/ |
| Caithness Biotechnologies Ltd.–Phytotitre Natural Product Extract Library | A unique library of extract, fraction of traditionally reported plant material information of DMSO stock and microplate format | http://www.caithnessbiotechnologies.com/ |
| ChromaDex®Natural Compound Library | Pure compound library of important reference standards | https://chromadex.com/natural-product-libraries/ |
| Developmental Therapeutics Program – The National Cancer Institute at the National Institutes of Health | 230,000 crude extracts and 400 pure compounds from plant, marine, and microbial sources | https://dtp.nci.nih.gov/organization/npb/introduction.htm |
| Greenpharma | Pure compounds from natural resources | http://www.greenpharma.com/ |
| InterBioScreen | Library of compounds isolated from different natural sources such as plants, fungi, molds, insects, and marine organisms, or produced by complete synthesis | https://www.ibscreen.com/natural-compounds |
| InterLink Biotechnologies | More than 200,000 microbial and plant extracts and purified compounds. Microbial strains | http://www.interlinkbiotech.com/natural-products.html |
| Magellan BioScience | Collection of marine microbes and plant tissues | http://www.magellanbioscience.com/libraries/divisions.html |
| Natural Products Discovery Institute – A Division of the Baruch S. Blumberg Institute | Crude and fractions of plants and microbes | http://www.npdi-us.org/ |
| NatureBank, Griffith Institute for Drug Discovery, Griffith University | Marine- and plant-derived extract (>18,000), fractions (90,000), and pure compounds (100) | http://www.griffith.edu.au/institute-drug-discovery/unique-resources/naturebank |
| Sequoia Sciences | Pure isolate of plant | https://www.sequoiasciences.com/compound-libraries/ |
| Specs | Pure compounds and derivatives of plants, fungi, bacteria, and sea organisms | https://www.specs.net/page.php?pageid=2004111115344639 |
| Target Molecule Corp. | Pure molecules from plant, animal, microorganism, marine species, etc. accordingly classified as alkaloids, flavonoids, glycosides, phenols, | http://www.targetmol.com/screening2/Natural-Compound-Library.html |

(continued)

**Table 1.1** (continued)

| Library name and/or company | Materials available in the library | Website link |
|---|---|---|
| | phenylpropanoids, quinones, saccharides, steroids, terpenoids, etc. | |
| TimTec | Compounds sourced from plants, bacteria, fungus, and animal sources | http://www.timtec.net/natural-compound-library.html |

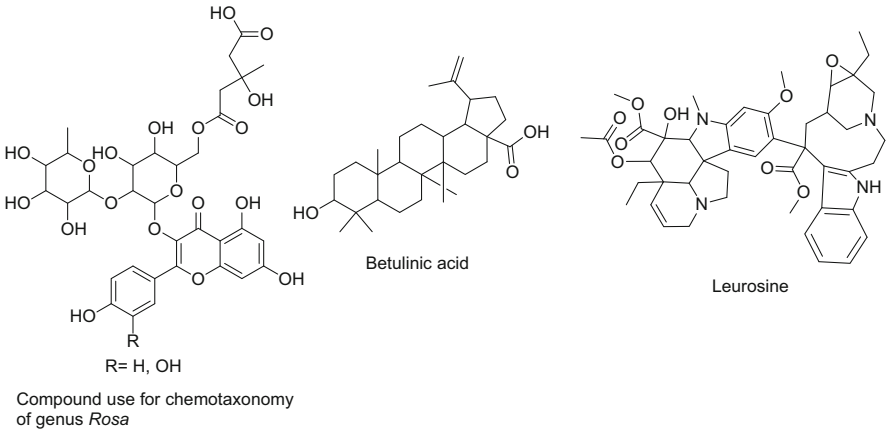


**Fig. 1.3** Well-recognized examples of bioassay-guided isolation

GC-MS, LC-PDA, LC-MS, LC-FTIR, LC-NMR, LC-NMR-MS, and CE-MS, are available for dereplication and other purposes in research. This accelerates the entire progression of natural product-based drug discovery drastically (Sarker and Nahar 2012). Dereplication is a technique that uses spectral archives, computer science, intelligent inference tools, and hyphenated analytical instruments as shown in Fig. 1.4 (Michel et al. 2013). Usually, this technique employs different steps: (1) chemical profiling of extract or bioactive fraction using LC-hyphenated technique (HPLC/UPLC-detection techniques; PDA, MS, HRMS, NMR, etc.) (Sarker and Nahar 2012), for example, HPLC coupled with PDA, gives UV information of chromophore which is an important aspect of basic scaffold of compound; similarly, MS, HRMS, and MS/MS provide mass information which can be useful for the information of known and unknown compounds; (2) informatics or database (Corley and Durley 1994).

These databases can be scanned with the minimum amounts of structural, physical, and/or biological information: Chemical Abstract Service (CAS) Registry File, available on the scientific and technical network, STN International, SciFinder, Dictionary of Natural Products (DNP) (250,000 natural products) (Jain et al.

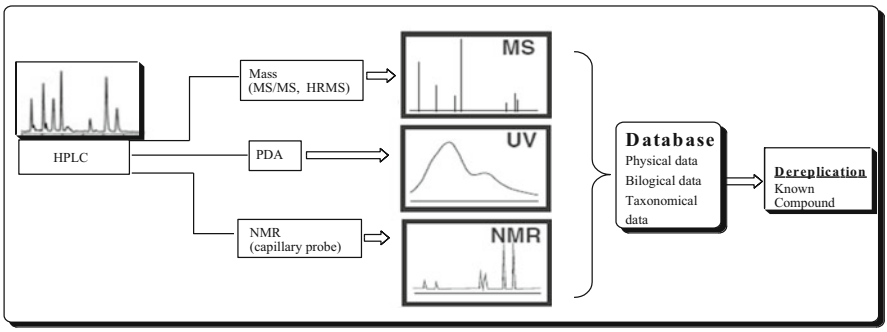


**Fig. 1.4** Schematic representation of experimental setup used for LC/UV/MS and LC/UV/NMR analyses

2013), MarinLit (approximately 40,000 marine compounds) (Lang et al. 2008; Mitova et al. 2008), and StreptomeDB (10,000 microbial isolates) (Lucas et al. 2013).

Using similar strategy various new compounds have been isolated (VanMiddlesworth and Cannell 1998; Bobzin et al. 2000; Bobzin and Kasten 2000; Lambert et al. 2005; Lang et al. 2008; Mitova et al. 2008; Michel et al. 2013; Tawfike et al. 2013). Such analytical methods are fully integrated into the process of isolation in the Hostettmann's group laboratory and are used to screen crude plant extracts in conjunction with online or at-line bioassays, to quickly locate and identify new bioactive compounds (Hostettmann et al. 2001). The various public and commercial databases listed by Füllbeck et al. (2006) that are available for dereplication are provided in Tables 1.2 and 1.3.

### 1.8.1 Liquid Chromatography–Mass Spectrometry

Liquid chromatography-mass spectrometry (LC-MS) is the most widely used system in chemistry in which HPLC is tethered with MS detector, and the separated components from HPLC column enter MS detector and are fragmented. Based on the fragments, the components can be identified. The mass spectra obtained provide the indispensable information about the separated molecule. Even if the molecule is known, its structure can be reconstructed by analyzing the mass spectra. The important advantage of MS analysis is that minute quantity of sample is required for complete analysis. Generally, soft ionization techniques are used in MS, which produces molecular ion peaks. However, the tandem mass spectrometry (MS-MS) produces collision-induced fragments of the produced molecular ion. Hyphenated techniques such as LC-UV and LC-MS have been extensively used in combination with biological screening for the discovery of natural products (Wolfender et al. 2000; Gaudêncio and Pereira 2015; Table 1.4).

**Table 1.2** Commercial database utilized for dereplication

| Database | No. of compounds | No. of natural products | Structure search | Mol. Wt. | UV | NMR | Mass spectra | Bioactivity | Source organism | Access | Contents |
|---|---|---|---|---|---|---|---|---|---|---|---|
| AntiBase | >43,700 | >43,700 | Yes | Yes | Yes | Yes | Yes | Yes | Yes | DVD | Antimicrobial activity |
| CAS | >73,000,000 | NA | Yes | Yes | | | | Yes | Yes | | |
| Dictionary of Natural Products | >250,000 | >250,000 | Yes | Yes | | | | | Yes | CD-ROM | All sources |
| The Merck Index | >11,500 | N.A. | Yes | Yes | | | | Yes | | Book and online | All sources |
| MarinLit | ~24,000 | ~24,000 | Yes | Yes | Yes | Yes | | Yes | Yes | Online | Marine organisms |

**Table 1.3** Public database which can be utilized for dereplication

| Name | Home page | No. of compounds | No. of natural products |
|---|---|---|---|
| ChEBI | https://www.ebi.ac.uk/chebi/ | >50,000 | >3500 |
| ChemBank | chembank.broad.harvard.edu | >1,600,000 | N. A. |
| ChemIDplus | chem.sis.nlm.nih.gov/chemidplus | >400,000 | >9000 |
| NCI | cactus.nci.nih.gov/ncidb3/download_ncidb3.html | >260,000 | N. A. |
| PubChem | pubchem.ncbi.nlm.nih.gov | >90,000,000 | N. A. |
| Super Natural II | http://bioinf-applied.charite.de/supernatural_new/index.php | >325,000 | >325,000 |
| NPACT | https://webs.iiitd.edu.in/raghava/npact/index.html | 1574 | 1574 |

**Table 1.4** Novel compounds isolated by using LC-MS-based dereplication strategies

| Identified chemical classes and natural sources | Analytical technique | References |
|---|---|---|
| Alkaloids from *Cimicifuga racemosa* (black cohosh) | LC-MS | Nikolić et al. (2012) |
| Coumarins from *Kielmeyera albopunctata* (bark) | LC-MS | Scio et al. (2003) |
| Luteolin, quercetin, and kaempferol from *Punica granatum* (peel) | LC-MS (online) | van Elswijk et al. (2004) |
| 16,23-Epoxycucurbitacin derivatives from *Elaeocarpus chinensis* | LC-MS | Pan et al. (2012) |
| Bioactive flavaglines from *Aglaia perviridis* | LC-MS | Pan et al. (2013) |
| Orientin and other compounds from *Trigonella foenum-graecum* seed extract | LC-MS | Singh et al. (2014) |
| Limonoids from *Azadirachta* extracts (stem bark) | LC-MS | Cui et al. (1998) |
| Catechols from *Semecarpus anacardium* | HPLC-MS | Shin et al. (1999) |
| Prenylated flavonoids from *Artocarpus kemando* (stem bark) | HPLC-MS | Eun-Kyoung et al. (2003) |
| Compounds from *Petiveria alliacea* | LC-MS | Urueña et al. (2008) |

### 1.8.2 Capillary Electrophoresis–Mass Spectrometry (CE-MS)

Capillary electrophoresis (CE) is also a powerful technique used to separate electrically charged molecule under electric field. When it is coupled with MS, the technique is called CE-MS. This hyphenated technique is used for the separation and analysis of biomolecules, including natural products yielding the molecular weight and structural properties of separated molecules. A theoretical library of 171 di-substituted xanthene derivatives was analyzed by CE-MS, and 160 of the expected compounds were found to be present. This hyphenated technique shows the promising results for the analysis of small combinatorial libraries containing up to 1000 molecules (Dunayevskiy et al. 1996).

### 1.8.3 Gas Chromatography–Mass Spectrometry (GC-MS)

The volatile components of the mixture are separated by GC and detected by MS. Fragments formed by MS detector provide useful structural information about the components. Mass spectra are compared with that of the compound library for the identification of separated components based on fragmentation patterns (Table 1.5).

### 1.8.4 Chemical Derivatization

Some natural products are derivatized to improve their ionization properties and volatility. The most common functional groups include alcohol (OH), carboxylic acids (COOH), amines (NH), carbonyl groups, esters, and amides. Common derivatization method is the conversion of the component to trimethylsilyl, acetyl, and methyl derivatives. For example, the extract of *Croton cuneatus* was found to be active in phorbol dibutyrate (PDBu) receptor binding assay and was found to contain seven compounds on HPLC-UV analysis. But, on hydrolysis, and acetylation of active fraction followed by GC-MS analysis, only two peaks were identified as phorbol tetraacetates by comparison with phorbol triacetate (standard) and were confirmed as esters of hydroxylated phorbol analogs (Beutler et al. 1990). Figure 1.5

**Table 1.5** Examples of compounds identified by using GC-MS

| Identified chemical classes and natural sources | Analytical technique | References |
|---|---|---|
| 1-Heneicosyl formate, β-sitosterol, stigmasterol, and diosmetin from *Premna odorata* leaves | GC-MS | Lirio et al. (2014) |
| Isomeric esters of long-chain alcohols from the essential oil of *Scandix pecten-veneris* | GC-MS | Radulović et al. (2014) |
| Sesquiterpene glycoside from pericarp of *Sapindus rarak* | GC-MS | Chung et al. (1997) |

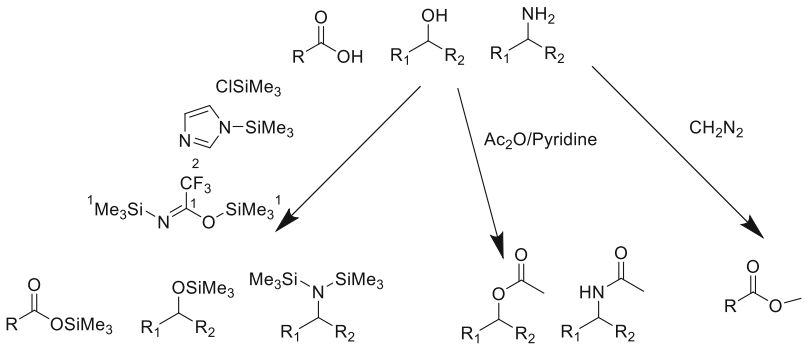


**Fig. 1.5** Common derivatization methods

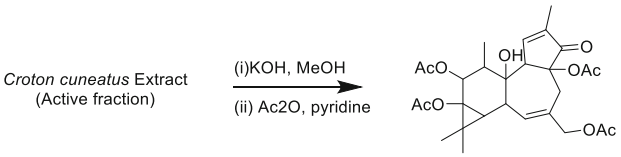


**Fig. 1.6** Identification of phorbol bioactive by GC-MS after derivatization

depicts the most common derivatization methods. Figure 1.6 shows the identification of phorbol bioactive by GC-MS after derivatization.

### 1.8.5 Liquid Chromatography–Photo Diode Array Detector (LC-PDA)

HPLC coupled with UV diode array detection has been one of the important techniques in the identification of the purified natural products. The presence of known compounds in the plant extract can be identified by comparing the $\lambda_{max}$ and $R_t$ of unknown components obtained by crude plant extracts. Some novel natural products identified by this technique are naphthgeranine F (similar to naphthgeranine E), juglomycin Z (similar to juglomycin C), and echinoserine (similar to echinomycin).

### 1.8.6 Liquid Chromatography–Infrared Spectroscopy (LC-IR)

The separation technique HPLC coupled with IR spectrometer as detection system makes this hyphenated system. Since a lot of organic compounds produce peaks at characteristic wavelengths, FTIR is a useful detection technique. But, due to the presence of solvents producing intense peaks in the same region, this technique is much less sensitive than other detection methods like UV and MS.

### 1.8.7 Liquid Chromatography–Nuclear Magnetic Resonance Spectroscopy (LC-NMR)

NMR is a sensitive spectroscopic technique providing useful information for the structure elucidation of natural products. HPLC separation followed by NMR analysis (using solvent suppression technique) is a potential technique for the investigation of natural products (Wolfender et al. 2000; Table 1.6).

**Table 1.6** Identification of novel compounds by LC-NMR-based dereplication strategies

| Identified chemical classes and natural sources | Analytical technique | References |
|---|---|---|
| Spiro compounds from *Carthamus oxyacantha* (wild safflower) | LC-NMR | Johansen et al. (2013) |
| Allopyranosides and phenolic compounds from *Cimicifuga heracleifolia* (rhizomes) | LC-NMR, LC-MS | Soon-Ho et al. (2012) |
| Tilirosides and flavonoid glycosides from *Lasiopetalum macrophyllum* | Online LC-NMR, offline LC, NMR, MS | Timmers and Urban (2011) |

**Table 1.7** Isolation and characterization of novel compounds by using multiple hyphenated techniques

| Identified chemical classes and natural sources | Analytical technique | References |
|---|---|---|
| Flavonol glycosides and cardenolides from extract of *Kanahia laniflora* | LC/DAD/SPE/NMR | Clarkson et al. (2005) |
| Quinolinone alkaloids from *Haplophyllum acutifolium* | LC/DAD/MS/SPE/NMR | Staerk et al. (2009) |
| Spiro compounds from *Carthamus oxyacantha* | HPLC-PDA-HRMS-SPE-NMR | Johansen et al. (2011) |
| Ecdysteroids from *Lychnis flos-cuculi* | HPLC/UV/NMR/IR/MS | Louden et al. (2001) |
| Identification of xenobiotic metabolites in maize plants | HPLC/NMR/MS | Bailey et al. (2000) |

## 1.9 Multiple Hyphenated Techniques

Combination of HPLC with a series of spectroscopic detectors like PDA-MS-SPE-NMR has become a dereplication technique of choice for the analysis of crude natural product extracts, allowing the conclusive data about different known and unknown natural products present in crude extracts. HPLC-SPE-NMR has an important advantage over HPLC-NMR in that the compounds are accumulated on SPE cartridges by repetitive adsorption. Further, the analytes are extracted by deuterated solvent, so that compounds are concentrated in detection cells, resulting in increased sensitivity of this hyphenated technique (Jaroszewski 2005; Staerk et al. 2009). Table 1.7 provides with isolation and characterization of novel compounds by using multiple hyphenated techniques.

## 1.10 Case Study

Dereplication of an antifungal compound from *Swertia calycina* (Gentianaceae): *Swertia calycina* dichloromethane extract showed strong activity against *Cladosporium cucumerinum* and *Candida albicans* fungi. Dereplication of extract was carried out to locate the compound accounting for antifungal activity. LC-UV chromatogram showed three peaks. Further, TLC-autobiography with

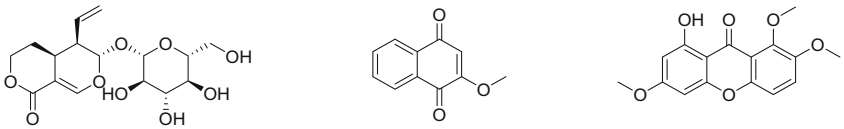


**Fig. 1.7** Isolation and identification of secoiridoids, antifungal naphthoquinone, and xanthones from *Swertia calycina*

*C. cucumerinum* revealed that antifungal activity of the plant extract was due to compound 2. The LC-UV-MS analysis showed that compound 3 produced four absorption band in UV spectra characteristic of xanthone, and MS spectrum showed $[M+H]^+$ peak at 303 (i.e., M.W. = 302) which on comparison with in-house UV spectra library confirmed compound 3 as decussatin. Online data obtained for compound 1 indicated the presence of secoiridoid-type molecule with 358 Da molecular weight, which is a characteristic feature of Gentianaceae family. The extract was submitted to online LC-NMR to confirm the above findings and to obtain more structural data. Compound 1 was confirmed as sweroside, a popular secoiridoid of the Gentianaceae. Compound 3 was confirmed as decussatin, and compound 2, responsible for antifungal activity, was finally identified as the known 2-methoxy-1,4- naphthoquinone (Rodriguez et al. 1995). Figure 1.7 shows the isolation and identification of secoiridoids, antifungal naphthoquinone, and xanthones from *Swertia calycina* as a case study.

## 1.11 Impact on Synthetic Medicinal Chemistry

Different aspects of synthetic chemistry can be utilized to produce natural product-like compounds, although natural products frequently display highly potent and selective bioactivity, they did not experience adaptive selection to function as human therapy and were therefore not fine-tuned to attain the required potency, selectivity, and pharmacokinetic properties in a clinical molecule. Optimization also requires modifying, replacing, or adding functional groups and stereo centers or extreme remodeling of the fundamental scaffold to optimize physico-chemical and pharmacokinetic properties. Thus, natural products are guiding principles or natural product-based design and synthesis of compound libraries (Rolf et al. 2002; Camp et al. 2012). To make discussion simpler, we categorized synthesis under the following categories:

- Semi-synthesis and derivatization
- Synthesis inspired by natural products
- Diverted synthesis

### 1.11.1 Semi-synthesis and Derivatization

Semi-synthesis is simplest and useful approach for lead optimization (Fig. 1.8). A large number of derivatives can be generated rapidly. The application of synthetic chemistry to modify the leads of the natural product has long been used to produce compounds with improved pharmacological properties. This semi-synthetic approach has been widely used either to generate more similar compounds randomly or to improve lead natural product candidates (Kennedy 2008). For example, aspirin is the best and first example; it was synthesized from salicin. Similarly, the cholesterol lowering semi-synthetic simvastatin is more potent than parent lovastatin (Alberts 1990). Taxol is one of the most effective anticancer drugs ever developed. It accumulates at a very low concentration in inner bark of *Taxus* species, the increasing demand for taxol greatly exceeds the supply which isolation from its natural source can sustain, and alternative sources of the medication are being pursued (Cragg et al. 1993; Exposito et al. 2009).

In 1992, Holton patented an improved semi-synthetic process for taxol from 10-deacetylbaccatin isolated from the needles of the European yew (Holton 1993).

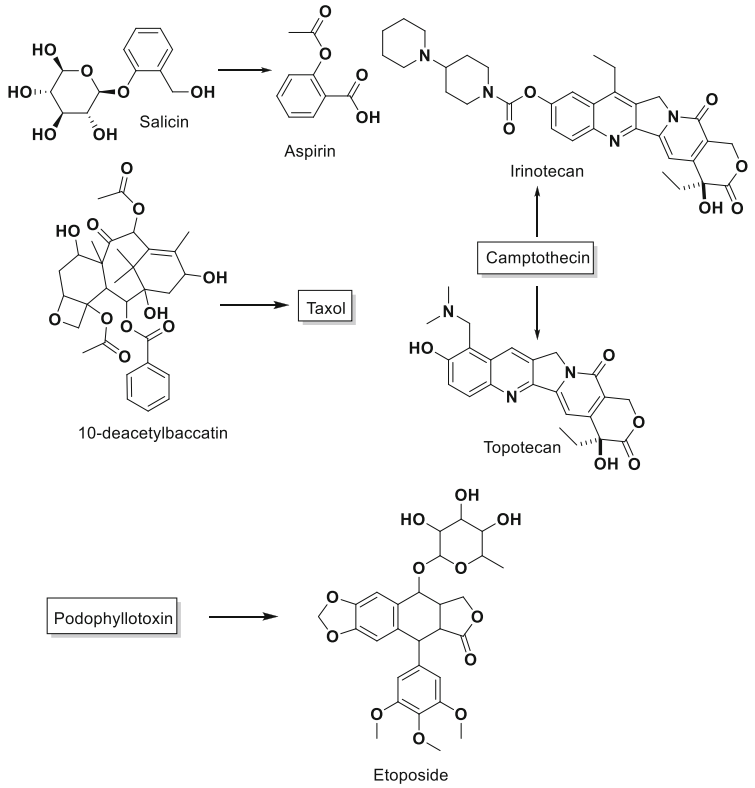


**Fig. 1.8** Semi-synthesis and derivatization

However, current Taxol supply is based on plant cell fermentation (PCF) technology developed by BMS; specific *Taxus* cell line is propagated in large fermentation tanks in aqueous medium with the endophytic fungus *Penicillium raistrickii*. Paclitaxel is then directly extracted and purified. Similarly, camptothecin (CPT) was identified as potent anticancer agent; later the solubility issue was resolved by semi-synthesizing potent anticancer compounds topotecan and irinotecan that are widely used for cancer treatment. Supply of starting material CPT depends on its biological source: *Camptotheca acuminata* (Nyssaceae); *Nothapodytes foetida*, *Pyren acanthaklaineana*, *Merrilliodendron megacarpum* (Icacinaceae); *Ophiorrhiza pumila* (Rubiaceae), *Ervatamia heyneana* (Apocynaceae); and *Mostuea brunonis* (Gelsemiaceae) (Lorence and Nessler 2004). Another example is clinically useful anticancer etoposide, a semi-synthetic derivative of podophyllotoxin which can be isolated from mayapple tree or American mandrake, *Podophyllum hexandrum* (Kingston 2008). There are a number of potential candidates of natural origin used for semi-synthesis-based natural product libraries. Here, we understand that semi-synthesis can lead to a wide range of natural product–like analogues rapidly, and subsequent improvement in the understanding of structure-activity relationships (SAR) can lead to molecules which are pharmacologically improved.

## 1.11.2 Synthesis Inspired by Natural Products

Adequate supply can be a significant limiting factor in the preclinical and clinical development of certain naturally derived products, and the emphasis of many leading synthetic organizations is on the development of economically feasible synthetic strategies.

Sometimes, the total synthesis revealed the correct structure of natural product, for example, diazonamide A; such type of case studies has been reviewed by Nicolaou (Nicolaou and Snyder 2005). One of the excellent examples of natural product inspired total synthesis is the discovery of flavopiridol (Alvocidib) and P-276 (Fig. 1.9). These two molecules are totally synthetic, but their novel structure is based on the natural product rohitukine isolated from *Dysoxylum binectariferum* (Jain et al. 2012), which is phylogenetically related to the plant, *D. malabaricum*,

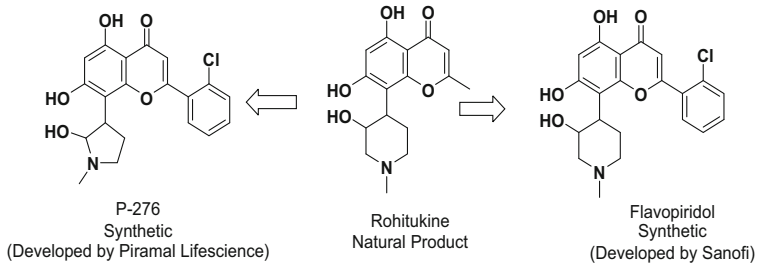


**Fig. 1.9** Natural product inspired synthesis

used for rheumatoid arthritis. Rohitukine was isolated as the constituent accounting for anti-inflammatory and immunomodulatory activity. For structure-activity relationship studies, a synthetic campaign was performed which culminated in flavopiridol; both flavopiridol and P-276 are in clinical trial for cancer treatment (Naik et al. 1988; Kattige et al. 1990).

### 1.11.3 Diverted Synthesis

The sub-structural portion which is essential for biological activity is called pharmacophore. This information allows the molecular editing of unnecessary structural complexity, resulting in simpler analogues with better activity (Wilson and Danishefsky 2006). Cragg et al. (2009) show the best example of diverted synthesis is artemisinin (Fig. 1.10). Various other examples have been reviewed by Szpilman et al. (Szpilman and Carreira 2010).

## 1.12 Conclusion

Conventionally, drug discovery from plants was a linear procedure where authentic plant material was subjected to extraction, fractionation, isolation, and purification of all possible compounds, most importantly, structure elucidation of purified compounds after acquiring all 2D NMR data, etc., and then biological evaluation. This is a laborious and time-consuming procedure. Hyphenated techniques–based dereplication and early identification of known compounds in crude/fraction combined with bioassay-guided fractionation drastically enhanced the drug discovery outcomes. LC-MS is now considered as a major hyphenated technique widely used in academia and industry. LC-MS-based metabolomic profiling and MS-MS fragmentation provide robust structural information of metabolites present within the crude. This technique gained immense popularity in the last 5–10 years, and hence the creation of databases or repository of MS-MS fragment-based mass spectrometry data has gained popularity in the field of natural products dereplication. The standard MS-MS fragmentation of a particular metabolite remains same under the same experimental conditions which can be considered as a signature or fingerprint of that particular metabolite. These signature profiles help in the identification of known metabolites within the crude. Various dereplication workflows have been proposed and reported where a hyphenated technology or multiple hyphenated

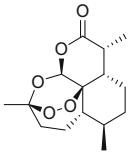


**Fig. 1.10** Artemisinin as an example of diverted synthesis

technology combined with a suitable database provides structural information fully or partially at the early stage of discovery.

**Acknowledgment** The author with BG and BS acknowledge IIT (BHU), Varanasi, for providing teaching assistantship.

**Conflict of Interest** The authors declare no conflict of interest.

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# Herbal Medicines as a Rational Alternative for Treatment of Human Diseases

2

Anand Kumar Chaudhari, Somenath Das, Bijendra Kumar Singh, Jitendra Prasad, Nawal Kishore Dubey, and Abhishek Kumar Dwivedy

**Abstract**

Herbal medicines (HMs) are receiving considerable attention as the complementary drugs throughout the world due to their cost effectiveness, low toxicity, and therapeutic potential against wide range of human illness. These plants possess a wide range of bioactive principles which alone or synergistically act on different targets. Even in the modern era of medicine and technology, more than 80% of the modern medicines currently available and one-third population of the developing countries largely depend on plant products either directly or indirectly for their primary health care. Several plants such as *Aegle marmelos*, *Atropa belladonna*, *Azadirachta indica*, *Catharanthus roseus*, *Camptotheca acuminata*, *Colchicum autumnale*, *Curcuma longa*, *Digitalis lanata*, *Eclipta alba*, *Ocimum sanctum*, *Papaver somniferum*, *Phyllanthus emblica*, *Rauvolfia serpentina*, *Taxus brevifolia*, and several other high value plants have been well acknowledged for its pharmacological importance to treat important human diseases like diabetes, cancer, dementia, epilepsy, hepatitis, fever, kidney stone, malaria, mouth ulcer, and other important disorders in indigenous system of medicine. In spite of their great potential against different human diseases, the HMs have faced several acceptance issues for the practical application due to lack of scientific and clinical evidence regarding their biochemical mode of action on particular cells, tissues, or organs. Therefore, a mass-scale clinical trials and specific documentation on its molecular mode of action is needed. Based on the aforementioned background, the present chapter describes the documentation of important medicinal plants and their derived bioactive principles, potential to combat important human diseases with underlying mode of action to facilitate

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_2

direction for reproducible drug discovery, which are safe, cost effective, and rational alternatives to the modern remedies.

**Keywords**

Herbal medicine · Mode of action · Pharmacological properties · Drug discovery · Bioactive principles

## Abbreviations

| | |
|---|---|
| CVDs | Cardiovascular diseases |
| HMs | Herbal medicines |
| TCMs | Traditional Chinese medicines |
| SCM | Sasang constitutional medicines |
| ROS | Reactive oxygen species |
| HIV | Human immunodeficiency virus |
| AIDS | Acquired immune deficiency syndrome |
| EOs | Essential oils |
| CAM | Complementary and alternative medicine |
| IPR | Intellectual property right |

## 2.1 Introduction

Since last few decades, people throughout the world and especially in developing countries have faced several challenges associated with health care. Many diseases like diabetes, tuberculosis, epilepsy, asthma, Alzheimer's, cancers, Parkinson's, cardiovascular diseases (CVDs), hepatitis, and multiple sclerosis, which are untreatable and directly affect the county's economy and the development (Wieland et al. 2005; Ahmed et al. 2017; Kocher et al. 2018). Although the current population of the world uses modern therapeutic approach to treat these diseases, still many traditional practitioners use plant-derived products to treat, prevent, and cure these ailments. These are called herbal medicines (HMs). HMs have long history as most of the cultures throughout the world use HMs for the treatment of diseases from time immemorial (Sahoo et al. 2010). It is estimated around 25% of the drugs launched worldwide and more than 70% population of the developing countries basically rely on plant products for their preliminary health care or as a supplement for health (WHO 2002; Sahoo et al. 2010). According to WHO, it has been estimated that 250,000–500,000 species of plants exist on the earth, up to now, only around 10% or 21,000 plants have been used as medicines by humans to treat these complications. Among them, more than 200 species are found only in India; however, only 150 species are being used commercially as medicinal plants (Seth and Sharma 2004). In 2005, the global market of herbal drugs was worth an estimate of $18 billion, which was extended to nearly $19 billion in 2006 and more than

$26 billion by 2011 and subsequently increases according to demands (McWilliams 2006; Saklani and Kutty 2008). Nowadays, HMs have been trending as alternative or complementary medicines in view of their safety profile, cost effectiveness, strength, effectiveness, and ecofriendly therapeutic potential. Many plant-derived bioactive compounds, such as paclitaxel and camptothecin from *Taxus brevifolia* and *Camptotheca acuminata* having anticancerous, artemisinin from *Artemisia annua* bearing antimalarial, forskolin from *Coleus forskohlii* against obesity and arthrosclerosis, antidiabetic compound steviol from *Stevia rebaudiana*, galantamine from *Galanthus nivalis* against Alzheimer's, and apomorphine, a semisynthetic analogue of morphine derived from *Papaver somniferum* with Parkinson's treating properties, have been well explored and reported since last few decades (Veeresham 2012; Boyle and Ondo 2015; Jain and Jain 2018; Zaidan et al. 2019). In spite of their appreciated potential against different human diseases, their exploration as medicine directly to human has faced several challenges due to lack of scientific and clinical evidence regarding their biochemical mode of action on particular cells, tissues, or organs as well as standardization issues. Based the aforementioned background, the present chapter deals with exploration of some important medicinal plants for the development of drugs, which are useful for treatment of severe human's ailments. Further, the chapter provides an emphasis on therapeutic properties of these HMs with possible mechanism of action against important diseases, and at the end, the safety profile of HMs has been incorporated so as to exploit them as an alternative medicine in the era of modern therapeutic approach.

## 2.2 Traditional Knowledge of Herbal Medicines (HMs)

The humans have used plant products since antiquity to relieve and treat the diseases. Some fossil records reveal that humans were using plants to cure diseases back at least 60,000 years (Fabricant and Farnsworth 2001; Shi et al. 2010). The knowledge of thousands of years of traditionally used herbal medicines can be used to overcome several medical problems of present generations. The development of medicine for the early humans was not an easy task; several of them have sacrificed their lives during the course of testing of plants with some beneficial medicinal importance. Some plants might be poisonous, harming their lives. HMs are still the centerpiece as about 80% population of developing countries requires them for the primary health care due to lesser side effects, cultural acceptability, higher efficacy, and availability (Gupta and Raina 1998; Kamboj 2000; Ekor 2014). Medicinal plants are the major source of traditional medicines as well as some modern medicines. The earlier records of HMs suggest their use for 5000 years in Greek, Chinese, Indian, Egyptian, Roman, and Syrian literature (Pal and Shukla 2003). The old texts of India such as Atharvaveda, Rigveda, Sushruta Samhita, and Charaka Samhita deal with HMs, and all these were derived from very rich scientific devise and early civilization (Kamboj 2000). Many forms of traditional HMs exist in the world; some of which are listed as follows.

### 2.2.1 Ayurveda

Ayurveda is one of the ancient medicinal systems of Indian civilization since prehistoric time. Etymologically, Ayurveda is the combination of two Sanskrit words "Ayur" meaning life and "Veda" dealing with science or knowledge, which means "the science of life." The philosophy behind Ayurveda is living a long life without unnecessary suffering and bringing balance as well as consonance between three paradigms of life, that is, spirit, mind, and body. Ayurveda is considered as holistic in sense that it balances and integrates the spirit, body, and mind to cope with illness and some diseases. On the basis of origin of medicines, Ayurvedic medicines are divided into three categories which are herbal, mineral, and animals. Among all, HMs gained a great deal of attention due to their safety profile (Parasuraman et al. 2014). The Indian subcontinent is one of the mega biodiversity centers with about 45,000 plant species, which contribute to be a reservoir of herbals (Hasan et al. 2009). From India, about 15,000 medicinal plants have been documented, out of which 7000 are being used by several communities for curing different kinds of diseases, and there are almost 700 types of plants listed in the Ayurvedic system of medicine (Meena et al. 2009).

### 2.2.2 Traditional Chinese Medicines (TCMs)

This originated thousands of years ago in China. It comprises of four elements: monarch, minister, assistant, and servant according to their roles in the formula. TCMs are based on the concept of Yinyang and Wuxing, and formula includes a group of several kinds of drugs which function together to show a synergistic action. TCMs are a part of people medicine system in China. In recent years, TCMs have gained an approval to be used as alternative and complementary medicine in Western countries also. It is estimated that about 1.5 billion people are being cured by TCMs throughout the world (Dobos et al. 2005; Qi et al. 2013). TCMs are being used in the treatment of allergic responses along with some other diseases which put burden on the economic trade due to poor health condition of the country (Lin et al. 2019). The pharmacology of TCMs has evolved with time, and several scientific communities are trying to make an effort to understand the molecular mechanism of action of TCMs so as to incorporate these formulation into modern medicines.

### 2.2.3 Kampo

Kampo evolved around fifth and sixth century in Japan, and it came into existence from China via the Korean peninsula. It focuses on patient as a whole in place of the disease only. TCMs were altered and some new modifications were done accordingly and adapted by the Japanese medical practitioners which evolved as Kampo (Yakubo et al. 2014). The herbal formulations of Kampo medicine system are being governed in the same way as that of the other conventional medicines of the world,

and it is considered to be safe. Currently, about 90% of the medical professionals in Japan prescribe Kampo medicine in spite of Western medicines. Physicians in Japan use Kampo medicine with chemotherapy or radiation therapy for the treatment of patients suffering from cancer (Okamoto et al. 2014; Sahashi 2005). Kampo medicine system does not discriminate the symptoms as physical and psychiatric; the reason behind such concept is the thought of the Kampo system which considers human beings a complete, self-controlled unit. Both body and mind control each other and get affected by each other.

### 2.2.4 Unani Medicines

This is an important traditional HM system, popular in India and originated from Greek- Arabic medicine system around 2000 years back in Arabian civilization. In this system of treatment, whole body is treated as a single unit and its mind, soul, and body as a whole (Lone et al. 2012). Unani medicine is also recognized by WHO as alternative medicine system for curing the sufferers. The body temperature of a human being shows its well-being and mental and physical status. Alteration in the body temperature makes the human body susceptible to many kinds of diseases. Many of the bioactive components of mangrove forests have been isolated and being used in Unani medicine system since long time (Govindasamy and Kannan 2012; Jabin 2011).

### 2.2.5 Russian Herbal Medicines

This originated in Russia in the tenth century and was also introduced in Europe and Asia. Russia has large size of land and variety of soil composition which favors the growth of large numbers of medicinally important plants. So, herbal medicine is quite popular out there. A recent survey done by an authority found that about 44% people of Russia use HMs occasionally and about 12% use it frequently (Shikov et al. 2014). There is a separate branch of phytotherapy in Russia for the treatment of patients.

### 2.2.6 Africa Traditional Herbal Medicines

It originated in Africa and was based on holistic view of HMs which inherited from rich cultural heritage. About 80% people of Africa use herbal medicines to get rid of the disease (WHO 2002). The herbal medicines are easily accessible by the people of Africa, so this remedy is the most popular one in that area. The traditional medicine system is well integrated in the scheme of national healthcare system of the country and well organized (Boakye et al. 2015).

### 2.2.7 Traditional Medicines of Korea: Sasang Constitutional Medicines (SCM)

It was first introduced in nineteenth century in Korea, and SCM is also a part of Korean traditional medicine. A Korean medical doctor named Lee proposed the concept of SCM about 100 years ago. SCM is very holistic and patient specific, and every patient may be categorized into one, on the basis of inherited characters out of four Sasang Constitution (SC). The four types of SC are TaeEum (TE), So-Eum (SE), So-Yang (SY), and Tae-Yang (TY). The TE (TY) type is called liver (lung) type. The other two, SE and SY are called kidney and pancreas types, respectively (Kim et al. 2013). It is applicable in private and public hospitals in Korea. In 2006, the scientific proof of SCM was provided by Lee Jama project and also taken cared by the government of Korea.

## 2.3 Past, Present, and Future Scenario of Human Disease Prevention by Plant Products

Plant products are the natural source of several biologically active substances, which encompasses different properties that play major role in disease prevention caused either by biological or by non-biological agents. It has been proven that allopathic medicines are more toxic to the nontarget tissues and alter some other related biological activities and are more expensive. On the other hand, HMs show lesser or no side effects and are available in affordable price too (Alzohairy 2016). Plants have several secondary metabolites in the form of phytochemicals which protects plants from invasion of pests and infection from microorganisms. These phytochemicals have some active ingredients which can be used in the form of drug for the treatment and prevention of disease (Shakya 2016). Phenolic compounds of plants are most valuable among the secondary metabolites because of their active role in morphological development, reproductive process, and physiological responses. There are about 8000 structures of different plant phenolic compounds known to us. The central skeleton of phenolic compounds is formed by one phenolic ring, and hydrogen is replaced by more active residues like hydroxyl, acetyl, and methyl groups. This is the reason behind wide spectrum of biological properties of phenolic compounds. In plants, these phenolic compounds are assembled in phenolic rings and are called polyphenols (Del Rio et al. 2013; Laura et al. 2019). Phenolic compounds containing plants have high antioxidant properties. Plants absorb the radiation of sun and produce oxygen as a by-product. Oxygen gets activated by UV light and heat generated by sunlight and produces reactive oxygen species (ROS), which interfere with cellular entities and alter them leading to cellular damage (Singh et al. 2019). Many studies show that high amount of consumption of fruits and vegetable containing phenolic compounds reduces the risk of diabetes and risk of cardiovascular diseases (Nöthlings et al. 2008).

## 2.4 Prevention of Important Diseases Using Herbal Medicines (HMs)

Prevention of infectious as well as noninfectious diseases in healthcare setting is the primary goal of the medical authority throughout the world. There are several diseases caused by microbial (bacterial, fungal, and viral) infection while others are caused due to disregulation of primary metabolic and body defense systems, which are evolved to protect body from suffering. There are many examples, where plant products have made their irony contribution toward disease management (Mitchell et al. 2016). Some of the important untreatable diseases and their possible preventive measures reported using plant products are discussed below in the following sections.

### 2.4.1 HMs Against Malaria

Malaria is considered as the disease of global importance with more than 3 billion people in tropical and subtropical countries are at risk with the estimated death of 6 lac as recorded by World Health Organization in 2015 (Cowman et al. 2016). The infection initiated when sporozoites produced by *Plasmodium* spp. enter the host body through female anopheles mosquito vector feeding human blood. The accompanying chronic symptoms appear in the form of rigors, nausea, headache, body pain, etc. The first affordable and safe plant-derived compound discovered in 1820 against malaria was quinine, which was obtained from the bark of *Cinchona* tree found abundantly in high altitudes of the South Africa (Achan et al. 2011). In 1940, another antimalarial drug called chloroquine was synthesized and used for the treatment of malaria; however, due to their synthetic origin, the pathogen develops resistance, which was more problematic (Mukherjee 1991). At the same time, a group of Chinese chemists extracted the drug artemisinin from the warm wood plant *Artemisia annua*, which has proved to be very effective against both the chloroquine-resistant and chloroquine-sensitive *Plasmodium falciparum* pathogen (Spooner and Harvey 1976; Meshnick and Dobson 2001). Krettli et al. (2001) reported the antimalarial property of freshly prepared extract of *Bidens pilosa* and *Ampelozizyphus amazonicus* against erythrocyte stage sporozoites of *Plasmodium*. In addition, the essential oil obtained from the leaves and stems of some plants like *Myrtus communis* and *Rosmarinus officinalis* was reported to inhibit the active growth of *Plasmodium falciparum* under in vitro condition (Milhan et al. 1997; Hennia et al. 2019). Later on many other plants such as *Azadirachta indica*, *Asparagus africanus*, *Bixa orellana*, and *Clerodendrum viscosum* as a whole or some plant parts such as leaf of *Jasminum syringifolium*, root of *Plumbago zeylanica*, flower of *Corymbia watsoniana*, seed of *Cuminum cyminum*, and the fruit of *Citrus limetta* have been well explored and reviewed for their potential antimalarial properties (Kaur and Kaur 2017). In addition, some plant-specific compounds like vasacine from *Adhatoda vasica*, barberine from *Berberis aristata*, calusamine from *Clausena anisata*, caesalpin from *Caesalpinia sappan*, piperine

from *Piper nigrum*, and tinosporine or withanine from *Tinospora cordifolia* and *Withania somnifera*, respectively, showed antimalarial properties (Sankhala et al. 2012; Uddin et al. 2012; Damanhouri and Ahmad 2014; Kaur and Kaur 2017).

### 2.4.2 HMs Against Diabetes

Diabetes is a class of metabolic syndrome rather than disease characterized by low glucose level in blood due to alteration in insulin secretion, insulin action, or both from the β-cells of pancreas affecting large number of people throughout the world (Patel et al. 2012). Generally, it is evident that the diabetic people have more chances of cardiovascular attacks than the nondiabetic ones (Kannel and McGee 1979). Up to now only plasma-mediated transfusion of drugs are available to treat diabetes due to their sensitivity toward digestive enzymes, which inactivates them upon consumption when taken through oral routes; therefore, it is desirable to think toward some oral form of alternatives. In this context, plants can be the best alternatives, and more than 800 plants are available and reported to show antidiabetic properties without causing any complication (Trojan-Rodrigues et al. 2011). *Eugenia jambola*, a plant from family Myrtaceae was reported to contain many phytochemicals such as ellagic acid, isoquercetin, kaempferol, myricetin, and alkaloid jambosine, which all exhibited antidiabetic action upon consumption (Ayyanar and Subash-Babu 2012). Whole plant part of holy basil (*Ocimum sanctum*) was reported to show antihyperglycemic effect, and after their chemical standardization, it was found that the antidiabetic action of this plant was due to eugenol (Pattanayak et al. 2010). *Stevia rebaudiana*, which was reported to be more than hundred times sweeter than the sugar, possesses hypoglycemic action in diabetic patients (Shivanna et al. 2013). Likewise, many more plants such as *Aegle marmelos*, *Acacia arabica*, *Andrographis paniculata*, *Aloe barbadensis*, *Juglans regia*, *Momordica charantia*, *Terminalia chebula*, *Tinospora cordifolia*, and *Withania somnifera* were reported to show antidiabetic action either due to dowregulation of the blood glucose level by improving the action of insulin or by some other metabolic functioning (Stanely et al. 2000;, Kumar et al. 2006; Udayakumar et al. 2009; Tripathi and Chandra 2010; Naveen and Baskaran 2018).

### 2.4.3 HMs Against Cancer

According to an estimate of WHO, more than 200 different types of cancer have been identified, and cancer is considered as one of the second leading causes of death throughout the world (Bray et al. 2018). Several chemopreventive measures have been undertaken to treat cancers; however, their cost effectiveness and adverse effects on healthy cells or tissue restrict their successful utilization. To combat these effects, many plant-derived compounds like vincristine from *Catharanthus roseus*, taxol from *Taxus brevifolia*, camptothecin from *Camptotheca acuminata* have been well documented in literature to show their broad term action against

cancer or cancer cell lines under in vitro condition. The possible mechanism of action of taxol was believed to occur due to arrest of cells in G2/M phase of the cell cycle, while camptothecin exhibits their action by inhibiting the activity of topoisomerase I enzymes involved in DNA replication (Yeung et al. 1999; Liu et al. 2000). Several other compounds like topotecan, irinotecan, docetaxel, podophyllotoxin, teniposide, and elliptinum have been documented to showed anticancerous properties (Cragg and Newman 2005). Although they possess novel mechanism of action against different cancer cell lines, in order to recommend or commercialize them as possible alternative to the harmful and costly anticancerous drugs, it is desirable that the scientific community should work more on their action.

### 2.4.4 HMs Against Alzheimer's and Parkinson's

Alzheimer's and Parkinson's have been considered as the most prevalent form of late life mental complications in humans due to irreversible loss of neurons. The clinical symptoms of Alzheimer's appear in the form of impairment in memory, judgment, decision making, orientation to physical surroundings, and language, while Parkinson's showed symptoms in the form of Parkinsonism, that is, resting tremor, bradykinesia, rigidity, and postural instability (Hoehn and Yahr 1967; Nussbaum and Ellis 2003). One of the most promising approaches used to treat the Alzheimer's and Parkinson's is to increase the level of acetylcholine by acetyl cholinesterase inhibitors. Several plant-derived bioactive compounds like galanthamine, donezepil, rivastigmine, physostigmine, and morphine have been reported to act as potential inhibitor of acetylcholine and therefore help in the treatment of Alzheimer's complications (Barbosa Filho et al. 2006; Saklani and Kutty 2008). Some other approaches have also been hypothesized; however, due to lack of authentic evidences, the complete cure of these neurodegenerative disorders remains unresolved.

### 2.4.5 HMs Against HIV

AIDS is a well-known sexual disorder causing mortality and morbidity throughout the world. Since its discovery, there are no effective vaccines developed to cure HIV infection. The antiretroviral therapy is the most significant approach used for their treatment, and it is recommended that all patients with detectable HIV infection should be treated with antiretroviral therapy to prevent their subsequent progression as well as to reduce transmission (Günthard et al. 2016; Salehi et al. 2018). Several plants such as *Artemisia annua*, *Astragalus membranaceus*, *Calendula officinalis*, *Chelidonium majus*, *Galanthus nivalis*, *Helichrysum populifolium*, and *Hypericum perforatum* have been tested and reported for their antiretroviral properties against HIV infection. *Galanthus nivalis* performed its action by preventing the binding of viral envelop protein to the host cell's receptor, therefore inhibiting host-specific recognition (Magadula 2010). Some others like *Chelidonium majus* and *Calendula*

*officinalis* showed their action by preventing infection of $CD^{4+}$-T cells and inactivating the action of viral-specific reverse transcriptase (Asres and Bucar 2005; Salehi et al. 2018).

### 2.4.6 HMs Against Bacterial Food Poisoning and Fungal Mycotoxicosis

Several species of bacteria like *Staphylococcus*, *Clostridium*, *Campylobacter*, *Shigella*, and *Bacillus* as well as fungi such as *Aspergillus*, *Fusarium*, and *Penicillium* have been reported to produce most frequent group of bacterial and mycotoxins in food items, which upon consumption can cause severe case and food-borne poisoning and mycotoxicosis. The important disease caused by bacteria include diarrhea, tuberculosis, typhoid, and many more, while fungi caused mycotoxicosis, which is characterized by immune suppression, liver cirrhosis, abnormal fetus development, stunted growth, and sometimes cancers (Newell et al. 2010; Dwivedy et al. 2017; Chaudhari et al. 2019; Hashempour-Baltork et al. 2019). Different chemical preservatives and shelf life enhancers have been used since long time to eradicate these contaminants and their toxins; however, their indiscriminate use may lead to the development of resistance stain of these contaminants and residual toxicity to the nontarget organisms and to the environments (Linke et al. 2018; Chaudhari et al. 2019). In this context, many authors have claimed the importance of plant-derived essential oils (EOs) and their bioactive compounds as safer candidate for the preservation of these food items. Some of the important essential oils showing broad term toxicity against food-borne bacterial and fungal pathogens are allspice EO isolated from *Pimenta dioica*, lemon balm EO from *Cistus ladanifer*, basil EO from *Ocimum sanctum*, rosemary EO from *Rosmarinus officinalis*, citrus EO from *Citrus citrata*, mint EO from *Mentha spicata*, and *Cymbopogon citratus* (Chao et al. 2000; Burt 2004; Prakash et al. 2012; Kedia et al. 2016; Chaudhari et al. 2018; Upadhyay et al. 2018). These EOs contain different bioactive components, and each of them has different mode of action; therefore, the development of resistant strain among pest's population is hindered, and hence it is recommended as the safer alternative to the chemical preservatives for the preservation of food items from bacterial and fungal contamination as well as against their toxic metabolites.

### 2.4.7 HMs Against Tuberculosis

It is estimated that throughout the world, around 8 million people are suffering with the death of around 2–3 million due to tuberculosis. Although few slow working drugs are available against tuberculosis causing bacteria, development of resistance due to their multiple uses may further increase the risk (Keshavjee and Becerra 2000). To combat this issue, finding new drugs which have desirable capacity to fight with multiple sites of action is required; therefore, several tremendous researches throughout the world by different groups of scientists have been carried

out in the search of novel anti-tuberculosis agents of plant origins. Several reports are available regarding the anti-tuberculosis activity of plants such as extracts of *Curcuma longa*, *Allium cepa*, *Terminalia glaucescens*, *Leucophyllum frutescens*, *Chrysanctinia mexicana*, and *Schinus molle* (Newton et al. 2002; Molina-Salinas et al. 2007; Ibekwe and Ameh 2014); however, despite of their novel activity against tuberculosis and their causing agent *Mycobacterium tuberculosis*, none of the drugs are currently being used due to lack of scientific evidence and standardization of their exact mode of action. Hence, it is suggested that the scientists should work more on their mechanism of action, so as to utilize them as possible candidate for the prevention of tuberculosis.

### 2.4.8 HMs Against Epilepsy

After Alzheimer's and Parkinson's, epilepsy is the third important class of serious brain disorder occurring in people of all age groups from childhood to old. Currently, many of the used drugs suppress epileptic seizures without influencing the underlying tendency to generate seizures and are effective in more than 70% of individuals tested (Duncan et al. 2006). A lot of plants, namely, *Hypericum perforatum*, *Ginkgo biloba*, *Allium sativum*, *Piper methysticum*, *Illicium anisatum*, *Ephedra sinica*, and *Bacopa monnieri*, have been used by patients without consultation of physician to treat epilepsy (Samuels et al., 2008; Schachter 2009). Although these plants and their bioactive principles possess therapeutic properties against epilepsy, however, they also have some adverse effects as many of them induce seizure due to the neurotoxic action of their active principles; hence, it is recommended to utilize these plants or their derived products only after complete profiling and pharmacological action recognition by the experts.

### 2.4.9 HMs Against Hepatitis

Hepatitis is a serious complication of liver caused mainly by hepatitis virus or toxic substances (alcohol, drugs, or aflatoxin) or due to immune suppression. It is also believed that aflatoxin along with hepatitis virus is the serious cause of liver cancer (Henry et al. 2002). Several antiviral proteins (interferons) are developed, and currently two important proteins that is, pegylated interferon-α (PEG IFN- α) and ribavirin (RBV) have been in use to treat this hepatitis; however, they have certain limitations based on genotype, which could force the scientist to develop antiviral proteins without adverse effects. In the last two decades, herbal formulations have been proven to act as effective strategies, and many plant bioactive compounds, namely, epigallocatechin gallate from tea, quercetin from onion and apple, acacetin from black locust, and genistein from bean plants have been proved to have antiviral and hepatoprotective properties (Loa et al. 2009; Stagos et al. 2012; Rojas Rojas et al. 2018). These substances can inhibit the proliferation of virus either by altering their genetic machinery or inhibiting them to bind with the receptor proteins present

on the host surface. Due to their activity relationship with aflatoxins, it is also possible to reduce the chances of hepatitis infection by inhibiting the level of aflatoxins. In this respect, many plant products, especially EOs, have been reported to show anti-aflatoxigenic properties, and thus foods preserved with EOs are free from aflatoxins. The consumption of these foods may passively reduce the chances of hepatitis.

### 2.4.10 HMs Against Multiple Sclerosis

Multiple sclerosis is a common non-injurious T-cell-mediated disability arising due to inflammation, demyelination, and axonal injury or due to childhood obesity, low level of vitamin D, smoking, or infection by Epstein–Barr virus (Dobson and Giovannoni 2019). Current marketed drugs for multiple sclerosis therapies include different injectable compounds such as interferon beta, glatiramer, and natalizumab as well as oral drug formulations like fingolimod, dimethyl fumarate, and teriflunomide (Brandstadter and Sand 2017; Guarnera et al. 2017; Yu et al. 2019). None of the medicines discovered till date have been reported to completely reverse multiple sclerosis; however, sometimes it is believed that the utilization of plant-derived compounds can be the effective strategies, and this may restore the functioning of neurons inflammation. In this regard, some important plants and their derivatives have been used and reported by many workers against multiple sclerosis. For example, cannabinoides from *Cannabis sativa*, icariin from *Herba epimedii*, plumbagin from *Plumbago zeylanica*, salvianolic acid from *Salvia miltiorrhiza*, eriocalyxin from *Isodon eriocalyx* (Ingram and Pearson 2019; Yu et al. 2019).

## 2.5 Complementary and Alternative Medicine (CAM): Modern Technological Platform for Reverse Pharmacology

Traditional medicines based on the herbal materials have now been clustered into "complementary and alternative medicines" with integrative health benefits and significant pharmacological properties, especially, the drugs derived from botanical formulations made of plant extract, aromatic essential oil profile, powders, and whole parts with different health benefits such as anticarcinogenic, anticonvulsant, antipyretic, and vasodilatory actions (Patwardhan et al. 2008). Recent report suggested that approximately 75% of the world population relies on the application of medicinal plant-based formulation in different health and healing effects. Newman and Cragg (2007) focused on the utilization of 47% of natural medicinal plant products in the major areas of cancer research. Different national and internationally recognized pharmacopoeia's have focused on the safety evaluation of these natural drugs with their potential therapeutic effects. Now, in the era of current generation, different accidental serendipity of excessive dose of plant drugs was also reported from different regions of the world, which ultimately led to a common

decision on their dosage identification and advanced combinatorial medicinal chemistry and system biology (Takenaka 2001).

Recently, the trends on medicinal plants research have a specific paradigm shifting with major biotechnological approaches and post marketing innovations on multiple modes of action. Pharmacological industries have faced critical challenges for practical application of medicinal drugs in terms of their safety issues and expenses (Surh 2011). Modern scientific communities and medicinal boards have decided a fast track platform with trans-disciplinary innovations on "Reverse Pharmacology" to improve the cost, toxicity exposure, and time management. Patwardhan and Mashelkar (2009) reported the documentation of medicinal formulations and group lineage through modern "Omic" technologies facilitating the transcriptomics, metabolomics, and operon dynamics. The modern dynamic technologies have moved on opposite directions of the traditional herbal cure methodologies with much faster reverse pharmacological approaches (Takenaka 2001). Screening, standardization, and modified clinical observation have biodynamic and exploratory potentiality for optimizing the level of acceptability and bio-accessibility. Moreover, the reverse pharmacology also signified the selective bioprospection of active constituents of plant parts and exclusion of poisonous ingredients with safety issues (Singh et al. 2018). Potential interest on active components of medicinal plants and their targeted mode of action having technological advances were first reported by Sir Ram Nath Chopra and Gananath Sen with documented discovery of tranquilizing and antihypersensitive agents from *Rauvolfia serpentina* (Patwardhan et al. 2008). Recently, the developing countries have developed "drug act" for the inclusion of active phyto-ingredients of different medicinal plants with pluralistic and opportunistic healthcare complexes (Rajapakse and Davenport 2019).

The process of reverse pharmacology follows targeted proteinaceous action and emerging dimension in sequence technology with target mining actions. Distant homologies and ligand fishing can be identified during drug moderation, and mechanistic basis of indexed biological processes is involved (Cavalli et al. 2008). It is an inter-bridging community between the molecular genomics and pathophysiology for the development of superior drug candidate. Today, there is actual need of linkage between Ayurvedic pharmacokinetics and modern systemic therapeutic approach which endeavors the direction for development of novel drugs. In India, Research and Development (R&D) authorities, Council of Scientific and Industrial research (CSIR), and Ministry of AYUSH actively participate in this direction and standardize several protocols for successive identification of plant parts and their rational drug development.

Case studies on *Withania somnifera* have suggested the significant modulation on the toxicity of cyclophosphamide and lipid peroxidation of stressed animals as well as chemo-protectant activity of the compound methylguanine DNA methyltransferase (MGMT) (Niture et al. 2006). Root extract of *W. somnifera* contains glycowithanolides as a protective measure against iron toxicity. Mechanistic details of *W. somnifera* fruits on interferon, interleukin, and macrophage-modulating factors have also been studied (Davis and Kuttan 1998). Flavones,

isoflavones, and saponins of *Asparagus racemosus* exhibited active inhibition of neuromuscular disorders, macrophage modulatory activities, and myelo-suppressive effects. Extracts of the dried roots caused targeted inhibition of neuroendocrinal effects (Dahanukar and Thatte 1988). Tumor necrosis factor-α and interleukin-1 stimulation by ochratoxin have been actively modulated by bio-components of *A. racemosus*. Different antioxidative compounds such as plumbagin, silymarin, and glycyrrhizic acid have been isolated from roots of *A. racemosus* with significant antitumor and cytotoxic activities (Patwardhan et al. 2008). Different active ingredients, namely, cordifoliside, columbin, berberine, and cordioside isolated from *Tinospora cordifolia* exhibited potent free radical scavenging, antipyretic properties, and modulate metabolic enzymes of liver. Bio-ingredients of *T. cordifolia* actively inhibited chloroquine-induced splenomegaly in mice (Singh 2005). Alkaloids, namely ajmaline and reserpine, and several active biocomponents such as bromoergocryptine and L-Dopa extracted from *Rauvolfia serpentina* inhibited depression and toxicity in human and modulated ATP-dependent cellular metabolism at the transcription level (Patwardhan et al. 2008; Singh 2017). Currently, the effect of alkaloids on H-2 receptor, inflammation inhibitory, and pharmaco-vigilance activity has been the focus of different researchers throughout the world. The *Argemone mexicana* decoction, which is in progress of being approved for the preparation and utilization as an antimalarial drug in Mali (Willcox et al. 2011). Different phytomedicines (P-glycoproteins, allocryptopines, and berberine) prepared through synergistic and additive activities of extracts of *Artemisia annua*, *Argemone mexicana*, *Withania somnifera*, and *Cassia auriculata* with effective drug phramacokinetics have been utilized as traditional remedial measures (Willcox et al. 2011; Visavadiya and Narasimhacharya 2007; Annie et al. 2005). Bioactive pharmacological ingredients extracted from *Commiphora wightii* (guggul) exhibited different cellular and molecular level pharmacodynamic and pharmacokinetic properties in human (Vaidya 2006; Shishodia et al. 2008). Technological advancement through proteinaceous metabolites of *Commiphora* extract modulates the hypolipidemic profile in animals and human being. Recent study of Upadhyay et al. (2013) suggested active participation of Picrosides I and II isolated from dried rhizome and roots of *Picrorrhiza kurroa* for inhibition of respiratory tract infection, renal disorders, and dyspepsia in different animal models. Steps involved in reverse pharmacology with the active participation of different bioactive components and biotechnological approaches are presented in a flow diagram in Fig. 2.1.

Moreover, in addition to different healthy aspects of reverse pharmacology, each and every country has now secured its traditional herbal knowledge through the guidelines of Intellectual Property Right (IPR) and developed a digital library and patent consistency. The traditional knowledge along with modern pharmacodynamics could integrate the knowledge of ambitious scientific innovation and novel clinical approaches.

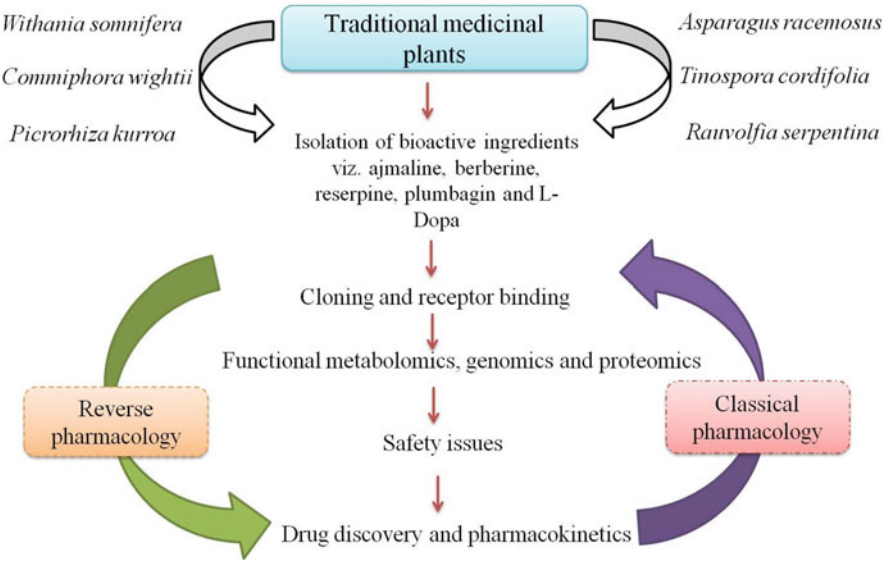


**Fig. 2.1** Successive stages of reverse pharmacology involving the bioactive ingredients of medicinal plants and modern technologies

## 2.6 Safety Paradigm of Traditional Herbal Medicine

Herbal medicines are being used since very long time, and selection and cultivation of herbal medicines totally depend on the selection of right specimens. There are chances of alterations in the samples which could lead to a threat to consumer's health. In recent years, there is increase in the consumption of the herbal medicines due to its more safety values than that of allopathic medicines. So, it leads to greater chances of adulteration of herbal medicines (Zhang et al. 2015). Very less research has been done in case of safety of herbal medicines. Processing stage is the step where the herbal medicines can get contaminated even by slightly miss caring. Moreover, most of the natural products do not get proper evaluation at the laboratory level by the regulatory authorities, as result of which these products fail to prove their efficacy at the molecular level (Booker and Heinrich 2016). There are variabilities that are found in the herbal medicinal products at the inherited level. Also, variability is found at the batch-to-batch level, and absence of proper standard reference material also adds some more difficulties in the quality control assessment of herbal products (Ghosh 2018). It is believed that risks associated with herbal medicines are very low, but this is not the case, there are risks associated with the contamination in herbal products.

## 2.7 Conclusion and Future Prospective

From time immemorial, man has sought to prevent disease caused by different biological and nonbiological agents using various means among which the use of HMs containing different forms of plant secondary metabolites is very common. These HMs possess pharmacological activities against malaria, diabetes, Alzheimer's, Parkinson's, HIV, tuberculosis, hepatitis, cancers, multiple sclerosis, and many more caused by bacterial and fungal toxins without posing any desirable impact on body due to safety profile as approved by international as well as national authorities. In spite of their proved therapeutic potential, global utilization remains limited due to the fact that their mode of action and standardization were not tested during application. Further, by using reverse pharmacological tools, the promotion of HMs may be approved. Therefore, based on overall reports, this chapter concludes that the herbal medicines can be utilized as an alternative medicine after complete profiling and testing the exact mode of action.

**Acknowledgments** Anand Kumar Chaudhari, Somenath Das, and Jitendra Prasad are thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India, and Bijendra Kumar Singh is thankful to the Department of Biotechnology (DBT), New Delhi, India, for research fellowship.

**Conflict of Interest** No conflicts of interest were reported by the authors.

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# Effect of Natural Products on Improvement of Blood Pathophysiology for Management of Sickle Cell Anemia

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Abhishek Gour, Ashish Dogra, Shipra Bhatt, and Utpal Nandi

**Abstract**

Sickle cell anemia (SCA) is an inherited disorder in the β-globin chain of hemoglobin that affects millions of people around the world, especially children. This disease prevalently occurs in some Mediterranean and Saharan Africa. For the treatment of SCA patients, a wide range of drugs have been explored by targeting antisickling activity, γ-globulin induction, antiplatelet effect, etc., but hardly a few drugs have shown potential to combat with this complex disease phenomenon. In spite of unprecedented advances in modern system of medicine, people in the disease-prone area have been taking traditional medicinal plants or plant-derived products to increase the life span of patients. Moreover, numerous clinical trials have been going on for the use of natural products under the purview of symptomatic management of SCA. This chapter is focused on the effect of natural products in pure form or characterized phytoconstituents on particularly inhibition of hemoglobin polymerization. This summarized information will be beneficial for further exploration of new therapeutics in the treatment arena of SCA.

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_3

**Keywords**

Sickle cell disease · HbS polymerization · Antisickling agent · Phytoconstituents · Natural products

## Abbreviations

| | |
|---|---|
| SCA | Sickle cell anemia |
| SCD | Sickle cell disease |
| WHO | World Health Organization |
| HbS | Sickle hemoglobin |
| RBC | Red blood cell |
| HbF | Fetal hemoglobin |
| USFDA | United States Food and Drug Administration |
| P-selectin | Pan selectin |
| NO | Nitric oxide |
| NAD | Nicotinamide adenine dinucleotide |
| MDA | Malondialdehyde |

## 3.1 Introduction

Sickle cell anemia (SCA) is the most common form of sickle cell disease (SCD) which is a group of an inherited disorder of hemoglobin (Piel et al. 2017). SCA is considered as the "first molecular disease" in 1949 (Pauling et al. 1949). Millions of people around the world, especially children, have been affected by SCA (CDCP 2011; WHO 2006). This prevalently occurs in Sub-Saharan Africa and in some parts of Greece, Turkey, Saudi Arabia, and India (WHO 2006; Frenrtte and Atweh 2007). This disease is recognized as a global public health problem by the World Health Organization (WHO) whereas some countries consider it as a rare disease. This global burden is a growing concern nowadays as the yearly increase of newborns with SCA is expected from around three to four lakhs between 2010 and 2050 (Weatherall 2010; Piel et al. 2013). SCA, congenital hemolytic anemia, is caused by a single amino acid substitution (valine instead of glutamic acid) at the sixth position of the β-globin gene (Pauling et al. 1949). This shows the way for polymerization of deoxygenated sickle hemoglobin (HbS) inside the red blood cell (RBC) which is the crucial step in the molecular pathogenesis of SCA.

Polymerization of HbS in a circulating sickle RBC can occur in different rate and extent depending on factors like the degree of oxygenation, hemoglobin concentration at intracellular level, availability of fetal hemoglobin (HbF), etc. (Steinberg 1999). This HbS polymerization is reversible at early stage but changes with repeated deoxygenation/reoxygenation. The term "delay time" can be demonstrated as the time required for HbS polymerization. The increase in the transit time of RBC in the microcirculation enhances the chances of HbS polymerization which also

involves the lowering of oxygen tension (Mozzarelli et al. 1987). This polymerization alters the RBC rheology by changing its surface property, membrane damage, and dehydration of RBC. Potassium chloride co-transport and calcium-activated potassium efflux are generally involved during the process of RBC dehydration (Lew et al. 1985, 2002). During this process, shape of RBC is changed from round disk to crescent moon–like structures. These dehydration events further show the way to reduce the RBC volume with rise in hemoglobin concentration at intracellular level in parallel (Ataga and Stocker 2009). This conformational change in RBC directs toward hemolysis and vaso-occlusion leading to ischemia followed by pain crisis. Generation and impairment of oxidative stress is involved during the above-mentioned process (Chirico and Pialoux 2012). A series of complications turn out because of vaso-occlusion like acute painful episodes, acute chest syndrome, splenic sequestration, etc. and/or due to hemolysis like pulmonary hypertension, stroke, glomerulopathy, cholelithiasis, etc. (Ataga and Stocker 2009). These cause mortality where treatment targets are to increase life expectancy. Gene therapy can provide a proper solution to tackle the above-mentioned pathophysiological conditions, but currently, research including clinical trials have been ongoing (NCT02247843, NCT02186418). A vast range of drugs have been explored for symptomatic management of these disease conditions that are discussed below. As natural products have immense potential for the treatment of a broad range of disease conditions, supplementation/adjuvant therapy with natural products can be a suitable alternative. This is also evidenced by a number of research works in preclinical and clinical settings.

## 3.2 Current Treatment Options of SCA

SCA patients are associated with acute and chronic complications leading to mortality. The main treatment targets (Fig. 3.1) can be categorized in the following manner to improve the survival of the patients under such complex phenomenon.

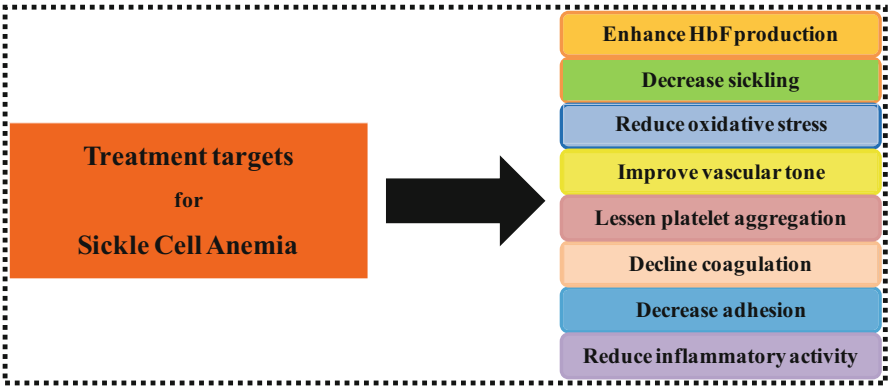


**Fig. 3.1** Different pharmacological approaches for the management of SCA

Use of antisickling agents is one of the key therapeutic targets to treat SCA patients. This broad term includes induction of HbF, protection from dehydration of RBC by slowing down the production of deoxy-HbS, improvement in the affinity of hemoglobin for oxygen, and availability of ligands to prevent/reverse the HbS polymerization.

Hydroxyurea is the first-ever approved drug by United States Food and Drug Administration (USFDA) for the management of SCA (Segal et al. 2008). Hydroxyurea is an anticancer agent that inhibits ribonucleotide reductase. It is reported that it helps to increase HbF synthesis to decrease HbS polymerization, reduce RBC membrane damage to diminish hemolysis, enhance hemoglobin synthesis, elevate NO release to decline endothelial activation, and lessen the accumulation of molecules responsible for vaso-occlusion. Despite several advantageous effects of hydroxyurea, the major limitation is its myelosuppression effects (Verma et al. 2018). Histone deacetylase inhibitor is another class of drug that increases HbF production. Azacytidine, decitabine, and pomalidomide are the representatives of this class of molecules (Desimone et al. 2002; Moutouh-de Parseval et al. 2008). Zileutin, a 5-lipoxygenase inhibitor, was found to induce HbF where the mechanism of action is different from hydroxyurea (Haynes et al. 2004). Senicapoc (ICA-17043) has been reported to prevent dehydration of RBC by interfering with the Gardos channel available in the membrane of RBC (Ataga and Stocker 2009).

Voxelotor (GBT440) is a candidate under phase-3 investigation that prevents sickling of RBC and increasing affinity for oxygen after binding to N-terminal of α-chain of hemoglobin. This molecule has been reported to extend half-life of RBC (Oksenberg et al. 2016, Lehrer-Graiwer et al. 2018). Panobinostat (NCT01245179) and sanguinate (NCT02411708) are the antisickling agents which are under clinical trials (Kato et al. 2018). Inhibition of platelet aggregation is beneficial to prevent vaso-occlusion. Therefore, antiplatelet agents have been investigated for the management of SCA. Eptifibatide is a platelet glycoprotein aIIbb3 antagonist that inhibits platelet aggregation and alters inflammatory mediators. Investigation of it was carried out earlier for SCA (Lee et al. 2007). Prasugrel is an antiplatelet agent and irreversible antagonist of $P2Y_{12}$ adenosine diphosphate receptors. It interferes with adenosine diphosphate–mediated platelet activation as well as aggregation. The ability of prasugrel to reduce the rate of vaso-occlusive crisis in the clinical trial is not promising for the treatment of SCA patients (Badawy 2016). Ticagrelor is a new antiplatelet agent under clinical investigation (Hsu et al. 2018). Anticoagulant medications are also used to treat and prevent blood clots.

Rivaroxaban (NCT02179177) and apixaban (NCT02179177) are the two anticoagulant drugs under clinical trial for SCA. Pan selectin (P-selectin) inhibitors are another class of molecule that mediates adhesion of RBC to vascular endothelium linked to vaso-occlusion (Manwani and Frenette 2013; Matsui et al. 2001). Therefore, blocking/depleting of P-selectin can be advantageous for SCA patients. Heparin was examined earlier because of its pharmacological actions like anti-inflammatory activity and P-selectin inhibition (Nelson et al. 1993). Tinzaparin is low-molecular-weight heparin and is used as antithrombotic drug. It is reported to decline the duration of vaso-occlusive crisis as well as lowering of brutal

hemorrhagic complications (Qari et al. 2007). There are several other molecules that have been investigated for the management of SCA because of their additional pharmacological activity instead of its current pharmacological use like simvastatin, a hypolipidemic agent that activates endothelial nitric oxide (NO) synthase (Hoppe et al. 2011), and propranolol, a nonselective β-adrenergic blocker that hinders sickle RBC adhesion (De Castro et al. 2012). Currently, anti-inflammatory agent namely regadenoson, is under clinical trial for SCA (NCT01788631). Rivipansel (GMI-1070) is a P-selectin inhibitor that acts by interfering with cell-cell interactions and adhesion. It is reported to prolong the survival time of the sickle cell mice (Chang et al. 2010).

Crizanlizumab (SEG101) is a humanized monoclonal antibody that blocks the action of P-selectin. It was found to be effective for prevention of vaso-occlusive crisis in SCA patients (Ataga et al. 2017). To improve the vascular tone and endothelial dysfunction, magnesium and sildenafil were investigated to reduce the frequency/duration of painful crisis, but results are not encouraging (Machado et al. 2011; Brousseau et al. 2015). Blood transfusion has been carried out in parallel with currently available therapy for symptomatic management of SCA (Kapoor et al. 2018). It involves hospitalization as well as is associated with some potential adverse effects like iron overload, alloimmunization, hemolytic transfusion reaction, etc. (Kapoor et al. 2018). Moreover, continuous blood transfusion to all patients is difficult in the developing countries because of the unavailability of blood and higher chances of infections (Guerrini et al. 2009).

## 3.3 Prospects of Natural Products for the Treatment of SCA

Based on the current therapeutic targets and their associated availability or ongoing therapy option, L-glutamine has been approved by USFDA in 2017 after two decades of hydroxyurea approval to replenish the erythrocyte reducing potential which is beneficial for the symptomatic management of SCA patients (Kapoor et al. 2018; Quinn 2018). Hence, the use of natural products for the above-mentioned pharmacological aspects can be a suitable alternative (Fig. 3.2). The effects of natural products for the symptomatic management of SCA are described below, starting with L-glutamine. L-glutamine is an amino acid precursor for nicotinamide adenine dinucleotide (NAD). It is needed for the generation of reduced NAD in sickle cell erythrocytes. In the patient with SCA, glutathione and glutamine levels are low in spite of increasing availability of glutamine. These events possibly lead to an increase of oxidative stress and episodes of pain crisis. Though NAD production is adequate, but glutathione and glutamine levels in the erythrocyte are significantly exhausted. This lessening in the L-glutamine level shows the way to drop NAD redox potential and erythrocyte integrity impairment, enhance hemolysis, and exhaust NO (Reid et al. 2006; Morris et al. 2008). Therefore, supplementation with L-glutamine helps to lessen oxidative stress, which is evidenced by a result of clinical trials in the presence or absence of hydroxyurea. It has been illustrated that supplementation with L-glutamine reduced the frequency of hospitalization in terms

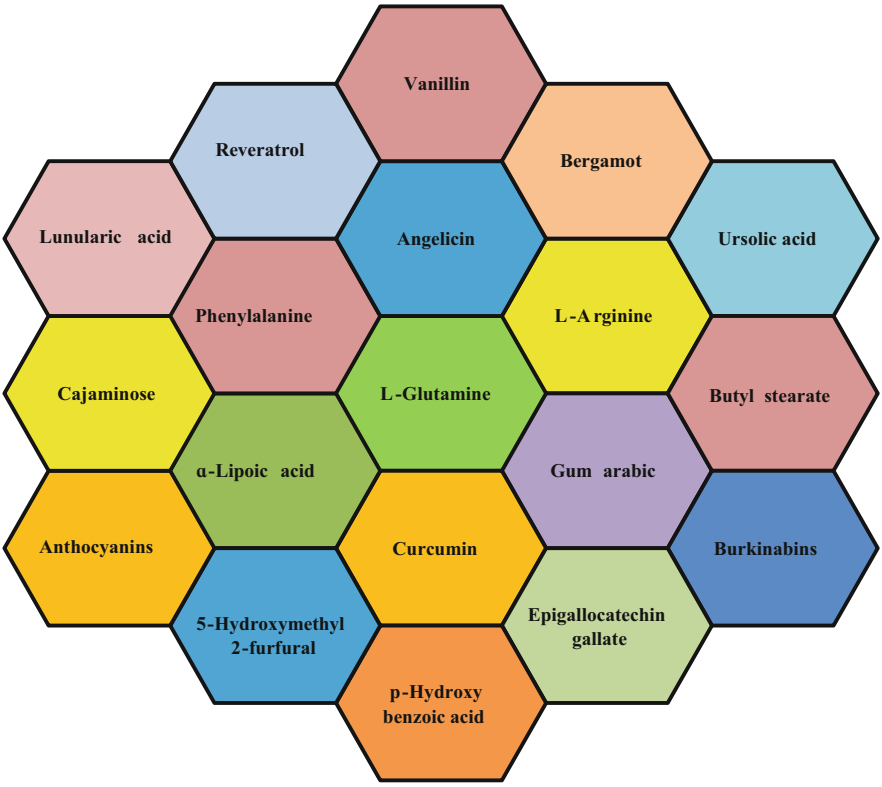


**Fig. 3.2** Representative natural products having promising action in preclinical or clinical model

of both number and duration of hospitalization as well as enhance median time to the painful crisis (Niihara et al. 2014, 2018). Several plant extracts as well as pure molecules have been explored under the wide range of pharmacological treatment perspectives to manage SCA patients. Here, we are focused on the inhibition of hemoglobin polymerization activity by the characterized components in plant extracts or pure natural candidates. Phenylalanine and p-hydroxy-benzoic acids are the two components present in the methanolic extract of seed from *Cajanus cajan* (Family: Leguminosae). These two components as individual (phenylalanine: 0.69 mg/ml or p-hydroxybenzoic acid: 10.5 μg/ml) as well as in combination at same concentration level exhibit significant protection against sickling of blood with respect to time (Akojie and Fung 1992). Ekeke et al. advocated that free amino acids mainly phenylalanine in the methanol water-soluble fraction from the same plant expressed antisickling activity with respect to time (Ekeke and Shode 1990).

In another study, aromatic amino acids like phenylalanine as well as tryptophan were investigated for the effect on gelation and solubility of HbS using blood from SCA patients (homozygous for HbS). Phenylalanine as well as tryptophan significantly inhibited the gelation and improved the solubility of HbS. Results indicate

that tryptophan has a better effect than phenylalanine (Noguchi and Schechter 1977). It is reported that cajaminose is present in the extract of *C. cajan* and can prevent sickling over time as well as reversal of sickling where already sickling occurred (Iwu et al. 1988). L-arginine is an amino acid that helps for the production of nitric oxide. In SCA, nitric oxide level depletes that link to the complication of pain crisis. Supplementation with L-arginine at the dose of 100 mg/kg thrice daily was given to the children with SCD, those who were hospitalized with vaso-occlusive pain episodes. This clinical investigation indicates that use of L-arginine by the patients can decrease the use of total parental opioids significantly to tackle pain crisis (Morris et al. 2013). Cepharanthine, a bisbenzylisoquinoline alkaloid, showed that it can reverse sickling but have no effect on oxygen affinity of sickle cell or delay the time in gelation of deoxy-HbS (Sato and Ohnishi 1982). 2-Hydroxy-1,4-naphthoquinone is one of the major ingredients from the extract of Henna plant that showed decrease in sickling of the irreversibly sickled cell at low oxygen tension where oxygen affinity of sickle cell blood was increased. Further investigation of this compound reveals that it can protect the erythrocyte membrane from damage by transient oxidative species (Clarke et al. 1986). Vanillin is a phenolic aldehyde mainly used as a flavoring agent/food additive. Abraham et al. (1991) demonstrated that vanillin could enter into RBC and react with hemoglobin in covalent fashion. These researchers also described that vanillin in adduct form with HbS could shift the oxygen equilibrium curve to left in dose-dependent manner that may improve the fraction of oxygenated HbS under hypoxic condition. Moreover, they also suggest that vanillin-HbS adduct may directly inhibit stereo-specific HbS polymerization. This dual mechanism of vanillin may be responsible for antisickling activity (Abraham et al. 1991). As vanillin is rapidly degraded in the upper gastrointestinal tract of oral administration, a prodrug of vanillin, namely, MX-1520, was explored in transgenic sickle mice that can develop pulmonary sequestrations under hypoxic condition. Treatment of MX-1520 significantly reduced the percentage of sickle cell in blood as well as prolonged the survival time after oral as well as intraperitoneal administration in severe hypoxic condition (Zhang et al. 2004).

It is reported that INN-312 and INN-298, pyridyl derivatives of vanillin, increased antisickling activity by 90-fold as compared to vanillin. Results reveal that these molecules preferentially bind to HbS to transform the oxygen affinity of hemoglobin. Moreover, results postulate that these compounds increase the soluble high affinity HbS and/or stereospecific inhibition of deoxygenated HbS polymerization that prevent sickling action (Abdulmalik et al. 2011). Mehanna et al. explored the HbS polymerization inhibition activity of vanillin and its structural-related aromatic aldehydes and carboxylic acids where three compounds, namely, 4-hydroxy-3-methoxymandelic acid, homovanillic acid, and 3-furfural, showed the ability to prevent polymerization of hemoglobin (Mehanna and Abdullah 2018). Research on antisickling activity by naturally occurring five-membered heterocyclic aldehydes evolve that **5**-hydroxymethyl-2-furfural (Aes-103) inhibit sickling of homozygous sickle red blood cells up to 90% at the concentration of 5 mM. Results illustrated that this molecule increased the oxygen affinity of hemoglobin and displayed strong antisickling activity (Safo et al. 2004).

Aqueous extract from stem bark and leaves of *Khaya senegalensis* (Family: Meliaceae) displayed a strong antisickling activity at a concentration lower than pentoxifyline as standard by 100-folds. The responsible phytoconstituent was identified as a rearranged limonoid (Fall et al. 1999). NIPRASIN (Nix-0699), a formulated extract, inhibits 50% of erythrocyte sickling at around 0.05 mg/ml. This contains ethanol/water extract from four indigenous plants of Nigeria like seeds of *Piper guineense* (Family: Piperaceae), stem of *Pterocapus osun* (Family: Fabaceae), fruit of *Eugenia caryophyllum* (Family: Myrtaceae), and leaves of *Sorghum bicolour* (Family: Poaceae). Chemical standardization of the extract demonstrated the presence of the main active constituents like β-caryophyllene, piperine, chavicine, capsaicin, cubebin, etc. It was observed that Nix-0699 prolonged the delay time prior to deoxy-HbS polymerization by 6-folds at the concentration level of 0.05 mg/ml. Moreover, significant improvement in the solubility of deoxy-HbS occurred due to Nix-0699 treatment.

Based on the results on oxygen affinity of HbS, it can be stated that the drug slightly shifted to the left in the oxygen dissociation curve of HbS where there was no apparent change in the Hill coefficient. Antisickling activity of Nix-0699 is possibly due to direct interaction with hemoglobin molecules. Nix-0699 decreased the percentage of sickle cell to 30% at 0.5 mg/ml compared to cells underwent sickling to 70% in 5 h in the absence of Nix-0699. A clinical trial to access the safety and efficacy of NIPRISAN was carried out. This phytomedicine is found to be safe in terms of liver and kidney function test. Moreover, it significantly lowered the frequency of pain crisis (Iyamu et al. 2002; Ameh et al. 2012; Wambebe et al. 2001). Burkinabins A/B/C, divanniloylquinic acid derivatives are present in the root bark extract of *Fagara zanthoxylum* (Family: Rutaceae). It showed antisickling activities where burkinabins C is the most active component (Ouattara et al. 2009). Citropan and bergamot are the two main pharmacological active components present in the extract from epicarp of fruit of *Citrus bergamia* (Family: Rutaceae). It is reported that these have the ability to induce differentiation and γ-globin gene expression in human erythroid cells (Guerrini et al. 2009). Butyl stearate isolated from acidified methanol extract of *Ocimum basilicum* (Family: Lamiaceae) leaves showed good antisickling activity based on Emmel test of the blood sample (Tshilanda et al. 2014). Further research by the same research group illustrated that ursolic acid is present in the same plant part. This phytochemical also exhibits antisickling activity based on Emmel test of blood samples (Tshilanda et al. 2015). A phytochemical, namely lunularic acid, was present in the characterized ethanol extract from *Noronhia divaricata* (Family: Oleaceae) and possessed dose-dependent antisickling activity using SCA patient blood in sodium metabisulfate-induced sickling model (Ngbolua et al. 2015). Chemical standardization of whole plant extract of *Mitracorpus villousus* (Family: Rubiaceae) revealed that psychorubrin, quercetin, and tarpene containing stigmasterol are the main constituents present in it. These showed dose-dependent and time-dependent inhibition of in vitro sodium metabisulfate-induced polymerization of hemoglobin using SCA patient blood samples.

Quercetin displayed a strong inhibitory effect on hemoglobin polymerization, whereas psychorubrin and stigmasterol possessed moderate and weak activity,

respectively (Elusiyan et al. 2018). Oduola et al. reported that caricapinoside is present in the methanol extract from unripe fruit of *Carica papaya* (Family: Caricaceae) that has a beneficial effect on hematological parameters of SCA patients (Oduola et al. 2012). SCA-101 is a botanical drug that exhibits both in vitro and in vivo antisickling activities. Clinical investigation depicts that it can able to reduce chronic pain, but limited data is available on its mechanism (Swift et al. 2016). Anthocyanin is a flavonoid class of compounds present in several plants. The aqueous and ethanolic extracts *from Vigna unguiculata* (Family: Fabaceae) showed antisickling activity in Emmel test using sickle blood erythrocytes. Dose-dependent activity on inhibition of sickling was attributed to anthocyanin (Mpiana et al. 2009). Anthocyanins obtained from the plant *Morinda lucida* (Family: Rubiaceae) also showed in vitro antisickling activity in sodium metabisulfate-induced blood sample. Ethanolic extract was found to be better compared to aqueous extract in terms of higher antisickling activity (Mpiana et al. 2010a).

Anthocyanins acquired from *Justicia secunda* (Family: Acanthaceae) also possess antisickling activity. The result illustrates that anthocyanin treatment enhanced the solubility of sickle deoxy-hemoglobin and reduced the osmotic fragility of sickle red cell (Mpiana et al. 2010b). Resveratrol, a natural dietary phytoalexin, seems to have a similar property to hydroxyurea toward erythroid differentiation of human erythroleukemic K562 cell lines and induction of HbF synthesis in the erythroid progenitor of SCA patient. It is reported that resveratrol induced more hemoglobin production than hydroxyurea with respect to erythroid differentiation which is linked to time and dose-dependent inhibition of cell proliferation. Properties of resveratrol like induction of nitric oxide synthase, inhibition of neutrophil/monocyte adhesion, and inhibition of platelet aggregation may be additionally advantageous to SCA patients (Rodrigue et al. 2001). Angelicin, structurally related to psoralens, is an inducer of erthyroid differentiation and γ-globin mRNA accumulation as compared to cytosine arabinoside, mithramycin, and cisplatin. The compound showed better activity than hydroxyurea for induction of HbF (Lampronti et al. 2003). Cucurbitacin D, an oxygenated tetracyclic triterpenoid, was found to induce HbF synthesis in K562 cells and human hematopoietic progenitors through activation of p38 pathway and stabilization of the γ-globin mRNA. Pharmacological reactivation of HbF is silenced in adults and increase in HbF reduced α-/β-globin chain imbalance (Liu et al. 2010).

The above discussion is related to antisickling activities, including polymerization inhibition of HbS and γ-globin induction by the natural products. The other natural products that act through different mechanisms are described as follows: α-lipoic acid is a natural antioxidant explored by Lal et al. for the effect on reversal of iron-induced oxidative stress in human fibroblasts (Lal et al. 2008). Iron overload leads to the generation of free radicals that subsequently cause oxidation of biomolecules and tissue damage. This occurs due to frequent blood transfusion in SCA patient. Result suggests that α-lipoic acid has the ability to improve cellular redox status and attenuate iron-mediated elevation of oxidant level dose-dependently. This molecule is given better activity in the presence of acetyl-L-carnitine (Lal et al. 2008). Curcumin is a natural phenol, obtained from the rhizome

of the plant *Curcumin longa* (Family: Zingiberaceae). Preclinical investigation of this compound was carried out to elucidate its effect on iron overload-induced hepatic and splenic abnormalities. Result suggests that it can able to reduce iron accumulation in the specified organs. Level of malonyldialdehyde (MDA) and NO was significantly lowered upon its treatment that can be beneficial in the pathological condition where hepatic iron accumulation occurs like SCA (Badria et al. 2015). Astaxanthin is a carotenoid from marine origin. It was investigated for incorporation into SCA patients RBC at oral daily dose of 8–12 mg for 3 months and results demonstrated that astaxanthin level in plasma and RBC was increased from the baseline during the treatment but asymmetric dimethylarginine level did not change. As slight reduction of reticulocyte count was observed after 3 months, it may indicate lower hemolysis (Ruiz-Nunez et al. 2013). Docosahexanoic acid is an omega-3-fatty acid which was explored for its effect on membrane flexibility of RBC in mice model of SCD. Result showed that stiffness of RBC decreased and a number of irreversibly sickled RBC was lowered upon treatment with docosahexanoic acid (Wandersee et al. 2015). Gum arabic is a natural antioxidant obtained from the plant *Senegalia senegal* (Family: Fabaceae). It was investigated clinically for its ability to decrease oxidative stress for SCA. Results indicate that gum arabic at the dose of 30 g/day for 12 weeks is able to significantly enhance the total antioxidant capacity and reduce the MDA as well as $H_2O_2$ which are the marker of oxidative stress (Kaddam et al. 2017). Epigallocatechin gallate is a flavonoid class of polyphenol having antioxidant activity. This is one of the key ingredients present in green tea extract. It has been found that epigallocatechin gallate at the concentration of 0.3 mg/ml inhibited dehydration of sickle red blood cell in in vitro model to interfere with potassium-chloride co-transport (Ohnishi et al. 2001).

Research has been going on for supplementation or adjuvant therapy to SCA patients using several compounds from natural as well as synthetic source for the symptomatic management. These include niacin/magnesium to improve blood flow (Kato and Gladwin 2008; Brousseau et al. 2015), N-acetyl-cysteine for the restoration of glutathione levels (Pace et al. 2003), and delphinidin chloride for reversing the red cell sickling (Basonbul 2016).

## 3.4 Conclusion

SCA is an inherited hemoglobinopathy disorder where sickled RBCs cause vaso-occlusion leading to painful crises and organ damage associated with acute and chronic complications that lead to frequent hospitalization. To combat with this situation, hydroxyurea is the only approved drug till date. L-glutamine has also been approved recently for the management of acute complications. A lot of research works in the preclinical as well as clinical level have been proceeding targeting the improvement in the pathophysiology of the patients. Though gene therapy can provide an ultimate solution, its therapy is not currently available. Research works on the use of natural products has been providing exciting results for symptomatic

management and can be a suitable option as adjuvant therapy toward the unmet medical need for SCA.

**Acknowledgment** AG, SB, and AD are thankful to DST, CSIR, and UGC (New Delhi, India), respectively, for providing fellowship to carry out research support.

**Competing Interest** Authors have no competing interest.

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# Anti-inflammatory Activity of Medicinal Plants: Present Status and Future Perspectives

4

Sonam Chouhan and Sanjay Guleria

**Abstract**

Inflammation, a kind of innate immunity, is a biological response of body tissues towards various harmful stimuli. It is known to be initiated as a normal body defense mechanism during injury, exposure to contaminants, radioactive substances, toxicants as well as allergens and infection by a plethora of agents like microbes, viruses. Inflammation is involved in a host of diseases like rheumatoid arthritis, atherosclerosis, obesity, and even cancer. Several inflammatory mediators are produced and secreted at the time of inflammatory responses of different kinds (interferons, interleukins, and tumor necrosis factor-α). Inflammation is associated with the characteristics like pain, swelling, redness, loss of function in the affected area and heat accumulation in the inflamed area. A significant role in human health is being played by natural products with respect to preventing and treating inflammatory conditions. Besides various synthetic anti-inflammatory agents (non-steroidal anti-inflammatory drugs (NSAIDs)) available, herbal medicine still plays a major role to cure various health conditions as large number of medicinal plants possess secondary compounds that retard the key steps of the inflammation pathway (the nuclear factor NF-κβ, lipoxygenase and cyclooxygenase). In addition, many of them exhibit excellent free radical scavenging properties. Many studies have reported about the potential role of herbal medicines in suspending inflammation. This communication summarizes the published literature regarding the anti-inflammatory activities of plant extracts, essential oils, and plant-derived compounds along with the underlying molecular mechanisms of their role in inflammation-mediated metabolic diseases. The huge range of research as well as review papers that have reported about the anti-inflammatory effects of essential oils, plant extracts,

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_4

and/or pure compounds derived from natural products has also been summarized in this chapter. Moreover, this chapter also presents some latest data on some traditionally used medicinal plants that were not investigated yet in this respect.

**Keywords**

Anti-inflammatory · Anti-oxidants · Reactive oxygen species · Inflammation · Immune cells

## Abbreviations

| | |
|---|---|
| NSAIDs | Non-steroidal anti-inflammatory drugs |
| NO | Nitric oxide |

## 4.1 Introduction

Inflammation is a pervasive process which occurs in the disturbed state of homeostasis-like injury, subjection to contaminating agents, and infection, the process being activated by the receptors of innate immune system for the elimination of pathogens upon their recognition (de Melo et al. 2014). Inflammation is a defensive mechanism in which both the innate and the acquired immune responses are involved having pain, swelling, redness, heat, and loss of function in the affected area, which is because of dilated blood vessels, and increased spaces between the cells, and thereby resulting in the movement of proteins, leukocytes, and fluids into the regions of inflammation (Fig. 4.1) (Leelaprakash and Dass 2011; Artis and Spits 2015; Azab et al. 2016a, b). The process of inflammation is activated once the body is subjected to lasting shock because of either exogenous or endogenous stimuli such as infectious pathogens, extreme temperature, physical force, irradiation, and irritants (Nathan 2002). During inflammation, a local accumulation of end products (inflammatory mediators) having small molecular weight occurs, which results in the marked increase of the osmotic pressure of the affected tissues along with the aggregation of fluid in excess amount along with raised temperature (Stankov 2012). The synthesis and the secretion of various inflammatory mediators occur during inflammatory reactions of various kinds (Vignali and Kuchroo 2012). Usually, the inflammatory reactions get triggered when immune cells such as neutrophils, dendritic cells and macrophages are activated due to the phagocytosis of pathogens by receptors such as toll-like receptors (TLRs), which are associated with the recognition of molecular patterns of pathogen-derived materials like lipopolysaccharide (LPS) (Hiraiwa and van Eeden 2013).

The inflammatory substances have been categorized as the pro and anti-inflammatory mediators, except for some mediators such as IL-12 (interleukin) that has both pro and anti-inflammatory properties (Vignali and Kuchroo 2012). Several studies associated with human pathological conditions have been carried out studying the inflammatory mediators and cellular pathways such as cytokines (e.g.,

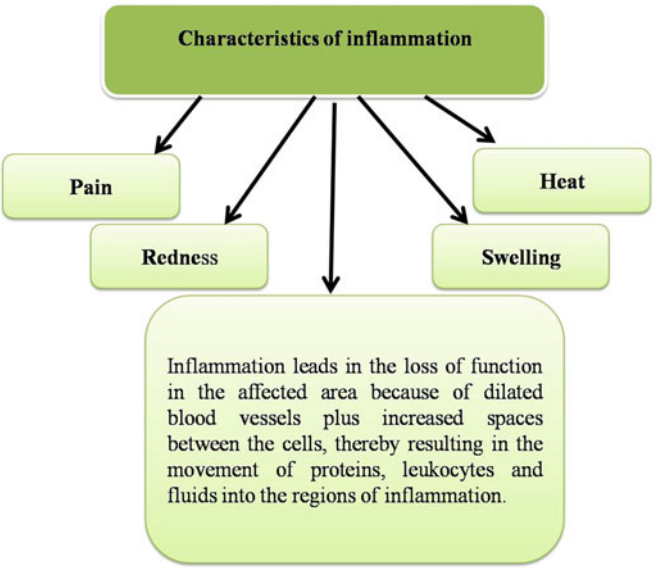



**Fig. 4.1** Characteristics of inflammation

interferons, interleukins, and tumor necrosis factor-α), chemokines (e.g., monocyte chemo attractant protein-1), eicosanoids (e.g., prostaglandins and leukotrienes) and the potent inflammation-modulating transcription factor nuclear factor (NF)-κβ (Azab et al. 2016a, b). When the inflammatory cells get activated, they result in elevated intracellular signaling by cascades that involve tyrosine kinases and inhibitor of κβ kinase (IKK), thereby, resulting in activation of the nuclear transcription factor-κβ and stimulated expression of several inflammatory genes like inducible nitric oxide synthase and cyclooxygenase-2 (Byeon et al. 2012; Yang et al. 2014). This in turn results in the release of several arbitrators of inflammation such as nitric oxide (NO), prostaglandin $E_2$ and pro-inflammatory cytokines, and this further activates the chemotactic reactions of other inflammatory cells, resulting in the synthesis of hydrolytic enzymes and cytotoxic molecules (Labow et al. 2001). The reaction of organisms towards this would be the migration of immune cells via the endothelial cells (Nathan 2002). Inflammation response results in the elimination of possible pathogens, thereby, returning the damaged tissue back to the condition of homeostasis (Lawrence and Gilroy 2007). Homeostasis is the tendency towards a relatively stable equilibrium between the interdependent elements and is maintained by physiological processes. The vital role being played by both the acute and the chronic inflammatory responses as a natural defense mechanism of the body's innate immune system for the maintenance of immune homeostasis (Barton 2008). However, if the inflammation is not controlled adequately, it can spread in the entire body and may result in several tissue damages including gastritis and other associated organs. Moreover, oxidative stress and inflammation are strongly correlated with each other (Ceriello and Motz 2004). In addition to this, inflammation results in the

aggravation of oxidative damage and reduces anti-oxidant capacity of cells by overproducing various inflammatory mediators through mast cells associated with the release of cytokines from macrophages as well as the production of neutrophils. Apart from this, inflammation is accompanied with the production of huge amounts of reactive oxygen species and nitrogen-derived free radicals, as a result of which tissues get severely destructed and DNA gets damaged. Increased amounts of reactive oxygen species can result into human disorders like cancer, cardiovascular disease and diabetes mellitus as well as neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Surh and Packer 2005; Bennett and Brown 2003).

The enzymes having characteristic feature of getting induced are the main targets of the anti-inflammatory drugs because these enzymes cause the production of a large number of pro-inflammatory mediators like the enzymes from the arachidonic acid pathways (phosphor-lipase, lipoxygenase and cyclo-oxygenase) and hyaluronidase. The steroidal or the non-steroidal anti-inflammatory drugs are used in treating inflammation but such drugs usually have unexpected side effects and they are also not regarded as a good clinical choice for chronic inflammatory disorders and these drugs exhibit their action through the inhibition of these enzymes via different mechanisms (Khansari 2009). Another mode of action for the anti-inflammatory drugs is by inhibiting the generation of reactive oxygen species or through their scavenging (Vane and Botting 1998). It has been demonstrated in different studies that there exists a strong bond between the anti-inflammatory and the anti-oxidant properties (Werz and Steinhilber 2005; Mateo Anson et al. 2011). Yet, the consumption of anti-inflammatory drugs over a prolonged period of time is associated with a great amount of secondary effects, thereby, increasing the costs in healthcare. Therefore, it should be of great interest to search novel natural alternative sources of drugs for treating chronic inflammatory pathologies. A significant role in human health is being played by natural products with respect to preventing and treating inflammatory conditions (D'Almeida et al. 2013). One of the most important aspects of complementary medicines is herbal medication, and the process of inflammation has several mechanisms and number of treatment methods consequently. A large numbers of cytokines participate in the activation of enzyme (like phospholipase A2), mediator release, fluid extravasation and vasodilation and cell migration (Fig. 4.2) (Ghasemian et al. 2016).

## 4.2 Anti-inflammatory Effects of Plant Extracts

Anti-inflammatory activity is one of the most reported effects among the different biological activities of natural plant products that have been published till the present time. The extract obtained from winter season collected *Calamintha nepeta* exhibited anti-inflammatory activities as it caused 40.10% inhibition of COX-2 production (Pacifico et al. 2014). The shoot methanolic extract of the halophyte *Limonium densiflorum* exhibited best anti-inflammatory activity by inhibiting 80% release of nitric oxide at a concentration of 160 μg/mL in LPS-stimulated RAW

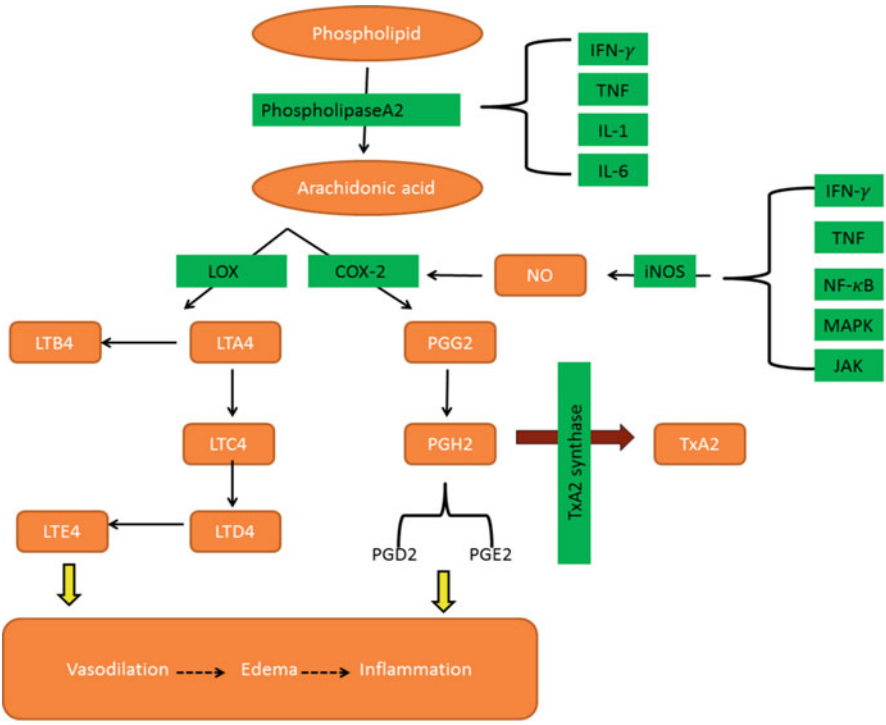



**Fig. 4.2** Inflammation pathway. COX, cyclooxygenase; LOX, lipoxygenase; PG, prostaglandin; LT, leukotriene; TX, thromboxane; NO, nitric oxide; iNOS, inducible NO synthase; IFN, interferon; TNF, tumor necrosis factor; NF-κB, nuclear factor-κB; MAPK, mitogen activated protein kinase; JAK, Janus kinase; IL, interleukin (Adapted from Ghasemian et al. 2016)

264.7 cells (Medini et al. 2015). The aqueous methanolic leaf extract of strychnine tree (*Strychnos nuxvomica*) exhibited promising anti-inflammatory activity (Omayma and Abdel-Daim 2015). Determination of the molecular mechanism behind the prevention of HCl/EtOH-instigated gastric ulcers in mice due to the methanol extract of *Persicaria chinensis* against lipopolysaccharide-instigated PGE2 and nitric oxide in RAW264.7 macrophages revealed that it remarkably reduced the expression of lipopolysaccharide-instigated pro-inflammatory cytokines including interleukin-$\beta$, interlukin-6, and tumor necrosis factor-$\alpha$. Also, the activation as well as phosphorylation of activator protein-1 and mitogen-activated protein kinase was decreased in both differentiated U937 cells as well as lipopolysaccharide-instigated RAW264.7 cells. Thus, these results were strongly indicative of methanolic extract of *P. chinensis* as a remedy that suppresses mitogen-activated protein kinase (MAPK)/activator protein (AP-1)-mediated inflammation processes (Hossen et al. 2015b). Across India, *Jasminum sambac* L. is cultivated and its roots and leaves are utilized traditionally to treat fever, pain and inflammation. It has been reported that its leaves exhibit remarkable anti-inflammatory activity. In the year 2015, Sengar et al. revealed that at a concentration of 400 mg/kg, the root extract of

*J. sambac* prepared in ethanol exhibited remarkable anti-inflammatory activity after 2nd, 3rd, 4th and 6th hours of treatment in carrageenan-instigated edema and a 33.58% inhibition in cotton pellet-instigated granuloma production was also found at the similar dosage amount. Moreover, this extract also remarkably ($p < 0.001$) produced inhibition in arthritis instigated by adjuvant (Sengar et al. 2015). *Phyllanthus acidus* is being utilized traditionally in the treatment of respiratory disorders, gastric trouble, hepatitis, bronchitis, rheumatism and asthma. In a study conducted by Hossen et al. (2015a), the methanolic extract of the aerial parts of *P. acidus* resulted in the suppression of nitric oxide and prostaglandin-E2 synthesis as well as caused prevention of morphological alterations associated with lipopolysaccharide-treated RAW 264.7 cells. Moreover, this extract resulted in the down-regulation of the expression of inducible nitric oxide synthase and cyclooxygenase-2 as well as caused reduction in the nuclear levels of NF-κβ. Among the flavonoids that were identified, quercetin and kaempferol were found to be partially active anti-inflammatory compounds in the methanolic extract of the aerial parts of *P. acidus*. Thus, it was concluded that the methanolic extract of the aerial parts of *P. acidus* exhibited anti-inflammatory effects in vivo as well as in vitro through the suppression of Syk, Src and their downstream transcription factor, NF-κβ (Hossen et al. 2015a). The anti-inflammatory activity of methanolic extracts from two different stages of *Dendropanax morbifera* (green and senescent leaves) revealed that they showed a strong suppression in the synthesis of LPS-instigated pro-inflammatory cytokines as well as mediators by suppressing the expression of inducible nitric oxide synthase and cycloxygenase-2 and also inhibited the ERK1/2 signaling pathway. Moreover, the analysis of phenolic compounds through high performance liquid chromatography (HPLC) revealed that the leaf extracts comprised of active phenolic compounds like myricetin, quercetin, rutin, chlorogenic acid, resveratrol, (+)-catechin and ferulic acid which were considered responsible for the anti-inflammatory properties (Hyun et al. 2015).

Inflammation and pain have been reported to be responsible for various pathological conditions. The extract prepared in methanol and ethyl acetate fraction of *Acacia hydaspica* exhibited anti-inflammatory effect in carrageenan-instigated paw edema in rats; 150 mg/kg of the dose was markedly efficient to a greater extent resulting in 91.92% suppression. Moreover, the methanolic extract and ethyl acetate fraction of *A. hydaspica* exhibited the highest suppression of edema at a dose of 150 mg/kg after 4 h on prostaglandin E2 (PGE2)-instigated edema in rats (Afsar et al. 2015). *Pistacia lentiscus* has been utilized in Algeria in treating burns, inflammation and gastrointestinal problems. Its leaf extract (100 g/mL) exhibited remarkable anti-inflammatory effect than acetylsalicylic acid (ASA) indicating that *P. lentiscus* extracts possessed anti-inflammatory property in line with its conventional utilization (Remila et al. 2015). The extracts of *Clausena anisata*, at a concentration of 6.25 μg/mL, caused 96% inhibitions in the synthesis of nitric oxide by RAW 264.7 macrophage cell lines in vitro (Adebayo et al. 2015). The extract of *Ocimum labiatum* showed potential to inhibit inflammation at a concentration of 25 μg/mL without any cytotoxic effect and caused remarkable ($p < 0.05$) inhibition in the synthesis of pro-inflammatory cytokines, interlukin-4, interlukin-2,

interlukin-17A and interlukin-6. (Kapewangolo et al. 2015). Crude extract obtained from the fruits of *Nitraria schoberi* exhibited potential to inhibit the inflammation of the order of 36.12%, 59.89%, and 88.33% at 100, 200 and 500 lg/mL, respectively (Sharifi-Rad et al. 2015). For mankind, roots as well as herbaceous parts of leguminous crops have not been utilized generally because these have been conventionally regarded as waste substances. Despites not regarded as consumable, such parts of leguminous crops have a complex chemical composition possessing remarkable biological potentials. Keeping in view such potentials, the herb extracts of *Phaseolus vulgaris* and *Cicer arietinum* were evaluated for anti-inflammatory potential and were found to be potent suppressors of TXS enzyme in cyclooxygenase pathway, making these extracts important sources of protective agents against inflammation, cardiovascular diseases and thrombosis. Therefore, these results were indicative of a strong capability of waste legume material as a potential source to isolate bioactive anti-inflammatory as well as anti-oxidant compounds to be utilized as therapeutic as well as dietary additives in the food as well as pharmaceutical industry (Sibul et al. 2016). The methanolic extract of the aerial parts of *Xanthium strumarium* exerted anti-inflammatory activity by suppressing the synthesis of nitric oxide as well as prostaglandin E2. Also, oral treatment with this extract improved HCl/EtOH-instigated gastric lesions. Hence, it was concluded that this extract exerted in vitro as well as in vivo anti-inflammatory potential through the inhibition of PDK1 kinase action, which in turn blocked the signal to its downstream transcription factor, NF-κβ (Hossen et al. 2016). In conventional system of medicine, *Saposhnikovia divaricata* is utilized for the treatment of inflammation, arthritis and pain. The potential of *S. divaricata* extract to inhibit inflammation was investigated in vitro in RAW 264.7 cells treated with lipo-polysaccharide. It was found that this extract exhibited anti-inflammatory activity through the production inhibition of prostaglandin-E2, tumor necrosis factor-$\alpha$, nitric oxide and interleukin-6 in RAW 264.7 cells instigated with lipopolysaccharide (LPS). Moreover, this extract also resulted in the inhibition of the synthesis of pro-inflammatory cytokines as well as arbitrators (Chun et al. 2016). In the folk medicines, *Salvia fruticosa* is utilized on a large scale. The ethyl acetate extracts of both the roots and the aerial parts of *S. fruticosa* exhibited significant anti-inflammatory potential which was partly correlated with the radical scavenging capacities of their polyphenolic constituents (Boukhary et al. 2016).

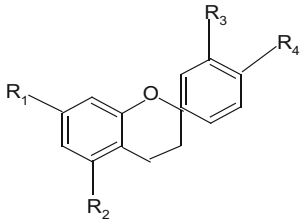
*Cymbopogon citratus* is among one of the known aromatic plants being used throughout world in the conventional system of medicine for curing inflammation associated conditions. The in vivo topical anti-inflammatory potential of *C. citratus* infusion (CcI) as well as *C. citratus* flavonoids fraction (CcF) and *C. citratus* tannins fraction (CcT) in the carrageenan-instigated rat paw edema model revealed that at a concentration of 4% and 1%, *C. citratus* infusion (CcI) exhibited an edema decrease of 43.18% and 29.55%, whereas, CcF + CcT decreased 59.09% edema separately (Costa et al. 2016). The extracts of *Lavandula dentata* and *L. stoechas* possess immunomodulatory potential because they in vitro down-regulated varying inflammatory arbitrators such as nitric oxide as well as cytokines. In addition to this, modulation of the expression of pro-inflammatory chemokines and cytokines was

also observed due to these extracts. It was concluded that the extracts of *L. dentata* and *L. stoechas* had impact to suppress inflammation and to inhibit inflammation in the region of intestine and other body parts, affirming their potent utilization as herbal remedies in the gastrointestinal ailments (Algieri et al. 2016).

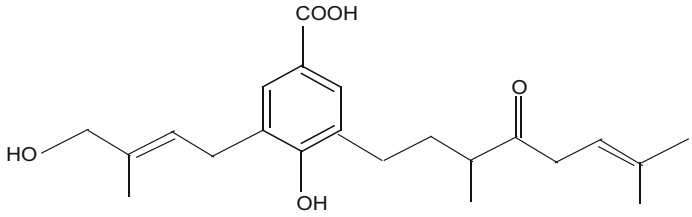
The species of *Stryphnodendron*, popularly named "barbatimao," have been utilized traditionally in Brazil as anti-inflammatory agents. The study conducted by Henriques et al. (2016) reported that the extracts of *Stryphnodendron obovatum*, *S. adstringens*, *Terminalia glabrescens* and *Campomanesia lineatifolia* inhibit tumor necrosis factor-$\alpha$ in a concentration dependent manner, thereby, revealing anti-inflammatory activity (Henriques et al. 2016). The anti-inflammatory activities of the bark ethanolic extract of *Mimosa tenuiflora* and solvent soluble fractions (hexane-H, DCM-D, EtOAc-E and BuOH-B) of the extract in vivo was indicative of the fact that the bark ethanolic extract exhibited anti-inflammatory potential through the reduction of neutrophil migration to the peritoneal cavity as well as in the plantar tissue which was determined via decreased myeloperoxidase activity, decreased IL-10 levels as well as expression of ICAM-1 in the peritoneal exudate and the mesentery. Moreover, the three soluble fractions (H, D, E) also showed good anti-inflammatory results (Cruz et al. 2016).

The anti-inflammatory activities of seeds, leaves and few Brazilian native fruits (*Eugenia myrcianthes, E. leitonii, E. brasiliensis* and *E. involucrata*) were determined in an in vivo model by utilizing the technique of carrageenan-instigated migration of neutrophil in the acute phase and it was observed that the seeds, pulp and leaves of these fruits decreased the influx of neutrophil by 40–64%. Therefore, these results were suggestive of the anti-inflammatory property of these fruits due to the modulation of neutrophil migration, retardation of chemokines, cytokines, and adhesion molecules. Hence, these fruits can be used in the production of food additives as well as functional foods (Infante et al. 2016). The hydroalcoholic extracts of *Frankenia triandra* prepared through maceration and soxhlet extraction exhibited a remarkable retardation of hyaluronidase as well as two enzymes of arachidonic acid pathway, that is, lipoxygenase and cyclooxygenase-2, thereby, exhibiting anti-inflammatory potential related to the anti-oxidant properties (Carro et al. 2016). The *Buddleja crispa* crude extract (50–200 mg/kg i.p.) as well as its hexane fraction inhibited carrageenan-instigated rat paw edema with a maximum retardation of 65% and 71%. Moreover, the anti-inflammatory capacity of its extract as well as the isolated pure compounds could be compared to that of diclofenac sodium (Bukhari et al. 2016). Analysis of the dried leaves of *Eriodictyon angustifolium*, a North American shrub, revealed eight active compounds that exhibited a profound potential to suppress inflammation (Fig. 4.3) (Walker et al. 2016).

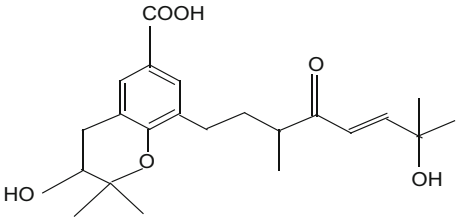
The leaf extract of *Anacardium occidentale* can be used to manage inflammation, and oleamide has been found to be one of the most bioactive component attributed for its anti-inflammatory potential (Awakan et al. 2018). It has been reported in a study that the extract of *Physalis angulata* calyces exhibited the highest in vivo anti-inflammatory potential in 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-instigated mouse ear edema and also exhibited impact on the synthesis of pro-inflammatory




$R_1$=$R_2$=$R_3$=$R_4$=OH ; Eriodictyol
$R_1$=$R_2$=$R_4$= OH, $R_3$= H ; Naringenin
$R_1$=$R_2$=$R_4$= OH, $R_3$= OMe ; Homoeriodictyol
$R_1$=$R_2$=$R_3$= OH, $R_4$= OMe ; Hesperetin
$R_1$= OMe , $R_2$=$R_3$=$R_4$=OH ; Sterubin
$R_1$=$R_2$= OH , $R_3$= H, $R_4$= OMe ; Isosakuranetin




Erionic acid C




Erionic acid B

**Fig. 4.3** Active anti-inflammatory compounds identified from *Eriodictyon angustifolium* (Walker et al. 2016)

mediators in vitro. Further, fractionation of this extract was carried and it was found that its dichloromethane fraction was the most potential fraction in vitro, suppressing the synthesis of prostaglandin E2, nitric oxide, tumor necrosis factor-α, monocyte chemotactic protein, interleukin (IL)-1b and IL-6. Moreover, this fraction significantly inhibited penetration inside tissue (Rivera et al. 2018). The in vitro potential of different concentrations of aqueous root extract of *Syzygium caryophyllatum* to inhibit inflammation through heat-instigated egg albumin denaturation bio assay process has also been established (Heendeniya et al. 2018). Among the crucial health issues are pain and inflammation that have been usually cured through conventional treatments chiefly through the help of medicinal crops. Anti-inflammatory effect of 80% methanolic leaf extract of *Leonotis ocymifolia* in rodent models decreased 75.88% paw edema after 6 h of instigation with carrageenan. Also, it was observed that all the studied doses of extract remarkably retarded the synthesis of granuloma

as well as inflammatory exudates (Alemu et al. 2018). The ethanolic extract and alkaloids total fraction obtained from the aerial parts of *Cissampelos sympodialis* possess the anti-inflammatory activity as they exhibited reduced amounts of tumor necrosis factor-α and interlukin-1β and elevated the amounts of interlukin-10 and glutathione–glutathione (de Sales et al. 2018).

Similarly, the ethanolic extract of *Ajuga laxmannii* exhibited the anti-inflammatory effect through the reduction of polymorpho-nuclear leukocytes, total leukocytes, oxidative stress and phagocytosis. The studies in comparison to diclofenac, 50 mg/mL of *A. laxmannii* extract exhibited better anti-oxidative stress and anti-inflammatory effects. Therefore, such findings were strongly suggestive of *A. laxmannii* as a precious source of bioactive compounds that can be further valued as anti-inflammatory agents in preparing various herbal drugs (Toiu et al. 2018). The polyphenol-abundant extract from the leaves of *Syzygium aqueum* exhibited promising anti-inflammatory activities in vitro by inhibiting lipoxygenase, cyclooxygenase-1 and cyclooxygenase-2 having a higher cyclooxygenase-2-selectivity as compared to diclofenac and indomethacin plus decreased the extent of erythrocytes lysis when incubated in hypotonic buffer. Moreover, the extract also remarkably decreased the amount of leukocyte having same activities to diclofenac in the rats that were treated with carrageenan. The observed anti-inflammatory activities were attributed to some affinity of the identified polyphenolics from the extract for the active pockets of cyclooxygenase-1, cyclooxygenase-2 and 5-lipoxygenase (Sobeh et al. 2018). Anti-inflammatory activity of the hydro-methanol leaves' extract of *Allophylus africanus* on laboratory rats has shown that doses of 250 and 1000 mg/kg resulted in remarkable anti-inflammatory activity at the 3rd, 4th and 5th hours having a dose-dependent effect at the 4th and 5th hours. Moreover, significant decrease of paw edema in the rats was also observed (Ibrahim et al. 2018). The plants *Rhus tripartitum* and *Periploca laevigata* possess anti-inflammatory activity as they reduced the release of nitric oxide as well as reactive oxygen species in J774A.1 macrophages, *P. laevigata* possessed more anti-inflammatory effect (Ncib et al. 2018). The ethanolic extract of *Ziziphus jujuba* possessed remarkable ability to cause inhibition of carrageenan-instigated paw edema in the female Wistar rats ($p \leq 0.05$) and it also had an effect on the paw volume as well as the thickness of both left and right paws than the negative control group (Mesaik et al. 2018). The flowers of *Opuntia ficus* resulted in significant inhibition of inflammation in carrageenan-instigated rat paw edema model which was affirmed through the histological and the hematological determination and this was associated with the decreased amount of malondialdehyde and elevation in the action of superoxide dismutase, reduced glutathione and catalase. Therefore, these results are indicative of the use of flowers of *Opuntia ficus* as a natural source to treat inflammatory disorders (Ammar et al. 2018). The photoprotective activity of hydroalcoholic extract of red propolis in a murine model when given topically and the protective mechanisms have been associated with the anti-inflammatory and anti-oxidant properties of compounds present (Batista et al. 2018). The high anti-inflammatory potential in the extracts of *Peganum harmala* and *Marrubium alysson* prepared in the methanol has also been observed (Edziri et al. 2018). The reverse

phase-HPLC was indicative of coumarin as the compound present in abundance in the extract of *Hertia cheirifolia* L. (53.80% made in methanol). When this extract was administered at 100 mg/kg, the maximum acute anti-inflammatory potential was observed in rats, thereby promoting the conventional utilization of this plant in treating diseases related to inflammation (Majouli et al. 2018).

Anti-inflammatory effects of the extracts of *Feretia apodanthera* against the right hind paw's edema of albino rats, thereby, could play the role of an efficient antioxidant (Owolabi et al. 2018). A total of 200 and 400 mg/kg doses of *Brucea antidysenterica* possess a significant anti-inflammatory effect (Tessema et al. 2018). The ethyl acetate fraction of the leaves of *Tetraclinis articulata* exhibited anti-inflammatory activity with an $EC_{50}$ value of129.67 μg/mL (Rached et al. 2018). The bio-available fraction from the species of *Origanum* significantly inhibited the secretion of TNF-α, IL-1β and IL-6 in the human THP-1 macrophages model (Villalva et al. 2018). Studies on the anti-inflammatory activity of *Scutellaria barbata* revealed that the ethanol fraction was comprised mainly of flavonoids and phenolics, whereas, the ethyl acetate fraction was comprised mainly of chlorophylls as well as carotenoids. Moreover, both the extracts were capable of remarkably inhibiting synthesis of lipopolysaccharide-instigated prostaglandin $E_2$, nitric oxide, IL-1 β and IL-6. Both the extracts were found to exhibit a dose-dependent anti-inflammatory potential on RAW 264.7 cells. Therefore, this study was suggestive of using *S. barbata* extract as an anti-inflammatory medium for the feasible biomedical utilization in the coming years (Liu et al. 2018).

The anti-inflammatory assay conducted on the oil/extracts of *Thymus vulgaris* and extracts of *Chlorella vulgaris* depicted a potential synergistic impact as they reduced the LPS-instigated increase of nuclear factor-κ, tumor necrosis factor-α, inducible nitric oxide synthase, cyclooxygenase-2, nitric oxide as well as oxidative stress. Hence, it was concluded that the Greek *T. vulgaris* extracts possessed anti-inflammatory activities that can be potentiated upon mixing with the extracts of *Chlorella vulgaris* (Habashy et al. 2018). The anti-inflammatory potential of *Bupleurum marginatum* methanolic and dichloromethane extracts carried in the in vitro and in vivo experiments revealed that the release of prostaglandin-E2 was decreased by 41.33% and 52.85% at a concentration of 25 μg/mL, whereas, 5-lipoxygenase was retarded with $IC_{50}$ values of 45.28 and 25.92 μg/mL. Moreover, it was also observed that the methanolic and dichloromethane extracts decreased the diameter of the carrageenan-instigated rat paws' edema by 50% and 70%, respectively (Ashour et al. 2018). Some typical flavanones or extracts obtained from the buds of *Populus* x *burliness* and *P. nigral* reduced the liberation of IL-1β as well as IL-6 in HGF-1 cells along with down-regulation of their mRNA (Pobocka-Olech et al. 2018). The extract of *Piper cubeba* prepared in methanol exhibits anti-inflammatory activity through action on Src/Syk in the nuclear factor-κB pathway (Qomaladewi et al. 2019). Likewise, another study revealed that the 80% extract obtained from the leaves of *Calpurnia aurea* prepared in methanol possess anti-inflammatory potential (Ayal et al. 2019). *Anthocleista vogelii* ethyl acetate fraction possesses the anti-inflammatory activity at 100 mg/kg and the results shows that it inhibited 37.8%, 62.5% and 69.7% of edema instigated through egg-albumin at the

2nd, 4th and 6th hours which was attributed to the non-cytotoxic terpenoids present in this fraction (Eze et al. 2019). The anti-inflammatory effect of the extract of *Portulaca oleracea* is due to the suppression of lung inflammation as it reduced interlukin-6, interleukin-β, prostaglandin-E2, tumor necrosis factor-α and transforming growth factor-β, whereas, it elevated the amounts of interlukin-10. Thereby, it was concluded that the extract of *Portulaca oleracea* exhibited anti-inflammatory potential towards lipopolysaccharide-instigated rat acute lung injury (Rahimi et al. 2019). Elicitation of *Levisticum officinale* leaves with 0.1% yeast extract and 10 μM jasmonic acid elevated the anti-inflammatory activity (Złotek et al. 2019). *Kalanchoe brasiliensis* and *K. pinnata* possess confined potential to inhibit inflammation and the formulations comprising aqueous extract of both these plants decreased ear as well as paw edema which was established through decreased activity of interlukin-1β, myeloperoxidase, interlukin-1β, and tumor necrosis factor-α amounts and elevated interlukin-10 amounts (de Araújo et al. 2019).

## 4.3 Anti-inflammatory Activity of Essential Oils

Essential oils (EOs) possess important volatile compounds with diverse bioactivities including anti-microbial potential and used in drugs, food, and cosmetics (Chouhan et al. 2017). EOs are complex mixtures of substances being biologically active that are classified as natural products possessing pharmacological activity that can be of therapeutic use to manage human diseases (Derwich et al. 2010). The EOs extracted from medicinal and aromatic plant species are among the natural compounds that are gaining particular attention as they possess radical scavenging activities (de Sousa Barros et al. 2015). The direct incorporation of aromatic plants' EOs to foodstuffs exhibit an anti-oxidant and anti-microbial effect (Costa et al. 2015). The oral administration of *Citrus limon* EOs at a dose of 50, 100 and 150 mg/kg remarkably decreased the number of writhes and the highest doses decreased the number of paw licks, thereby, exhibiting anti-inflammatory activity (Ficarra et al. 2015). The coriander oil exhibits anti-inflammatory potential in the ultraviolet (UV) erythema test in vivo (Reuter et al. 2008). The anti-inflammatory activity from EOs of *Origanum ehrenbergii*, *O. syriacum* and *O. ehrenbergii* was studied in lipopolysaccharide-instigated inflammation in RAW264.7 cells and a significant decrease in NO production was observed (Loizzo et al. 2009). The anti-inflammatory effect of *Cyperus rotundus* EOs in carrageenan-instigated rats revealed significant ($p < 0.01$) dose dependent reduction from 2nd hour after carrageenan injection in paw edema rats. This essential oil also resulted in the inhibition of inflammatory pain ($p < 0.01$) at a dose of 500 mg/kg, whereas, the pain due to inflammation was significantly ($p < 0.05$) blocked at lower doses (Biradar et al. 2010). The anti-inflammatory potential of cumin volatile oil in carrageenan-instigated rat paw edema indicated that the volatile oil of cumin exhibited dose-dependent inhibition of the rat paw edema at a dose of 0.1 mL/kg, i.p. than the control group. Moreover, the anti-inflammatory activity was found to be comparable with that of the standard drug, diclofenac

sodium (Shivakumar et al. 2010). Further in the same year, Chouhan et al. (2011) showed the dose-dependent reduction of carrageenan-instigated rat paw edema by the *Crotalaria juncea* seed oil. Also, remarkable ($p < 0.001$) anti-inflammatory effect was exhibited by *C. juncea* seed oil at a dose of 200 mg/kg during the late inflammation phase comparable to that of diclofenac sodium (Chouhan et al. 2011). The anti-inflammatory effect of extra virgin olive oil from *Olea europaea* in carrageenan-induced paw edema in rats was found similar to that of treatment with dexamethasone (Fezai et al. 2013). As revealed by real-time PCR tests, cumin EOs caused remarkable inhibition of the mRNA expressions of inducible nitric oxide synthase, cyclooxygenase-2, interleukin-1, and IL-6 in lipopolysaccharide-instigated RAW 264.7 cells. Further, Western blotting analysis indicated that cumin EOs caused blockage of LPS-induced transcriptional activation of nuclear factor kappa β (NF-κβ) as well as inhibition of the phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK). Hence, it was concluded that cumin EOs exhibited anti-inflammatory effects in LPS-stimulated RAW264.7 cells by inhibiting NF-κβ and mitogen-activated protein kinases ERK and JNK signaling (Wei et al. 2015). Garlic oil exhibited anti-inflammatory potential by inhibition of the assembly–disassembly processes of the cytoskeleton (Hussein et al. 2017). *Citrus limetta* EOs comprised of limonene as well as monoterpene hydrocarbon being the chief constituent. When macrophages were pre-treated with the EOs of *C. limetta*, inhibition in the synthesis of pro-inflammatory cytokines such as interlukin-6, tumor necrosis factor-α, interlukin-1β in the lipopolysaccharide-instigated inflammation and inhibition in the synthesis of reactive oxygen species in $H_2O_2$-instigated oxidative stress was also observed. On the other hand, in vivo study revealed that when the volatile oil was applied topically, it had the ability in reducing 12-*O*-tetradecanoylphorbol-13-acetate-instigated ear weight, ear thickness, synthesis of pro-inflammatory cytokines, lipid peroxidation as well as improved the histological damage in the ear tissue (Maurya et al. 2017). The two components cinnamaldehyde and linalool distilled and extracted from the fresh leaves of *Cinnamomum osmophloeum* have shown remarkable anti-inflammatory potential and affirm the potent utilization of this essential oil as an anti-inflammatory natural product as well as gave proof that linalool and cinnamaldehyde were the two potential compounds for prophylactic utilization in inflammations associated health issues which were assigned to the hyper-activated TLR4 and/or NLRP3 signaling pathways (Lee et al. 2017). The EOs from half ripe *Citrus myrtifolia* decreased the synthesis of nitric oxide as well as the expression of inflammatory genes, cyclooxygenase-2 and inducive nitric oxide synthase, cytokines, including interleukin-1 and interleukin-6, and chemokine monocyte chemotactic protein-1 by lipopolysaccharide-instigated RAW 264.7 macrophages. Moreover, the chief components that were identified in the EOs of *Citrus myrtifolia* were linalool, limonene, linalyl acetate and γ-terpinene (Plastina et al. 2018). $IC_{50}$ value of 0.97 μg/mL has been established regarding the in vitro anti-inflammatory activity of the volatile oil of *Siegesbeckia pubescens*, thereby reducing the capability of lipopolysaccharide-instigated RAW264.7 macrophages to liberate nitric oxide. On the other hand, the EOs of *Siegesbeckia orientalis* having an $IC_{50}$ value of 14.99 μg/

mL was able to inhibit the lipopolysaccharide-instigated liberation of cytokine interlukin-6 (Gao et al. 2018). The EOs of *Rosmarinus officinalis* also possess anti-inflammatory activity through the inhibition of NF-κβ transcription and suppression of arachidonic acid cascade (Borges et al. 2018). Likewise, *Pistacia lentiscus* fatty oil resulted in a remarkable reduction of interlukin-6, nitric oxide and tumor necrosis factor-α levels in the ex-plant culture supernatants. Moreover, the *P. lentiscus* fatty oil also decreased the expression of inducible nitric oxide synthase expression in the gastric mucosa (Boutemine et al. 2018). *Pimpinella anisum* (aniseed) EO non-toxic doses remarkably reduced the expression amounts of interlukin-1 as well as interlukin-8 along with the elevated secretion of Muc5ac lipopolysaccharide-treated tracheal epithelial cell lines (HBEpC and HTEpC). Moreover, EOs also exhibited a remarkable anti-inflammatory impact on both HBEpC and HTEpC cells together along with the increased secretion of mucus (Iannarelli et al. 2018).

The chemical composition of *Croton campestris* EOs comprised of 1,8-cineol (16.98%), caryophyllene (15.91%) and germacrene-D (14.51%) among the chief components. It was observed that 1,8-cineol and germacrene-D exhibited anti-inflammatory potential in the abdominal contortions, paw edema induced by carrageenan, histamine, dextran and arachidonic acid models, the formalin test, peritonitis test and vascular permeability. The β-caryophyllene, on the other hand, showed no remarkable impact on the granuloma assay (de Morais Oliveria-Tintino et al. 2018). It was observed that EOs of *Thymus camphoratus* exhibited more pharmacological potential than the essential oil of *T. carnosus* than the volatile oil of *T. carnosus* exhibiting inhibition ion potential towards nitric oxide synthesis at smaller concentrations of 0.16 μL/mL and a concomitant inhibition of the expression of two important pro-inflammatory proteins, cycloxygenase-2 and inducible nitric oxide synthase at a concentration of 0.32 μL/mL. As the quenching of nitric oxide activity was not observed, it was concluded that the anti-inflammatory potential of the two EOs occurred upstream of inducible nitric oxide synthase expression, probably by inhibiting relevant pro-inflammatory signal transduction pathways (Zuzarte et al. 2018). The first study on the leaf essential oil of *Psidium guineense* demonstrated that it possessed anti-inflammatory property when orally administered in mice, thereby, remarkably inhibited the carrageenan-instigated mice paw edema in pleurisy model (do Nascimento et al. 2018). Likewise, the first report on the anti-inflammatory potential of pure coconut oil was due to the suppression in the inflammatory markers (Varma et al. 2019). The anti-inflammatory potential of volatile oil extracted from the leaves of *Curcuma caesia* has been observed (($IC_{50}$value 182.5 μg/mL)) (Borah et al. 2019). The volatile oil of *Santolina corsica* possesses potential to inhibit and to suppress inflammation on the bronchial tract of hospitalized patients reported with respiratory ailments of hospitalized patients recovered with respiratory ailments (Foddai et al. 2019). It has also been established that EOs of *Rosmarinus officinalis* possess potential to inhibit inflammation against macrophages, thereby, making this oil as a potential anti-inflammatory medium (Lorenzo-Leal et al. 2019). The EOs of *Stachys subnuda* possess medium in vitro anti-inflammatory potential having an $IC_{50}$ value of 0.419 mg/mL (Sen et al. 2019).

## 4.4 Anti-inflammatory Effects of Isoflavones

The most absorbable and bioavailable flavonoids are isoflavones that have been originally considered as an anti-inflammatory agent as genistein causes down-regulation of cytokine-instigated events of signal transduction in immune system cells (Verdrengh et al. 2003; Yu et al. 2016). After this, large number of investigations frequently showed that isoflavones possess anti-inflammatory properties. The isoflavone genistein exhibited potential to inhibit inflammation in mouse models that affected monocytes, granulocytes and lymphocytes (Verdrengh et al. 2003). The diets comprising of isoflavone help in the prevention of inflammation-related instigation of metallothionein in the intestine and prevent inducing manganese superoxide dismutase in the mice liver injected with endotoxin lipopolysaccharide. It also inhibits the reaction of intestine to inflammation by altering the activity of pro-inflammatory cytokine interleukin-6 (Paradkar et al. 2004). The isoflavone powders (derived from processed soybean cake, a byproduct of the soybean oil industry) and genistein standard efficiently caused inhibition of lipopolysaccharide-instigated inflammation, reduction in the amount of leukocyte in mouse blood, as well as decreased the synthesis of interlukin-6, interlukin-1, prostaglandin-E2 and nitric oxide in the supernatant of peritoneal cells' exudates and the fluid of peritoneal exudates (Kao et al. 2007). Tests in human on the consumption of a soy nut diet for 8 weeks (340 mg isoflavones/100 g soy nut) revealed decreased markers of inflammation like interlukin-18 and C-reactive protein and elevated titers of nitric oxide levels in the plasma of postmenopausal women with metabolic syndrome (Azadbakht et al. 2007). Such in vivo observations have depicted that various isoflavones exhibit anti-inflammatory potentials consistently in multiple animal models, whereas, in vitro studies in various cultured cells have also revealed the anti-inflammatory capability of isoflavones. The pre-treatment with genistein decreased the lipopolysaccharide-instigated amount of cycloxygenase-2-protein and nitric oxide in the supernatant of primary cultures of human chondrocytes, with no effect on the amount of cycloxygenase-1 protein (Hooshmand et al. 2007). Genistein mediated inhibition of cycloxygenase-2 than cycloxygenase-1 is superior because suppression of COX-2 can decrease the synthesis of pro-inflammatory particles (Hooshmand et al. 2007). The methanol fraction of soybean comprising of isoflavones exhibits potential to inhibit inflammation in the experimental replica of ear edema instigated by croton oil (Carrara et al. 2008). Puerarin, a distinctive isoflavone, has been able to save the brain of rats from ischemic damage after middle cerebral artery blockage. This effect was attributed to the anti-inflammatory potential of puerarin through the expression inhibition of cycloxygenase-2 in microglia and astrocyte (Lim et al. 2013). It has been reported in a study that isoflavone daidzein stops tumor necrosis factor-α-instigated elevation in the expression as well as activity of pro-inflammatory chemokine Cxcl2, along with a marked inhibition of tumor necrosis factor-α-instigated protein poly-adenosine diphosphate-ribosylation in murine lung epithelial cells (Li et al. 2014). The THP-1 monocyte cells of humans have been reported to be suppressed by daidzein because of the LPS-instigated amounts of interlukin-6, interlukin-12, and tumor necrosis

factor-α (Tanaka et al. 2014). It has been found that the isoflavone-abundant soy foods too decrease the amount of serum C-reactive protein in the end-stage patients of renal failure as well as the amount of interferon in healthy volunteers (Ferguson et al. 2014).

Extensive epidemiological studies, together with in vivo and in vitro experiments in the current times, revealed that isoflavones give benefits to those patients having cardiovascular diseases, osteoporosis and cancer (Messina 2014). Pre-treatment with genistein decreased nitric oxide and prostaglandin-E2, and inhibited the synthesis of D-galactosamine-instigated pro-inflammatory cytokines, including tumor necrosis factor-α and interlukin-1β in the male Wistar rats (Ganai et al. 2015). Genistein stops homocysteine-instigated death of vascular endothelial cell, morphological alterations of cells and reactive oxygen species' synthesis, thus, indicating that genistein blocked injury of endothelial cell due to inflammation (Han et al. 2015). The anti-inflammatory characteristics of isoflavones have been addressed in cell cultures, animals and clinical trials with the underlying mechanisms being elucidated in many studies. Although, it is still unclear about the mechanisms of isoflavones actions, various possibilities have been well unrevealed (Yu et al. 2016).

## 4.5 Anti-inflammatory Effects of Polyphenols

The phenolics compounds having an aromatic ring possess one hydroxyl group while "poly-phenols" possess one or more aromatic rings having more than one hydroxyl group. The chemical structures of polyphenols are chiefly associated with their anti-oxidant, anti-inflammatory activities and other biological functions (Zhang and Tsao 2016). The dietary intake of foods enriched with polyphenols like fruits and vegetables reduces the chances of degenerative diseases due to oxidative stress and inflammation (Chuang et al. 2014). Inflammation as well as oxidative stress can result in the pathogenesis of chronic diseases and metabolic disorders. The anti-inflammatory potentials and the activity related to anti-oxidation stress due to phenolics can essentially affect similar biomarkers (Chuang et al. 2014). Supplementations of kaempferol or quercetin caused alteration of inflammation or insulin resistance in adipocytes by activating peroxisome proliferator-activated receptor-g, which is a nuclear receptor that regulates degradation of fatty acid and metabolism of glucose (Fang et al. 2008). *p*-coumaric acid is reported to cause inhibition of inflammasome-mediated secretion of interlukin-1β as well as the activation of caspase-1 in the macrophage cells of ex vivo inflamed mouse (Hori et al. 2013).

The flavonoids such as procyanidin β2 and apigenin have also been reported to be capable of inhibiting inflammasome activation and interlukin-1β secretion in the lipopolysaccharide-instigated human macrophages (Martinez-Micaelo et al. 2015). The stilbene phenolic compound named resveratrol present in red grapes also caused inhibition of NLRP3 activation-instigated autophagy for the preservation of mitochondrial function in both in vitro as well as in vivo studies. Moreover, resveratrol also improved hepatic inflammation in high-fat diet-instigated obesity mouse model

(Chang et al. 2015). The flavonoids and their metabolites are among the varieties of phenolic compounds that have been observed to agonistically control PPAR-g activation via ligand interaction (Wang et al. 2014). The phenolic compounds obtained from dietary sources like plant foods, herbs and spices can trigger PPAR-γ to reveal protagonist consequences on the transcription factors of inflammation, resulting in the repression of inflammation and inhibitory consequence on the metabolic diseases. A nicotinamide adenosine dinucleotide-dependent protein deacetylase sirtuin (SIRT)-1 control epigenetic gene silencing in response to stress causes the regulation of the NF-kβ signaling transductions as well as elevates insulin sensitivity (Anastasiou and Krek 2006). As PPAR-g coactivator (PGC-1a) and SIRT-1 interacts, activation of PPAR-g with flavonoids can thus effect SIRT-1-controlled signaling transductions comprising the transcriptional factor NF-κβ (Davis et al. 2009). Resveratrol has an agonistic action on SIRT-1 for the protection of cells from inflammatory damages (Chen et al. 2009). Since, the dietary polyphenols have less absorption rate, less amounts of such compounds having physiological relevance can still alter the expression of several inflammatory bio-markers through different signaling pathways. In order to produce effects, therapeutical drugs target these biomarkers, therefore, the capability of dietary phenolics on the same pro-inflammatory cytokines as well as other signaling molecules can have significant positive effect in preventing chronic non-communicable diseases due to oxidative stress. The protagonistic role of dietary phenolics is the topmost feature involved behind their anti-inflammatory mechanisms (Zhang and Tsao 2016).

## 4.6 Role of Fermentation in Increasing Anti-inflammatory Properties of Herbs

The easiest as well as safe traditional method for the enrichment of useful bioactive compounds is fermentation, as this method upgrades biological properties of herbs, vegetables and plants. This process is associated with the decomposition and/or biotransformation of complex substrates into the compatible constituents, thus, either alters the product' properties or the amount of some bioactive components (Chouhan et al. 2019). Accumulating evidences are indicative of enhanced anti-inflammatory potentials of herbs that have undergone through the process of fermentation. A rate-limiting enzyme, namely cycloxygenase-2, exhibits regulatory effect in the production of various inflammatory mediators that are active in biological systems, like prostaglandin-E2, which is also activated in various carcinomas, thereby, indicating its important part in inflammation as well as tumor genesis (Chouhan et al. 2019). The protein, namely cycloxygenase-2, and synthesis of prostaglandin-E2 in RAW 264.7 cells are triggered due to lipopolysaccharide (Mathers et al. 2006). The changed profile of secondary metabolites and changes in their mechanisms that affect biological activity having therapeutic enhancement are positively associated with fermentation because this process increases the amount of bioactive components like anti-oxidants, and also the anti-inflammatory activity of

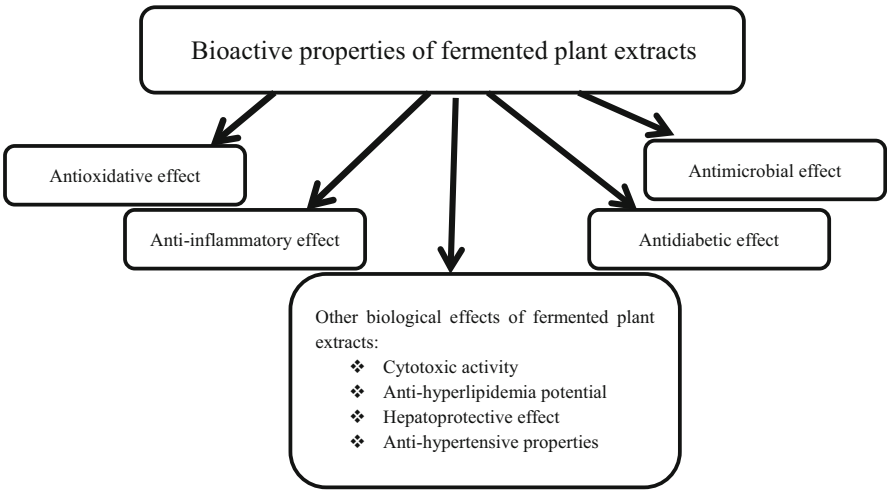



**Fig. 4.4** Bioactive properties of fermented plant extracts

several compounds gets ameliorated (Chouhan et al. 2019). Inhibition of the translocation of NF-κβ p65 occurred because of the fermented OY-reduced degradation of Iκβα and through the phosphorylation of extracellular kinases that are signal regulated, and p38 and c-Jun more strongly as compared to the unfermented OY. Thus, it was concluded from these findings that fermentation increased the potential of the anti-inflammatory effect of OY by obstructing NF-κβ and MAPK pathway in the macrophage cells (Seo et al. 2005). Obstruction of expression of cycloxygenase-2 due to BST204 is not dependent on NF-κβ and mTOR/p70 S6 kinase pathway and some other pathways of undetermined nature had been reported to be the mediators for this (Seo et al. 2005). Fermentation resulted in significant increase in the obstructive impact of Oyaksungisan because of fermentation against the expression or synthesis of numerous vital pro-inflammatory mediators, namely cycloxygenase-2, tumor necrosis factor-α, inducible nitric oxide synthase, nitric oxide, prostaglandin-E2 and interlukin-6 in RAW 264.7 cells (Oh et al. 2012). Fermented preparation of *Rhizoma atractylodis* macrocephalae remarkably reduced the potential of NF-κβ upon co-treatment of lipopolysaccharide-instigated RAW 264.7 cells (Bose and Kim 2013). The activity of NF-κβα gets inhibited through the fermented *Artemesia princeps* in the lipopolysaccharide-instigated peritoneal macrophages (Joh et al. 2010). The co-treatment of lipopolysaccharide-instigated RAW 264.7 cells with the leaf extract of guava that has undergone fermentation could significantly suppress the transcriptional activity of NF-κβ in a concentration-dependent manner (Choi et al. 2008). Therefore, it was suggested that fermented leaf extract of guava could obstruct the activation of NF-κβ through suppressing Iκβα deterioration stimulated due to lipopolysaccharide (Choi et al. 2008). A schematic presentation of bioactive properties of fermented plant extracts is shown in Fig. 4.4.

## 4.7 Conclusion

The information presented in this chapter indicates that many extracts, essential oils and compounds derived from natural products exhibit potent anti-inflammatory properties. In the recent studies, it has been established that these extracts, essential oils and compounds derived from natural products possess significant modulatory effect on cellular biomarkers that are related to oxidative stress and inflammation, which results in reducing the risk of many chronic diseases. By reviewing the potent role of plant extracts, essential oils and compounds derived from natural products along with their mechanisms on oxidative stress and inflammation-related biomarkers, it is hoped that future efforts in this respect can focus on increasing the bioaccessibility, bioavailability from processing and formulation of plant-based functional foods, and ultimately this will develop functional foods or nutraceuticals that will decrease health risk of chronic diseases because of their modulatory effects. Although production of drugs from the plant-based anti-inflammatory compounds may prove a difficult task, but plant extracts, essential oils and pure compounds of natural products may still open new areas for therapeutic interventions.

**Acknowledgement** The authors would like to thank all people who helped directly or indirectly in this manuscript.

**Competing Interest** The authors have no competing interest.

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# Cannabinoids as Promising Anti-inflammatory Agent

5

Nagma Banjare, Bhushan S. Bhale, and Prem N. Gupta

**Abstract**

Inflammation is a reaction of immune system to external stress conditions or any microbial attack on body, or it may be followed by autoimmune reaction. Different treatment strategies have been utilized to manage with this situation including use of different nonsteroidal anti-inflammatory drugs (NSAIDs) and other disease-modifying agents. In recent years, different newer natural agents have been screened for their anti-inflammatory activity, and one such agent is cannabidiol (CBD), which has significant activity against inflammation. CBD is an active constituent of *Cannabis sativa* and other species of *Cannabis*. Besides, anti-inflammatory activity, this plant species also possess different activities like neuroprotective, antiepileptic, hypoxia-ischemia, anxiolytic, antipsychotic, analgesic, anti-asthmatic, and antitumor properties. In this communication, anti-inflammatory effects of CBD are described along with its mechanism of action and pharmacokinetic studies.

**Keywords**

Cannabidiol · Anti-inflammation · Immune system · Cytokines · Immunomodualtion

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_5

## Abbreviations

| | |
|---|---|
| AD | Alzheimer's disease |
| AS | Atherosclerosis |
| CB | Cannabinoid |
| CBD | Cannabidiol |
| CNS | Central nervous system |
| COX | Cyclooxygenase |
| DM | Diabetes mellitus |
| GIT | Gastrointestinal tract |
| GPR | G-protein-coupled receptor |
| IFN | Interferon |
| IL | Interleukins |
| ILD | Lung inflammation disease |
| iNOS | Inducible nitric oxide synthase |
| MAPK | Mitogen activated protein kinase |
| MS | Multiple sclerosis |
| NF-kβ | Nuclear factor-kappa β |
| NO | Nitric oxide |
| NOD | Nonobese diabetes |
| PPAR | Peroxisome proliferators-activated receptor |
| RA | Rheumatoid arthritis |
| Th | T helper cell |
| THC | Tetrahydrocannabinol |
| TNF | Tumor necrosis factor |
| TRPM | Transient receptor potential of the melastatin |
| TRPV | Transient receptor potential of vanilloid |
| VCAM | Vascular cell adhesion molecule |

## 5.1 Introduction

The delimit reaction of living tissues to any trauma owing to any reason is referred to as inflammation. Body shows its defensive response to get rid of, or suppressing the expansion of, infectious agent and subsequent eradication of localized dead tissues. It is characterized by redness, swelling, heat, pain, and loss of function. Factors responsible for inflammation are classified into five different groups, as infective agents, immunological agents, physical agent, chemical agent, and inert material (Fig. 5.1) (Mohan 2010). Inflammation is a protective response by the body to variety of etiologic agents (infectious or noninfectious). Inflammation involves two basic processes with some overlapping, *viz*., early inflammatory response which mainly includes release of pro-inflammatory cytokines and chemokines, and followed by healing. Though both these processes generally have protective role against injurious agents, inflammation and healing may cause considerable harm to the body as well, for example, anaphylaxis to bites by insects or reptiles, drugs,

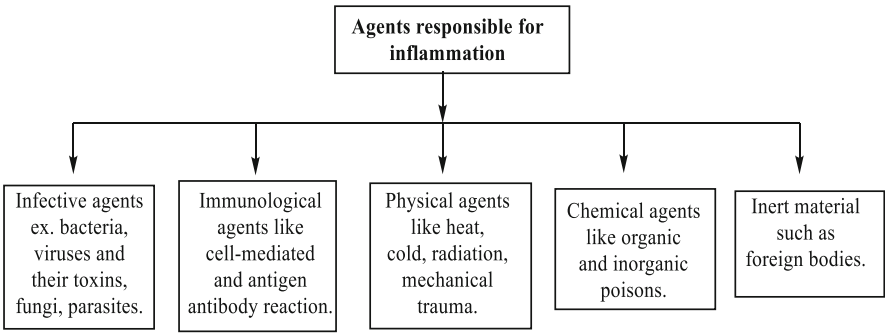


**Fig. 5.1** Agents responsible for inflammation

toxins, atherosclerosis, chronic rheumatoid arthritis, fibrous bands, and adhesions in intestinal obstruction (Netea et al. 2017; Katzung 2012). Treatment strategy for inflammation mostly targets toward the reduction in pain and swelling caused due to release of inflammatory immunological agents. Currently available agents include use of different classes of nonsteroidal anti-inflammatory agents like nonopioid analgesics (e.g., Aspirin), use of selective COX-2 inhibitors (e.g., celecoxib, meloxicam), nonselective COX inhibitors (e.g., ibuprofen, diclofenac, indomethacin) (Katzung 2012).

Ethnomedical uses include various medicinal preparations that contain *Cannabis sativa* (Fig. 5.2) due to its positive effect on rheumatism, antipyretic, analgesic, and anti-allergic effect. Cannabis is primarily used to manage back pain, sleeping disorders, depression, injury or accident-generated pain, and multiple sclerosis. Psychoactive and non-psychoactive constituents, *namely*, delta-1-tetrahydrocannabinol and cannabinol, respectively, are major components found in cannabis extract that exhibit in vivo analgesic activity. Cannabis can be administered by smoking, vaporization, and by oral route; however, smoking or vaporization releases high concentration of tetrahydrocannabinol (THC) into blood within minutes. Neuropathic pain occurs in different disease conditions like HIV, cancer, and rheumatoid arthritis. It is found that cannabis smoking improved the health in HIV-affected persons (Anilkumar 2010; Madras 2015).

Nowadays, researchers are more interested in use of natural disease-modifying agents as they significantly come up with less toxic effects and high potency. Cannabis is one of the natural agents, which is emerging as a potential anti-inflammatory agent, as *Cannabis sativa* traditionally has been used for its medicinal activity in managing with depression, pain, glaucoma, etc. Cannabis is a term used for chemical group present in different species, viz., *Cannabis sativa*, *Cannabis ruderalis*, and *Cannabis indica*; among those, cannabis sativa is abundantly used. In the mid-seventeenth century researchers focused on this plant for isolation of new compounds. Trans-1, 9-tetrahydrocannabinol [1, 9-THC] and cannabidiol (CBD) are the major components that have therapeutical potential in various conditions (Fig. 5.3) (Rong et al. 2017). The cannabis is well known for its psychoactivity,

**Fig. 5.2** Habit of *Cannabis sativa*, the source plant for cannabidiol (CBD) and tetrahydrocannabinol (THC)

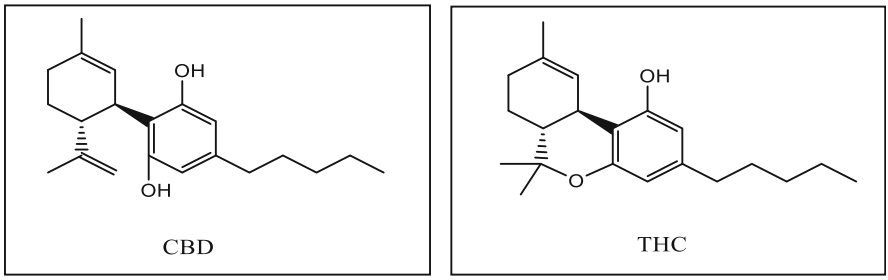


**Fig. 5.3** Structure of cannabidiol (CBD) and tetrahydrocannabinol (THC) compounds

which is observed due to the 1, 9-THC, whereas CBD is shown to have nonpsychoactive effect and the receptors responsible for their effect are cannabinoid type 1 (CB1) receptor, cannabinoid type 2 (CB2), G-protein-coupled receptor 55 (GPR55), nuclear peroxisome proliferator-activated receptor (PPAR), transient receptor potential of the melastatin type 8 (TRPM8); serotonin receptor (5-HT1a); and transient receptor potential of vanilloid type 1 and type 2 (TRPV1-V2) (Morales et al. 2017; Elsohly et al. 2017). This chapter mostly focuses on nonpsychotic effects of cannabis, so CBD is a compound of our interest. CBD is shown to have different effects like neuroprotective activity, antiepileptic, hypoxia-ischemia, anxiolytic, antipsychotic, analgesic, anti-inflammatory, anti-asthmatic, and antitumor properties

(Appendino et al. 2011). However, currently CBD is under extensive study for its anti-inflammatory activity. So in this chapter we will discuss anti-inflammatory effects of CBD and its mechanism of action along with pharmacokinetic studies.

## 5.2 Pharmacokinetic-Pharmacodynamic Profile

CBD has poor water solubility and shows unpredictable absorption throughout the gastrointestinal tract (GIT). Through oral route of administration the bioavailability is found to be ~6%.The high lipophilic nature of CBD governs the distribution pattern, and the volume of distribution is high in organs like adipose tissue, brain, and others. It shows high protein binding, and approximately 10% of CBD binds to RBC in circulatory system. CBD is extensively metabolized by cytochrome P450 and is converted to its hydroxylated CBD (7-OHCBD) metabolite. The metabolites are eliminated through urine (lesser extent than feces) and feces (high percent) (Grotenhermen 2003). The half-life is found to be 18–32 h in humans (Devinsky et al. 2014). Studies indicated that CBD is nontoxic to the normal cell (non-transformed cell), and does not show any changes psychologically and physiologically (i.e., body temperature, heart rate, and blood pressure). However, few studies showed side effects of CBD like decrease in drug metabolism and induction of infertility (Machado et al. 2011).

The anti-inflammatory role of CBD is very crucial in the context of immunomodulation. This immunomodulatory action is a result of its action on macrophages. It reduces T-cell responses along with declining the release of pro-inflammatory cytokines and bioactive TNF (Silva et al. 2019). The anti-inflammatory and neuroprotective action of CBD is due to increased signaling mechanism of adenosine followed by accumulation of adenosine and reduction in uptake of adenosine. This adenosine-based mechanism is thought to be the main process which addresses the inflammation and shows the anti-inflammatory effect (Ribeiro et al. 2012). Along with the prostaglandin-2(PGE-2) plasma levels, tissue cyclooxygenase (COX) activity and production of reactive oxygen species (ROS) and nitric oxide (NO) are decreased by the action of CBD (Costa et al. 2004; Malfait et al. 2000).

## 5.3 Uses in Different Inflammatory Disease Condition

CBD acts on immune responses by suppressing its effects and also reduces the production of pro-inflammatory responses which specify its uses in different inflammatory diseases (Zuardi 2008).

There are different promising therapeutic effects of CBD on inflammatory disease conditions, including rheumatoid arthritis (RA), Alzheimer's disease (AD), type 1 diabetes mellitus (DM1), edema and hyperalgesia, lung inflammation disease (ILD), multiple sclerosis (MS), and atherosclerosis (AS) (Fig. 5.4).

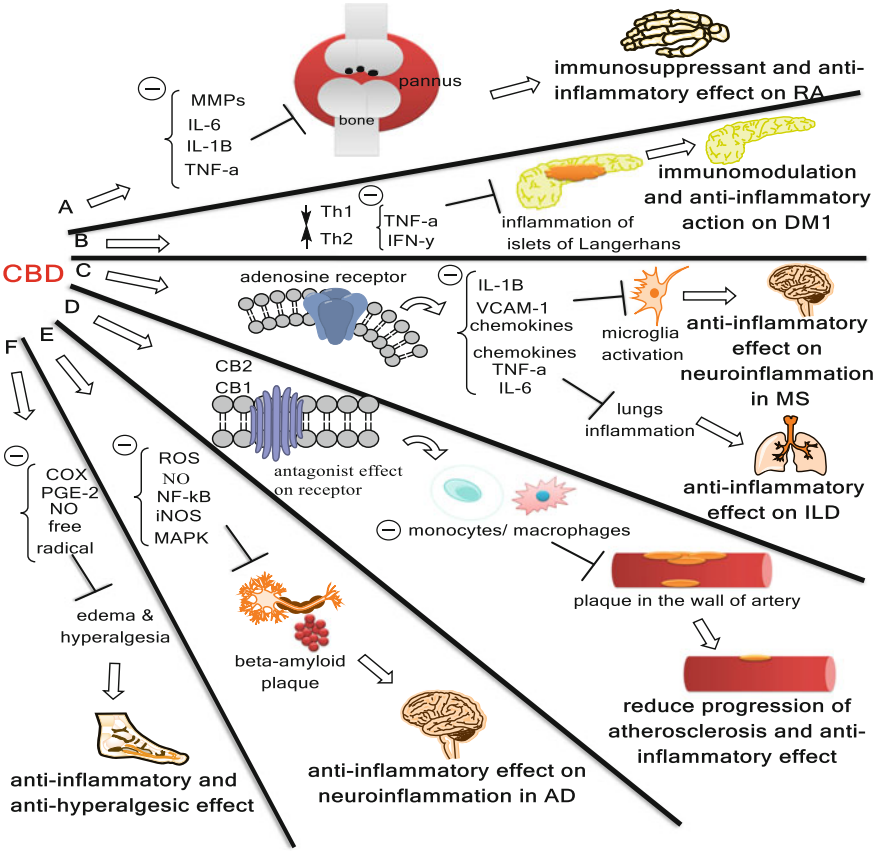


**Fig. 5.4** Effect of CBD on biological function. (*A*) CBD inhibits/decreases the production of MMPs, IL-6, IL-1β, and TNF-α which in result reduces the arthritic progression in RA and shows immunosuppressant and anti-inflammatory action on RA. (*B*) CBD balances the Th1 (downregulation) and Th2 (upregulation) cytokines followed by inhibition of the production of TNF-α and IFN-γ. Through this mechanism CBD reduces the inflammation of islets of Langerhans and shows immunomodulatory and anti-inflammatory effects on DM1. (*C*) CBD acts through adenosine receptors and shows inhibitory action on MS and ILD. In MS it reduces the microglia activation by inhibiting the expression of IL-1β, VCAM-1, and chemokines. In ILD it reduces inflammation by decreasing the production of chemokines, TNF-α, and IL-6. (*D*) CBD shows antagonist effect on cannabinoid receptors CB1 and CB2 which leads to decrease/inhibit the action of macrophages and monocytes which in turn results in reduction of oxidized lipoprotein plaque formation in the wall of artery followed by decrease in progression of atherosclerosis. (*E*) CBD decreases the activity of ROS, NO, NF-kβ, and MAPK and reduces the formation of beta amyloid plaque thereby play role in AD. (*F*) CBD shows anti-inflammatory and anti-hyperalgesic effects on edema and hyperalgesia by inhibiting the production of COX, PGE-2, NO, and free radicals (T-Indicates reduction in disease progression, and θ Indicates inhibition/decrease)

### 5.3.1 Rheumatoid Arthritis (RA)

RA is an autoimmune chronic inflammatory condition that mainly affects the joints followed by pannus formation. The inflammatory mediators, cytokines mainly TNF-α; interleukins like IL-1β, IL-6, and IL-17; and enzyme, that is, MMPs (matrix metalloproteinases) in synovium induce inflammation in a sustained manner which leads to pannus formation and degradation of articular cartilage (Navarini et al. 2019). CBD suppresses the release of TNF-α production and chemokine production by a human B cell. In a model for rheumatoid arthritis, when CBD was given intraperitoneally, or orally, it showed promising therapeutic effect; CBD also modulates T-cell responses. The effect of CBD in rheumatoid arthritis treatment is by mechanism of immunosuppression and anti-inflammatory responses (Zuardi 2008; Silva et al. 2019). For clinical application in RA, CBD should shows positive response against severe joint damage (Malfait et al. 2000).

### 5.3.2 Type 1 Diabetes Mellitus (DM1)

Nowadays, diabetes has become very common fascinating pathological manifesto in almost everyone's health. Starting from children to adult, ending to old persons, most of them are victimized by the severity of two categorical disease conditions. Insulin-dependent diabetes (DM1) is an autoimmune disorder that results in the obliteration of pancreatic β cells which produce insulin and an inflammation of islets of Langerhans. The promising anti-inflammatory action of CBD acts by reducing production of cytokines (IFN-γ and TNF-α) and inhibiting the proliferation of T cell. Some investigations of CBD on nonobese diabetic (NOD) mice revealed that the incidence of development of DM1 is reduced from 86% to 30%. There is also a reduction in plasma level of pro-inflammatory cytokines (IFN-γ and TNF-α) as a result of CBD treatment. The balance between the pro-inflammatory cytokines (reduction in IL-12 produced by splenocytes) and anti-inflammatory cytokines (enhance the level of IL-10) was observed after CBD treatment (Navarini et al. 2019; Zuardi 2008). CBD is able to downregulate T-helper type 1 cell (Th1) cytokines as well as upregulate T-helper type 2 cell (Th2) cytokines, probably it acts by immune-modulation (modulating the response from Th1 to Th2 dominance) (Mechoulam et al. 2007). The above data represents that CBD can possibly be used for the treatment of DM1.

### 5.3.3 Multiple Sclerosis (MS)

Multiple sclerosis (MS) is a disease of the central nervous system (CNS) with chronic inflammatory demyelinating condition. This inflammatory condition is a process that involves leukocytes migration by crossing the blood–brain barrier (BBB) and activates immune system (Vilela et al. 2015). In viral model of MS, CBD prevents expression of chemokines (CCL2 and CCL5), pro-inflammatory

cytokine, interleukin-1β (IL-1β), and vascular cell adhesion molecule-1 (VCAM-1) via downregulation followed by fading the microglia activation (Vilela et al. 2015). CBD shows long-lasting effect when administered at the time of infection. Adenosine receptors are involved in the reduction of VCAM-1 expression (via endothelial cells) when treated with CBD, and this shows an important mode of action of CBD in neuroinflammatory processes (Mecha et al. 2013).

### 5.3.4 Alzheimer's Disease (AD)

The inflammatory responses and oxidative stress play critical role in the AD pathophysiology (Pisanti et al. 2017). In the AD experimental model, the anti-inflammatory properties of CBD are based on glial fibrillary acidic protein. mRNA and protein expression (in beta-amyloid) were inhibited dose-dependently. Additionally, the expression of inducible nitric oxide synthase (iNOS) and IL-1β protein and release of NO and IL-1β are also reduced by CBD (Booz 2011; Pisanti et al. 2017). It is reported that CBD reduced phosphorylation of the stress-activated protein kinase and P38 mitogen-activated protein kinase (MAPK), which results in preventing the transfer of nuclear factor-kappa β (NF-kβ) into the nucleus and the successive pro-inflammatory genes transcription and also which encoded for iNOS protein (Iuvone et al. 2009). Above information suggest that CBD successfully inhibit neuroinflammatory responses and might be useful in the management of AD.

### 5.3.5 Edema and Hyperalgesia

Edema and hyperalgesia is an inflammation followed by increase in pain sensitivity as a result of injury or other environmental factor. It mainly affects joints, paw, and ankles. Carrageenan model of inflammation produces inflamed tissue followed by hyperalgesia because of production of different mediators, that is, histamine, prostaglandins, cyclooxygenase, and 5-hydroxytryptamine with enhanced activity (Malfait et al. 2000). This model drastically increased NO production, and free radicals were also reported (Burstein 2015). The anti-inflammatory and anti-hyperalgesic effects of CBD have been studied in carrageenan model which is due to decreased level of plasma PGE2, COX activity, and production of free radicals and nitric oxide. Thus, CBD exhibited a favorable action on inflammation-related process, that is, edema and hyperalgesia (Burstein 2015).

### 5.3.6 Inflammatory Lung Diseases (ILD)

Acute lung injury is a pathological condition which occurs due to many reasons like pneumonia, lung injury, abnormal gas exchange, asthma, chronic obstructive pulmonary diseases, etc., all of these lead to inflammation of lungs. Current treatment strategy is only supportive and not leads to effective relief from inflammation. As

CBD has promising anti-inflammatory and immunosuppressive activity, murine model was developed for investigation of anti-inflammatory effect. Inflammatory cells, myeloperoxidase activity, and albumin concentration in the bronchoalveolar lavage fluid are the parameters that were observed and analyzed by colorimetric assays, as well as production of cytokine/chemokine in lungs was analyzed by enzyme-linked immunosorbent assay. CBD decreased leukocyte migration, production of pro-inflammatory cytokines (TNFα and IL-6), and chemokines (MCP-1 and MIP-2) in lungs. It was also observed that adenosine A2A receptors were involved in the anti-inflammatory effects of CBD (Vilela et al. 2015; Ribeiro et al. 2012; Burstein 2015).

### 5.3.7 Atherosclerosis

Atherosclerosis is narrowing of arteries as a result of retention, accumulation, deposition of oxidized lipoprotein which leads to plaque formation followed by migration of inflammatory cells to the wall of artery (Hulsmans and Holvoet 2010). The production of reactive oxygen species and oxidized low-density lipoprotein is enhanced by inflammatory cells (Saha et al. 2009). Atherosclerosis is an inflammatory disease initiated and progressed by the monocytes/macrophages with activated CB2 and CB1 receptors, which shows early development of atherosclerosis and inflammatory response. The CBD has antagonistic effect on these receptors. Treatment with CBD may reduce the progression of atherosclerosis along with reduction in inflammation and oxidative stress (Booz 2011; Rajesh et al. 2010).

## 5.4 Conclusion

*Cannabis* can be used to treat various pathological conditions by acting as an antipyretic, anti–rheumatic, and analgesic. Inflammation causes pain and reduces efficiency of the individual, and sometimes if untreated it may lead to damage of affected tissue or joints. To treat such immunological conditions, different agents have been used like NSAIDs. Among the currently screened newer molecule, CBD is found to have significant anti-inflammatory effect. Its immunomodulatory effect is due to its action on macrophages. It reduces T-cell responses along with declining the release of pro-inflammatory cytokines and bioactive TNF. The anti-inflammatory and neuroprotective action of CBD is due to increased signaling mechanism of adenosine followed by accumulation of adenosine and reduction in uptake of adenosine. This adenosine-based mechanism is thought to be the main process which addresses the inflammation and shows the anti-inflammatory effect. CBD becomes a potential agent to deal with different inflammatory conditions like rheumatoid arthritis, Alzheimer's disease, type 1 diabetes mellitus, edema and hyperalgesia, lung inflammation disease, multiple sclerosis, and atherosclerosis. But use of CBD is lacking due to its poor water solubility, and it shows unpredictable absorption throughout the GIT. Through oral route it shows bioavailability about

6%, and the high lipophilic nature of CBD shows high volume of distribution in organs like adipose tissue, brain, etc. Different formulation strategies must be developed to improve the water solubility and bioavailability, which potentiate its use as a strong anti-inflammatory molecule. From this we may conclude that CBD possesses potential immunomodulatory activity and can be used to treat different inflammatory conditions alone or in combination with other agents for its synergistic effect.

**Acknowledgment** The authors are thankful to the Director of CSIR-IIIM Jammu for his initiatives to initiate research work in the area of cannabis.

**Competing Interest** Authors declare no competing interest for this communication.

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# Plant Volatile Organic Compounds and Neuroregenerative Health

6

Rekha Chouhan, Sajad Ahmed, and Sumit G. Gandhi

**Abstract**

Presently, neurological disorders form a major proportion of non-communicable diseases. Their incidence has increased due to several factors such as lifestyle changes, changes in dietary patterns, and increased psychological stress. Due to increase in awareness regarding these disorders by health-care professionals and general public, increasing number of cases are diagnosed with every passing year. This presents a challenge, especially in under-developed and developing countries, where the public health-care systems are not well established and penetration of health insurance cover is relatively much lower. Alternative medicine has traditionally been used in several cultures around the world to treat neurological problems. Essential oils and other plant volatiles have a long history of traditional use for ameliorating symptoms of neurological and psychological disorders. Essential oils of lavender, rose, lemon balm, etc. have shown good promise. Further, modern research has validated some of the claims with regard to relieving of neural and psychological issues by plant VOCs. Some of these have been shown to modulate key enzymes that are targets for depression therapy. In the present chapter we have presented an overview of above, and toward the end we have attempted to identify lacunas in this area which may help to formulate future research strategies.

**Keywords**

Neurological disorders · Health · Ethnopharmacology · VOCs · Essential oils

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_6

## Abbreviations

| | |
|---|---|
| 2-D GC-MS | 2-Dimensional gas chromatography-mass spectroscopy |
| AChE | Acetyl cholinesterase enzyme |
| AITC | Allyl isothiocyanate |
| CAT | Catalase |
| CNS | Central nervous system |
| $CO_2$ | Carbon dioxide |
| DART | Direct analysis in real time |
| FIS | Fast isoprene sensor |
| GABA | Gamma-amino butyric acid |
| GC | Gas chromatography |
| GC-EAD | Gas chromatography-electroantennogram detection |
| GC-FID | Gas chromatography-flame infrared detection |
| GC-MS | Gas chromatography-mass spectroscopy |
| GC-TOF-MS | Gas chromatography-time of flight-mass spectroscopy |
| GSH-Px | Glutathione peroxidase |
| Ig | Immunoglobulin |
| IL | Interleukin |
| LE | Liquid extraction |
| NMDA | *N*-methyl-D-aspartate |
| PA | Photoacoustic |
| PTR-MS | Proton transfer reaction-mass spectrometry |
| PTZ | Pentylentetrazol |
| SBSE | Stir bar sorptive extraction |
| SDE | Simultaneous distillation extraction |
| SHXW | Su-He-Xiang-Wan |
| SOD | Superoxide dismutase |
| SPE | Solid phase extraction |
| SPME | Solid phase microextraction |
| SPME-HS-GC-MS-O | Solid phase microextraction of headspace volatiles followed gas chromatography, mass spectrometry and olfactometry |
| TNF | Tumor necrosis factor |
| UV | Ultraviolet |
| VOCs | Volatile organic compounds |
| WHO | World Health Organisation |

## 6.1 Introduction

Neurological disorders, including dementia, epilepsy, Parkinson's disease, Alzheimer's, Huntington's disease, stroke-induced secondary neurodegeneration, migraine and common headache problems, depression, schizophrenia, and various

other psychotic disorders, have come up as common health issue these days due to the prevalent unhealthy lifestyle (Walsh 2011). They have contributed significantly to global burden of noncommunicable diseases worldwide (World Health Organization 2006). The lack of detailed knowledge behind the underlying causes of many neurological conditions poses major hurdle in the development of treatment strategies for these conditions. Also, the modern medicine and therapies developed for mental health and neurodegenerative diseases provide only symptomatic relief and possess multiple side effects (Miyamoto et al. 2005; Salomone et al. 2012). Thus, none of the existing pharmacotherapy is known to improve neural disorders effectively. It becomes even more challenging to deal with such health issues particularly for developing countries with limited resources. Further, there is often a social stigma associated with mental disorders and they frequently go unreported and untreated for several years (Corrigan and Watson 2002). The problem of neurological conditions is further accentuated due to malnutrition and lack of proper basic health-care facilities in underdeveloped and developing nations.

Alternative medicine and use of medicinal plants may provide a viable and economical option to deal with these prevalent medical conditions (Debas et al. 2006; Benzie and Wachtel-Galor 2011). Since long the role of plants has been known to meliorate several diseased conditions. Moreover, due to traditional use history and a positive public perception, particularly with regard to the lesser or no side effects, as compared to modern medicine, makes them a popular choice (Msomi and Simelane 2018). Aromatic and medicinal plants have played indispensible role in the human health and disease prevention among human population. Of 250,000 species of higher plants known on earth, around 5000 have been known to have medicinal properties associated with them (Roy et al. 2013). There is growing evidence that essential oils derived from plants have useful therapeutic properties. Inhalation and massages using essential oils of various aromatic and medicinal plants have been a common practice as complementary and alternative medicine (Lis-Balchin 1997). Essential oils are mixtures of volatile organic compounds produced by plants as secondary metabolites. They are considered to have healing properties significant for both mental and physical health (Guenther and Althausen 1948). Essential oils and volatile fractions from many medicinal plants are known to boost brain functioning, relieve stress and anxiety, and treat neuropsychiatric issues. They have been reported to be useful in the treatment of emotional stress and psychological disorders, particularly those associated with the neurodegenerative diseases (Castillo et al. 2018; Dobetsberger and Buchbauer 2011). Identification of neuroactive essential oil constituents and their functional characterization and standardization may facilitate their use in modern medicine as well.

The present chapter gives an overview of the knowledge base with regard to plant VOCs and their use to treat neural disorders.

## 6.2 Plant VOCs

### 6.2.1 Plant VOCs – Definition

Plants produce a vast array of secondary metabolites, which play important role in the survival and interaction in their habitat. Plant secondary metabolites chemically are classified as alkaloids, terpenoids, flavanoids, and phenolics (Kabera et al. 2014). They include substances that give color to fruits and flowers, and add to the characteristic fragrances and tastes of plant. The secondary metabolites that impart smell to the plants are typically volatile in nature. VOCs are low molecular weight (< 300 Da) organic compounds, lipophilic in nature with high vapor pressure and low boiling point. VOCs are emitted by various living organisms, including microbes, plants, humans, etc. Plants being the richest source constitute nearly 90% of all VOC emissions (Maffei 2010; Rowan 2011). VOCs constitute major fraction of plant essential oils (Laird and Phillips 2012). It has been estimated that plant utilizes 36% of their assimilated carbon for the production of volatiles (Maffei 2010) and over 1700 plant VOCs have been identified so far (Dudareva et al. 2013). The plants involved in the active volatile emission mainly belong to the families including Asteraceae, Brassicaceae, Fabaceae, Lamiaceae, Polygonaceae, Rosaceae, and Solanaceae (Vivaldo et al. 2017). The term volatilome has been introduced to refer to the complete VOC emanation of a plant (Maffei et al. 2007).

### 6.2.2 Biosynthesis and Chemical Nature of Plant VOCs

VOCs are produced and secreted from almost all plant parts (Vivaldo et al. 2017). The structure and physical characteristics of VOCs are such that they are easily released to the surroundings crossing the physical barriers (Rowan 2011). Also, these volatile emissions are highly regulated in response to changing environmental conditions. Many VOCs are constitutively synthesized in plants but there levels vary in particular biotic and abiotic conditions, while several others may be only produced in response to specific stresses. Herbivory attack, pest or insect attacks, wounding, and other surrounding ecological conditions (light, temperature, drought, pH, and salinity changes) are the main factors that influence the synthesis, composition, and emission of VOCs in plants (Holopainen and Gershenzon 2010). The synthesis of VOCs diverges from many primary metabolic pathways as is true for other secondary metabolites as well. Thus, the availability of carbon, nitrogen, sulfur sources, and energy from primary metabolism also regulates the levels of secondary metabolites in any organism (Dudareva et al. 2013).

Plant synthesizes and accumulates VOCs as inert molecules or in specific secretory structures (trichomes, ducts, cavities) to avoid cellular damage to self. On the basis of their biosynthesis and structure, plant VOCs can be classified into several groups, including terpenoids – homoterpenes, monoterpenes, diterpenes, sesquiterpenes or isoprenoids (e.g., linalool, β-ionone); aromatic VOCs involving phenylpropanoids and benzenoids (e.g., eugenol); fatty acid derivatives-green leafy

volatiles (e.g., hexenals); and oxygenated VOCs (e.g., methanol, acetone). These classes of VOCs are produced via isoprenoid: 2-*C*-methyl-D-erythritol 4-phosphate and mevalonic acid, phenylpropanoid/benzenoid, and lipoxygenase pathways, respectively. In some plant species, other specialized biosynthetic pathways may also contribute to the VOC fraction of secondary metabolites (Dudareva et al. 2013; Maffei 2010).

### 6.2.3 Analysis of Plant VOCs

Different sampling techniques and detection methods are in use for the analysis of VOCs produced by plants. Various methods including Liquid Extraction (LE), Simultaneous Distillation Extraction (SDE), Soxhlet Extraction, Solid-Phase Extraction (SPE), Solid-Phase Microextraction (SPME), and Stir Bar Sorptive Extraction (SBSE) are known for plant VOCs extraction (Gyawali and Kim 2012; Kusano et al. 2013; Lubes and Goodarzi 2017; Jabbar et al. 2019). The extraction and analysis of VOCs can be done separately using Gas Chromatographic (GC) techniques such as GC-mass spectrometry (GC-MS), GC-time-of-flight-mass spectrometry (GC-TOF-MS) (Ieri et al. 2019), GC-flame ionization detection (GC-FID) (Cheng et al. 2012), GC-electroantennogram detection (GC-EAD), and 2- D GC-MS (GC × GC-MS). While technologies including Fast Isoprene Sensor Chemiluminescence detection (FIS), laser-based infrared Photoacoustic (PA) spectroscopy, or proton transfer reaction–mass spectrometry (PTR-MS) techniques enable the real-time detection of changing VOC emissions in dynamic sampling experiments. Further the annotation of spectral data collected involves the comparison with reference libraries – such as Terpenoids Library, Adams Library, NIST/EPA/NIH Mass Spectral Library, and VocBinbase (Kusano et al. 2013). Hydrodistillation followed by GC and GC/MS analysis was used for detection of volatile compounds from the leaves of Tunisian *Eucalyptus erythrocorys* L. (Ghnaya et al. 2013). Headspace-SPME-GC-MS method was employed in studying the effects of drying on the volatile fraction of *Laurus nobilis*, *Rosmarinus officinalis*, *Salvia officinalis*, and *Thymus serpyllum* (D'auria and Racioppi 2015). Direct analysis in real time (DART) allowed the identification of VOCs of various *Eucalyptus* species with temperature changes (Maleknia et al. 2009). Super critical $CO_2$ extraction method is also in use nowadays for extraction of VOCs at low temperature without the use of solvents. It reduces impurities and improves extraction efficiencies. Solid phase microextraction of headspace volatiles followed by gas chromatography, mass spectrometry, and olfactometry (SPME-HS-GC–MS–O) detection was used to study the aroma profiles of cultivars of *Viburnum opulus* and *V. opulus* var. *sargentii* fruits (Kraujalyte et al. 2012).

### 6.2.4 Functions of Plant VOCs Within Plant and Habitat

VOCs form major fraction of plant chemical constituents and play important role in their interactions and existence with their immediate surroundings. These emissions are responsible for several above ground and below ground effects while released from aerial parts and roots, respectively. The key roles of plant VOCs include herbivore defense, plant–plant signaling, attraction of pollinating agents, and repulsion to pathogenic microbes (Baldwin et al. 2002; Farre-Armengol et al. 2016; Schulz-Bohm et al. 2018; Ueda et al. 2012). Plant VOCs are known to function in plant communications with microbes, animals, and other plants in the ecosystem. They have been reported to play roles in determining the microbiome that inhabits plant surface. Some of these metabolites may serve as energy sources for microbes, while others may exert antimicrobial effects. These chemicals are also reported to aid in plant allelopathic mechanisms and are decisive in determining the consortia of other plants that grow in surroundings (Baldwin et al. 2006). Besides this, plant VOCs are known to exhibit protective roles to abiotic stresses in the host plant. They constitute important UV absorbing compounds, and thus prevent serious photo damage to the plants producing them. They are known to provide thermo-tolerance to plants. They also protect plants from various oxidative stresses (Dudareva et al. 2013).

## 6.3 Effects of Plant VOCs on Human Health

There have been several evidences of the pharmacological applications of VOCs and essential oils from different aromatic and medicinal plants. Since ancient times the smoke and fragrances from various plants have been explored for their medicinal and healing characteristics (Mohagheghzadeh et al. 2006). Inhalation of burnt plant parts, fragrances of essential oils, local application, oil steam, and baths are the major practices used in aromatherapy. The volatiles present in the oils stimulate central nervous system by olfaction (Nan et al. 2013) or through topical applications and may relieve numerous ailments like headache, stress, depression, insomnia, respiratory disorders, digestive problems, muscular and joint pains, and skin problems (Ali et al. 2015; Buckle 2014). Here we discuss some important therapeutically active plant oils and VOCs.

### 6.3.1 Antimicrobial Effects

The antimicrobial effects of different essential oils have been evaluated against numerous pathogenic bacteria and fungi. Terpenes and terpenoids fraction of essential oils from aromatic herbs are reported to be antibacterial (Nazzaro et al. 2013; Solorzano-Santos and Miranda-Novales 2012). Penalver et al. (2005) confirmed the antimicrobial activity of essential oils of *Coridothymus capitatus*, *Origanum vulgare*, *Satureja montana*, *Thymus mastichina*, and *T. zygis* against different strains

of *Escherichia coli*, *Salmonella choleraesuis, S. enteritidis*, *S. essen*, and S. *typhimurium* (Penalver et al. 2005). Various gram-positive and gram-negative human bacterial and fungal pathogens were found to be susceptible to *Cedar* leaf oil and vapors of *Thuja plicata* (Hudson et al. 2011). Lavender and neroli essential oils have shown antibacterial activities and are regarded as natural antibacterial agents (He et al. 2010). The essential oil of aromatic plant *Aristolochia delavayi* had shown resistance to the growth of various bacteria (*E. coli, Providencia stuartii*) and fungi (*Candida glabrata, C. guilliermondii*, and *Cryptococcus neoformans, Trichophyton ajelloi and T. terrestre*) (Li et al. 2013). *Petroselinum crispum* oil, rich in volatiles like apiol; 4-methoxy-6-(2-propenyl)-1,3-enzodioxole; 1,2,3-trimethoxy-5-(2-propenyl)benzene; 2,6,6-trimethyl- bicycloheptane; α-pinene, also possesses effective antimicrobial properties against several human pathogens (Mustafa 2017).

### 6.3.2 Respiratory Ailments

The healing action of aromatic essential oils from *Eucalyptus citriodora, E. globulus, Mentha piperita, Origanum syriacum*, and *Rosmarinus officinalis* in respiratory disorders is well documented (Ben-Arye et al. 2011). The volatile turmeric oil is also considered to be very effective in the treatment of respiratory disorders. Inhaling of turmeric oil vapors removes sputum, relieves cough, and asthma-related problems (Chengxiu et al. 1998). VOCs from *Chamaecyparis obtusa, Larix kaempferi*, *Pinus densiflora*, and *P. koraiensis* are also known to control the production levels of IL-4, IL-9, IL-13, and TNF-a and show anti-asthmatic effects (Ahn et al. 2018a).

### 6.3.3 Skin Healing Properties

Aromatherapy is also known to be very effective in skin allergies. Volatile compounds of *Chamaecyparis obtusa* have been proved to possess therapeutic effects against atopic dermatitis, eczema, and various other skin diseases. Volatiles from the plant reduced skin lesions by decreasing the serum IgE levels and infiltration of mast cells to the dermal and subcutaneous layers (Yang et al. 2015).

### 6.3.4 Antioxidant Properties

Many aroma components of essential oil from mint species, black cumin, cinnamon bark, ginger, thyme, and clove possess antioxidant potential (Shaaban et al. 2012). The essential oils from medicinal plants like *Artemisia dracunculus, Origanum vulgare*, *Thymus serpyllum*, and *Trianthema portulacastrum* are also regarded as natural antioxidants (Jabbar et al. 2019; Miron et al. 2010).

### 6.3.5 Quality of Life Improvement in Cancer Patients

Aromatherapy using essential oils from different medicinal plants have shown relaxing effects in cancer patients by reduced anxiety, stress, and depression (Boehm et al. 2012). Studies have reported that volatile organic compounds present in extracts of kaffir lime (*Citrus hystrix* DC.) leaves show cytotoxic effects with potential in cancer treatment (Dertyasasa and Tunjung 2017). Allyl isothiocyanate (AITC) present in various crucifers exhibits cancer chemopreventive properties (Zhang 2010).

### 6.3.6 Pain and Inflammation

The root oil of *Oxytropis glabra* DC. possesses immunomodulatory functions (Mehtab et al. 2018). The oils of *Chamaecyparis obtusa* and *Pinus densiflora* are reported to show anti-inflammatory response through their immunosuppressive activities (Ahn et al. 2018b). The volatile compounds of *Citrus aurantifolia* also possess anti-inflammatory properties (Chumsuwan 2011). The essential oils of *Chamaecyparis obtusa* are also known for their therapeutic value. The monoterpene richness in the volatile environment of *Chamaecyparis obtusa* tends to provide analgesic, antinociceptive, and anti-inflammatory effects on animal models (Park et al. 2015). The essential oils of *Lippia gracilis* have also been experimentally proved to be effective in inflammatory and nociceptive problems, mediated by cholinergic receptors and inhibition of nitric oxide and prostaglandin E2 production (Guilhon et al. 2011). Inhaling of Lavender essence is seen to be useful for the treatment of pain after cesarean section (Olapour et al. 2013).

### 6.3.7 Other Health Benefits

The sniffing of oils provides mental and physical reliefs (Kim et al. 2018). Essential oil of *Alpinia zerumbet* is known to have antihypertensive effects (Lahlou et al. 2003). Inhalation of volatile organic compounds extracted from *Chrysanthemum indicum* decreased the blood pressure and heart rate in subjects. Blood oxygen saturation increased, systolic and diastolic blood pressure and heart rate reduced on inhaling volatiles from *C. deodara*, thereby providing relaxing effects (Song et al. 2016). Essential oils are also known to pacify gastrointestinal problems and ulcers (Rozza and Pellizzon 2013). Histopathological studies have indicated the role of essential oil of fennel (*Foeniculum vulgare*) in the treatment of liver damage as well (Ozbek et al. 2003). Another study has shown the relaxing effects of volatiles from *Platycladus orientalis* (Wang et al. 2010).

## 6.4 Neurological Health and Plants VOCs

### 6.4.1 Neurological Disorders

Increasing incidence of noncommunicable diseases especially neurological and behavioral disorders has forced new challenges on health-care systems. The mental and neurological disorders often remained neglected as traditionally mortality impact of disease was given due importance. This has resulted in lack of facilities related to the neurological disorders at all levels of health care. It is only recent that the neurological and psychological health issues have been considered by policy makers and health-care units as important causes of disabilities around the world (World Health Organization 2006).

As per the world health report, 2001 presented by WHO on mental health and neurological disorders, there are approximately 450 million people around the globe suffering from one or other neurological problem representing about 12% of global load of diseases. Further, the proportion of neurologic and psychiatric disorders is expected to rise up to 14.7% by 2020 (Menken et al. 2000). Neurological disorders account for 31% of all years of life lived with a disability. The common neuropsychiatric conditions include depression, schizophrenia, epilepsy, Alzheimer's, bipolar disorders, and other dementias (Saraceno 2002). Strokes are major cause of mortality among neurological disorders and are considered as the third most common cause of deaths in western countries next to coronary heart disease and cancer (Poungvarin 1998).

The statistics of these disorders are even worse in developing countries due to malnutrition and cognitive problems related to parasitic infections (Bergen and Silberberg 2002; Singhal 1998). It is estimated that primary epilepsy alone affects 37 million people globally, and more than 80% of them belong from developing countries (Leonardi and Ustun 2002). The lack of treatments in developing nations further accentuates the problem. Majority of persons suffering from mental health issues can lead a normal life if properly treated. Thus, it points out to an immediate need to seek new remedies which are cheap and affordable to tackle these problems especially in developing countries of the world. Plant-based medicine could offer economical ways to deal with such issues as phytomedicines have served as major resources in health-care practices since time immemorial.

### 6.4.2 Essential Oils, and Their Volatiles – Role in Neurological Health

Ethnobotanical evidences have suggested the role of many plants and their parts in human neurological health. There are rising evidences for the use of phytochemicals and plant-derived products in psychological problems related to neurodegenerative diseases (Amoateng et al. 2018; Balkrishna and Misra 2018; Santos-Neto et al. 2006). Many essential oils and their volatile active constituents are known to improve brain functioning and possess neuroprotective effects (Fig. 6.1, Table 6.1)

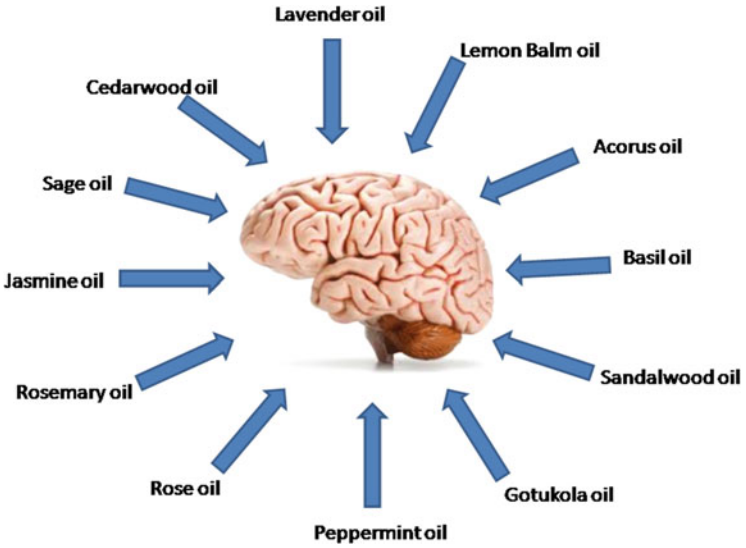


**Fig. 6.1** Essential oil with neuroactive properties

(Abuhamdah and Chazot 2008; Kuriyama et al. 2005). Some of the most effective commonly used oils in neurotherapies include essential oils from lavender, thyme, sage, cedarwood, and lemon balm (Koutroumanidou et al. 2013; Orhan et al. 2008).

#### 6.4.2.1 Acorus

The essential oils of *Acorus gramineus* and *Acorus calamus* are rich in volatiles: β-asarone and α-asarone (Cho et al. 2002; Raina et al. 2003). They are used to treat insomnia, melancholia, neurosis, epilepsy, hysteria, loss of memory, depression, and other mental disorders. Inhalation of volatile oil of *Acorus gramineus* enhances the learning capabilities in Alzheimer's induced rats. It brings about the reduction in malondialdehyde content and increases superoxide dismutase and glutathione peroxidase activities (Liu et al. 2010). The oil inhalation or oral dosage causes sedative effects. It inhibits central nervous system by GABAergic system causing increase in GABA levels by inhibiting GABA transaminase and causes anticonvulsive effects by inhibition of lipid peroxidation (Koo et al. 2003). The neuroprotective potential of essential oils from *A. gramineus* was studied on cultured cortical neurons. It was concluded that these effects resulted by the blocking of NMDA (*N*-methyl-D-aspartate) receptors (Cho et al. 2001). Essential oil of *Acorus calamus* is experimentally proved to possess acetylcholine esterase (AChE) inhibitory potential. They show memory enhancement and repair cognitive dysfunctioning in several dementia (Mukherjee et al. 2007).

#### 6.4.2.2 Basil

Essential oil of holy basil, *Ocimum basilicum*, is also well credited for its neuroprotective functions. GC/MS analysis of essential oils of *O. basilicum* has

**Table 6.1** Essential oils – their source plants, volatile constituents, and their neuroactive properties

| Essential oil | Plant source | Volatile component/s | Therapeutic/biological activity | References |
|---|---|---|---|---|
| Lavender | *Lavandula angustifolia* | Linalool and linalyl acetate | Inhibits the release of acetylcholine and changes ion channel functions at the neuromuscular junctions; relieves tension and stress; acts as sedative: anticonvulsive, anxiolytic, analgesic and neuroprotective agent | Cavanagh and Wilkinson (2002), Koulivand et al. (2013) and Sayorwan et al. (2012) |
| *Lemon balm* | *Melissa officinalis* | Citronellal, citronellol, b-caryophyllene, (E)-citral, (Z)-citral, geraniol, (Z)-b-ocimene and 1-octen-3-ol. | Relieves severe dementia, insomnia, anxiety and stress-related problems; used in the treatment of Alzheimer's disease | Bagdat and Cosge (2006), Ieri et al. (2017), Miraj et al. (2016) and Soodi et al. (2014) |
| Acorus oil | *Acorus gramineus* and *Acorus calamus* | β-asarone and α-asarone | Shows anticonvulsive effects, central nervous system inhibitory effects, acteylcholinesterase inhibitory potential; treats insomnia, melancholia, neurosis, epilepsy, hysteria, loss of memory, depression, and other mental disorders | Cho et al. (2002), Koo et al. (2003) and Mukherjee et al. (2007) |
| SuHeXiang Wan (Stroax pill) | Made of 15 crude herbs – mainly *-Liquidambar orientalis, Saussurea lappa, Aquilaria agallocha, Santalum album, Boswellia carterii, Eugenia caryophyllata, Styrax benzoin, Dryobalanops aromatica* and *Cyperus rotundus* | Endo-borneol, borneol, eugenol, eugenyl acetate, benzyl benzoate, fenchol, camphor, octyl acetate, b-caryophyllene, benzyl cinnamate | Treats epilepsy, convulsions, depression, seizures and strokes; has inhibitory action on CNS via the GABAergic system | Hong et al. (2011), Koo et al. (2004) and Liang et al. (2018) |

(continued)

**Table 6.1** (continued)

| Essential oil | Plant source | Volatile component/s | Therapeutic/biological activity | References |
|---|---|---|---|---|
| Basil | *Ocimum basilicum* | 1,8-cineole, eugenol, linalool, methyl chavicol, methyl cinnamate, bergamotene, α-humulene, α-bergamotene, α-terpineol, caryophyllene, limonene, α-pinene, β-pinene, myrcene, camphene, bornyl acetate, 2-carene, | Possesses anticonvulsant, antidepressant, antianxiety, anti-headache, neuroprotective activities | Askari et al. (2016), Cha et al. (2010), Ismail (2006), Rabbani et al. (2015) and Tadros et al. (2014) |
| Sandalwood | *Santalum album* | Z-α-santalol, epi-β-santalol, α-santalene, β-santalene | Treats insomnia and stress; enhances memory potential; has sedative, calming, and relaxing effect | Kumar et al. (2015), Misra et al. (2013), Misra and Dey (2013), Okugawa, et al. (1995) and Subasinghe et al. (2013) |
| Gotukola | *Centella asiatica* | a-humulene, b-caryophyllene, bicyclogermacrene, germacrene B, gemacrene D, myrcene, γ-terpinene, *α.*-pinene, p-cymene | Promotes dendrite arborization and elongation; prevents apoptosis in neurons, energizes nervous system, is antiepileptic, and antidepressant | Ahuja et al. (2017), Francis and Thomas (2016), Gohil et al. (2010), Oyedeji and Afolayan (2005) |
| Peppermint | *Mentha* × *piperita* | Menthol, methyl acetate, neomenthol, menthone, isomenthne, menthofuran | Relieves anxiety, stress, and common headache problems | Ayaz et al. (2017), Edris and Farrag (2003), Gobel et al. (1994), Meamarbashi (2014) and Rohloff (1999) |
| Rosemary | *Rosmarinus officinalis* | 1,8-cineol, camphor, borneol, α-terpineol, bornyl acetate, β-caryophyllene and δ-cadinene | Enhances mental health; relieves stress, anxiety, and improves self-esteem | Boutekedjiret et al. (2003), Pengelly et al. (2012), Rho et al. (2006) and Zanella et al. (2012) |
| Rose | *Rosa damascene* | Citronellol, nonadecane, geraniol, ethanol, heneicosane, nerol and 1nonadecene | Affects sympathetic activity; possesses anticonvulsant and relaxing properties; shows protective effects in neuritic atrophy | Baydar (2006), Bayrak and Akgul (1994), Boskabady et al. (2011), Hongratanaworakit (2009) and Zhu et al. (2017) |

| | | | | |
|---|---|---|---|---|
| Sage | *Salvia officinalis; Salvia lavandulaefolia* | α-thujone, β-thujone, camphor and 1,8-cineole | Treats depression, dementia, and memory loss; effective in Alzheimer's | Asllani (2000), Hamidpour et al. (2014), Savelev et al. (2004) and Sharma and Schaefer (2019) |
| Jasmine | *Jasminum gradiflorum* | Benzyl acetate, benzyl benzoate, phytol, linalool, isophytol, geranyl linalool, methyl linoleate, eugenol, cis-jasmonate and methyl jasmonate | Shows tranquilizing effects on brain activity | Adebesin et al. (2017), Bera et al. (2015), Jirovetz et al. (2007) and Umukoro et al. (2018) |
| Cedarwood | *Juniperus virginiana* | a-cedrene, b-cedrene, thujopsene, cedrol, widdrol | Possesses sedative effects and affects central nervous system by modulating dopamine and 5-hydroxytryptamine levels | Dayawansa et al. (2003), Eller and King (2000) and Zhang and Yao (2018) |

shown linalool, 1,8-cineol, eugenol, methyl cinnamate, α-caryophyllene, and α-cubebene as the major components (Ismail 2006; Mahajan et al. 2015). It is effective against variety of neurodegenerative problems like strokes, anxiety, headaches, nervous pain, and convulsions, and plays role in memory enhancement and motor coordination. Such effects are induced by the essential oils of basil by the reduction in cerebral infract size and lipid peroxidation, and increased glutathione levels (Bora et al. 2011). The efficiency of *Ocimum basilicum* oil was observed to treat depression in animal models by behavioral, biochemical, and histopathological changes (Ali et al. 2017). The extracts of the plant are known to potentiate anxiolytic and sedative effects as well (Rabbani et al. 2015). Central nervous system depressant and anticonvulsive properties of oil are mediated through central GABAergic receptors (Oliveira et al. 2009). Aromatherapies using the essential oils of *Ocimum basilicum* result in the effective decrease of headaches, stress, and anxiety, and reduce serum cortisol levels (Cha et al. 2010). The oil is effective in treating migraine, insomnia, and depression (Marwat et al. 2011). The essential oil from the plant alleviates neuronal atrophy, increases neurogenesis in hippocampal regions, and induces antidepressant effects (Ayoub et al. 2017). Volatile oils from the plant are also experimentally proved to be effective against Alzheimer's disease by the inhibition of acetylcholinesterase (AChE) (Tadros et al. 2014). Moreover, *Ocimum basilicum* is known to induce all the neuroprotective roles without exerting any type of cellular toxicity (Askari et al. 2016).

#### 6.4.2.3 Cedarwood

*Juniperus virginiana* (Cedar wood) oils contain a-cedrene, b-cedrene, thujopsene, cedrol, and widdrol as important volatile compounds (Eller and King 2000; Zhang and Yao 2018). The oil and its active constituent, cedrol, have got sedative effects and also affect autonomic nervous system (Dayawansa et al. 2003; Kagawa et al. 2003). The essential oil from *Juniperus virginiana* has shown anxiolytic effect in mice by affecting dopamine and 5- hydroxytryptamine levels (Zhang and Yao 2018).

#### 6.4.2.4 Gotukola

Therapeutically used as a brain tonic in Ayurvedic medicine, *Centella asiatica* (gotukola) is rich in volatile compounds including a-humulene, b-caryophyllene, bicyclogermacrene, germacrene B, gemacrene D, myrcene, γ-terpinene, α.-pinene, and p-cymene (Francis and Thomas 2016; Oyedeji and Afolayan 2005). *Centella asiatica* facilitates neuroprotection through different modes of action. It is known to prevent formation of amyloid plaques in Alzheimer's and reduces dopamine neurotoxicity in Parkinson's disease. It also reduces oxidative stress and improves brain functioning (Chandrika and Kumara 2015; Orhan 2012; Tiwari et al. 2011). It is used to treat mental illness, various nervine problems, Parkinsonism, insomnia, hysteria, epilepsy, depression, and headache (Hashim 2011; Jamil et al. 2007; Singh et al. 2010). *C. asiatica* has been shown to repair oxidative damages causing cognitive deficits and Alzheimer's disease (Veerendra Kumar and Gupta 2003). Gotukola is seen to exhibit anxiolytic properties in humans (Bradwejn et al. 2000; Gohil et al.

2010; Wijeweera et al. 2006). Extracts of *C. asiatica* show anticonvulsant activity through cholinergic system by maintaining the concentrations of acetylcholine and acetylcholinesterase (Visweswari et al. 2010). It promotes neural regeneration, dendrite elongation, and arborization; prevents apoptosis in neurons; and energizes the brain and nervous system (Soumyanath et al. 2005). *C. asiatica* is known to affect brain functioning, shows memory improvement, neuroprotective and neuroregenerative activities (Lokanathan et al. 2016; Prakash and Kumar 2013). The herb is known to act through several mechanisms including reduced phospholipase $A_2$ activity, altered acetylcholinesterase activity, increased GABA levels, reduced ß-amyloid content, and enhanced oxidative defense (Ahuja et al. 2017; Gray et al. 2017; Puttarak et al. 2017).

#### 6.4.2.5 Jasmine

Essential oils from *Jasminium gradiflorum* (Jasmine) are rich in volatile components like benzyl acetate, benzyl benzoate, phytol, linalool, isophytol, geranyl linalool, methyl linoleate, eugenol, cis-jasmonate, and methyl jasmonate (Bera et al. 2015; Jirovetz et al. 2007). The olfactory stimulation of Jasmine oils generates relaxing and antidepressant effects by triggering neuroendocrine response (Aswini Dutt et al. 2011). Volatile components of jasmine essential oils are reported to alleviate stress depression by modulating corticosterone and oxidative stress in mice. They are known to decrease neuroinflammation as well (Adebesin et al. 2017). The plant oil reported to have tranquilizing effects on brain upon inhalation by numerous neurochemical mechanisms (Hossain et al. 2004; Umukoro et al. 2018).

#### 6.4.2.6 Lavender

The neurological effects of lavender oil are well known since ages. Lavender includes many species of genus *Lavandula* (family – Lamiaceae), of which the most commonly cultivated as well as therapeutically important species is *Lavandula angustifolia* (Cavanagh and Wilkinson 2002; Shahdadi et al. 2017; Woronuk et al. 2011). The major volatile components present in the essential oils of lavender are linalyl acetate and linalool (Prusinowska and Smigielski 2014; Tschiggerl and Bucar 2010; Worounk et al. 2011). Inhaling lavender oil is known to have relaxing effects. It possesses excellent mood stabilizing and sedative properties (Hanson et al. 2013; Koulivand et al. 2013). The neuroprotective potential of lavender oil has been shown in cerebral ischemia/reperfusion injury in mice (Wang et al. 2012). Lavender oil affects brain activity and autonomous nervous system (Sayorwan et al. 2012). It reduces mental stress, convulsions, anxiety, and depression (Field et al. 2005; Motomura et al. 2001). The mode of action of lavender oil in brain functioning is proposed to be related to the intensification of antioxidant defense like rise in the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) caused by its active biomolecules (Vakili et al. 2014). Besides the antioxidant properties, the oil components are also known to modulate NMDA receptors and serotonin transporters (Lopez et al. 2017).

#### 6.4.2.7 Lemon Balm

*Melissa officinalis* has got many historical evidences of its medical uses. These aspects of the plant are attributed to presence of active volatile principles like citronellal, α-citral, and β-caryophyllene (Ieri et al. 2017). Neurological effects of lemon oil treatment are very well studied. Balm tea is known for affecting numerous behavioral aspects by modulating brain functioning. The plant is widely applied as anti-anxiolytic and anti-Alzheimer's drug. It is effective against seizures, headaches, insomnia, and epilepsy (Miraj et al. 2016; Zarei et al. 2015). It is shown that *M. officinalis* can control both the mood and cognitive functions as it has got cholinergic receptor-binding properties (Kennedy et al. 2003). Many clinical trials have evaluated the positive effects of *M. officinalis* on emotional problems in patients suffering from dementia (Abuhamdah and Chazot 2008; Bagdat and Cosge 2006). Research has suggested that the essential oil from *M. officinalis* enhances cell proliferation and neuroblast differentiation in hippocampus part of mouse brain by reduction in serum corticosterone levels as well as increase in GABA (gamma-amino butyric acid) neurotransmitter (Yoo et al. 2011). Lemon balm acts as a neuroprotective agent of cognitive and behavioral dysfunctions by inhibiting acetylcholinesterase enzyme (AChE) (Eudes et al. 2017; Zarei et al. 2015). The memory improving effects of the plant are also experimentally proved (Soodi et al. 2014).

#### 6.4.2.8 Peppermint

Peppermint (*Mentha* × *piperita*) is known to possess numerous volatile compounds, mainly menthol, menthyl acetate, neomenthol, menthone, isomenthone, menthofuran, α-pinene, β-pinene, limonene, and 1,8-cineol (Edris and Farrag 2003; Rohloff 1999). Essential oils from mint are known to effectively relax mental fatigue and possess psychoactive potential (Umezu et al. 2001). Aroma of peppermint enhances concentration and helps in improvement of working memory, virtual recognition memory, and visual-motor responses (Meamarbashi 2014). The hot water infusions of leaves of peppermint have been shown to affect motor coordination, behavior, motility, and barbiturate induced sleep in mice (Della Loggia et al. 1990). Consumption of peppermint tea suggests its analgesic and sedative effects on the central and peripheral nervous system (McKay and Blumberg 2006). Peppermint oils relieve from headache and migraine issues and show antidepressant effects (Abbasi-Maleki et al. 2017; Gardier 2000; Gobel et al. 1994). They help to repair cognitive dysfunctioning through cholinergic effects, calcium regulation, and $GABA_A$/nicotinic receptor binding properties (Kennedy et al. 2018). Peppermint shows inhibition of AChE activity and oxidative stress suggesting their possible application in the treatment of various neurodegenerative disorders (Ayaz et al. 2017; Vladimir-Knezevic et al. 2014).

#### 6.4.2.9 Rose

*Rosa damascena* is a source of compounds like citronellol, nonadecane, geraniol, ethanol, heneicosane, nerol, and 1-nonadecene (Babu et al. 2002; Baydar 2006; Bayrak and Akgul 1994; Naquvi et al. 2014). In traditional system of folklore

medicines rose oils were known to have CNS modulatory effects. Inhalation of floral essence of roses influences physiological and psychological behaviors (Hongratanaworakit 2009; Igarashi et al. 2014). Rose oils are long been used to treat depression, stress, and grief. They are known to relieve nervous tension. Therapies using rose oil vapors heal headaches and migraine problems. Rose oils are reported with hypnotic, anxiolytic, and anticonvulsant properties (Boskabady et al. 2011; Bradley et al. 2007). *R. damascena* is known to possess neuroprotective abilities. Rose essence and extracts are known to treat epileptic seizures and prevent the formation of dark neurons in hippocampus regions in pentylentetrazol (PTZ)-induced rats (Homayoun et al. 2015; Kheirabadi et al. 2008). In stressed rats, olfactory stimulation by rose essential oils was reported to inhibit stress-induced hypothalamic paraventricullar nucleus activity and hypothalamo-pituitary-adreno-cortical axis activation (Fukada et al. 2011; Matsukawa et al. 2011; Rakhshandah et al. 2010). Also, CNS modulatory effects of rose oils were attributed to its GABAergic activity (Maleki et al. 2013; Nyeem et al. 2006). Clinical trials have shown that the application of rose oil improves sexual dysfunctioning in males suffering from depression (Farnia et al. 2015). Rose essential oil has also been shown to possess protective effects on neuritic atrophy associated with neurodegenerative diseases like Alzheimer's, through inhibition of Aβ deposition and SKN-1 signalling pathway (Zhu et al. 2017).

#### 6.4.2.10 Rosemary

*Rosmarinus officinalis* essential oils are rich in volatile compounds like 1,8-cineol, camphor, borneol, α-terpineol, bornyl acetate, β-caryophyllene, and δ-cadinene (Boutekedjiret et al. 2003; Ibanez et al. 1999; Porte et al. 2000). Rosemary is known to be effective against anxiety, depression, and insomnia (Nematolahi et al. 2018). A research carried on a group of secondary school students with essential oil of rosemary had shown positive influence of inhalation on memorization of images and numbers (Filiptsova et al. 2017). *R. officinalis* maintains the levels of AChE and avoids hypoglycemia resulting in antidepressant effects in mice. It suggests the possible role of the plant for the treatment of depression and stress (Machado et al. 2012). The low doses of rosemary are also reported to be effective in enhancements of memory and cognition abilities over a long time (Pengelly et al. 2012). The olfactory stimulation with rosemary oil improves mental health, cognitive performance and relaxes from anxiety. Aromatherapy massages using the oils of rosemary positively modulate mood and self-esteem (Moss et al. 2003; Rho et al. 2006). In another study, extracts of rosemary have been shown to influence short-term and long-term memory in mice in social recognition and inhibitory avoidance tasks. It thus improves learning and memorizing ability (Zanella et al. 2012). Rosemary essential oils also possess anti-inflammatory and antinociceptive activities (Takaki et al. 2008). Inhalation of rosemary aroma was seen to improve the cognition performance by different neurochemical ways (Moss and Oliver 2012).

#### 6.4.2.11 Sage

*Salvia officinalis* and *Salvia lavandulaefolia* (sage) oils contain major volatiles including α-thujone, β-thujone, camphor, 1,8-cineole, and pinenes (Asllani 2000; Bernotiene et al. 2007; Herraiz-Penalver et al. 2010; Perry et al. 1999; Pierozan et al. 2009; Tucker and Maciarello 1990). Various studies have suggested the pharmacological role of *Salvia* species against depression, anxiety, and dementia (Hamidpour et al. 2014; Kennedy et al. 2006). Aromas and extracts of *Salvia* species have profound effects on cognitive performances, memory retention, and mood (Eidi et al. 2006; Moss et al. 2010; Tildesley et al. 2005). The individual components of *Salvia* species and their extracts have been shown to inhibit butyrylcholinesterase and acetylcholinesterase, which is helpful in maintaining good psychological health. It potentiates its use for the treatment of dementia caused due to Alzheimer's (Akhondzadeh et al. 2003; Kennedy et al. 2011; Savelev et al. 2004). *S. lavandulaefolia* and *S. officinalis* oils have been shown to reduce various neuropsychiatric symptoms (Perry et al. 2003; Perry et al. 2018; Sharma and Schaefer 2019).

#### 6.4.2.12 Sandalwood

Essential oils of sandalwood (*Santalum album*) have a long history of being used as medicine. The sandalwood oils are described to be rich in aromatic compound santalols (Subasinghe et al. 2013). Analysis of sandalwood essential oils have shown the presence of oxygenated sesquiterpenoids, that is, Z-α-santalol and epi-β-santalol as the major volatile fraction (Celedon et al. 2016; Hongratanaworakit et al. 2004; Misra et al. 2013). The scent of sandalwood is widely used to release nervous tension and relax mind. Sandalwood has been used in traditional systems (Ayurveda, Unani, Siddha) of medicine to treat depression, anxiety, stress, insomnia, and mental disturbances. It is also known to have memory enhancement potential (Kumar et al. 2015; Misra and Dey 2013). Sandalwood constituents are reported to affect central nervous system by effectively increasing the concentrations of homovanillic acid, 3,4-dihydroxyphenylacetic acid, and/or 5-hydroxyindoleacetic acid in the brain of mice (Okugawa et al. 1995). They are also known to decrease the enhanced levels of prostaglandin E2 and thromboxane B2 in stress and depression (Rajsmita and Keshavamurthy 2019).

#### 6.4.2.13 Su-He-Xiang-Wan

Su-He-Xiang-Wan (SHXW or storax pill) is a Chinese traditional herbal formulation used for the treatment of convulsions, seizures, strokes, pains, loss of consciousness and related problems. The formulation is made up of extracts from 15 different medicinal plants, mainly *Aquilaria agallocha, Boswellia carterii, Cyperus rotundus, Dryobalanops aromatic, Eugenia caryophyllata, Liquidambar orientalis, Santalum album, Saussurea lappa*, and *Styrax benzoin.* It contains numerous volatile active constituents of which endo-borneol and borneol are most abundant (Koo et al. 2004; Wang et al. 2013). The essential oil from storax pill inhibits central nervous system via GABAergic mechanism. The inhalation causes sedative effects, inhibits lipid peroxidation, and is effective for epilepsy and convulsions (Koo et al. 2004). It is

used to treat seizures, strokes, depression, and stress. The formulation had shown positive effects on the neurological health of Alzheimer's affected *Drosophila* models (Hong et al. 2011; Park et al. 2013). SuHeXiang Wan essential oil was seen to alleviate amyloid-β induced memory impairments by inhibiting the phosphorylation of tau protein in mice (Jeon et al. 2011a, b). SHXW inhalation shows excellent antidepressant and anxiolytic properties (Liang et al. 2018). SHXW shows effective neuroprotection against Aβ-induced toxicity. It possesses antioxidative effect and suppresses apoptosis. It can thus be used against many neurodegenerative disorders (Hur et al. 2013).

## 6.5 Conclusion

Increasing incidences of neurodegenerative problems and no effective treatments against them have forced the need of exploring novel resources for drug designing. The small lipophilic volatile organic compounds (VOCs) from plants offer vast scope to treat brain health related problems. Due to their small size, plant VOCs effectively penetrate through blood–brain barriers and mediate neural affects. Essential oils rich in VOCs from many plants are reported to be in use for many mental and psychological issues. Thus, there is need to identify and isolate such plant VOCs and evaluate their bioactivities. To find novel psychoactive drugs, studies are needed to find out the mechanism of action of active plant constituents, particularly responsible for neuroprotective and neuroregenerative potential of various medicinal herbs. Furthermore, new strategies need to be designed to overcome the limitations associated with plant VOCs emissions. Variations in the plant VOCs profiles due to the extraction methods, spatial and temporal effects, influence of geographical parameters, and several other factors is a common phenomenon. Thus, standardization of these influencing factors is needed to obtain desired plant metabolite in sufficient extents. Also the use of genetic engineering tools on medicinal plants to obtain a targeted product can facilitate designing of reliable and cost-effective modern medicine. The blending of traditional knowledge of plants from ethnobotanical studies, recognition of therapeutically active molecules, and research on basic aspects of brain functioning in normal and diseased conditions could pave a way to remediate neurological problems.

**Acknowledgments** RC and SA are thankful to CSIR for Senior Research Fellowship. SGG acknowledges CSIR-IIIM for support in compilation of the manuscript. RC is also thankful to Ms. Mansavi Bhardwaj and Ms. Sonali Thakur for their help in formatting the references.

**Conflict of Interest** The authors declare no conflict of interest.

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# Medicinal Plants and Their Role in Inflammation: A Close Look on Future Drug Discovery

7

Gifty Sawhney, Satinder Kaur, Asha Bhagat, and Zabeer Ahmed

**Abstract**

Inflammation is a salubrious process resulting from a number of perturbances. It plays a protective role in our body and in some conditions engenders some negative effects such conditions include the inflammatory disorders rheumatoid arthritis, osteoarthritis, inflammatory bowel diseases, retinitis, multiple sclerosis, psoriasis, and atherosclerosis. For surmounting this quandary, the search for more incipient drugs is very requisite and obligatory, and there are many of phytochemical constituents present in plants which are playing a very paramount role in the treatment of inflammation. The present chapter shows some plant phytochemicals which are having anti-inflammatory activity that has been tested in inflammatory models utilizing the modern scientific techniques. In this chapter, the inflammatory activity of different species of medicinal plants are explained which mainly include Apiaceae, Asteraceae, Berberidaceae, Burseraceae, Caesalpinaceae, Capparidaceae, Chenopodiaceae, Combretaceae, Euphorbiaceae, Lamiaceae, Lauraceae, Moringaceae, Myricaceae, Myrtaceae, Oleaceae, Papaveraceae, Pinaceae, Ranunculaceae, Rutaceae, Sapindaceae, Thymelaeceae, and Verbenaceae. The major chemical constituents present in these anti-inflammatory plant species include azadiradione, flavonol, gallic

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_7

acid, gentisic acid, kaempferol, nimbin, 3-o-galloyl-(-)-epicatechin-4-benzylthioether, pinene, ricinoleic acid, thujone, and several other important bioactive compounds. These compounds play a significant role in current research and help a lot in developing new formulations for herbal botanicals and ongoing current pharmacological research.

**Keywords**

Medicinal plants · Inflammation · Phytochemicals · Biological activities

## Abbreviations

| | |
|---|---|
| % | Percentage |
| i.e. | That is |
| μM | Micromolar |
| μl | Microliter |
| ml | Milliliter |
| cm | Centimeter |
| kg | Kilogram |
| Bcl-xL | B-cell lymphoma-extra large |
| BCL2 | B-cell lymphoma 2 |
| COX | Cyclooxygenase |
| IL-1 | Interleukin-1 |
| LTC4 | Leukotriene C4 |
| LTB4 | Leukotriene B4 |
| LPS | Lipopolysaccharide |
| NSAIDs | Nonsteroidal anti-inflammatory drugs |
| NF-Πb | Nuclear Factor kappa-light-chain-enhancer of activated B cells |
| p38 MAPK | p38 mitogen-activated protein kinases kinase |
| pTEN | Phosphatase and tensin homolog |
| PI3K/ATK | Phosphatidylinositol 3-kinase/protein kinase B |
| PPAR | Peroxisome proliferator-activated receptors |
| PGE2 | Prostaglandin |
| PGI2 | Prostacyclin |
| TPA | Tissue plasminogen activator |
| TNF-α | Tumor necrosis factor-A |
| VEGFR-2 | Vascular endothelial growth factor receptor-2 |
| WHO | World Health Organization |

## 7.1 Introduction

The World Health Organization (WHO) estimated 80% of developing nations rely on traditional medications for their main health care (Palhares et al. 2015). In the health care systems of the remaining 20% population, plant products also play a

significant role among citizens of advanced nations (Palhares et al. 2015). The scientific data produced by plant studies is a useful instrument for defining plant species and characterizing the active pharmacological constituents for their biological activities. It is always essential to screen a new plant in search of their medicinal purpose. Once the plant has been recognized for positive biological activity, it is essential to obtain supporting scientific data produced by investigating plant's pharmacognostic and phytochemical characteristics.

Inflammation is the reactive state of hyperemia and blood vessel exudation with consequent redness, heat, swelling, and pain manifested by tissue in replication to physical or chemical injury or bacterial incursion. It is a body-to-injury tissue reaction and involves a complex range of activation of the enzyme, mediating release, fluid extravasations, cell migration and repair, a tissue reaction by the body toward injury. The most common form of chronic inflammatory joint disease is rheumatoid arthritis. Arthritis is one of the medical practice's most distressing and disabling syndromes. It affects an estimated 1–2% of the adult population (Okoli et al. 2003). About 0.1% of the population experiences rheumatoid arthritis in childhood in the Coalesced States. Steroids, for example, betamethasone, and nonsteroidal anti-inflammatory drugs (NSAIDs), for example, acetylsalicylic acid, are the pillars of inflammatory and inflammatory disease treatment/management (Allison et al. 1992; McCarthy 1991). These agents, however, are burdened with severe adverse effects such as steroid adrenal suppression, gastric ulceration, and NSAID perforation. Most NSAIDs are known to affect the gastrointestinal tract in potentially adverse manner. These have severely restricted the use of these agents in the treatment of inflammation and inflammatory diseases. There have been several efforts to reduce the negative effects of NSAIDs. Cyclooxygenase (COX) enzyme is now accepted as being present in two isoforms: COX-I (constitutive) and COX-II (inducible). NSAIDs therapeutic activities are attributed to COX-II inhibition.

Therefore, an ideal anti-inflammatory drug is expected to inhibit COX II-mediated synthesis of prostaglandin while reducing COX-I inhibition, which is believed to mediate the side effects. Just as selective COX-II inhibitors such as Celebcoxib and Rofecoxib appear to be cost-effective for patients at high risk of ulcer complications, serious theoretical concerns exist due to the potential risk of thrombosis (Smith et al. 1994; Brooks and Day 2000). And so, although arthritis is one of the oldest known diseases, there is still no drug that leads to permanent cure without adverse effects. Nature provides medicinal plants to the world to take care of health needs. Plant potentials have long been recognized as sources of drugs. In traditional medicine, several species of medicinal plants are commonly used as inflammatory remedies. In approximately every family in the plant kingdom, there are representative anti-inflammatory herbs. Many of these plants have proven their use in the treatment of inflammatory disorders in conventional medicine by oral and documented evidence. For some plants, other identified, pharmacological activity's cognate to modulation of the intricate inflammatory replication innate anti-inflammatory activity is inferred. There is currently mounting scientific evidence for many herbs' anti-inflammatory activity. Figure 7.1 demonstrates the pictorial representation of the technique involved in the pharmacological research.

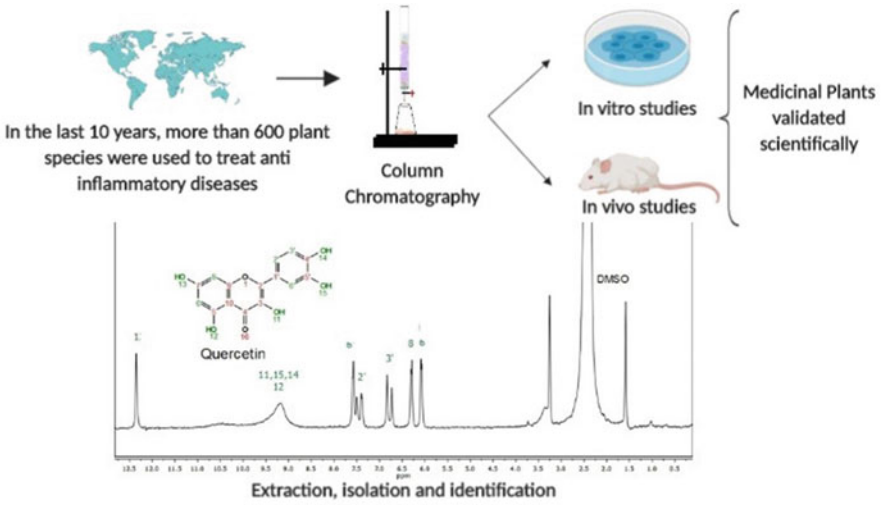



**Fig. 7.1** Pictorial representation of technique involved in pharmacological research

## 7.2 Research Methodology

The information presented were investigated from high valued medicinal plants growing in India and validated by research published and recorded through searched engines such as PubMed, ScienceDirect, and Google Scholar. The entire articles related to our objective were collected and classified on the basis of evidence, which showed the highest values in systematic reviews and randomized control studies. The information regarding accepted and valid names of different plant species with the reported anti-inflammatory activity were cross-checked with the web-database, Tropicos (www.tropicos.org) and The PlantList (www.theplantlist.org). The chemical structures of various phytoconstituents were drawn with the help of chemical and biological drawing software, namely ChemDraw Ultra 12.0. As indicated in Fig. 7.1, the pictorial representation of technique involved in pharmacological research was drawn with the help of a web-based tool, namely BioRender (https://biorender.com).

## 7.3 Results and Discussion

### 7.3.1 Plants Used as Anti-inflammation

Overall, this study demonstrates the significant anti-inflammatory activity of various important medicinal plant species. Nature provides medicinal plants to the world in order to take care of health-care needs. Several species of medicinal plants are commonly used as inflammatory remedies in conventional medicine. Some widely

distributed species of medicinal plants is listed in Table 7.1. A number of active principles have been isolated and recognized in an ongoing attempt to create more efficient anti-inflammatory agents from plant sources. Figure 7.2 depicts various chemical structures like azadiradione, flavonol, gallic acid, gentisic acid, kaempferol, nimbin, 3-o-galloyl-(-)-epicatechin-4-benzylthioether, pinene, ricinoleic acid, and thujone present in various anti-inflammatory plants.

#### 7.3.1.1 *Achillea millefolium*

This plant species is a common herb of both dry and wet regions, such as roadsides, meadows, fields, and coastal areas and is native to temperate northern hemisphere regions in Asia, Europe, and North America. The leaves are distributed evenly along the stem, the largest being the leaves close to the middle and bottom of the stem. The leaves are 5–20 cm long, bipinnate or tripinnate, nearly feathery, and spirally arranged on the stems. The degrees of hairiness (pubescence) of the leaves vary from altitude to altitude. Yarrow oil (*Achillea millefolium*) has a good anti-inflammatory activity and is used for the treatment of rheumatism, muscle aches, and inflamed cuts or wounds. Its phytochemical component, Flavonoids, has anti-inflammatory activity along with an effect on prostaglandin production. Azuline is another powerful anti-inflammatory component which accounts for nearly half of the chemical composition of *A. millefolium* (Tunón et al. 1995).

#### 7.3.1.2 *Aconitum heterophyllum*

This plant species has a tremendous amount of medicinal consequences. It has been used in some formulations in India's traditional healing scheme, that is, Ayurveda, for very old ages. It is reported to have used in treating patients with urinary infections, diarrhea, and inflammation. It also has been used as an expectorant and for the promotion of the hepatoprotective activity. The plant's chemical analyses have shown that different sections of the plants produce alkaloids, carbohydrates, proteins, and amino acids, saponins, glycosides, quinones, flavonoids, terpenoids, and so on. In Chinese and Bhutanese herbal medicines, the parts of *Aconitum* species are also used. In Indian English, this tree is also regarded as "atees" and "atis" root; in Sanskrit as "ativisha," "shuklakanda," "aruna," and "vishada"; in Urdu as "atees"; in Hindi as "atis" and "atvika; in Bengali as "ataish"; in Telugu as "ati vasa"; in Gujarati as "ativakhani"; in Marathi as "ati vish"; in Kannada as "ati-vishsa"; in Malayalam as "ati-vidayam"; and in Punjabi as "atis." *A. heterophyllum* has different forms of medicine. Antidiarrheal activity is documented when accomplished with fine powder of fresh ginger, Beel ("Bellpetra" in India) or Nutmeg ("Jaiphal" in India). The root's sap works as an expectorant when consumed with dairy. As a diuretic, the seeds are used. The plant is also used to treat people with sexual illnesses and has hepatoprotective, antipyretic and analgesic, antioxidant, alexipharmic, anodynamic, anti-atrabile, antiflatulent, antiperiodic, anti-phlegmatic, and carminative characteristics. The anti-inflammatory activity of *A. heterophyllum* was confirmed by Verma et al. by using cotton-pellet induced granuloma method. Their investigations showed that *A. heterophyllum* tuber (Ethanolic extract) has significant anti-inflammatory activity, thereby providing scientific evidence for a

**Table 7.1** Plant species used in anti-inflammatory activities

| Scientific name | Family | Common name | Global distribution |
|---|---|---|---|
| *Achillea millefolium* L. | Asteraceae | Common Yarrow, Queen Anne's Lace | Argentina, Australia, Bolivia, Brazil, Canada, Caribbean, Chile, China, Colombia, Costa Rica, Ecuador, Greenland, Guatemala, Honduras, Mexico, Mongolia, New Zealand, Nicaragua, Peru, Russian Federation, South Africa, United States, Venezuela |
| *Aconitum heterophyllum* Wall.ex Royle | Ranunculaceae | Atis | India |
| *Aegle marmelos* (L.) Correa | Rutaceae | Bael, Bhel, Golden Apple, Stone Apple, Wood Apple, Japanese Bitter Orange, Bengal Quince | Burma, Cambodia, China, Honduras, India, Laos, Thailand, Vietnam |
| *Apium graveolens* L. | Apiaceae | Wild Celery | Belize, Bolivia, Canada, Chile, China, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Italy, Mexico, Peru, South Africa, United States |
| *Annona squamosa* L. | Annonaceae | Sitaphal, Sugar-Apples, Sweetsops | Belize, Bolivia, Caribbean, China, Colombia, Costa Rica, Ecuador, El Salvador, Gabon, Guatemala, Honduras, Madagascar, Mexico, Panama, Peru, United States |
| *Azadirachta indica* A. Juss. | Meliaceae | Neem, Nimtree, or Indian lilac | Colombia, Ecuador, El Salvador, India, Pakistan, United States, Venezuela |
| *Berberis vulgaris* var. *lutea* DC. | Berberidaceae | Common barberry, European barberry | England, Europe, New Mexico, Missouri, and South Carolina |
| *Beta vulgaris* L. | Amaranthaceae | Sea Beet, Sugar Beet, Spinach beet | Belize, Bolivia, Colombia, Costa Rica, Ecuador, Honduras, Madagascar, Mexico, New Zealand, Saudi Arabia, United States |
| *Boswellia serrata* Lam. | Myricaceae | Indian frankincense | India, Punjab (Pakistan) |

(continued)

**Table 7.1** (continued)

| Scientific name | Family | Common name | Global distribution |
|---|---|---|---|
| *Bridelia ferruginea* Benth | Phyllanthaceae | Guinea | Gabon, South Africa |
| *Bryophyllum pinnatum* (Lam.) Kurz | Crassulaceae | Air plant, Cathedral bells, life plant, miracle leaf | Belize, Ecuador, Guatemala, Honduras, Madagascar, Mexico, Peru, United States |
| *Cassia fistula* L. | Fabaceae | Golden tree, golden shower tree, cassia stick tree, golden pipe tree, golden rain tree, Indian laburnum, Pudding-pipe tree, and Purging cassia | Argentina, Belize, Bolivia, Brazil, Caribbean, China, Colombia, Costa Rica, Egypt, El Salvador, Guatemala, Honduras, India, Madagascar, Malaysia, Mexico, Nicaragua, Panama, Peru, United States, Venezuela |
| *Chenopodium botrys* L. | Amaranthaceae | Jerusalem oak goosefoot, sticky goosefoot | Canada, Saudi Arabia, United States, Equatorial Guinea |
| *Combretum molle* R. Br. ex G. Don | Combretaceae | Velvet bushwillow | South Africa |
| *Commiphora myrrha* (T. Nees) Engl. | Burseraceae | African myrrh, herabol myrrh, gum myrrh | Arabian Peninsula (Oman, Yemen), Africa (Djibouti, Ethiopia, Somalia, Northeast Kenya) |
| *Consolida regalis* S. F. Grey | Ranunculaceae | Forking larkspur, Rocket-larkspur, and Field larkspur | Canada, United States |
| *Conium maculatum* L. | Apiaceae | California fern (English, United States) | Bolivia, Brazil, Canada, Chile, China, Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Mexico, Peru, South Africa, United States |
| *Coptis chinensis* Franch | Ranunculaceae | The Chinese goldthread | China |
| *Crataeva religiosa* Hook. f. &Thomson | Capparaceae | Temple Plant | China, Indonesia, India, Malaysia, Myanmar, and Sri Lanka |
| *Crataeva nurvala* Buch.-Ham. | Capparaceae | Varuna, Three-leaved caper | India, Southeast Asia, China |
| *Emblica officinalis* Gaertn. | Phyllanthaceae | Gooseberry, Phyllanthus Emblica, Emblica, Indian Gooseberry, Amla | China, India, Malaysia, Sri Lanka |

(continued)

**Table 7.1** (continued)

| Scientific name | Family | Common name | Global distribution |
|---|---|---|---|
| *Eucalyptus camaldulensis* Dehn | Myrtaceae | River Red Gum | Australia, Bolivia, Caribbean, China, Colombia, Costa Rica, Ecuador, El Salvador, Honduras, Italy, Mexico, Nicaragua, Panama, United States |
| *Erythrophleum lasianthum* Corbishley | Fabaceae | Maputaland ordeal tree (e) | Southeast Africa |
| *Felicia muricata* Nees | Asteraceae | Wild Aster | Angola, Kenya, Zimbabwe, Botswana, Lesotho, Swaziland and Mpumalanga, Free State, North-West, Eastern Cape, and Northern Cape South Africa |
| *Gmelina arborea* Roxb. | Lamiaceae | Beechwood, Gmelina, Goomar tree, Malay beechood, White teak, Yemane | India, Myanmar, Thailand, Laos, Cambodia, Venezuela, Vietnam, China |
| *Glaucium flavum* Crantz | Papaveraceae | Yellow hornpoppy, Sea-poppy, or Yellow horned poppy | Canada, United States |
| *Heteromorpha trifoliata* (Wendl.) Eckl. and Zeyh. | Apiaceae | | South Africa |
| *Hedera rhombea* (Miq.) Bean | Araliaceae | Japanese ivy, Songak | China, Japan, South Korea |
| *Isopyrum thalictroides* L. | Ranunculaceae | Isopyrum | North America |
| *Jasminum officinale* L. | Oleaceae | Jasmine | Bhutan, China, India, Mexico, Nepal, Peru, Tajikistan. |
| *Lactuca sativa* L. | Asteraceae | Garden lettuce | Argentina, Australia, Belize, Bolivia, Brazil, Canada, Caribbean, Chile, China, Colombia, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Mexico, New Zealand, Nicaragua, Panama, Paraguay, Peru, South Africa, Tanzania, United States, Venezuela, Zaire |

(continued)

**Table 7.1** (continued)

| Scientific name | Family | Common name | Global distribution |
|---|---|---|---|
| *Marrubium peregrinum* L. | Lamiaceae | Horehound | Europe, Balkans, Asia Minor |
| *Mentha piperita* L. | Lamiaceae | Peppermint | Mexico |
| *Moringa pterygosperm* Gaertn. | Moringaceae | Moringa, Drumstivk tree, Benzoil tree | India, Europe, Myanmar |
| *Olea europaea* L. | Oleaceae | Olive Tree | Afghanistan, Bolivia, China, El Salvador, India, Mexico, Nepal, Pakistan, Unites States |
| *Phellodendron amurense* Rupr. | Rutaceae | Amur cork tree | China, Japan, Russian Federation, South Korea |
| *Piper ovatum* Vahl | Piperaceae | Pepper plants, Pepper vines | Brazil, India, Venezuela |
| *Piper longum* L. | Piperaceae | Indian long pepper, Pipli | China, India, Madagascar, Nepal, Sri Lanka, Vietnam |
| *Pluchea indica* (L.) Less. | Asteraceae | Indian camphorweed, Indian fleabane, and Indian pluchea | Australia, Burma, Cambodia, China, India, Japan, Laos, Malaysia, Philippines, Singapore, Thailand, United States, Vietnam |
| *Ptaeroxylon obliquum* (Thunb). Radlk. | Sapindaceae | Sneezewood Tree | South Africa |
| *Premna integrifolia* L. | Lamiaceae | Agia, Arni, Agnimath | Madagascar, Papua New Guinea, India and Andaman coast |
| *Ruta graveolens* L. | Rutaceae | Rue, Common Rue or Herb-of-grace | Bolivia, Canada, Chile, China, Ecuador, Madagascar, Mexico, South Africa, United States, Venezuela |
| *Ricinus communis* L. | Euphorbiaceae | Castor bean, Castor Oil Plant | Belize, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Gabon, Guatemala, Guyana, Honduras, India, Madagascar, Mexico, Panama, Peru, South Africa, Suriname, United States, Venezuela |
| *Senna occidentalis* (L.) Link | Fabaceae | Coffee senna, Coffeeweed, Mogdad coffee, negro-coffee, Stephanie coffee, | Argentina, Australia, Belize, Bolivia, Brazil, Caribbean, China, Colombia, Costa Rica, |

(continued)

**Table 7.1** (continued)

| Scientific name | Family | Common name | Global distribution |
|---|---|---|---|
| | | Stinkingweed, Bana Chakunda | Ecuador, El Salvador, French Guiana, Guyana, Honduras, India, Malaysia, Mexico, Panama, Paraguay, Peru, Sri Lanka, Suriname, United States, Uruguay, Venezuela |
| *Scutellaria baicalensis* Georgi | Lamiaceae | Baikal skullcap, Chinese skullcap | China, Japan, Mongolia, Russian Federation, South Korea |
| *Sida cordifolia* L. | Malvaceae | Flannel weed, Bala, Country Mallow, Heart-leaf sida | Australia, Belize, Bhutan, Bolivia, Brazil, Caribbean, China, Colombia, Ecuador, El Salvador, French Guiana, Gabon, Guatemala, Guyana, Honduras, India, Madagascar, Mexico, Nepal, Pakistan, Peru, Philippines, South Africa, Sri Lanka, Suriname, Thailand, United States, Venezuela |
| *Swertia chirata* Buch.-Ham. ex C.B. Clarke | Gentianaceae | Chirayta | India |
| *Tanacetum vulgare* L. | Asteraceae | Common Tansy | Argentina, Australia, Bolivia, Brazil, Canada, Caribbean, Chile, China, Colombia, Ecuador, Japan. Kazakhstan, Mexico, Mongolia, North Korea, Peru, Russian Federation, South Korea, Turkmenistan, United States, United States California, United States Georgia, United States Kansas, United States Mississippi, United States North Carolina, United States North Dakota, United States Washington, Venezuela |
| *Thalictrum minus* L. | Ranunculaceae | Lesser Meadow-Rue | China, South Africa, United States |
| *Thespesia populnea* (L.) Sol. ex Correa | Malvaceae | Portia Tree, Pacific rosewood, Milo, Indian Tulip Tree | Aldabra, Belize, Bolivia, Borneo, Brazil, Cambodia, Caribbean, Chile, China, |

(continued)

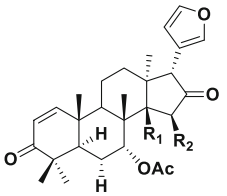
**Table 7.1** (continued)

| Scientific name | Family | Common name | Global distribution |
|---|---|---|---|
| | | | Colombia, Costa Rica, Fiji, Gabon, Gilbert, Guyana, Hawaiian, Honduras, India, Japan, Madagascar, Maldives, Mexico, Moluccas, Panama, Philippines, Phoenix Isl, Society Islands, Solomon Isl, Sri Lanka, Suriname, Thailand, Tonga, United States, Venezuela, Vietnam |
| *Zanthoxylum zanthoxyloides* (Lam.) Zepern & Timler | Rutaceae | Senegal prickly-ash or Artar Root | South Africa, Madagascar |
| *Zingiber officinale* Roscoe | Zingiberaceae | Ginger Root | Australia, Belize, Bhutan, Bolivia, Burma, Cambodia, China, Colombia, Costa Rica, Ecuador, Gabon, Guatemala, Honduras, India, Japan, Laos, Madagascar, Mexico, Nicaragua, Panama, Philippines, Sri Lanka, Thailand, Vietnam |

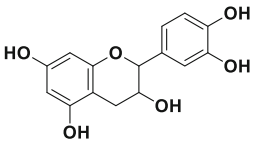
traditional medicinal claim as "*shotha/shophahara karma*" (anti-inflammatory action) (Verma et al. 2010).

#### 7.3.1.3 *Azadirachta indica*

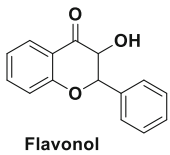
This plant species is attributed to its role as a health-promoting effect due to its rich antioxidant source. It grows to an average height of 30 cm from medium to large. In Sanskrit, it is known as "arishtha" meaning "perfect, complete, imperishable," "reliever of sickness," so many names such as "Sarbarogaribarini" and "wonder tree" have known it (Naik et al. 2014). It has been widely used in the treatment and prevention of various diseases in Chinese, Ayurvedic, and Unani medicines worldwide, particularly in the Indian subcontinent. Earlier findings confirmed that *A. indica* and its constituents play a role in free radical generation scavenging and pathogenesis prevention of disease. Studies based on animal model have shown that *A. indica* and its major constituents play a pivotal role in the management of anticancer by modulating different molecular pathways including p53, pTEN, NF-πB, PI3K/Akt, Bcl-2, and VEGF (Alzohairy 2016). It is considered as one of the safe medicinal plants and modulates the numerous biological processes without any deleterious effect. Earlier findings have also shown antipyretic, anti-



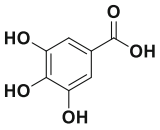

Azadiradione



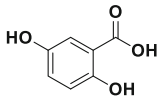

Catechin



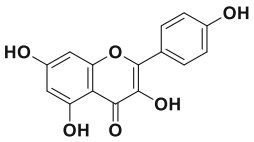

Flavonol



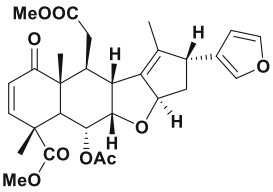

Gallic acid



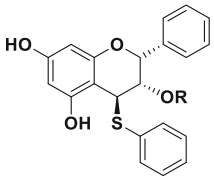

Gentisic acid




Kaempferol

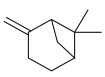



Nimbin

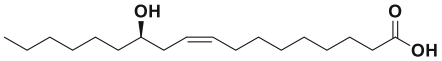



R= galloyl

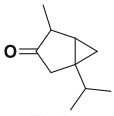
3-O-galloyl-(-)-epicatechin-4-benzylthioether



Pinene




Ricinoleic acid




Thujone

**Fig. 7.2** Major chemical bioactives present in Indian plants used as anti-Inflammatory agents. (**a**–**d**) Azadiradione, catechin, flavonol, and gallic acid present in *Azadirachta indica*; (**e**–**f**) gentisic acid and kaempferol in *Ricinus communis*; (**g**) nimbin in *Azadirachta indica*; (**h**) 3-O-galloyl-(-)-epicatechin-4-benzylthioether in *Thespesia populnea*; (**i**–**k**) pinene, ricinoleic acid, and thujone reported in *Ricinus communis*

inflammatory activity, and immunomodulatory effect of bark and oil seed extracts from the neem tree. Experiments were conducted to evaluate the analgesic activity of neem seed oil on albino rats and the study results showed that neem seed oil seemed to have a significant analgesic effect in the 1 and 2 ml/kg dose and that oil had dose-dependent analgesic activity. As discovered by phytochemical analysis; triterpenes, flavonoids, flavonol, tannins, saponins, nimbin, sodium nimbidate, gallic acid, catechin, and polysaccharides are the major anti-inflammatory constituents of neem. In the in vivo model, anti-inflammatory activity was observed using the carrageenan-induced paw edema model. The results concluded that the animals treated with 100 mg/kg dose of carbon tetrachloride extract and azadiradione exhibited significant antinociceptive and anti-inflammatory activities (Ilango et al. 2013). This study has rationalized the ethnomedicinal use of the plant for wound, burns, and injury by the tribal population.

#### 7.3.1.4 *Aegle marmelos*

This tree species is indigenous to the Indian subcontinent and Southeast Asia. The leaves, bark, roots, berries, and grains are widely used in Ayurveda's Indian traditional medicine scheme as well as in numerous folk medicines to cure countless diseases. Bael vegetables are used for nutritional purposes and the fruit pulp is used to make delicacies such as murabba, puddings, and juice. The aqueous extract of the root bark of Bael was prepared and tested for anti-inflammatory activity in albino rats using carrageenan-induced paw edema model and cotton pellet induced granuloma with the standard drug being taken as indomethacin and Bael. The result revealed that the *Aegle marmelos* expressed the anti-inflammatory inhibition in the carrageenan-induced paw edema as well as in the cotton pellet induced granuloma model (Benni et al. 2011).

#### 7.3.1.5 *Annona squamosa*

This species of plant is a tiny, well-branched tree or shrub with edible fruits called sitaphal, sugar apples, or sweetsops. It tolerates a humid tropical climate stronger than its parents, *Annona reticulata* and *Annona cherimola*. It is a multipurpose fruit tree having around 50–80% of the edible fruit. Its fruits are frequently referred to as edible custard apples. The pulp can be used as a flavor in ice cream. The content of vitamin C (35–42 mg/100 g) is appreciable and slightly greater than that of grapefruit. There is also an important nutrient value of thiamine, potassium, and dietary fiber. This species is known to possess multiple chemical compounds such as alkaloid, leaf isomeric hydroxyl ketones, acetogenin, samaquasine, seed annonacin, as well as anonastatin, acetogenin, and bark squamone. Different trials have documented the antibacterial, antidiabetic, antitumor, antimalarial, anthelmintic, anti-genotoxic, and hepatoprotective role of *Annona squamosa*. The leaves are used as a vermicide to treat cancerous tumors and are also applied to abscesses, insect bites, and many other skin problems. To overcome the hysteria and fainting spells, the broken leaves were picked and they were also applied to the ulcers and wounds. Root-bark scrapings are used for toothache. Powdered plants are used to destroy head-lice and fleas but consideration should be given not to touch the eyes as

this creates tremendous pain. It has been noted that crude samples of various components and mere small phytoconstituents of its fruits produce antidiabetic, antiviral, antioxidant behavior, respiratory stimulant during pregnancy, and diuretic properties, which are very helpful in strengthening the immune system, the nervous system, and also in developing the brain in the fetus. Sitaphal can be the most efficient solution of selection for different illnesses, and this fresh study will definitely assist humanity to live a good, disease-free existence. For anti-arthritic activity, the histopathological evidence revealed the reality that the reduction in neutrophils infiltration, pannus formation, and bone of the animal was massively reduced when treated with the plant extract. The extract reproached that it has dose-dependent analgesic and anti-inflammatory activities relative to the conventional target drugs, pethidine sulfate, and indomethacin (Ruckmani et al. 2018).

#### 7.3.1.6 *Bryophyllum pinnatum*

Ojewole et al. investigated the anti-inflammatory potential of this plant species. The study was undertaken in experimental animal models to investigate anti-inflammatory and plant leaf aqueous extract. This was verified using the control drug, Diclofenac 100 mg/kg by fresh egg albumin-induced pedal (paw) edema model. The results of that experimental animal study showed that the aqueous extract of *Bryophyllum pinnatum* leaf had anti-inflammatory properties. The various herbal flavonoids, polyphenols, are speculated to account for the plant's observed anti-inflammatory activity (Ojewole 2005).

#### 7.3.1.7 *Cassia fistula*

Over the past two decades, research results have validated the therapeutic effect of *C. fistula* via modulation of biological activities due to the rich source of antioxidants. An important finding has shown that *C. fistula* bark extracts were found to possess significant anti-inflammatory effect in both acute and chronic models. The water extract of dried fruits of *Solanum xanthocarpum* and dried pulp of *C. fistula* Linn was prepared. The anti-inflammatory activity was then measured and results revealed that among the different dose combinations of both extracts, the 1:1 combination at the 500 mg/kg dose showed maximum percentage inhibition of 75%, which was comparable with the positive control, diclofenac sodium, which showed 81% inhibition (Rahmani 2015). Another research was conducted to evaluate the anti-inflammatory effects of *C. fistula* against phenylbutazone using carrageenan, histamine, and dextran-induced paw edema in rodents and anti-inflammatory activity against all phlogistic substances. The anti-inflammatory activity of aqueous and alcoholic extracts of *C. fistula* confirmed that extracts in both air pouch granuloma and cotton pellet granuloma models showed significant anti-inflammatory effect (Rahmani 2015).

#### 7.3.1.8 *Emblica officinalis*

This tree species has been used for anti-inflammatory and antipyretic activities in subtropical and tropical parts of China, India, Indonesia, Sri Lanka, Thailand, and Malaysia. In the experimental studies, the anti-inflammatory activity was found in

the water fraction of methanol extract of plant leaves of *E. officinalis*. Fractional effects have been tested on inflammation mediator synthesis like leukotriene B4, platelet activating factor (PAF), and thromboxane. The water fraction of methanol extract inhibited migration of human PMNs in relatively low concentrations (Asmawi et al. 1993).

#### 7.3.1.9 *Hedera rhombea*

This plant species was formerly called *Hedera pedunculata*. The anti-inflammatory activity from the leaves of *Hedera rhombea* was found in the methanol, butanol, and ether fractions and was further investigated by using carrageenan-induced edema test (Vishal et al. 2014).

#### 7.3.1.10 *Pluchea indica*

The anti-inflammatory activity of the methanolic fraction of a chloroform extract of *Pluchea indica* roots was investigated and evaluated by Sen 1991 by using chloroform extract (Sen and Chaudhuri 1991). The extract showed significant inhibitory activity against urate-induced pedal inflammation of carrageenan, histamine, serotonin, hyaluronidase, and sodium, and also inhibited granuloma induced by carrageenan and cotton pellets.

#### 7.3.1.11 *Piper ovatum*

Since old times, piper plants have been used by human beings as condiment and medication. This is one of the most significant plant classes. *Piper ovatum* leaves are known as "joao burandi" or "anestesica" in folk medicine and in traditional Brazilian medicine are used to cure inflammatory diseases. Hydroalcoholic sample, fractions, and a combination of piperovatine and piperlonguminine in a 2:3 ratio extracted from *P. ovatum* were tested for anti-inflammatory action through carrageen-induced pleurisy in rats and croton-oil-induced edema in mice (Rodrigues Silva et al. 2008). Black-pepper preparations or its primary effective amide, Piperine, can induce digestive enzymes to improve digestion in particular and prevent certain illnesses of this organ. Apart from this, it has also shown anti-inflammatory, thermogenic, stimulating development, antithyroid, and chemopreventive operations.

#### 7.3.1.12 *Piper longum*

This plant species is one of the Ayurveda's primary Rasayana (rejuvenator) medicines and is commonly used in the therapy of multiple illnesses, particularly in the therapy of respiratory disorders. This plant's root is recognized in Ayurveda as Pippali Mula and its flowers (Spike) are primarily used for purposes of Rasayana. Several biological activities such as immune-stimulatory, antiulcer, anti-amoebial, antioxidant, hepatoprotective, and anti-inflammatory operations have been recorded on the fruit of this plant. In India's Ayurvedic form, Pippali is used in 324 formulations and is one of Trikatu churna's components (Kumari et al. 2012). Two kinds of Pippali in nature are Chhoti Pippali and Badi Pippali. Although both types are used for medicinal reasons, Chhoti Pippali is more preferred by physicians. It is a well-established fact that Pippali is one of the most important drugs in

bronchial disease therapy (Tamaka Shwasa), which should have anti-inflammatory efficacy in the treatment of asthma.

#### 7.3.1.13 *Ricinus communis*

This plant species belongs to the family Euphorbiaceae is a permanent floating plant species. It is the only species in the monotypic genus *Ricinus*, and *Ricininae* subtribe. This plant is native to the southern Mediterranean Basin, East Africa, and India but is commonly used as an ornamental plant in tropical areas. The plant is considered to exhibit antimicrobial action and has been used to manage various ailments. The plant has big, palm-lobed leaves and is a vigorous annual (northern) or hybrid (tropical and subtropical) medicinal herb. It is grown and rarely flees and persists in pine forests, disposal areas, and roadsides. Its leaves, stem, and plant juice is used in the treatment of inflammation, liver disease, hypoglycemia and as a laxative. The existence of multiple phytochemicals such as alkaloids, flavonoids, terpenes, saponins, phenolic compounds such as ricin, rutin, kaempferol, lupeol, ricinoleic acid, pinene, thujone, and gallic acid, and gentisic acid has been described in several study publications (Verma et al. 2010). These phytochemicals are shown to be targeting peroxisome proliferator-activated receptor (PPAR), nuclear factor NF-μ-B, cytochrome p450, P38 mitogen-activated protein kinases kinase (p38 MAPK), tumor protein P53, B-cell extra-large lymphoma (Bcl-xL), and vascular endothelial growth factor receptor-2 (VEGFR-2) (Abdul et al. 2018). Taking into account its broad range of phytochemicals, pharmacological activity, and subsequent clinical trials, *R. communis* might be a nice option to find new supplementary drugs.

#### 7.3.1.14 *Senna occidentalis*

As an alternative remedy, the plant holds the important traditional history. It is known that the plant has hepatoprotective, hypoglycemic, antimalarial, anti-inflammatory, immunosuppressive, hypolipidemic, anti-atherosclerogenic, and anti-pyretic activities. The current research on *Senna occidentalis* L. extract has shown that this plant has important analgesic and anti-inflammatory characteristics and justifies this plant's traditional use in treating multiple kinds of pain and inflammation. The anti-inflammatory activity of *Senna occidentalis* was tested using ear edema induced by hyaluronidase, lipoxygenase, xanthine oxidase assays, and an animal model of 12-0-tetradecanoyl phorbol-13-acetate (TPA). All the extracts of this plant were tested to find out some inhibitions against all the enzymes. The most significant activity was found in ethyl acetate and dichloromethane extracts, which inhibited 100% and 99% of lipoxygenase (100%) and hyaluronidase enzymes at 100 μg/ml, respectively. The hexane extract also showed a profound activity in both assays, while the ethyl acetate extract was the only one found to be effective with inhibition in the xanthine oxidase assay. Low to moderate activity was observed for all the extracts in the TPA-induced ear edema model, with the dichloromethane extract being the most active with 73% inhibition at 2 mg/ear concentration (Hani Idayu et al. 2009). This study has provided a scientific basis for using this plant to cure diseases associated with inflammation.

#### 7.3.1.15 *Sida cordifolia*

*Sida cordifolia* is widely used in conventional medical systems (TSM). *S. cordifolia* exhibited analgesic and anti-inflammatory activities (Sutradhar et al. 2006); hepatoprotective activity (Silva et al. 2006); antidiabetic property (Ahmad et al. 2015); and anticancer activity (Srinithya and Muthuraman 2014) in various animal models. In folk medicine, this plant species was used to treat mouth mucosal inflammation, blenorrhea, asthmatic bronchitis, and respiratory congestion. It has been studied as an anti-inflammatory drug to avoid cell proliferation and to promote the growth of the liver by EM Franzotti (Franzotti et al. 2000). *S. cordifolia* accelerates delays in wound healing induced by the dexamethasone, steroidal drug.

#### 7.3.1.16 *Swertia chirata*

In the Indian Ayurvedic Herbal System, *Swertia* is used to treat fever as in Laghu Sudarshana churna, Maha Sudarshan churna, and Tibetan folk medicine. However, xanthones are the main bioactive of *Swertia*. Other secondary metabolites such as flavonoids, iridoid glycosides, and triterpenoids are also important components of this genus. In biological activities such as hepatoprotective, antihepatotoxic, antimicrobial, anti-inflammatory, anticarcinogenic, antileprosy, hypoglycemic, and antimalarial, these secondary metabolites perform an important part (Negi et al. 2011).

#### 7.3.1.17 *Thespesia populnea*

It is a tiny tree or arborescent shrub with a pantropical range that is discovered on the world's shores. Phytochemical tests have shown that the ethanolic extract of bark of *Thespesia populnea* contains alkaloids, carbohydrates, protein, tannin like 3-O-galloyl-(-)-epicatechin-4-benzylthioether, phenols, flavonoids, gums, mucilage, saponins, and terpenes (Vasudevan et al. 2007).

#### 7.3.1.18 *Zingiber officinale*

This plant species exhibits a suppressive impact on acute and chronic inflammation, and the anti-inflammatory impact appears to involve inhibiting macrophage activity. The anti-inflammatory impact of *Zingiber officinale*'s was studied by Shimoda. They produced 40% ethanol sample from raw green pepper and assessed its anti-inflammatory action using acute and chronic inflammation models (Shimoda et al. 2010).

### 7.3.2 Mechanism of Inflammation

The inflammatory process is an amalgamation of many pathways like synthesis of prostaglandin, interleukin or another chemo toxin, adhesive protein receptor action, platelet-activating factors. All can act as chemotactic agonists. Inflammation initiates with any accentuate on the membrane or by other trigger or stimuli, which activate hydrolysis of membrane phospholipid by phospholipase A into arachidonic acid that further substrate for cyclooxygenase and lipoxygenase enzyme and the by-product of these are prostaglandins PGE2, PGH2, and leukotrienes like LTC4, LTB4, etc. Several cytokines, in particular, interleukin-1 (IL-1) and tumor necrosis factor-a

(TNF-a), also play essential roles in orchestration of the inflammatory process. The main mediators of biological responses to bacterial lipopolysaccharide (LPS) are IL-1 and TNF. Monocytes, macrophages, adipocytes, and other cells secrete IL1 and TNF. Working together and with different cytokines and growth factors (including IL-8 and colony-stimulating granulocyte-macrophage), they induce gene expression and protein synthesis in a variety of cells to mediate and promote inflammation. Prostaglandin (PGE2) or prostacyclin (PGI2) release increases blood flow as well as the permeability of the blood vessels by helping to release nitric oxide from the endothelium-derived releasing factor that causes vasodilatation and helps stick platelets and other chemical toxins (bradykinin, histamine). LTB4 stimulates the aggregation of polymorphonuclear leukocytes at higher concentrations and promotes degranulation and superoxide generation. LTB4 promotes adhesion of neutrophils to vascular endothelial cells and their trans-endothelial migration and stimulates the synthesis of pro-inflammatory cytokines from macrophages and lymphocytes (Medzhitov 2008).

### 7.3.3 Generic Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

All NSAIDs decrease pain and inflammation, but the intensity of relief is different from individual to individual. Some NSAIDs may have fewer side effects than the others. Aspirin, Celecoxib, Diclofenac, Etodolac, Fenoprofen, Flurbiprofen, Ibuprofen, Indomethacin, Ketoprofen, Ketorolac, Meloxicam, Nabumetone, Naproxen, Oxaprozin, Piroxicam, Salsalate, Sulindac, Tolmetin are some NSAIDs available in the market. NSAIDs can increase the risk of a heart attack or stroke, particularly at greater doses. They can also trigger bleeding from the stomach and are the safest if taken for short periods at low doses. Side effects can most frequently occur if large amount of doses are taken over a lengthy period of time (months or years).

### 7.3.4 Future Drug Perspectives

Indian medicine system involves different methods like Naturopathy, Unani, Siddha, Yoga, Homeopathy, and Ayurveda. Since the old times, plants have served a significant impact in human health care. This chapter indicates that Indian plants have an ancient history of anti-inflammatory, antioxidant, chemopreventive, and chemotherapeutic behavior in Indian and Chinese medicine systems in various diseases. In the emergence of fresh drugs, traditional plants perform a very considerable part. Nowadays, inflammation is a very big challenge of mankind. The synthetic drugs that are presently used demonstrate adverse effects and also change the different biological processes. Due to their side effect characteristics, alternative medicine has opened up a fresh window in health management.

### 7.3.5 Patents (Table 7.2)

**Table 7.2** Patents available on anti-inflammation report from plants

| Title | Patent no. | Published | Inventor | Date of publication | References |
|---|---|---|---|---|---|
| Compositions comprising nonsteroidal anti-inflammatory drugs and methods for use thereof | US9084769 | US20130142869, US20150342989 | Philip Alex, Ben Johns | July 21, 2015 | Alex and Johns (2015) |
| Pharmaceutical compositions for the coordinated delivery of NSAIDs | US9161920 | | John R Plachetka | October 20, 2015 | Plachetka (2015) |
| Therapeutic herbal composition | US5707631 | | Chaim J. Lieberman | January 13, 1998 | Lieberman (1998) |
| Combination of compounds isolated from *Curcuma* spp. as anti-inflammatory agents | US5120538 | DE69007960, DE69007960, EP0440885, EP0440885 | Ban Liang Oei | June 9, 1992 | Oei (1992) |
| Herbal compositions and their use as anti-inflammatory agents for alleviation of arthritis and gout | US6274176 | | Onkar S.Tomer, Peter Glomski, Kripanath Borah | August 14, 2001 | Tomer et al. (2001) |
| Herbal compositions for prevention and treatment rheumatic and inflammatory diseases and method of preparing the same | US20050276873 | | Yan Xue Li | December 15, 2005 | Li (2005) |
| Anti-inflammatory compounds | US9326977 | EP2766019, US9717718, US20140309258, US20160317513, WO2013054070 | Robert James Nash | May 3, 2016 | Nash (2016) |
| Anti-inflammatory herbal composition and method of use | US6387416 | CA2442964, CA244296, CN126571, CN1499933, DE60228560, EP1383386, WO2002080682 | Thomas Newmark, Paul Schulick | May 14, 2002 | Newmark and Schulick (2002) |
| Polyherbal composition as anti-inflammatory agent | WO2005120529 | | Tapan Kumar Chatterjee | December 22, 2005 | Chatterjee (2005) |
| Anti-inflammatory creatine composition comprising acetylated fatty acid | EP2283836 | US20110039928, US20150011631 | Jeffrey M. Golini | February 16, 2011 | Golini (2011) |

## 7.4 Conclusion

The existence of various targets for drug action in the inflammatory response pathway provides countless sites of action for the number of effective constituents of these medicinal plants. These plants have continued to serve as alternative and complementary therapies due to their effectiveness in the herbal treatment of inflammatory disease circumstances. This reality has continued to be credited with the mounting of experimental proof and to create a rationale for ethnomedicinal use. Furthermore, these medicinal plants will continue to serve as a reservoir for powerful drug development with less severe and life-threatening adverse effects. Recent studies based on in vivo and in vitro have confirmed that Indian medicinal plants have a great role in diseases inhibition via modulation of various physiological and biochemical processes. The mechanism of action in the prevention of diseases is still not fully understood. In order to confirm the accurate mechanism of action of medicinal plants in disease management, detailed research on molecular pathways should be carried out. The demand for medicinal plant-based medicines is increasing at a pace of 15%–25% annually and, according to WHO estimates, the demand for medicinal crops is expected to boost by more than 5 trillion USD in 2050. In India, the trade in medicinal crops is estimated to be at around USD 1 billion per year. Hence the use of herbal medicines poses less or no side effects and is less costly. Various herbal medicines in combination to conventional medicines can also be made so as to reduce the side effects of conventional medicines. So in this article, on behalf of their anti-inflammatory medicinal properties, some herbal plants have been included which may be effective in inflammation.

**Acknowledgment** The authors would like to express their utmost gratitude and appreciation to Director, CSIR-Indian Institute of Integrative Medicine, Jammu. We also thank the Department of Science and Technology- India for providing research fellowship to G. Sawhney *via* fellowship code no. DST/INSPIRE Fellowship/2017/IF170212.

**Conflict of Interest** The authors declare that they have no conflicts of interest regarding the publication of this chapter.

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# 8 Phytochemistry and Pharmacological Activities of *Rhodiola imbricata* Edgew., a High Value Medicinal Herb of Cold Desert Himalaya

Venugopal Singamaneni, Upasana Sharma, Bashir Lone, and Prasoon Gupta

**Abstract**

This present review deals with chemical constituents, biological activities and traditional uses of *Rhodiola imbricata* Edgew. in traditional and modern medicines. This species of plant grows at higher altitude zones of Himalayan such as in Ladakh and Kashmir regions. In total, there are 30 compounds reported from the roots and rhizomes of this plant, and major components include phenylpropanoids, flavonoids, terpenoids, phenolic acids, and phenylethanol derivatives. Pharmacological studies confirmed that *R. imbricata* exhibits adaptogenic, cardioprotective, anti-stress, and anti-inflammatory activities. The compounds present in plant are known to increase physical endurance, work productivity and longevity. It has been also used to treat fatigue, asthma, hemorrhage, impotence, gastrointestinal ailments, decreasing depression, preventing high altitude sickness and for stimulating the nervous system. In conclusion, *R. imbricata* has lots of folklore and pharmacological evidence to deal with stress and fatigue. Also there is emerging evidence for supporting radioprotective activity.

**Keywords**

Himalayan plant · *Rhodiola imbricata* · Phenylpropanoids · Anti-stress · Radioprotective

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_8

## Abbreviations

| | |
|---|---|
| HPLC | High-performance liquid chromatography |
| GC/MS | Gas chromatography/mass spectrometry |
| IL | Interleukin |
| TNF | Tumor necrosis factor |
| PBMCs | Peripheral blood mononuclear cells |
| LPS | Lipopolysaccharides |
| ELI spot | Enzyme-linked immune absorbent spot |
| FACS | Fluorescence activated cell sorting |
| ELISA | Enzyme linked immunosorbent assay |
| MCH | Mean corpuscular hemoglobin |
| MCV | Mean corpuscular volume |
| RDW | Red blood cell distribution width |
| SOD | Super oxide dismutase |
| MCHC | Mean corpuscular hemoglobin concentration |
| CAT | Catalase |
| GPx | Glutathione peroxidase |
| GSH | Reduced glutathione |
| SGPT | Serum glutamic pyruvic transaminase |
| SGOT | Serum glutamic oxaloacetic transaminase |
| ALP | Alkaline phosphatase |
| ME | Methanolic extract |
| Aqu. | Aqueous |
| EE | Ethanolic extract |

## 8.1 Introduction

The trans-Himalayan regions are very exceptional in the world with extreme temperature variation, high ultraviolet radiation, and low availability of oxygen which are difficult for human survival and results in cognitive dysfunctions, high altitude region maladies, and lowered performance due to alteration in biological functions. However, the Himalaya region is also a reservoir of diverse flora and fauna and to fight and service with these problems in these difficult situations. Plants found in high region above 3000 m of Himalayas are extensively used in ethnomedicine/traditional medicinal system, both as prophylactics remedy and therapeutics. The valuable effects of these herbal drugs/supplements in combating the adverse effects of high altitude region have been deeply investigated in recent years. The plant species is important due to the efficient medicinal properties with slightest or no side effects.

*Rhodiola imbricata* Edgew. species known to contain the active chemical compounds of the class flavonoid glycosides and coumarins (Khanum et al. 2005). Phenylpropanoids and phenylethanol derivatives are the major compounds from these *Rhodiola* plants, particularly roots or rhizomes, which have been shown to

have various biological activities (Kelly 2001; Yousef et al. 2006). Anti-inflammatory, cardioprotective, antipyretic, anti-stress, and adaptogenic effects are the major pharmacological activities exhibited by *R. imbricata* (Chawla et al. 2010; Gupta et al. 2010; Mishra et al. 2012; Tayade et al. 2013a, b, c). The rhizomes exhibits wound healing (Gupta et al. 2007), radioprotective (Arora et al. 2005), antioxidant, cytoprotective (Kanupriya et al. 2005), adaptogen (Gupta et al. 2008; Spasov et al. 2000), immunomodulatory (Mishra et al. 2006), neuroprotective (Mook-Jung et al. 2002), and antiproliferative activities (Senthilkumar et al. 2013).

## 8.2 Morphology and Distribution of Golden Root

*Rhodiola imbricata* (Synonyms: *Sedum imbricatum* (Edgew.) Walp.) is a high altitude perennial herb of Crassulaceae family, commonly known by different names such as rose root, arctic root, golden root, Himalayan stone crop, shrolo marpo, and lalphool. There are about 200 species of *Rhodiola* distributed worldwide. This species grows usually on rocky substratum on slopes and also prefers sandy area at high altitudes of Asia and Europe (Chaurasia and Singh 1996). In India, *R. imbricata* is mainly distributed in the Trans-Himalayan Cold Desert at the altitude ranges between 4000 and 5000 m above sea level in Leh-Ladakh region in Himalaya (Chaurasia and Singh 1996). A schematic representation of habit, part used, and active marker compounds present in *Rhodiola imbricata* is given in Fig. 8.1a–c.

## 8.3 Traditional Uses

Usually the genus *Rhodiola* root has been used in folklore medicine to increase physical endurance, longevity, to treat impotence, and gastrointestinal disorders (Kelly 2001). This species is considered as one of the major ingredient of local tea (herbal), acting as antioxidant biological function (Mishra et al. 2008). It is widely used as food as well as medicine for curing common diseases around the world. This plant species is used in Chinese phytotherapy and Amchi system to cure various ailments and helps to maintain body function. In Leh-Ladakh (UT), this species is

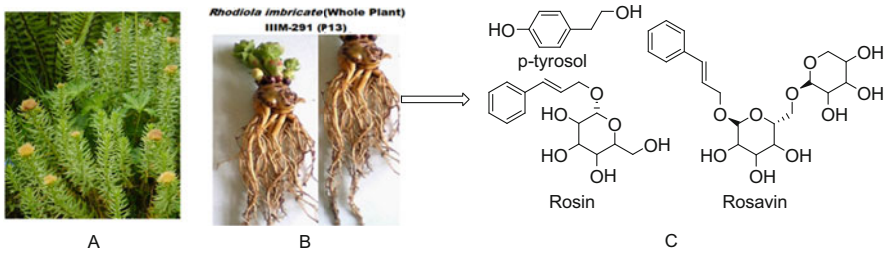


**Fig. 8.1** Morphology and chemical markers of *Rhodiola imbricata* ((**a**). Habit of *R. imbricata* in wild, (**b**). Part used, (**c**). marker compounds)

used as wild edible, fodder, and an ornamental plant. The boiled shoots with water used and mixed with yogurt to make tantur, a local delicious food item.

## 8.4 Phytochemistry

Several secondary metabolites such as phenylethanol derivatives, phenylpropanoids, phenolic acids and flavonoids were reported from *R. imbricata*. HPLC analysis of acetone extract established the occurrence of gallic acid and rutin (Senthilkumar et al. 2014)) in the plant. The aqueous extract reported with major bioactive compounds such as rosin, rosavin, and p-tyrosol (Mishra et al. 2008). The GC-MS analysis of methanolic extract (ME) showed phytosterols, phenols, alkyl halide and fatty acid esters (Tayade et al. 2013a, b, c). *Rhodiola imbricata* possesses phenolic compounds like salidroside and p-tyrosol as primary and secondary metabolites (Guo et al. 2014). Total of 19 compounds have been isolated (Figs. 8.2a and 8.2b) from ethyl acetate and n-butanol extracts through preparative HPLC (Choudhary et al. 2015). Chemometric profile of the *n*-hexane, $CHCl_3$, $CCl_4$, EtOAc, $CH_3OH$, and 60% $C_2H_5OH$ root extracts through GC-MS technique revealed the occurrence of 63 phyto-chemotypes (Tayade et al. 2013a, b, c).

## 8.5 Phyto-chemotypes Identified through GC-MS

### 8.5.1 Phyto-chemotypes Identified from *n*-Hexane Root Extract

The root extract in *n*-hexane was identified to contain the major compound as 28.2% 1-pentacosanol; other constituents include, 17-pentatriacontene (7.1), stigmast-5-en-3-ol, (3β,24S) (13.4), 1-tetracosanol (9.3), 1-henteracontanol (8.5)and 13-tetradecen-1-ol acetate (6.4), 1-heptacosane (3.5), 1-hentriacontane (3.6), 1-tericosanol (2.5), α-tocopherol-β-D-mannoside (0.7), 13-docosan-1-ol, (Z) (2.2), eicosen-1-ol, cis-9 (1.9) stigmast-4-en-3-one (1.3), bis (2-ethylhexyl) phthalate (1.2), hexadecanoic acid (1.2), 1- tetrateracontane (0.9), campesterol (0.9), stigmastanol (0.7), and 3-methoxy-5-methylphenol (0.5). The structures of phyto-chemotypes identified from *R. imbricata* are given in Figs. 8.3a, 8.3b, 8.3c, 8.3d and 8.3e.

### 8.5.2 Phyto-chemotypes Identified from Chloroform and Dichloromethane Root Extract

In the root extract with chloroform, the chemical compounds characterized were 24.30% stigmasterol, 14.64% methyl tri-butyl $NH_4Cl$, and 11.50% bis (2-ethylhexyl) phthalate; other identified compounds were 7,8-dimethylbenzocyclooctene (7.97), ethyl-linoleate (4.75), 3-methoxy-5-

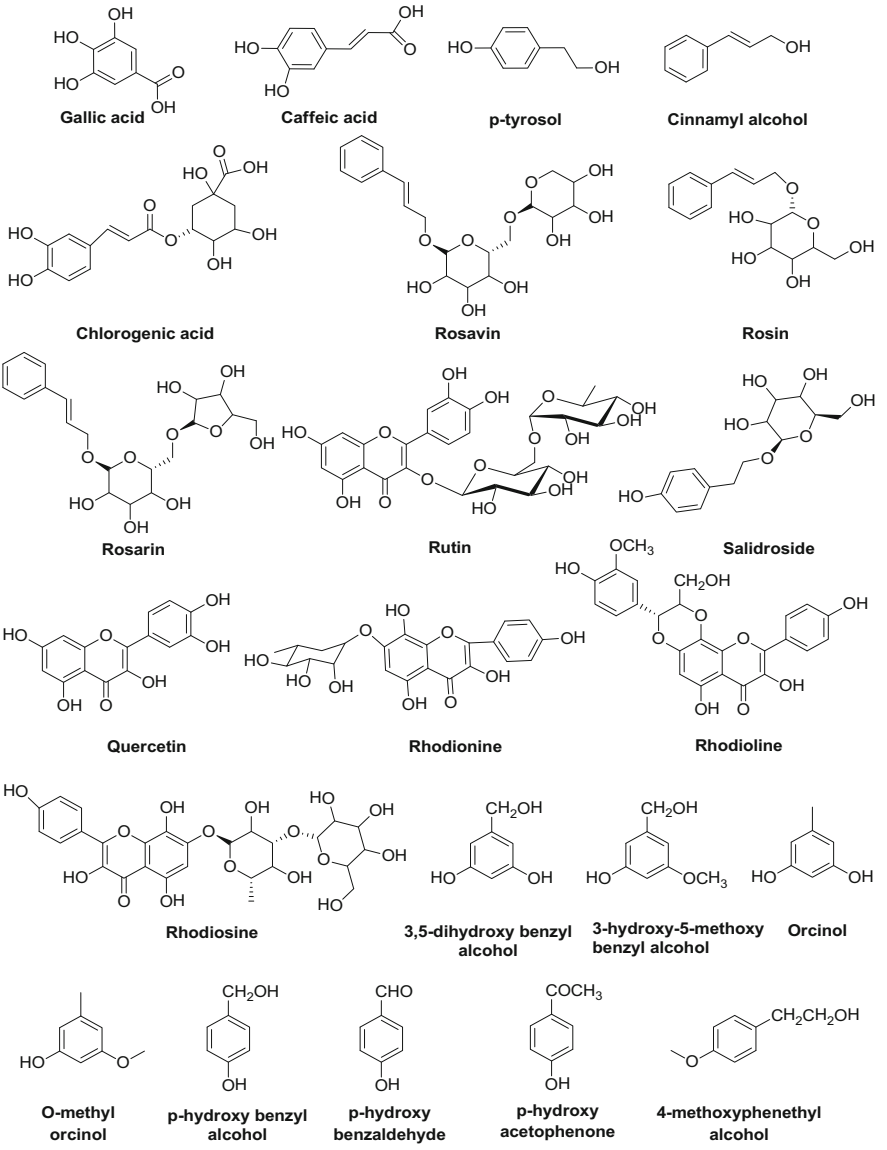


**Fig. 8.2a** Chemical constituents of *Rhodiola imbricata*

methylphenol (4.16), and hexadecanoic acid (4.13) were found in major amount while campesterol (3.94), benzene methanol, 3-hydroxy-5-methoxy (2.62),17-pentatriacontene (3.38), benzene sulfonic acid, 4-amino-3-nitro (3.21), orcinol (2.93), 1- tetracosanol (1.86), stigmast-4-en-3-one (1.82), α-tocopherol (1.31), and eicosen-1-ol, cis-9 (1.13). In dichloromethane root extract, the most dominant

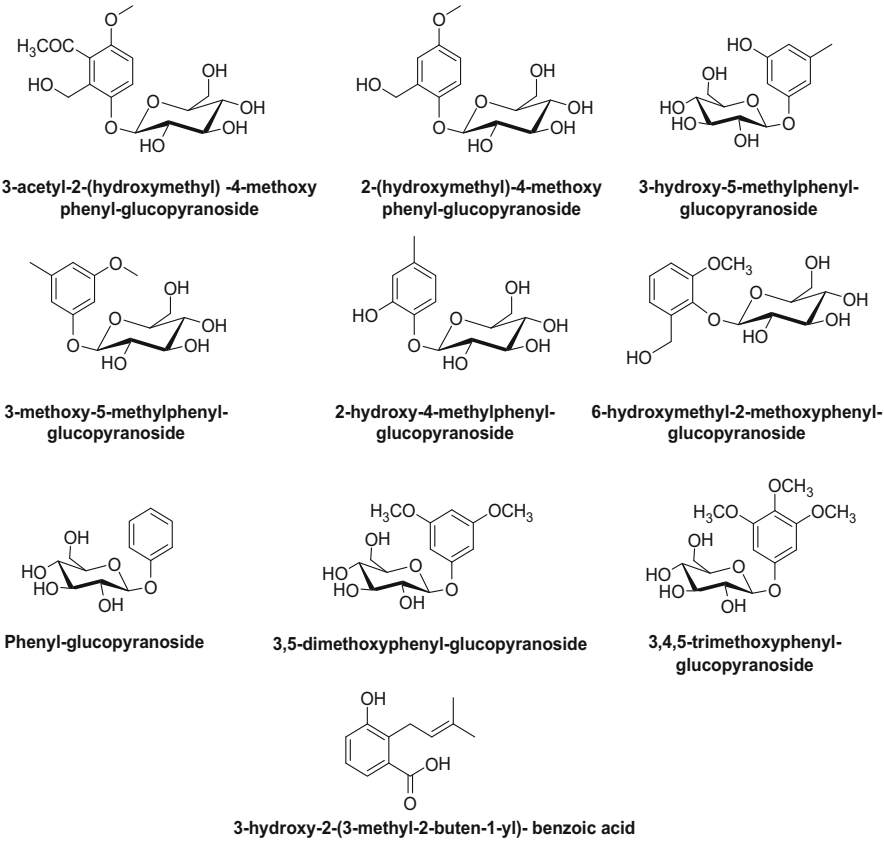


**Fig. 8.2b** Chemical constituents of *Rhodiola imbricata*

isolates were 17.78% camphor and 15.42% stigmast-5-en-3-ol(3β,24S); other metabolites were mostly identical to chloroform extract in different ration.

### 8.5.3 Phyto-chemotypes Identified from Ethyl Acetate Root Extract

In ethyl acetate extract, the major isolates were 1,3-dimethoxybenzene and 1,3-benzenediol, 5-pentadecyl which were represented by 27.6 and 16.9%, respectively; other chemical constituents include 1,3-benzenediol, 5-methyl (8.4), 3-methoxy-5-methylphenol (10.1), benzenemethanol, 3-hydroxy, 5-methoxy (5.7), cholest-4-ene-3,6-dione (5.7), dodecanoic acid, 3-hydroxy (4.5), 7,8-dimethylbenzocyclooctene (3.6), 3,5-dimethoxyphenyl acetate (3.4), 1-dodecanol, 3,7,11-trimethyl (0.6), stigmasterol (2.2), hexadecanoic acid (1.8) and oleic acid (1.4).

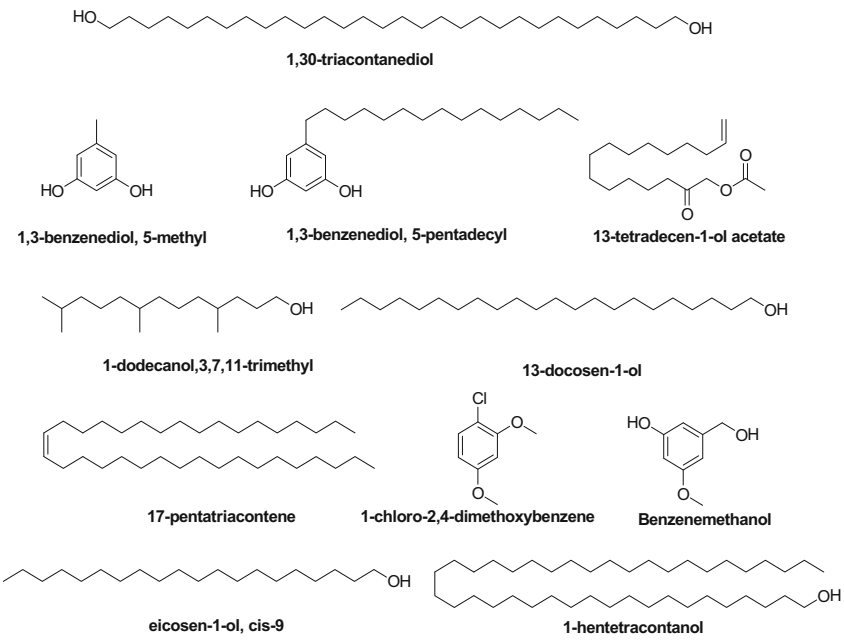


**Fig. 8.3a** Phyto-chemotypes identified through GC-MS from *R. imbricata*

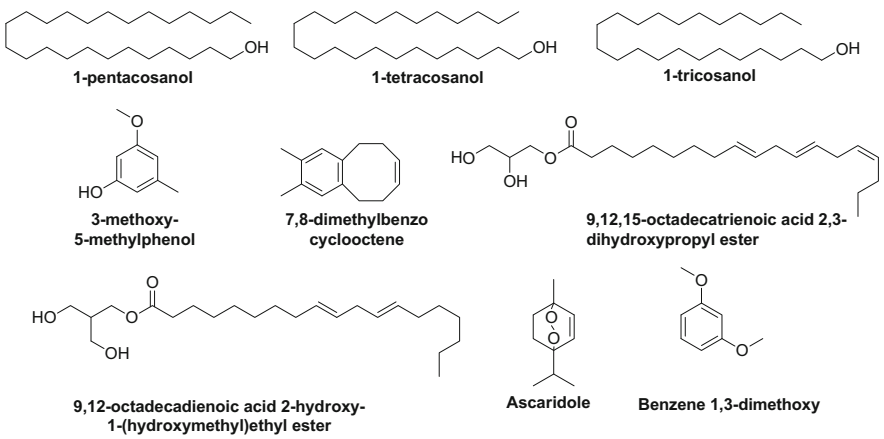


**Fig. 8.3b** Phyto-chemotypes identified through GC-MS from *R. imbricata*

## 8.5.4 Phyto-chemotypes Identified from Ethanol Root Extract

In this root extract, the major constituents were dotriacontane and heptadecane, 9-hexyl represented by 5.7 and 5.4%; others in trace amount include hexadecanoic

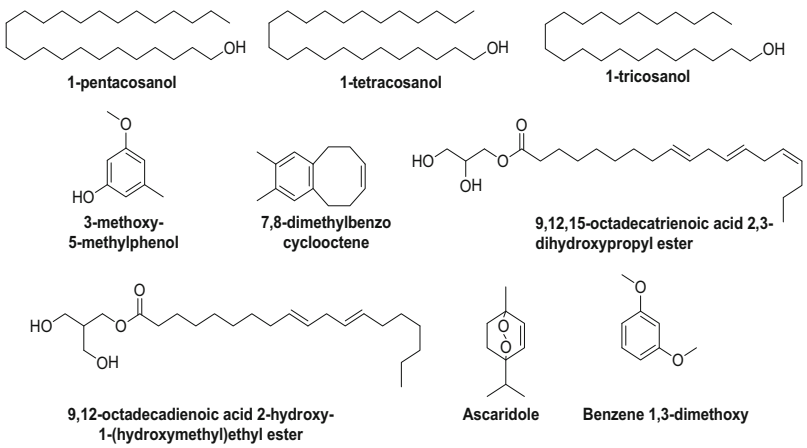


**Fig. 8.3c** Phyto-chemotypes identified through GC-MS from *Rhodiola imbricata*

acid, methyl ester (2.3), bis (2-ethylhexyl) phthalate (3.6), and dibutyl phthalate (1.3).

Total of six fat-soluble vitamins (A, E, D2, D3, K1, K2) and nine water-soluble vitamins (B1, B2, 2 number B3, B5, B6, B7, B9, B12) were analyzed from the root of *R. imbricata* by the RRLC-MS/MS (Tayade et al. 2013a, b, c).

## 8.6 Biological Aspects

### 8.6.1 Immunomodulatory Activity

#### 8.6.1.1 IL-6, TNF-α, and NO Production

Aqueous extract (AE) causes increase in TNF-α and IL-6 production in PBMCs (human) and RAW 264.7 cells. AE also causes significant increase in NO- and LPS-induced production when human cells are incubated for 24 h (probability $<0.05$).

#### 8.6.1.2 Phosphorylated IκB in PBMCs

The expression of p-IκB increased when the cells were exposed with AE at concentration of 250 μg/ml after 40 min, while decreased expression of p-IκB was observed after 60 and 80 mins exposure to the extract.

#### 8.6.1.3 Activation of Transcription Factor NF-κB in PBMCs

Aqueous extract (250 μg/ml) accelerates the nuclear translocation of NF-κB in human PBMCs (Mishra et al. 2006).

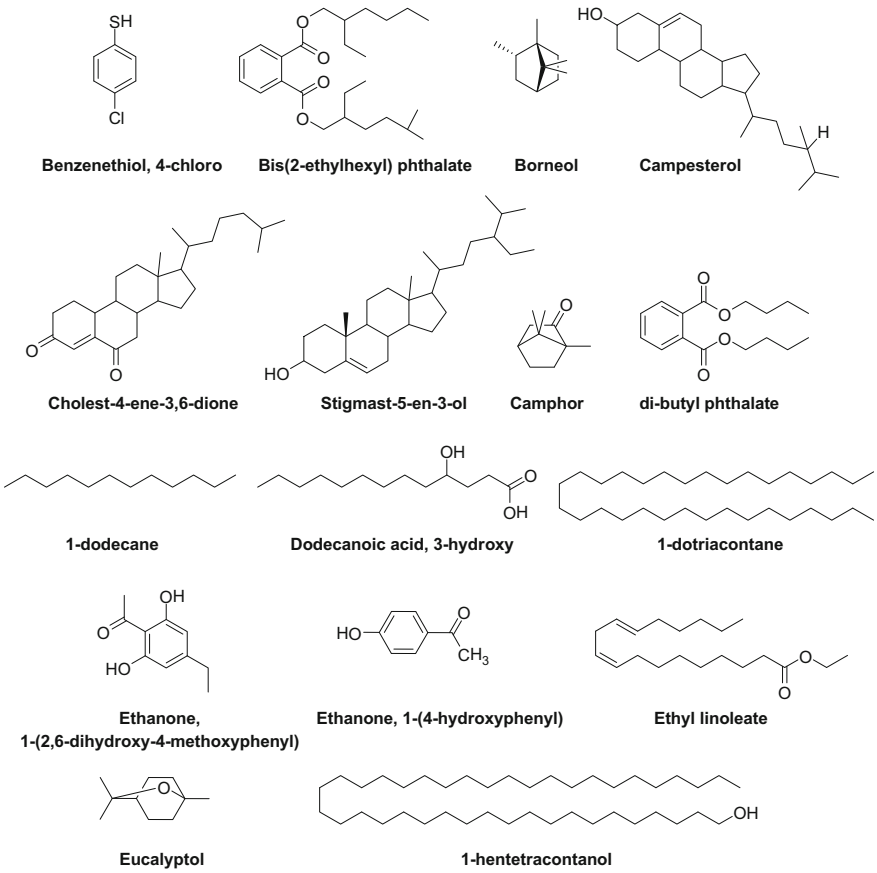


**Fig. 8.3d** Phyto-chemotypes identified through GC-MS from *R. imbricata*

## 8.6.2 Radiomodulatory and Free-Radical Scavenging Activity

### 8.6.2.1 Electron Donation Ability of Hydro-alcoholic Fractionated Extract

A dosage-dependent increase was observed in electron donation ability of extract as does the absorbance. Higher reducing power was achieved at lower AUV.

### 8.6.2.2 O•-2 Quenching Ability of Hydro-alcoholic Fractionated Extract

The O•-2 scavenging potential of extract was significantly higher ($p < 0.05$) between 0.025 and 0.250 mg/ml. Both exhibit >60% activity beyond this concentration ($IC_{50} \leq 0.025$ mg/ml).

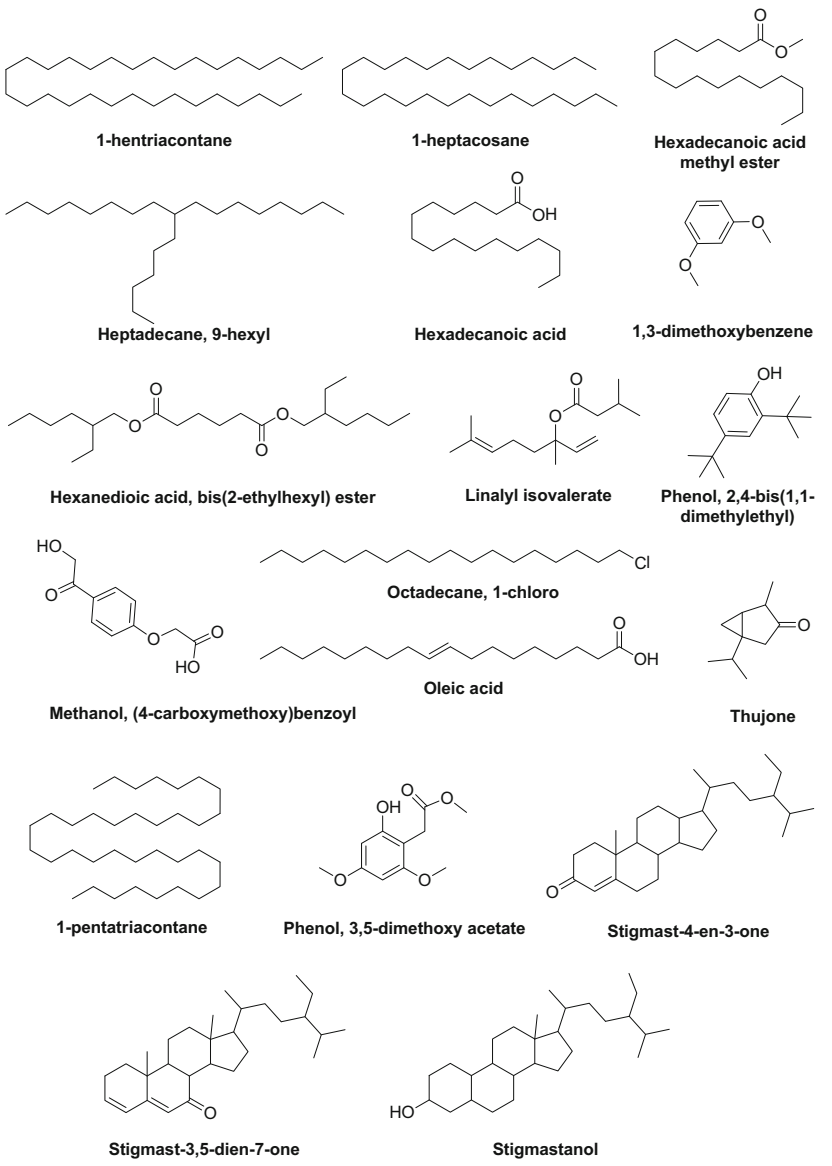


**Fig. 8.3e** Phyto-chemotypes identified through GC-MS from *R. imbricata*

### 8.6.2.3 NO Scavenging Potential of Hydro-alcoholic Fractionated Extract

A dose-dependent increase in NO radical scavenging potential of extract was observed, which was considerably better as compared to standard drug ascorbic acid (vitamin C) at all the tested concentrations. Vitamin C indicated lowering

properties at dosages of 0.75 mg/ml. The higher activity was observed at 2 mg/ml (60.38 ± 1.84%) ($IC_{50}$ = 0.5 mg/ml).

#### 8.6.2.4 Erythrocyte Protection Potential of Hydro-Alcoholic Fractionated Extract

There was 80% hemolysis inhibition with 100 μg/ml. But beyond 500 μg/ml, the extract showed rise of hemolysis upto 2 mg/ml (Arora et al. 2008).

### 8.6.3 Anti-cellular and Immunomodulatory Activity

#### 8.6.3.1 Hemolytic Activity

Aqueous extract did not show any hemolytic effect on RBCs of human blood samples when tested in various concentrations.

#### 8.6.3.2 Effect on HL60 and EL-4 Cells Proliferation

Proliferation of erythroleukemic HL60 cells was significantly inhibited by the aqueous extracts (100 and 200 μg/ml) in dose-dependent manner.

#### 8.6.3.3 ELISPOT Assay for TNF-α

Enhanced TNF-α spots were observed when human PBMCs were treated with the extract by ELISPOT assay.

### 8.6.4 Immunopotentiating Activity

Detectable antibody response to tetanus toxoid (TT) and ovalbumin (OVA) was observed with CFA and aqueous extract treated rats. Antibody titers after 27 days of immunization generated were >1:20,000 against TT which was considerably higher than PBS immunized control rats. Antibody response observed with OVA and CFA was detectable up to dilutions of 1:100; however, aqueous extract in combination with OVA had shown superior response at higher dilution (1:5000). The efficacy of aqueous extract was determined by using strong antigen TT and weak antigen OVA. Therefore, this study suggested that aqueous extract had adjuvant/immune potentiating activity in terms of humoral as well as cell-mediated immune response against strong antigen like TT and weak antigen like OVA (Mishra et al. 2010).

### 8.6.5 Anticancer Activity

#### 8.6.5.1 Effect on Cell Viability

Aqueous extract of 200 μg/ml has no adverse effect on human erythro-leukemic K-562 cells viability in comparison to untreated controls.

#### 8.6.5.2 Effect on Proliferation of K-562 Cells

Dosage-dependent inhibition in erythro-leukemic K-562 cells proliferation was observed when treated with aqueous extract, and decrease in proliferation at 100 and 200 μg/ml was observed.

#### 8.6.5.3 Measurement of ROS in K-562 Cells

A significant increase in reactive oxygen species (ROS) generation was recorded when cells were treated with aqueous extract (200 μg/ml) as compared to control.

#### 8.6.5.4 Effect on Apoptosis

There was a marked increase in apoptosis (17%) in K-562 cells when treated with 200 μg/ml aqueous extract compared to untreated control (11%) with Annexin V FITC and PI after staining.

#### 8.6.5.5 Effect on Cell Cycle

Increase in G2/M phase cells percentage was observed significantly when K-562 cells treated with aqueous extract (200 μg/ml), and aqueous extract arrests cell cycle progression in G2/M phase.

#### 8.6.5.6 Effect on NK Cell Cytotoxicity

About 200 μg/ml aqueous extract of this species significantly observed to increase NK cell cytotoxicity with effector/target ratio of 50:1, and suggested potent anticancer properties useful in treatment of leukemia cancer (Mishra et al. 2008).

### 8.6.6 Radioprotective Activity

#### 8.6.6.1 Maximum Tolerated Dose (MTD)

Aqueous and aqua-alcoholic extract single dosages administered to mice was well tolerated up to 1.1 and 1.3 gm/kg of body weight, respectively, without any noticeable adverse effect with 3 days. However, beyond these levels, mortality was observed in a dose-dependent manner.

#### 8.6.6.2 Hemopoietic Stem Cells Protection

CFU counts in treated control mice, 7.5Gy have $1.91 \pm 0.15$. The aqueous extract pretreated irradiated mice had higher CFU ($17.3 \pm 0.7$) than the aqua-alcoholic pretreated irradiated mice ($15.6 \pm 0.6$). The relative increases over untreated irradiated control were 9.1- and 8.3-fold, respectively. The radioprotective effects generated in terms of survival and increased hemopoiesis led to high curative gains which indicated the potential of this species in the development of a radioprotector (Goel et al. 2006). The extract up to 1400 mg/kg BW was well tolerated by mice without any adverse effect, except for the mice being a little drowsy for 3–5 mins, while in higher doses mortality was observed in a dose-dependent manner. Mice exposed to 10Gy gamma-radiations died before 12th post-irradiation day (Arora et al. 2005).

### 8.6.7 Dermal Wound Healing

1.0% w/v of extract causes significant wound healing when applied topically, 20 µl two times daily for 1 week (Gupta et al. 2007).

### 8.6.8 Immunological Properties of *R. imbricata* Aqueous Extract

#### 8.6.8.1 TLR4-MD2 Expression by Flow Cytometry

The splenocytes dosages with aqueous extract of 25 and 50 µg/ml indicated noticeable increase in the expression of TLR4-MD2 compared to the control group.

#### 8.6.8.2 Enhanced Intracellular Granzyme-B Production

The PE-granzyme-B fluorescence intensity normally used to examine the expression of intracellular granzyme-B in splenocytes. Dosages of aqueous extract at 50 µg/ml for 3 days induced a major increase in expression of Granzyme-B, and this indicated helpful in killing of cancerous cells infections.

#### 8.6.8.3 Increased Production of TH1 Cytokines

PBMCs of human used for evaluating the effect of aqueous extract on production of TNF-γ, IL-1β, IL-2, IL-6, IL-8, TNF-α, IL-10, IL-4, and TNF-β. The hPBMCs treating with aqueous extract at 50, 25, and 12.5 µg/ml records increasing IL-2, IL-1β, IL-6, and TNF-α levels (Mishra et al. 2009).

### 8.6.9 Antiproliferative Activity

It was found that 84% acetone and $CH_3OH$ extracts inhibited the proliferation of HT-29 cells treated at 200 µg/ml concentration (Senthilkumar et al. 2013).

### 8.6.10 Radioprotective Activity

Methanolic fraction exhibited significant antioxidant activity at 250 µg/ml and contributed to radioprotective efficacy. The solvent extraction and dose are essential in bioactivity modulation; this plant species could be developed as a possible prophylactic radiation counter measure for nuclear and radiological emergencies (Chawla et al. 2010).

### 8.6.11 Safety Study

The higher dosages of 250 and 500 mg/kg extract increase BW of rats (both sexes). Increase in physiological parameters, that is, plasma glucose and protein levels, was

recorded at both the dosages, which were observed to be restored to normal after 2 weeks withdrawal of treatment (Tulsawani et al. 2013).

### 8.6.12 Antioxidant Activity

The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 515 nm induced by antioxidants. The DPPH activity of the plant extract was increased at a dose of 0.1–1.2 mg/ml in a dose-dependent manner, and found between 39.55 and 70.76% as compared to standard ascorbic acid 46.78–81.47%. The inhibitory concentrations ($IC_{50}$) of methanol extract (ME) in DPPH radical, nitric oxide, and OH radical were observed to be 0.33, 0.47, and 0.58 mg/ml, respectively (Kumar et al. 2010a, b). Total flavonoid and flavonol contents were probable to be 30.2, 17.67, 20.68, and 7.38 mg quercetin equivalent/g of the plant extract, respectively. The ME exhibits higher antioxidant capacity as compared to aqueous extract (Tayade et al. 2013a, b, c). Senthilkumar et al. (2013) recorded methanol ($IC_{50}$ 62.80 μg/mL) and acetone ($IC_{50}$ 63.80 μg/mL) extracts exhibit the maximum DPPH radical scavenging activity, which is similar to the reference standard BHT ($IC_{50}$ 45.56 μg/mL). At different concentrations (100–1000 μg/ml), *R. imbricata* demonstrated powerful reducing capacity, and root extract records the maximum antioxidant capacities (Kumar et al. 2010a, b).

#### 8.6.12.1 Free Radical Scavenging Activity

Quercetin $IC_{50}$ value, aqueous extract, and BHT were found to be 3.824, 4.391, and 4.743 μg/ml, respectively, and the extract exhibited significant dose-dependent inhibition.

#### 8.6.12.2 Effect on Anti-Lipid Peroxidation Activity

Aqueous extract at 500 μg/ml concentration exhibits the maximum scavenging activity, while the least scavenging activity was observed at 0.5 μg/ml concentration. The $IC_{50}$ values of aqueous extract and α-tocopherol were found to be 5.12 and 4.89 μg/ml, and this suggested inhibition of lipid peroxidation due to free radical scavenging properties.

#### 8.6.12.3 Total Phenolic Content

Gallic acid equivalent of phenols present in 1 g of aqueous extract is 240.0 ± 10 mg (Gupta et al. 2009a, b).

#### 8.6.12.4 Hydrogen Peroxide Radical Scavenging Activity

Aqueous extract had effective $H_2O_2$ scavenging activity (3.254 μg/ml) as compared to standard (4.004 μg/ml).

#### 8.6.12.5 Total Flavonoid Content

The total flavonoid content was found to be 66.7 μg quercetin equivalent/mg of aqueous extract.

### 8.6.13 Antioxidative Effect during Cold, Hypoxia, and Restraint Exposure

Antioxidative potential of *R. imbricata* root aqueous extract (100 mg/kg) was examined in rats. It was administered orally 30 min prior to cold (5 °C)–hypoxia (428 mmHg)–restraint (C–H–R) exposure. There was restricted increase in blood MDA, GSH (glutathione),) and SOD (superoxide dismutase) activity with limited rise in blood, muscle, and liver LDH; improved muscle and liver SOD on attaining $T_{rec}23$ and $T_{rec}37$ °C; liver CAT on attaining $T_{rec}23$ °C and liver GST during recovery when administered with single dose of aqueous extract.

#### 8.6.13.1 Adaptogenic Activity

Studies by Gupta et al. (2008) record 100 mg/kg drug dosages significantly provide the maximum resistance to C–H–R induced hypothermia (103.9%) and speed up the recovery rate by 36.8%. One dose of plant extract considerably increased time required to attain $T_{rec}23$ °C during C-H-R exposure, and it also decreased time recover ($T_{rec}37$ °C) after C-H-R exposure as compared with experimental controls (Gupta et al. 2009a, b).

#### 8.6.13.2 Acute Toxicity

There was no mortality when rats were treated at oral doses of 1, 2, 5, and 10 g/kg with aqueous extract.

### 8.6.14 Cytoprotective Activity

An increase in cytotoxicity and apoptosis was recorded significantly over control cells in tert-BHP presence (Table 8.1). Both aqueous and alcoholic extracts (250 μg/ml) were observed to inhibit tert-BHP induced free radical production and cell death. These results clearly suggested *R. imbricata* had marked cytoprotective and antioxidant activities (Kanupriya et al. 2005).

**Table 8.1** *Rhodiola imbricata* effect on tert-BHP induced cytotoxicity

| Groups | Live cells (%) |
|---|---|
| Control | 102 ± 7.4 |
| tert-BHP | 66 ± 9.0 |
| Rh (aq) | 99 ± 8.2 |
| Rh (aq) + tert-BHP | 83 ± 8.0 |
| Rh (alc) | 95 ± 6.5 |
| Rh (alc) + tert-BHP | 90 ± 7.3 |
| Vit C + tert-BHP | 82 ± 7.1 |

### 8.6.15 Hepatoprotective Activity

#### 8.6.15.1 Acute Toxicity

Acetone extract at 2000 mg/kg of root in Swiss albino mice did not indicate any mortality.

#### 8.6.15.2 Effect on Hematological Parameters

Paracetamol causes hepatic injury with a decrease in erythrocytes, leucocytes, and platelets. Administration of acetone extract at 400 mg/kg exhibited increased levels of hematological parameters (Table 8.2).

#### 8.6.15.3 Effect on Serum Biochemical Markers

Administration of *R. imbricata* acetone extract at 400 mg/kg decreased the liver markers SGPT (78.92 ± 0.4 U/l), SGOT (89.51 ± 0.1 U/l), and ALP (192.47 ± 0.4 U/l) which was comparable to standard silymarin (25 mg/kg) (Table 8.3).

#### 8.6.15.4 Effect on In Vivo Antioxidant Activity

The use of 400 mg/kg, p.o. acetone extract increases the total proteins, enzymatic antioxidants SOD, and GPx (Table 8.4).

#### 8.6.15.5 Histopathological Examination

Significant reduction of serum enzyme markers, which was elevated by paracetamol, was observed when acetone extract of this plant species was administered at concentrations of 200 and 400 mg/kg, p.o. continuously for 2 weeks and found comparable to silymarin effect. At a dose of 400 mg/kg the acetone extract observed to have the highest protection (Senthilkumar et al. 2014).

**Table 8.2** Effect on hematological parameters

| Parameter | Control | Paracetamol | Silymarin, 25 mg/kg | 200 mg/kg | 400 mg/kg |
|---|---|---|---|---|---|
| RBC count | 6.9 ± 0.3 | 3.4 ± 0.4 | 7.2 ± 1.3 | 5.3 ± 0.1 | 7.2 ± 0.6 |
| Hemoglobin | 12.8 ± 0.4 | 7.4 ± 1.3 | 12.6 ± 2.3 | 10.9 ± 2.3 | 11.4 ± 1.1 |
| Hematocrit | 31.89 ± 1.5 | 16.9 ± 2.3 | 32.1 ± 1.2 | 25.3 ± 0.1 | 29.3 ± 1.4 |
| MCV | 46.8 ± 0.4 | 55.2 ± 0.1 | 46.5 ± 1.2 | 31.2 ± 0.2 | 34.8 ± 2.4 |
| MCH | 18.8 ± 0.3 | 22.9 ± 0.1 | 18.0 ± 2.1 | 18.2 ± 0.4 | 16.7 ± 1.3 |
| RDW | 10.8 ± 1.2 | 13.8 ± 3.2 | 10.4 ± 2.3 | 8.4 ± 1.5 | 9.6 ± 1.3 |
| WBC count | 7200 ± 1.0 | 2500 ± 2.0 | 7000 ± 1.0 | 6000 ± 1.0 | 5700 ± 1.0 |
| Polymorphs | 8.3 ± 1.1 | 10.4 ± 0.1 | 7.5 ± 1.4 | 6.2 ± 2.3 | 6.4 ± 1.3 |
| Lymphocytes | 86 ± 0.4 | 92 ± 10.2 | 76 ± 1.4 | 66 ± 0.6 | 72 ± 0.1 |
| Platelets | 525 ± 1.2 | 81 ± 2.4 | 406 ± 0.1 | 374 ± 1.3 | 395 ± 2.3 |

Units: RBC count, mill/cmm; Hemoglobin, gm percentages (%); Hematocrit, percentages (%); MCV, fl; MCH, pg; RDW, percentages (%); WBC count, cells/cmm; Polymorphs, percentages (%); Lymphocytes percentages (%); Platelets, thou/cmm

**Table 8.3** Effect on serum biochemical markers

| Parameter | Group-I (control) | Group-II (Paracetamol) | Group-III (Silymarin, 25 mg/kg) | Group-IV (200 mg/kg) | Group-V (400 mg/kg) |
|---|---|---|---|---|---|
| Creatinine (mg/dl) | 0.72 ± 0.06 | 0.74 ± 0.03 | 0.65 ± 0.04 | 0.74 ± 0.05 | 0.73 ± 0.01 |
| Bilirubin (mg/dl) | 0.18 ± 0.04 | 0.13 ± 0.05 | 0.18 ± 0.04 | 0.13 ± 0.02 | 0.18 ± 0.06 |
| SGOT (U/l) | 38.81 ± 0.6 | 162.36 ± 0.7 | 54.81 ± 0.6 | 122.90 ± 0.4 | 89.51 ± 0.1 |
| SGPT (U/l) | 33.22 ± 0.4 | 147.50 ± 0.3 | 46.21 ± 0.6 | 114.28 ± 0.6 | 78.92 ± 0.4 |
| ALP (U/l) | 157.35 ± 0.2 | 262.55 ± 0.2 | 166.69 ± 0.2 | 223.52 ± 0.5 | 192.47 ± 0.4 |
| Cholesterol (mg/dl) | 53.98 ± 0.4 | 54.78 ± 0.3 | 54.53 ± 0.5 | 54.62 ± 0.5 | 55.13 ± 0.2 |
| Triglycerides (mg/dl) | 56.23 ± 0.4 | 52.42 ± 0.6 | 55.23 ± 0.4 | 52.41 ± 0.5 | 54.12 ± 0.4 |

**Table 8.4** Effect on in vivo antioxidant activity

| Biochemical markers | Control | Paracetamol | Silymarin, 25 mg/kg | 200 mg/kg | 400 mg/kg |
|---|---|---|---|---|---|
| Total protein | 0.232 | 0.078 | 0.241 | 0.108 | 0.192 |
| SOD | 0.480 | 0.186 | 0.506 | 0.239 | 0.318 |
| CAT | 229.27 | 97.9 | 230.31 | 146.65 | 184.07 |
| GPx | 22.52 | 13.13 | 22.91 | 15.17 | 18.98 |
| GSH | 18.93 | 10.65 | 18.08 | 15.05 | 15.94 |

Note: unit of protein, μg/10 mg of tissue; unit of SOD, units/min/mg/protein; CAT unit, μmole of $H_2O_2$ consumed/min/mg protein; unit of GPx, mg GSH consumed/min/mg protein; unit of GSH, μmol of GSH/mg protein

## 8.7 Future Direction

The usage of herbal medicine for the cure of various illnesses has increased greater concern about the safety and least or no side effects. *R. imbricata* is used in several folklore local medicines. Many scientific studies have established that *R. imbricata* possesses unique cardioprotective and adaptogenic functions. The importance of this species mentioned in this communication recommended that the species of this plant increase resistance to stress and salidroside, responsible for preventing oxidative stress. Considering the importance of the plant, further biological studies should be conducted to identify the compounds responsible for pharmacological properties, so that the active molecules present in this species of plants could be utilized for future drug development programs.

**Acknowledgment** Authors are thankful for the financial assistance from SERB, DST, Government of India (EMR/2016/002584), and IIIM project (MLP4011).

**Conflict of Interest** Authors declare no conflict of interest.

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# 9 *Phyllanthus amarus* Schum. and Thonn. as Herbal Medicine: Ethnobotany, Phytochemistry, and Pharmacology Aspects

Sunil Kumar

**Abstract**

*Phyllanthus amarus* Schum. and Thonn. (family Euphorbiaceae) is a small herb well known for its potent medicinal properties and widely used worldwide. *P. amarus* is an important plant reported in Indian Ayurvedic system of medicine to treat various problems related to the stomach, kidney, genitourinary system, liver, and spleen. It is bitter, astringent, diuretic, febrifuge, stomachic, and antiseptic. The whole plant is used in menorrhagia, gonorrhea, and other genital affections. The present chapter covers the ethnobotany, phytochemistry, and pharmacology of *P. amarus*. The lignans, flavonoids, hydrolysable tannins (ellagitannins), polyphenols, triterpenes, sterols, and alkaloids are class of phytochemical reported in the different plant parts of *P. amarus*. The extracts, their fractions, and the phytochemicals isolated from *P. amarus* show a wide spectrum of pharmacological activities such as antiplasmodial, antibacterial, antimalarial, antimicrobial, anti-inflammatory, antiviral, anticancer, hypolipidemic, antidiabetic, hepatoprotective, nephroprotective, antioxidant, and diuretic properties. The present communication summarizes information concerning the ethnobotany, ethnopharmacology, phytochemistry, and biological activities of *P. amarus*.

**Keywords**

*Phyllanthus amarus* · Traditional uses · Pharmacological activities · Phytochemical analysis

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_9

## Abbreviations

| | |
|---|---|
| cm | Centimeter |
| HBV | Hepatitis B virus |
| HIV | Human immunodeficiency virus (HIV) |
| TLC | Thin-layer chromatography |
| HPLC | High-performance liquid chromatography |
| GC | Gas chromatography |
| GC-MS | Gas chromatography–mass spectrometry |

## 9.1 Introduction

*Phyllanthus* L. (Euphorbiaceae) is a large genus having more than 800 species (Chaudhary and Rao 2002). This genus was described by Carolus Linnaeus for the first time in 1737 (Webster 1956). Phyllanthus, derived from Greek meaning "leaf and flower," is named so because the leaf, flower, and fruit appear fused. Plants of this genus are distributed throughout tropical and subtropical countries of the American, African, and Asian continents and occur as trees, shrubs, and herbs (Webster 1994). Some of these are also known by different local names such as stonebreaker, carry me seed, leafflower, wind breaker, chanca piedra, smartweed, chamber bitter, gripeweed, Bhumi Amalaki, dukung anak, keezharnelli, meniran, *niruri*, paraparai mi, phyllanto, seed-under-leaf, Tamalaka, Yaa Tai Bai, zhu zi cao, and others (Natural Medicines 2018). *P. debilis*, *P. urinaria*, *P. virgatus*, *P. maderaspatensis*, *P. amarus*, *P. acidus*, *P. emblica*, *P. niruri*, *P. reticulates*, *P. fraternus*, *P. simplex*, *P. rheedii*, and *P. polyphyllus* are reported in India. More than 50 species of *Phyllanthus* including *Phyllanthus amarus* are found in India (Tharakan 2011). *P. amarus* is an erect herb that grows (30–40 cm tall) throughout India along roadsides and valleys, on riverbanks, and near lakes (Fig. 9.1). It has tiny greenish leaves, stems, and fruits. It is widely distributed in subtropical and tropical areas of China, India, Indonesia, and Malay Peninsula (Zhang et al. 2003; Khan 2009). The fruits have immense medicinal value and hence have been consumed as nutritional food and used in traditional medicines for thousands of years in India, China, and Southeast Asian countries (Singh 2019).

## 9.2 Ethnobotany

Herbs being easily accessible to mankind have been widely explored for their medicinal properties. The cultural tradition associated with the use of medicinal plants for treating various disease conditions is one of the oldest, richest, and most diverse in India. This traditional knowledge is codified in the millennia-old Indian system of medicine, Ayurveda. Several *Phyllanthus* species are used widely in traditional medicine for the treatment of diabetes, jaundice, gallbladder, and liver

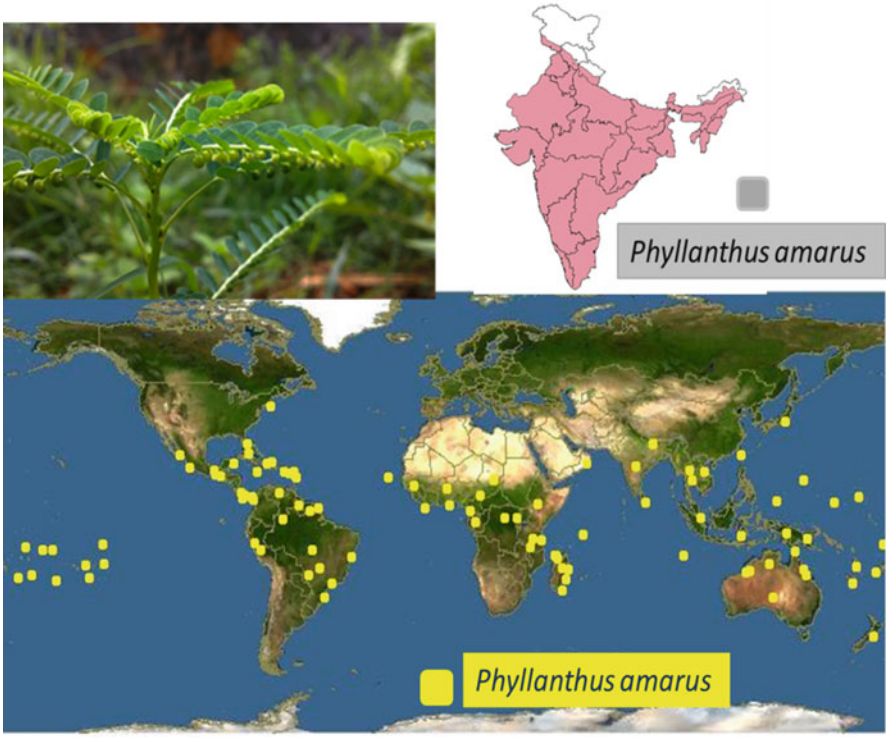



**Fig. 9.1** Habitat of *Phyllanthus amarus* worldwide

diseases (Calixto et al. 1998; Dhiman and Chawla 2005; Shirani et al. 2017). *Phyllanthus* has remarkable antiviral, antioxidant, antidiabetic, hepatoprotective, and anticancer activities and a long history of use in the treatment of intestinal parasites and liver, kidney, and bladder diseases (Kirtikar and Basu 1933; Nadkarni 1976; Kuttan and Harikumar 2012). As a traditional medicine, it is used to cure jaundice and other liver diseases. Folk medicine and local herbal practitioners vouch for the healing powers of the genus for diseases ranging from headache, asthma, diarrhea, skin diseases, diabetes, malaria, indigestion, wounds, and a host of others (Mao et al. 2016). It is used in Ayurveda for digestive, genitourinary, respiratory, and skin diseases. Several factors such as the therapeutic potential of *Phyllanthus* for the management of many diseases, widespread availability of many species in tropical and subtropical regions, and the diversity of secondary metabolites contribute to their increasing use as traditional medicine. The infusion of leaves, stems, and roots of most species of *Phyllanthus* has been used in folk medicine for the treatment of kidney and bladder stones, urinary tract-related diseases, intestinal infections, diabetes, and hepatitis B. Seeing the popularity of herbal medicines, the modern society is now eager to resort to green medicines which are without adverse side effects.

*P. amarus* is one of the most important plants traded as a raw herbal drug in India (Ved and Goraya 2008). Several herbal drug formulations involving *Phyllanthus* species are in the market of India. For example, for jaundice and other liver ailments in general, herbal drug formulations such as Hepex, Liv 52, Livomap, Liv D, Liv Plus, Vimliv, Nirocil, Livocin, Livcure, Livol, etc. are very popular. *P. amarus*, an important herb of Indian Ayurvedic system, is bitter, astringent, stomachic, diuretic, febrifuge, and antiseptic and used traditionally as hepatoprotective, expectorant, diuretic, anti-inflammatory, antiseptic, and antispasmodic. The Indian Ayurvedic system describes its use in problems of the stomach, genitourinary system, liver, kidney, and spleen (Singh and Bedi 2017). It is also used to treat flu, dropsy, jaundice, diabetes, irregular menstruation, intestinal parasites, asthma, bronchial infections, bladder problems, hepatic, and urolithic diseases (Patel et al. 2011). The other activities reported include antipyretic, antibacterial, antioxidant, anticancer, and hepatoprotective (Barik et al. 2006). It is also reported to inhibit hepatitis B virus (HBV) and human immunodeficiency virus (HIV) (Thyagarajan et al. 1990; Kiemer et al. 2003; Notka et al. 2004; Srividya and Periwal 1995). It is predominantly used as a cure for liver disorders in India (Nadkarni 1976; Kritikar and Basu 1993; Thyagarajan and Jayaram 1992; Thakur et al. 2020). Its increasing use can be attributed to activities against hepatitis B, HIV and other viral diseases, jaundice, kidney stones, gallbladder stones, and cancer (Singh and Borthakur 2011). It has potent antioxidant property and could scavenge superoxides and hydroxyl radicals (Lim and Murtijaya 2007; Maity et al. 2013).

## 9.3 Phytochemistry

More than 500 compounds comprising alkaloids, flavonoids, glycosides, lignans, phenols, sterols, tannins, and terpenoids have been isolated from the genus *Phyllanthus* (Calixto et al. 1998; Qi et al. 2014; Mao et al. 2016; Nisar et al. 2018). Phenolic compounds such as flavonoids, phenolic acids, tannins are abundant in *Phyllanthus* species (Nisar et al. 2018). Lignans exist abundantly in the *Phyllanthus* genus. Phyllanthin is an active lignan isolated from various *Phyllanthus* species. The phytochemical diversity in *Phyllanthus* is evident from the classes of compounds identified. Alkaloids, flavonoids, glycosides, lignans, phenols, phenylpropanoids, tannins, terpenes, and saponins are the major classes of bioactive compounds found in *Phyllanthus*. The unique structural diversity found among the *Phyllanthus* compounds and their strong bioactive nature make *Phyllanthus* genus of great commercial value (Fig. 9.2).

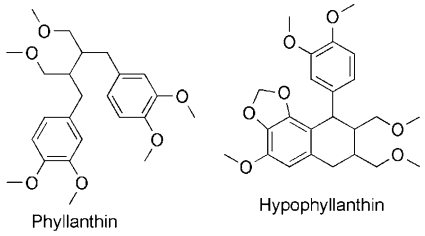



**Fig. 9.2** Marker compounds in *Phyllanthus amarus*

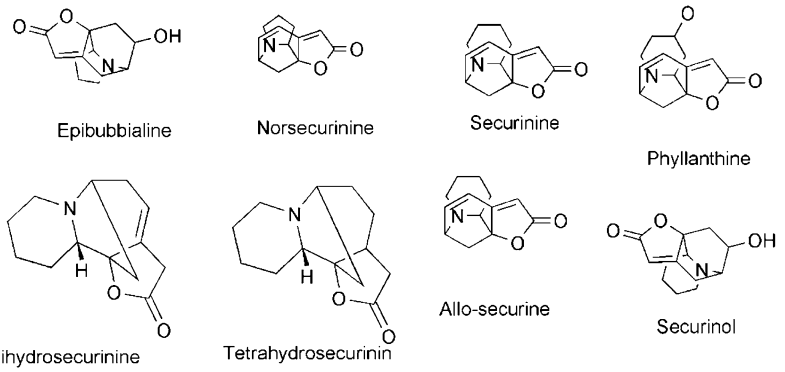
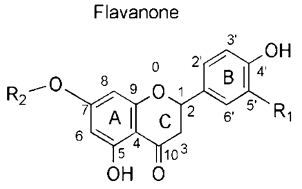


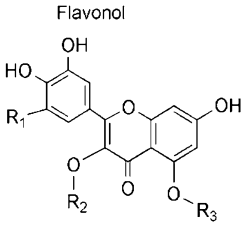

**Fig. 9.3a** Structure of alkaloids in *Phyllanthus amarus*

The phytochemicals in *P. amarus* comprise different classes of organic compounds of medicinal importance. These include alkaloids, flavonoids, hydrolysable tannins (ellagitannins), major lignans, polyphenols, triterpenes, sterols, and volatile oil (Patel et al. 2011; Verma et al. 2014). Active compounds such as phyllanthin, hypophyllanthin, niranthin, nirtetralin, phyltetralin, phyllangin, nirphyllin, phyllnirurin, and corilagin have been isolated from *P. amarus* (Figs. 9.3a, 9.3b, 9.3c, 9.3d, 9.3e, 9.3f, 9.3g, and 9.3h).

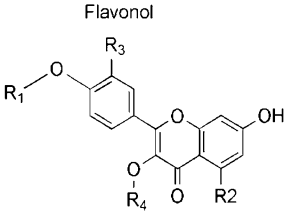
The leaves contain several lignans such as phyllanthin (a bitter constituent), hypophyllanthin (a non-bitter constituent), niranthin, nirtetralin, and phyltetralin (Rastogi and Mehrotra 1991). Isobubbialine and epibubbialine, two new securinega-type alkaloids, were isolated from the leaves of *P. amarus* along with three known alkaloids, phyllanthine, securinine, and norsecurinine (Houghton et al. 1996). From the polar extractives of the aerial parts of *P. amarus*, geranic acid and other tannins including a novel cyclic hydrolysable tannin, amarulone, were isolated (Foo and Wong 1992; Foo 1993, 1995). Flavonoides and sterols were identified in *P. amarus* hexane extract, whereas sterols and triterpenes were identified in the methanol extract (Zubair et al. 2016). An unusual flavonoides in natural products, quercetin-3-*O*-*β*-D-glucuronopyranoside, was detected in the aqueous extract of *P. amarus* (Sprenger and Cass 2013). The phytochemicals similar to those found in *P. amarus* are also present in *P. niruri* and include bioactive molecules, such as lignans, flavonoids, glycosides, tannins, alkaloids, ellagitannins, triterpenes, phenylpropanoids, and steroids (Bagalkotkar et al. 2006). Seventeen lignans have been identified from *P. niruri*. Phyllanthin, hypophyllanthin, niranthin, phyltetralin, nirtetralin, isonirtetralin, hinokinin, phyllnirurin, lintetralin, isolintetralin, demethylenedioxy-niranthin, and 5-demethoxy niranthin are among the lignans isolated from *P. niruri*. The number of alkaloids from *P. niruri* is considerably more than that from *P. amarus*. These alkaloids include 4-hydroxysecurinine, 4-methoxy-dihydro-norsecurinine, 4-methoxynorsecurinine, 4-methoxytetrahydrosecurinine, allosecurinine, dihydrosecurinine, ent-norsecurinine, epibubbialine, isobubbialine, nirurine,



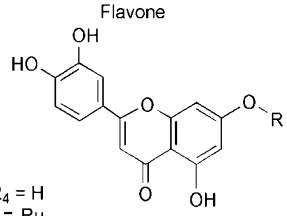

Prunin: $R_1$ = H, $R_2$ = glc
Eriodictyol: $R_1$ = OH, $R_2$ =H
Naringenin:$R_1$ = H, R = H



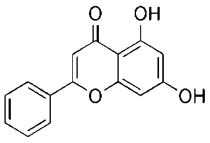

Quercetin diglycoside: $R_1$ = H, $R_2$ = $R_3$ = glc
Quercetin-3-O-hexoside: $R_1$ = H, $R_2$ = glc, $R_3$ = H
Rutin: $R_1$ = H, $R_2$ = Ru, $R_3$ = H
Myricitrin: $R_1$ = OH, $R_2$ = Rh, $R_3$ = H
Quercetin-3-*O*-l-rhamnoside(quercitrin): $R_1$ = H, $R_2$ = Rh, $R_3$ =H
Quercetin:$R_1$ = H, $R_2$ = H, $R_3$ = H



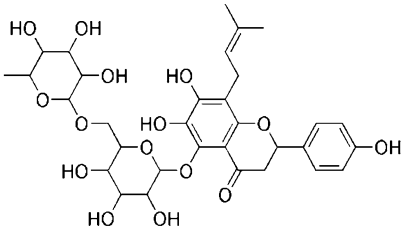

Kaempferol-4'-*O*-l-rhamnoside: $R_1$= Rh, $R_2$ = H, $R_3$ = H, $R_4$ = H
Kaemferol-3-O-rutinoside: $R_1$= H, R2 = OH, $R_3$ = H, $R_4$ = Ru
Fisetin- 4'-glucoside: $R_1$ = glc, $R_2$ = H, $R_3$ = OH, $R_4$ = H
Kaempferol: $R_1$ = H, $R_2$ = OH, R = H, $R_4$ = H



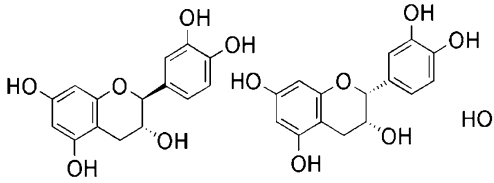

Luteolin-7-*O*-rutinoside: R=Ru
Luteolin-7-*O*-glucoside R=glc
Luteolin: R=H



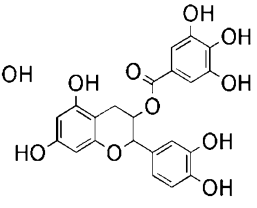

Chrysin




Nirurin




Catechin Epicatechin




Catechin 3-gallate

**Fig. 9.3b** Structure of flavonoids in *Phyllanthus amarus*

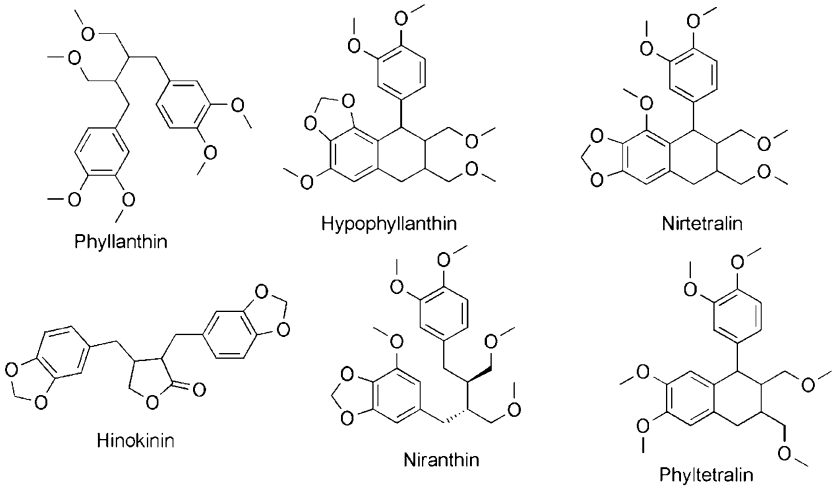



**Fig. 9.3c** Structure of lignans in *Phyllanthus amarus*

norsecurinine, 4-methoxy-nor-securinine, phyllanthine, securinine, securinol A, securinol B, and tetrahydrosecurinine (Table 9.1).

## 9.4 Pharmacological Activity

The medicinal plants under the genus *Phyllanthus* produce diverse classes of secondary metabolites including alkaloids, flavonoids, lignans, phenolic acids, and tannins and are used for treating a variety of medical conditions (Calixto et al. 1998). Pharmacological screening revealed that phyllanthin is antioxidant, hepatoprotective, anticancer, antidiabetic, immunosuppressant, and anti-inflammatory. Phyllanthin and hypophyllanthin show antitumor activities. It is reported that the aqueous extract of several species of *Phyllanthus* exhibits potent in vitro and in vivo inhibition against hepatitis B virus (Syamsunder et al. 1985; Thyagarajan et al. 1988; Shead et a1. 1992). The effectiveness and biosafety of the genus *Phyllanthus* for chronic HBV infection were reported (Liu et al. 2001). It is an effective remedy for urolithiasis, one of the oldest known diseases (Lopez and Hoppe 2010). The presence of a large number of polyphenols in *Phyllanthus* species is the reason why they are strong antioxidants (Joy and Kuttan 1995).

Studies carried out by Patel et al. (2011) indicated several pharmacological activities such as antibacterial, anticancer, antidiarrheal, gastroprotective, antiulcer, antifungal, analgesic, anti-inflammatory, antiallodynic, antinociceptic, antioxidant, antiplasmodial, antiviral, aphrodisiac, contraceptive, diuretic, hepatoprotective, immunomodulatory, nephroprotective, and radioprotective, and spasmolytic is exhibited by *P. amarus*. The aqueous extract of *P. amarus* was found to be active against 20-methylcholanthrene (20-MC)-induced sarcoma development

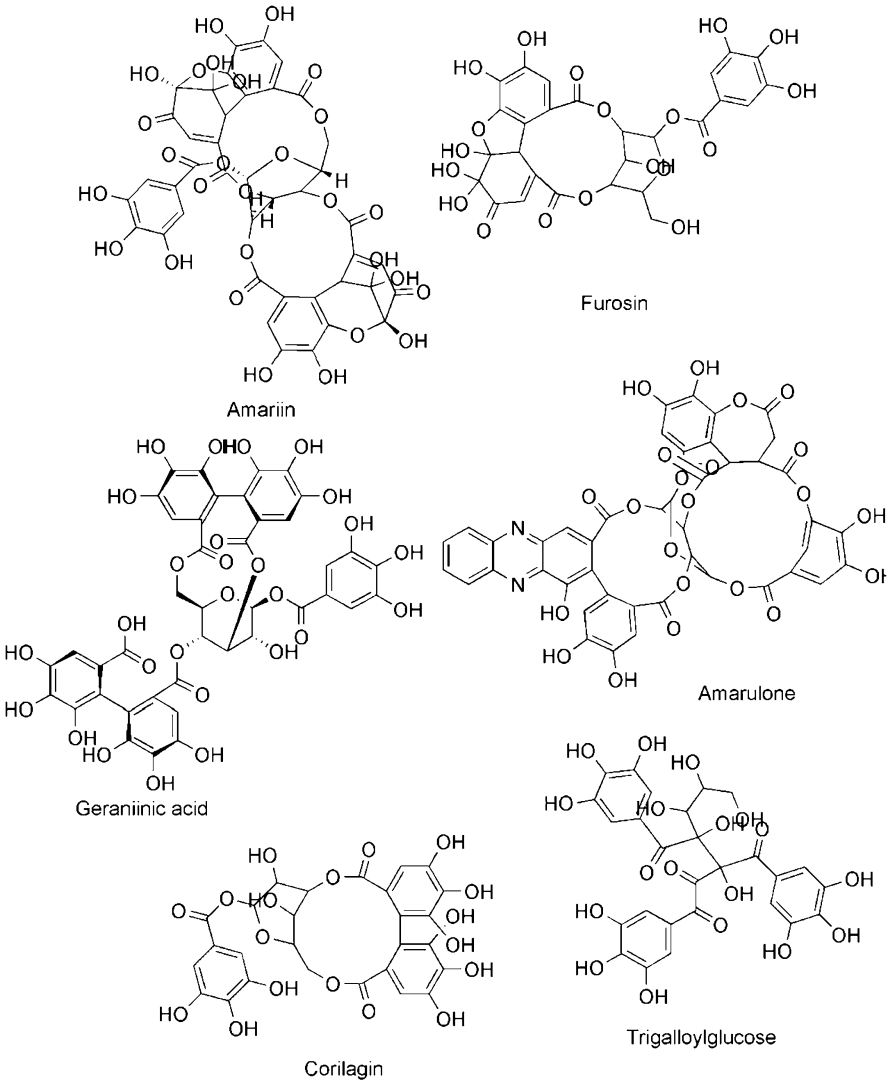



**Fig. 9.3d** Structure of tannins in *Phyllanthus amarus*

(Rajeshkumar et al. 2002). Administration of methanolic extract of *P. amarus* significantly reduced the toxic side effects of cyclophosphamide in mice without interfering with its antitumor efficiency (Kumar and Kuttan 2005). Lignans have shown antimitotic, antitumor, antiviral (MacRae and Towers 1984), and anti-HIV (Schroder et al. 1990) activities and protective effects against hormone-related cancers due to their antioxidant effects (Lee and Xiao 2003). Methanol extract of *P. amarus* was found to have very high inhibitory activity against bacterial species such as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis,*

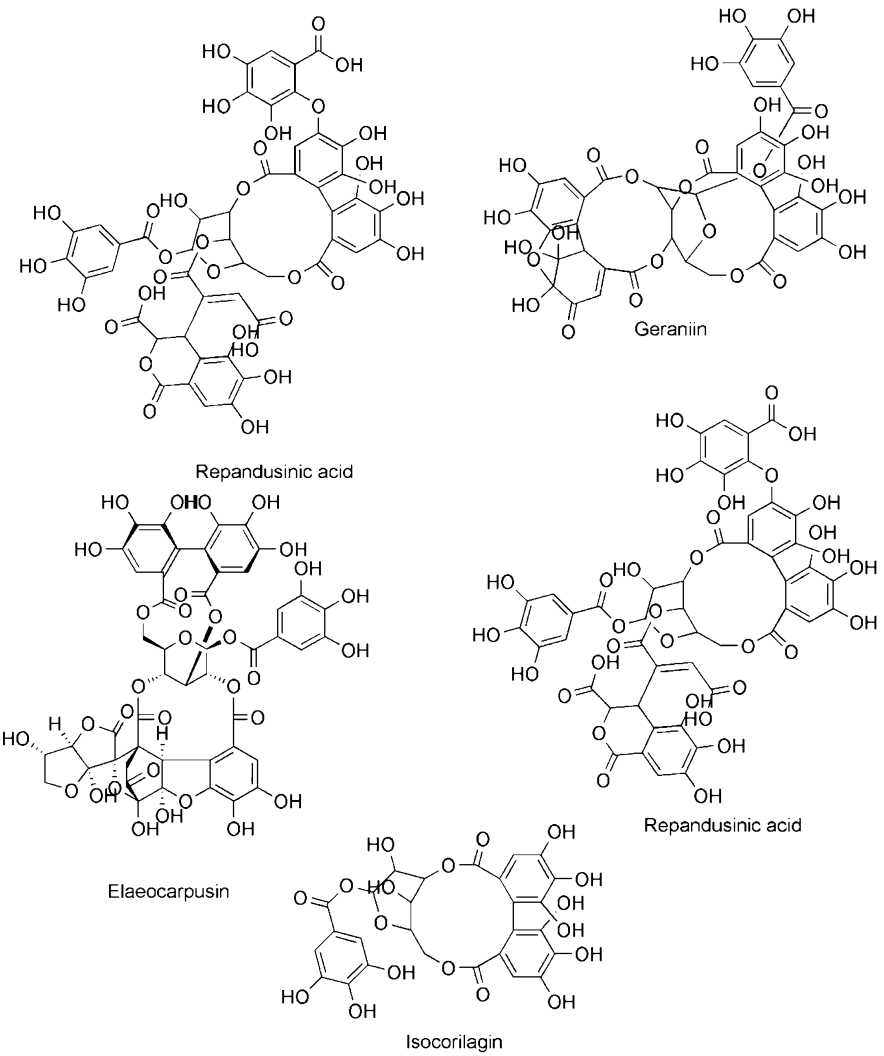



**Fig. 9.3e** Structure of tannins in *Phyllanthus amarus*

*Streptococcus faecalis, Enterobacter* species, *Serratia marcescens, Staphylococcus aureus*, and *Escherichia coli.* The aqueous extract of *P. amarus* and its ethanol fraction showed hypotensive effect in rabbits (Amonkan et al. 2013). Inhibitory action was also noticed for aqueous and methanol extracts against *leptospira*. Methanol extract of the leaves of *P. amarus* has great anti-inflammatory and analgesic potential (Ofuegbe et al. 2014). *P. amarus*, widely used in Ayurvedic medicine for the treatment of liver diseases, has shown both in vitro and in vivo activities against hepatitis B virus. A correlation between total phenolic content and

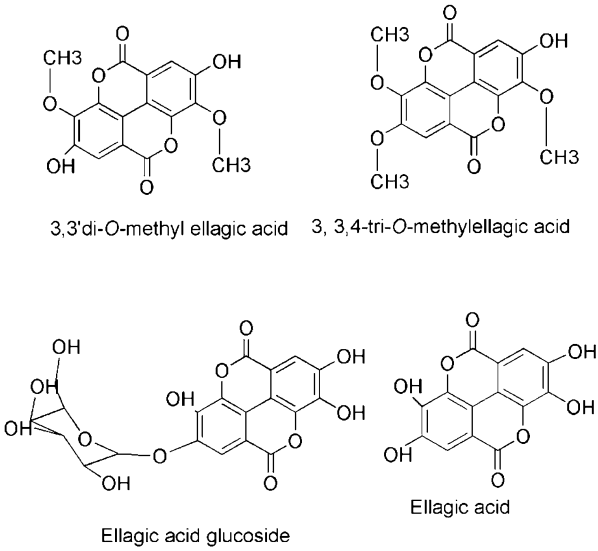



**Fig. 9.3f** Structure of ellagic acid and derivatives in *Phyllanthus amarus*

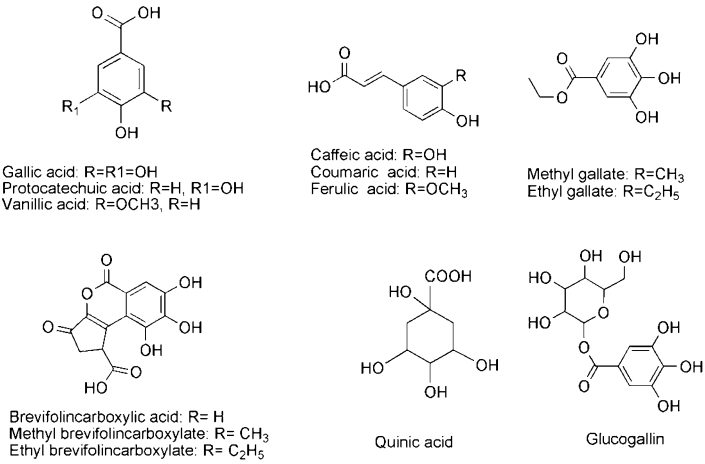



**Fig. 9.3g** Structure of phenolic acid and others in *Phyllanthus amarus*

antioxidant activity was observed for methanolic extract of *P. amarus* (Kumaran and Karunakaran 2007).

Hepatoprotective effect of the aqueous extract and antidiabetic effect of the ethanolic extract have been reported (Pramyothin et al. 2007; Thyagarajan et al. 1988). Ellagitannins and flavonoids from *P. amarus* relieved the oxidative stress after radiation (Londhe et al. 2009). Ellagitannins geraniin and amariin were found to protect the liver cell from ethanolic cytotoxicity (Londhe et al. 2012). Aqueous

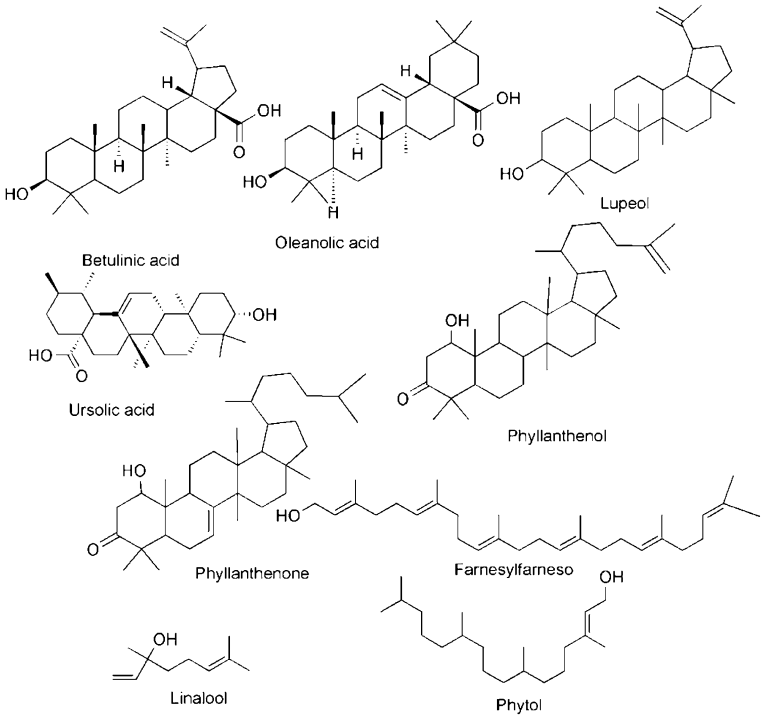



**Fig. 9.3h** Structure of terpenoids and oils in *Phyllanthus amarus*

ethanol extract of *P. amarus* was found to block HIV-1 attachment both in vitro and in vivo (Notka et al. 2004). The lignan niranthin exhibited anti-inflammatory and antiallodynic activities (Kassuya et al. 2006). It was suggested that the polyphenols and lignans in *P. amarus* could be the major contributors to the immunomodulatory effect of the plant (Jantan et al. 2014). Methanolic extract of *P. amarus* showed significant concentration-dependent antibacterial activity particularly against gram-negative microbes (Mazumder et al. 2006). Silver nanoparticles synthesized from plant of *P. amarus* extract showed excellent antibacterial potential against multidrug resistant strains of *P. aeruginosa* from burn patients (Singh et al. 2014). Extensive studies on *P. amarus* have demonstrated it to be antiviral against hepatitis B and C viruses, hepatoprotective, immunomodulating, and anti-inflammatory (Thyagarajan et al. 2002).

## 9.5 Phytochemical Analysis by Analytical Techniques

Herbal raw materials are influenced by identity of the plant, geographical location, seasonal variation, and drying and storage conditions. There is increasing concern regarding adulteration and species admixture in the raw herb trade as the adverse

**Table 9.1** Phytochemicals in *Phyllanthus amarus*

| Class | Compounds |
|---|---|
| Alkaloids | Epibubbialine, norsecurinine, securinine, phyllanthine, dihydrosecurinine, tetrahydrosecurinine, allosecurinine, securinol |
| Flavonoids | Prunin, eriodictyol, naringenin, quercetin-3,4′-di-*O*-glucosides, quercetin-*O*-hexoside, rutin, quercetin-*O*-hexoside (isomer), myricitrin, quercitrin, kaemferol-3-O-rutinoside, fisetin-*O*-hexoside, kaempferol-*O*-hexoside, quercetin, kaempferol, luteolin-O-dihexoside, luteolin-*O*-hexoside, luteolin, nirurin, chrysin, catechin, epicatechin |
| Lignans | Phyllanthin, hypophyllanthin, nirtetralin, phyltetralin, nirtetralin, hinokinin, niranthin |
| Tannins and other phenols | Amariin, amariinic acid, amarulone, corilagin, geraniin, geraniinic acid B, furosin, phyllanthusiin A, phyllanthusiin B, phyllanthusiin C, phyllanthusiin D, repandusinic acid, elaeocarpusin, 4-galloyl quinic acid, gallic acid, ellagic acid, *β*-glucogallin, trigalloylglucose, castalin, protocatechuic acids, methyl gallate, p-coumaric acid, caffeic acid, syringing, 4-hydroxybenzoic acid, ellagic acid-O-hexoside, di-*O*-methyl ellagic acid, tri-*O*-methylellagic acid |
| Terpenoids | Betulinic acid, oleanolic acid, lupeol, phyllanthenol, phyllanthenone 2*Z*, 6*Z*, 10*Z*, 14*E*, 18*E*, 22*E*-farnesyl farnesol, linalool, phytol |
| Others | Quinic acid, brevifolin carboxylic acid, phloridzin, tuberonic acid hexoside, methylbrevifolincarboxylate, dimethylbrevifolin carboxylate |

consequences on the health and safety of consumers cannot be ignored (Srirama et al. 2017). In order to make herbal medicine more credible and acceptable, a comprehensive herbal product authentication has to be evolved. Qualitative and quantitative analysis of a number of marker compounds is the basis of quality control of herbal medicines. This approach fails when there are no suitable markers. Moreover, selecting a few compounds from a complex mixture may not reflect the real picture as synergistic effects are not taken into account. Herbal drugs contain diverse compounds in complex matrices, and the overall therapeutic efficacy may depend not on a single compound but on the synergy of several components.

Chemical fingerprinting or evaluation of a product in its entirety can be effectively used to describe the complexity of herbal medicines (Liang et al. 2004). It serves as a tool for identification, authentication, and quality control of herbal drugs all over the world. Analysis of such complex data requires special software, and principal component analysis (PCA) is one such software used for chemometric analysis, an approach to the interpretation of patterns in multivariate data, to evaluate similarities and to discriminate herbs from closely related species and from adulterants (Martins et al. 2011; Goodarzi et al. 2013; Gad et al. 2013). TLC, HPLC, GC, GC-MS, and HPLC-MS data can be used to generate a chemical fingerprint. Because of its simplicity and reliability, chromatographic and spectral fingerprinting technique is a popular and potent tool for quality control of herbal medicines (Balammal et al. 2012; Joshi 2012).

Most of the active components in *Phyllanthus* belong to phenolics and lignans. Until recently their analysis had been carried out using HPTLC, HPLC, GC, and

GC-MS. But lately, because of the extremely high sensitivity and specificity, LC-MS or LC-MS/MS has found its place in the analysis of phytoconstituents of *Phyllanthus*. There are several reports of use of HPTLC for the analysis of *Phyllanthus* constituents, especially lignans (Annamalai and Lakshmi 2009; Tripathi et al. 2006). HPTLC analysis was used by several workers for the estimation of lignans and other bioactive components (Nayak et al. 2010; Mehta et al. 2013), alkaloids, flavonoids, glycosides, and saponins (Shah et al. 2017) in *Phyllanthus*. A TLC image analysis method was developed for detecting and quantifying bioactive phyllanthin in *Phyllanthus amarus* and commercial herbal drugs (Ketmongkhonsit et al. 2015). Comparative quantification of phyllanthin in different organs of *P. amarus* was achieved by HPTLC and HPLC (Annamalai and Lakshmi 2009). Simultaneous determination of four lignans from *Phyllanthus* was carried out by an HPTLC method using chiral TLC plates (Srivastava et al. 2008). Phytochemical diversity of *P. amarus* was assessed by HPTLC fingerprints (Khan et al. 2011). Twelve lignans originating from *Phyllanthus* were separated by micellar electrokinetic chromatography (Kuo et al. 2003).

Several bioactive components were identified from *P. amarus* extracts by GC-MS (Arun et al. 2012; Mamza et al. 2012). Hexane and methanol leaf extracts were analyzed by GC-MS (Zubair et al. 2016). Nine compounds, mainly flavones and sterols, were identified from hexane fraction and 14 compounds including steroids and triterpenoids were identified in the methanol extract. More than 50 phytocompounds were detected from the methanolic extract of *P. fraternus* by GC-MS (Singh 2016). Terpenes, phytosterols, and terpenoids were identified in ethyl acetate extract of *P. emblica* by GC-MS (Deepak and Gopal 2014; El Amir et al. 2014). GC-MS analysis of the chloroform extract of the leaves of *P. amarus* identified a few fatty acids, esters, and hydrocarbons (Igwe and Okwunodulu 2014). H-NMR-based metabolomics approach using partial least-square (PLS) results showed that phytochemicals, including hypophyllanthin, catechin, epicatechin, rutin, quercetin and chlorogenic, caffeic, malic, and gallic acids were correlated with antioxidant and α-glucosidase inhibitory activities of *P. niruri* extract (Mediani et al. 2017).

Several HPLC methods are reported for the estimation of phyllanthin in *P. amarus* (Hamrapurkar and Pawar 2009). An HPLC method was developed for the quantification of phyllanthin and hypophyllanthine in *P. amarus* (Murali et al. 2001). A validated HPLC method was reported for the estimation of phyllanthin in *P. amarus* extract (Alvari et al. 2011). The leaves of *P. amarus* were found to contain the highest amounts of phyllanthin (0.7% w/w) and hypophyllanthin (0.3% w/w) as compared to the other parts of the plant based on a RP-HPLC method (Sharma et al. 1993). Phyllanthin and hypophyllanthin in herbal dosage forms containing *P. niruri* extract were determined by HPLC-UV detection (Rai et al. 2009). Authentication of *P. niruri* from related species was done using HPLC fingerprint and simultaneous quantitative analysis of phyllanthin and hypophyllanthin (Nasrulloh et al. 2018). Using corilagin as a marker, a validated HPLC method was reported for the standardization of *P. niruri* herb and commercial extracts in Brazil (Colombo et al. 2009). A sensitive HPLC-fluorescence detection method was reported for the simultaneous determination of four lignans, phyllanthin, hypophyllanthin, phyltetralin,

and niranthin, from *P. niruri* in rat plasma (Murugaiyah and Chan 2007). Phenolic constituents in the aqueous ethanolic extracts of *P. niruri* and *P. urinaria* were identified by HPLC assay (Mahdi et al. 2011). An HPLC method was developed for the simultaneous estimation of ascorbic acid and gallic acid in *P. emblica* fruit extract prepared from freeze-dried juice (Sawant et al. 2010). In connection with a study on the antioxidant activity of *P. emblica* fruit juice, six polyphenol compounds were identified by HPLC analysis (Liu et al. 2008). The phyllanthin, hypophyllanthin, niranthin, and the antioxidant ellagic acid in methanolic extracts of *P. amarus* were quantified by LC-MS (Muthusamy et al. 2018). The simultaneous determination of six lignans of therapeutic importance (heliobuphthalmin lactone, virgatusin, hypophyllanthin, phyllanthin, nirtetralin, and niranthin) in four *Phyllanthus* spp. using HPLC-PDA-MS was reported (Shanker et al. 2011). LC-MS/MS analysis of the ethyl acetate fraction of 70% ethanolic extract of *P. amarus* led to the identification of 28 different phenolic compounds (Maity et al. 2013). Phytochemical analysis of *P. amarus* using HPLC-UV-MS and LC-MS led to the identification of a number of phenolic acids, flavonoids, and phytosterols (Corciova et al. 2018). A comparison of four *Phyllanthus* species including *P. amarus* and *P. niruri* was reported by characterizing the chemical profiles of their aqueous extracts using LC-ITMS$^n$ (Sprenger and Cass 2013). Twenty phenolic compounds were identified in enriched phenolic extracts of *P. acuminatus* by UPLC-ESI-MS (Navarro et al. 2017), whereas eight phenolic compounds were simultaneously quantified in *P. simplex* using HPLC-DAD-ESI-MS (Niu et al. 2012) and four lignans from *P. urinaria* were simultaneously determined in rat plasma (Fan et al. 2015). During HPLC-MS/MS analysis of *P. emblica* extract and fractions, 144 peaks were detected, of which 67 were tentatively identified mostly as ellagitannins, flavonoids, and simple gallic acid derivatives (Yang et al. 2012). Tannins, organic acids, and flavonoids of *P. emblica* extract could be identified by HPLC-ESI-MS/MS method during a run time of 120 min (Wang et al. 2017). LC-MS analysis showed that gallic acid, quinic acid, quercetin, and other flavonoids were the major constituents in *P. emblica* extracts (Packirisamy et al. 2018). Geraniin, quercetin 3-$\beta$-D-glucopyranoside, kaempferol 3-$\beta$-D-glucopyranoside, isocorilagin, quercetin, and kaempferol were identified by spectral methods (Liu et al. 2008).

Recent reports from our laboratory describe the effective use of high-pressure liquid chromatography hyphenated with quadrupole time-of-flight mass spectrometry (HPLC-ESI-QTOF-MS/MS) for the identification and characterization of 52 phenolics and lignans in the ethanolic extract of *P. amarus* (Kumar et al. 2015). Six bioactive compounds were quantified using UPLC-ESI-MS/MS in a run time of 3.6 min with an LOQ of 0.44–3.82 ng/mL. HPLC-ESI-QTOF-MS/MS was used for the identification and characterization of phenolics and terpenoids from ethanolic extracts of four *Phyllanthus* species tentatively identifying 30 compounds and unambiguously identifying 21 compounds by comparison with their authentic standards (Kumar et al. 2017a). Using UPLC-ESI-MS/MS, 23 targeted bioactive compounds in ethanolic extracts of *P. amarus*, *P. niruri*, *P. fraternus*, and *P. emblica*

and their herbal products were quantified simultaneously in polarity switching MRM mode (Kumar et al. 2017b).

Geographical variation of phytoconstituents in *P. amarus* was investigated by a rapid and versatile direct analysis in real time-time of flight-mass spectrometry (DART-TOF-MS) method (Kumar et al. 2017c). Sixteen constituents including alkaloids and lignans were identified and the ten chemical markers identified by PCA could discriminate among the samples of different geographical locations. PCA was carried out to differentiate different species, locations, and herbal formulations. These studies form part of our attempt to chemical fingerprinting (identify, characterize, and quantify the phyto-constituents) of *P. amarus*, *P. niruri*, *P. fraternus*, and *P. emblica*. Along with the species identity, geographical variations influence the efficacy of herbal drugs. Development of discriminative analytical methods for authentication, phytochemical fingerprinting, and geographical distribution is absolutely necessary for the quality control of raw and processed herbal products. Details of these investigations are discussed.

## 9.6 Patents

The treatment of hepatitis by an enriched fraction prepared from *Phyllanthus amarus* was patented (Janardhan et al. 2000). Method for producing extract powder of *P. amarus* (Ichiba 2007) and different types of extraction and isolation of phyllanthin was patented in the USA (Ghosal et al. 2013; Chaudhuri et al. 2000).

## 9.7 Conclusion

*Phyllanthus amarus* has extensive biological potential. It is strongly believed that detailed information as presented here on the phytochemical and various biological properties. It has various traditional uses worldwide for the treatments of jaundice, diabetes, dysentery, fever, gonorrhea, syphilis and stomachache, and skin diseases. There is high demand to standardize the various properties of *P. amarus* extracts and their detailed clinical trials. Pharmacological and chemical studies have confirmed that the extracts of the plant possess various biological activities, and extracts have been evaluated in human trials for the treatment of HIV, jaundice, hypertension, and diabetes due to the impressive preclinical therapeutic potential. *P. amarus* is reported to contain lignans, flavonoids, hydrolysable tannins (ellagitannins), polyphenols, triterpenes, sterols, and alkaloids. The lignans phyllanthin, hypophyllanthine, nirtetralin, phyltetralin, or niranthin are isolated from *P. amarus*. The antitumor, antimitotic, and antiviral activities were reported due to presence of various lignans. The presence of high contents of phenolic compounds in the aqueous extract of *P. amarus* was found to have strong and significant antioxidant activity. Phyllanthin showed very high antioxidant activity. Similarly, amariin, repandusinic acid, and phyllanthusiin D showed higher antioxidant activity. A gallotannin containing fraction and the isolated ellagitannins geraniin and corilagin were exhibited to be

the most potent mediators of antiviral activities. Mixture of phyllanthin and hypophyllanthin (1:1) showed antitumor activity against EAC in Swiss albino mice. Lignans nirtetralin, niranthin, or phyllanthin exerted cytotoxic effects on K-562 cells. Phyllanthin demonstrated its role in protection by antagonizing rat liver cell injury induced by ethanol. It effectively alleviated the changes induced by $CCl_4$ in a concentration-dependent manner. Three pure pentacyclic triterpenoids, oleanolic acid, ursolic acid, and lupeol, inhibited amylase. Thus activity-guided phytochemical and phytoanalytical information seems to be very useful and might lead to development of novel agents for various disorders and could be explored further for commercial purposes. However, there are many aspects, which required to be explored like well-controlled clinical trials using huge sample size for the efficacy and toxicity and the mechanism of biological activity of active constituents present in the plant. *P. amarus*, crude extract, and derived phytochemicals and their uses as pharmacological agents in traditional and modern research are possible but will first require more clinical trials and product development on the basis of biological activities. This could be achieved by molecular modeling studies involving interaction of bioactive phytochemicals from *P. amarus* with their respective molecular targets, and the extract of *P. amarus* could be further explored in the future as a source of useful phytochemicals for the pharmaceutical industry.

**Acknowledgements** The author is grateful to Principal of MK Government Degree College, Ninowa, Farrukhabad, for support.

**Competing Interest** Author has no competing interest.

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# Medicinal Applications of Cannabidiol from the Genus *Cannabis* L.

10

Debojyoti Bag, Aliya Tabassum, Nidhi Arora, Praveen Kumar Verma, and Sanghapal D. Sawant

**Abstract**

*Cannabis* L. belongs to Cannabaceae family known as 'hemp' and has been used as a mind-altering drug as described in prehistoric societies of Eurasia and Africa. A total of 100 phytocannabinoids have been reported from *Cannabis sativa* L. till date, owing to their therapeutic values. The two primary constituents present in *Cannabis* are non-psychoactive cannabidiol (CBD) and psychoactive $\Delta^9$-tetrahydrocannabinol (THC). CBD has enormous potential for the development as a drug candidate as depicted by diverse clinical and preclinical studies for the treatment of various neuropsychiatric disorders, arthritis, cancer and other diseases. Regardless of its therapeutic importance and interaction with the endocannabinoid system (ECS), definite pharmacological mechanism is not clearly established. Apart from medicinal research, there are a number of aspects which should be taken under consideration such as opinion of FDA, legal aspects of cannabis and international scenario during the drug development based on this plant.

**Keywords**

*Cannabis* · Cannabinoids · Cannabidiol · Tetrahydrocannabinol · Stereochemistry · Endocannabinoid · Psychoactive · Pharmacology · Neurodegenerative

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_10

## Abbreviations

| | |
|---|---|
| AD | Anno Domini |
| AD | Alzheimer's disease |
| ASD | autism spectrum disorder |
| AS | ankylosing spondylitis |
| AIDS | acquired immune deficiency syndrome |
| APP | amyloid precursor protein |
| BCE | Before Common Era |
| CII | type 2 collagen |
| CBC | annabichromene |
| CBE | cannabielsoin |
| CBDA | cannabidiolic acid |
| CBT | cannabitriol |
| CBND | cannabinodiol |
| CBG | cannabigerol |
| CBL | cannabicyclol |
| CBN | cannabinol |
| CBLA | cannabicyclic |
| CBLV | cannabicyclovarin |
| CBM | cannabimovone |
| CBDA | cannabidiolic acid |
| CBDVA | cannabidivarinic acid |
| CBDV | cannabidivarin |
| CBDND | cannabinodivarin |
| CB1 | cannabinoid receptor type 1 |
| CB2 | cannabinoid receptor type 2 |
| CNS | central nervous system |
| CHO | Chinese hamster ovary |
| CYP | cytochrome P450 |
| CIA | collagen-induced arthritis |
| CBGA | cannabigerolic acid |
| DRN | dorsal raphe nucleus |
| DS | Dravet syndrome |
| DLPC | dorsolateral prefrontal cortex |
| Diagnostic and Statistical Manual of Mental Disorders | Fifth Edition |
| ECS | endocannabinoid system |
| ERK | extracellular signal-regulated kinases |
| EMA | European Medicines Agency |
| FDA | Food and Drug Administration |
| GABA | gamma-aminobutyric acid |
| GFAP | Glial Fibrillary Acidic Protein |
| GPCR | G-protein-coupled receptors |

| | |
|---|---|
| HD | Huntington disease |
| HPA | hypothalamic pituitary adrenal |
| HUVE | human umbilical vein endothelial |
| HIV | human immunodeficiency virus |
| HEK | human embryonic kidney |
| HL | human leukaemia |
| iNOS | inducible nitric oxide synthase |
| IS | infantile spasms |
| IFN-γ | interferon-gamma |
| IP | intraperitoneal injection |
| LGS | Lennox-gastaut syndrome |
| L-DOPA | Levodopa |
| mTOR | messenger ribonucleic acid |
| mPFCx | medial prefrontal cortex |
| MDA-MB cells | M.D. Anderson and MB stands for metastasis breast cancer cell lines |
| MS | multiple sclerosis |
| NMR | nuclear magnetic resonance |
| NOD | non-obese diabetic |
| NAA/tCr | N-acetylaspartate/total creatine |
| NFTs | neurofibrillary tangles |
| PS1 | Presenilin 1 |
| PTSD | post-traumatic stress disorder |
| PPARγ | peroxisome proliferator-activated receptor gamma |
| PrPres | Protease-resistant prion protein |
| PD | Parkinson disease |
| PNS | peripheral nervous system |
| RA | rheumatoid arthritis |
| RNA | ribonucleic acid |
| ROS | reactive oxygen species |
| SAD | social anxiety disorder |
| SPST | Simulation Public Speaking Test |
| SNpc | substantia nigra pars compacta |
| THC | tetrahydrocannabinol |
| THCS | tetrahydrocannabinolic acid |
| TNF | tumour necrosis factor |
| TRPV1 | transient receptor potential cation channel sub-family V member 1 |
| TRPV2 | transient receptor potential cation channel sub-family V member 2 |
| TSC | tuberous sclerosis complex |

## 10.1 Introduction

*Cannabis sativa* L. has a long history in India and earliest mention of this plant has been found in Hindu antique texts, Atharvaveda, composed courting from 1500 to 1000 BCE, hired in numerous rites and formalities (Russo 2005). Herodotus, in "Histories" (400 BCE), defines that *Cannabis* seeds were being burnt ceremonially throughout a Scythian funeral near the Black Sea and burnt the *Cannabis* seeds (Russo 2007). Physiological properties of *Cannabis* seeds and their medicinal application in the treatment of earache were described by Pedanius Dioscorides, a Greek physician between 40 and 90 AD, and supported by Claudius Galen (129–201/215 AD) (Brunner 1977; Baron 2015). In modern times, O'Shaughnessy in 1839 suggested its application for the treatment of tetanus and also its use as an analgesic (Russo 2005). During the 1970s, identification of novel cannabinoids from different *Cannabis* species (i.e. *Cannabis sativa* L., *C. indica* Lam. and *C. ruderalis* Janisch.) was given significant importance. Till date, mankind is connected with the use of more than 100 phytocannabinoids present in *C. sativa* owing to their therapeutic values. The endocannabinoid system associated with *Cannabis sativa* has been growing as an important goal of pharmacotherapy displaying very substantial physiological consequence (Mechoulam et al. 2014). Two primary constituents present in *Cannabis* are cannabidiol (CBD) and $\Delta^9$-tetrahydrocannabinol (THC). The psychoactive effects of *Cannabis* are attributed to the activation of CB1 receptors which are highly distributed in the central nervous system by $\Delta^9$ –THC, while CBD is considered as non-psychoactive. Presently, equimolar formulation of CBD and $\Delta^9$-THC (Sativex) is being used for dealing the neuropathic symptoms allied with multiple sclerosis (Fernández 2016). Other relevent literatures on *Cannabis* includes Ch et al. (1977), Claussen et al. (1966), Libro et al. (2016), Gaoni and Mechoulam (1966), Iskedjian et al. (2007), Parker and Mechoulam (2003), Pisanti et al. (2017), Rock et al. (2011, 2012) and Russo et al. (2005).

Despite its concrete definition in modern medicine, clinical and preclinical trials on cannabidiol have decorated encouraging results, and its application has been widened for the treatment of various neuropsychiatric disorders, arthritis and cancer. Regardless of its therapeutic importance and interaction with the endocannabinoid system (ECS), a definite pharmacological mechanism is not clearly established.

## 10.2 Morphology of the Genus *Cannabis*

### 10.2.1 Cannabis sativa

The plant is typically 3–4 m tall (Fig. 10.1) having hollowing stems with extended internodes and short petioles, sometimes with 5–9 leaflets per leaf. The leaflets are lance-like with largest leaflets underneath 13.6 cm. The racemes have long internodes with partly exposed achenes; achenes (seeds) are sometimes 0.4 cm long, slightly lens-shaped with a blunt base. The surface colour of the leaflets is dull light to dark green and usually un-marbled. The matured seeds are cultivated for fibre and oil and for therapeutic uses (Gloss 2015; Pollio 2016).

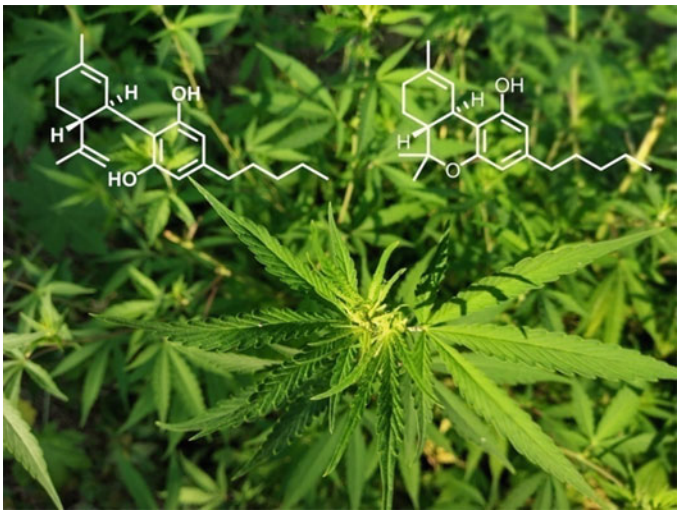


**Fig. 10.1** *Cannabis sativa*. (Photo from IIIM-Jammu Farm)

### 10.2.2 *Cannabis indica*

*The* plant usually reaches up to 3 m tall, relatively shorter in height, with nearly solid and smooth stems. The densely arranged branches have shorter internodes and petioles; a leaf has 7–11 number of narrow brownish-green leaflets, and surface of leaflets usually has marbled or un-marbled types and with or without an abscission layer and lanceolate with largest leaflets normally 92 mm long (l/w ratio is 10) and achenes averaging 3.7 mm long, less lens-shaped, with a more rounded base. Due to its therapeutic value and its use as fibre and oil, cultivation is carried out in large scale (Gloss 2015; Pollio 2016).

### 10.2.3 *Cannabis ruderalis*

The plant is extremely small in height (under 0.5 m tall) with smooth and hollow stems, irregularly unbranched, and shorter petioles. The leaflets are elliptic in shape, achenes are small with a pronounced abscission structure at the base, the surface of the leaflets is dull green and marbled and it have oil-producing cells. Seeds are readily shed from plant (Gloss 2015; Pollio 2016).

### 10.2.4 *Cannabis afghanica*

This plant is considerably shorter in height with ribbed solid stems and dense branches with short and long internodes and petioles, respectively. The leaves are dark green coloured; broadly oblanceolate, racemes have short internodes, and

achenes are not exposed; nested, sometime compound produce bracts; achenes usually <3.0 mm long, and its nearly round with a blunt base, and the colour of surface like shiny grey and marbled (Gloss 2015; Pollio 2016; Piomelli and Russo 2016).

## 10.3 Ethnobotanical Usages of *Cannabis*

*Cannabis* serves for diverse purposes such as fibre, oil and narcotics and hence is cultivated from over 4500 years. Traditionally, *Cannabis* is being used for fibre, followed by the use of its seeds for culinary purposes and for the betterment of numerous health issues. In countries like Afghanistan, China and India, the hot water infusion is used as a remedy to cure several ailments such as menstrual pain, rheumatism, to persuade abortion, to clear blood, to cool temperature and as narcotic and pain relievers. The oral intake of hot water extract of dried flower and leaf serves various purposes such as dyspepsia and gonorrhoea and as a nerve stimulant. The fresh leaves are taken for haemorrhoids, whereas its extract is used as insect repellent. The seeds are also useful for the treatment of hysteria, sleeplessness and diabetes and skin problems. Respiratory ailments such as cough and bronchitis are cured by the mixture of dry leaf powder and honey. The fluid extracts of the dried flowers and fruits are used as pain relievers in Iran. The seed oil is used to reduce cramps associated with lead poisoning related with constipation and vomiting. The hot water extract of the plant is taken orally for its antispasmodic effects and purification in Jamaica and Pakistan, respectively. In Mexico and Morocco, the aerial portions are smoked as a hallucinogen and taken as narcotic. In Nepal, decoction of leaves is used as anthelmintic. The leaf juice is used to treat wound and to treat swelling of wrenched joints. In the USA, fluid extract is taken orally as a narcotic, antispasmodic, analgesic and aphrodisiac. The dried aerial parts are smoked by both sexes as an aphrodisiac. In West Indies and Zimbabwe, hot water extract is used as antispasmodic and antimalarial, respectively (Ross 2007). The brief detail on ethnobotanical application of *Cannabis* is given in Table 10.1.

## 10.4 Chemical Constituents of *Cannabis*

The terpenophenolic natural products isolated from *Cannabis* are known as cannabinoids. Since the earliest findings of the chemical compositions of *Cannabis*, many cannabinoids (approximately 120) have been isolated till date, which can be divided into 11 general kinds as summarized in Table 10.2 (Kinghorn et al. 2017).

### 10.4.1 Tetrahydrocannabinol

The major chemical constituent is tetrahydrocannabidiol (Fig. 10.2). Both $\Delta^9$-THC and $\Delta^8$-THC are known for inducing natural synthesis of anandamide and 2-arach

**Table 10.1** Ethnomedicinal uses of *Cannabis* across the globe

| Part used | Disease | Mode of application | Country |
|---|---|---|---|
| Flowers | Induce abortion | Hot water extract of the resin, orally | Afghanistan |
| Seeds | Hallucinations, emmenagogue, rheumatism, migraine, cancer | Hot water extract, orally, for wasting diseases, blood purification, to reduce body temperature | China, Vietnam, Yugoslavia, Senegal |
| Leaves | Muscular pains, putrefacient, General weakness | Hot water extract of leaves, orally | Guatemala, Pakistan |
| Whole plants | Dysmenorrhea, dyspepsia, gonorrhoea, nerve stimulant, abortifacient, menstrual pain, inflammation, emmenagogue, piles, anthelmintic, sleeplessness, anticancer drugs, aphrodisiac, amorous prowess, haemorrhoids, stomach troubles and indigestion, earache, skin diseases, sleeplessness | Hot water extract of dried plant, orally | India |
| Flower, seeds, fruits | Abdominal pain, indigestion, cancer, rheumatoid arthritis, gastric cramps or neuralgia, coughing, whooping cough, hypnotic, tranquilizer, diuretic, analgesic, rheumatism, sedative, diaphoretic, hysteric conditions, gout, epilepsy, cholera, constipation, vomiting, urinary incontinency | Fluid extract of the dried flowers and fruits, orally | Iran |
| Flower, leaves, twig | Antispasmodic, anodyne, diabetes | Hot water extract of the flower, leaf and twig, orally<br>Hot water extract of the resin, orally | Jamaica |
| Aerial parts | Hallucinogen, narcotic | Smoking or orally | Mexico, Morocco |
| Leaves, stem, seeds | Anthelmintic, diarrhoea, applied externally, headache, antiseptic, wounds, sprained joints, dysentery | Decoction of the leaf, orally; leaf juice, externally; crushed seeds mixed with curd, orally; decoction of seed, orally; powdered seeds and sesame oil paste, intravaginally | Nepal |
| Whole plant, leaves | General weakness | Hot water extract of plant, orally | Pakistan |
| Aerial parts | Psychotropic, malaria | Aerial parts mixed with honey, sugar and nutmeg, orally; hot water extract of aerial parts, orally | Saudi Arabia Zimbabwe |

(continued)

**Table 10.1** (continued)

| Part used | Disease | Mode of application | Country |
|---|---|---|---|
| Whole plant, roots, seeds | Asthma, induce abortion, labour and menstruation pain | Hot water extract of plant, orally | South Africa |
| Fluid extract, flower, aerial parts | Narcotic, antispasmodic, analgesic, aphrodisiac | Fluid extract, orally; hot water extract of flowers, orally; one teaspoon of plant steeped in two cups of boiling water, and one tablespoonful is taken two to four times a day | USA |
| Whole plant | Antispasmodic | Hot water extract of plant, orally | West Indies |

Source: Medicinal plants of the world, Volume 3. chemical constituents, traditional and modern medicinal uses

**Table 10.2** Constituents of *Cannabis* by chemical class

| Cannabinoid type | Isolated cannabinoid |
|---|---|
| (−)-*trans*-$\Delta^9$ -tetrahydrocannabinol ($\Delta^9$-THC) | 23 |
| (−)-*trans*-$\Delta^8$ -tetrahydrocannabinol ($\Delta^8$-THC) | 5 |
| Cannabigerol (CBG) | 16 |
| Cannabichromene (CBC) | 9 |
| Cannabidiol (CBD) | 7 |
| Cannabielsoin (CBE) | 5 |
| Cannabinodiol (CBND) | 2 |
| Cannabicyclol (CBL) | 3 |
| Cannabinol (CBN) | 11 |
| Cannabitriol (CBT) | 9 |
| Miscellaneous types | 30 |

idonoylglycerol in the body and brain via intracellular CB1 activation. The absolute configuration of $\Delta^9$-THC was elucidated as *trans*-(6aR,10aR) by Gaoni and Mechoulam (Gaoni and Mechoulam 1964a, b).

## 10.4.2 Cannabinol

Till date, 11 cannabinol analogues (Fig. 10.3) have been isolated including 4-terpenyl cannabinolate, 8-hydroxycannabinolic acid, 8-hydroxycannabinol and recently discovered (1'S)-hydroxycannabinol (ElSohly and Slade 2005; Ahmed et al. 2015).

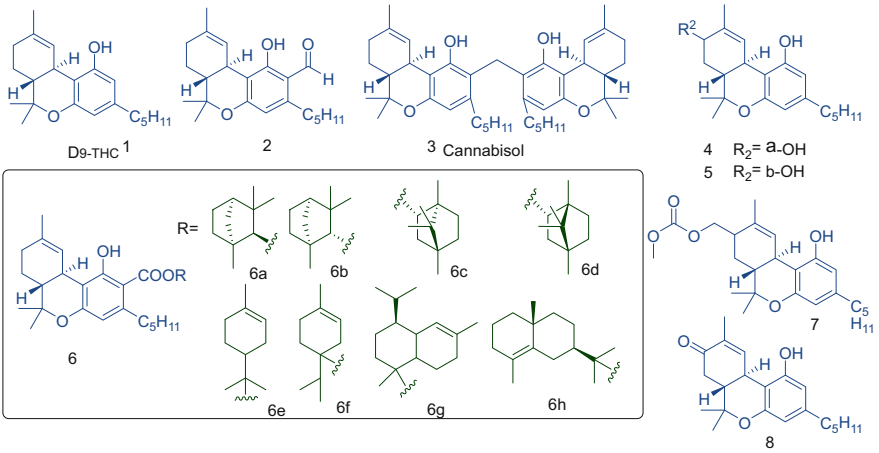


**Fig. 10.2** THC-type cannabinoids

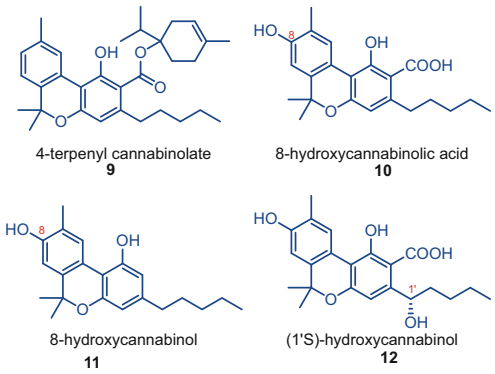


**Fig. 10.3** Cannabinol-type cannabinoids

### 10.4.3 Cannabigerol

Cannabigerol was first isolated from the resin of *C. sativa* (Gaoni and Mechoulam 1964a, b). Till date, a total of 16 cannabinoids of this type have been isolated. Even though cannabigerol is non-psychoactive, it devotes to the inclusive effect of *Cannabis*.

### 10.4.4 Cannabichromenes (CBC)

Cannabichromenes (Fig. 10.4) revealed by Claussen et al. and Mechoulam et al. are one of commonly found phytocannabinoids from *Cannabis*, produced

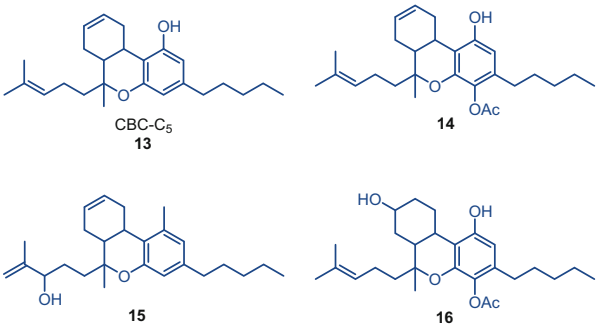


**Fig. 10.4** CBC-type cannabinoids

by the biosynthesis from cannabigerolic acid followed by cyclization and decarboxylation.

### 10.4.5 Cannabidiol

Cannabidiol (CBD) and cannabidiolic acid (CBDA) are the two main non-psychoactive variety of cannabinoids from *Cannabis*. Till date, seven cannabidiol-type cannabinoids have been isolated. Since 2005, no new cannabinoid in this type has been reported.

### 10.4.6 Cannabinodiol

Aromatized derivatives of cannabidiol-type cannabinoids are termed as cannabinodiol type. The two major cannabinoids, cannabinodiol (CBND-C5) and cannabinodivarin (CBND-C3), have been isolated from *Cannabis* plant (Turner et al. 1980; ElSohly and Slade 2005).

### 10.4.7 Cannabielsoin

Till date, five cannabielsoin (CBE)-type cannabinoids have been isolated to the absolute configuration (5aS, 6S, 9R, 9aR). They were also recognized as CBD of mammalian metabolites (Fig. 10.5).

### 10.4.8 Cannabicyclol

Cannabicyclol (CBL, Fig. 10.6), cannabicyclic acid (CBLA) and cannabicyclovarin (CBLV) are the phytocannabinoids of CBL type isolated from *Cannabis*.

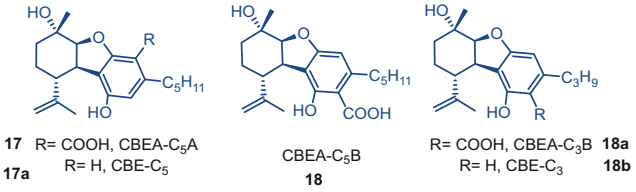


**Fig. 10.5** CBE-type cannabinoids

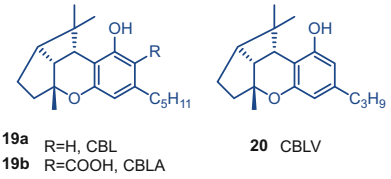


**Fig. 10.6** CBL-type cannabinoids

### 10.4.9 Cannabitriol

After its isolation by Obata and Ishikawa in 1966 (Obata and Ishikawa 1966), till date, nine phytocannabinoids of this type have been isolated including (−)-trans-cannabitriol (Fig. 10.7).

ElSohly and Slade briefed the remaining phytocannabinoids in a review (ElSohly and Slade 2005). Figures 10.8 and 10.9 summarize the cannabinoid present in this type. Recently in 2010, Taglialatela-Scafati et al. isolated cannabimovone (CBM), a new phytocannabinoid, from cannabis (Taglialatela-Scafati et al. 2010) (Table 10.3).

## 10.5 Origin and Chemical Structure of Cannabidiol

Even though historically, the cannabidiol (CBD) was first discovered in 1940 from Mexican marijuana by Roger Adams and from Indian charas by Alexander Todd (Todd 1946), its structure was not completely established till Raphael Mechoulam isolated CBD from Lebanese hashish and recognized its relative stereochemistry and structure by NMR analysis (Mechoulam and Shvo 1963) in 1963. The absolute stereochemistry of CBD was determined in 1967 by converting it into methane carboxylic acid which was a stereochemically well-recognized molecule (Mechoulam and Gaoni 1967). The knowledge obtained from the above discoveries helped in elucidating the structure and stereochemistry of $\Delta^9$-THC, as a major psychoactive constituent of *Cannabis*. The various steps involved in the transformation of CBD are given in Fig. 10.10.

In spite of their structural similarity, conformational constructions of CBD and $\Delta^9$-THC differ significantly. In 1977, by X-ray structure analysis, Jones et al. determined two independent arrangement of CBD having conformational difference

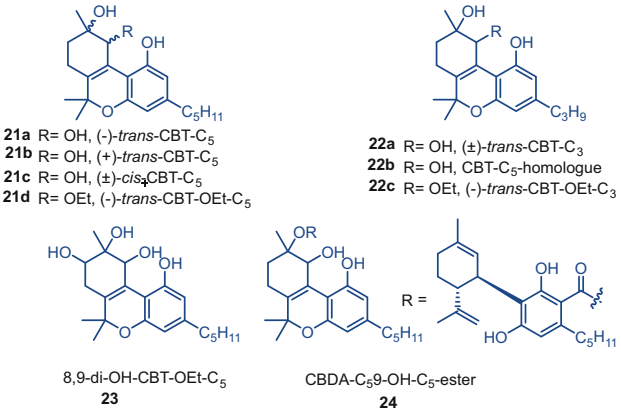


**Fig. 10.7** CBT-type cannabinoids

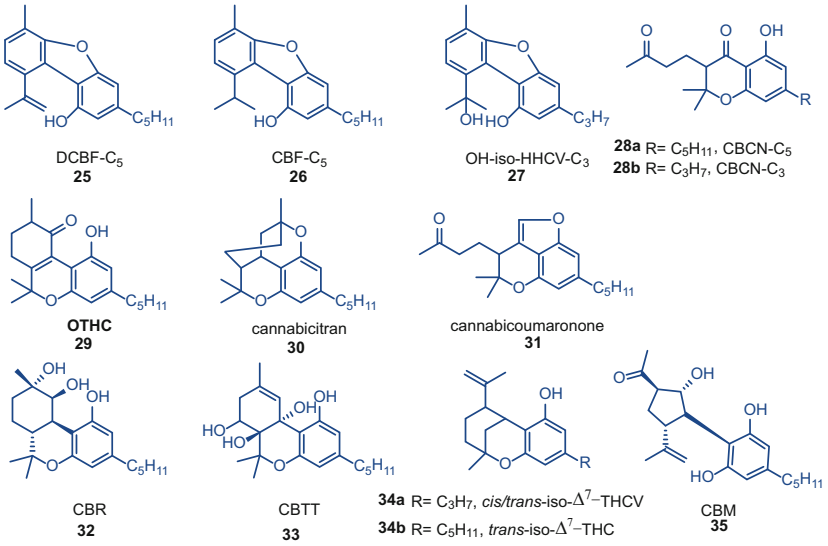


**Fig. 10.8** Cannabinoids of miscellaneous types

in the side chain (pentyl) and having the terpene segment and the aromatic ring almost perpendicular to each other (Jones et al. 1977), while $\Delta^9$-THC holds a planner geometry. Chemical nomenclature of CBD starts from the terpene segment, whereas the presence of pyran ring directs the nomenclature of $\Delta^9$-THC in a diverse way by providing different numbers to the same carbon atoms. The nomenclature of CBD and THC is presented in Fig. 10.11.

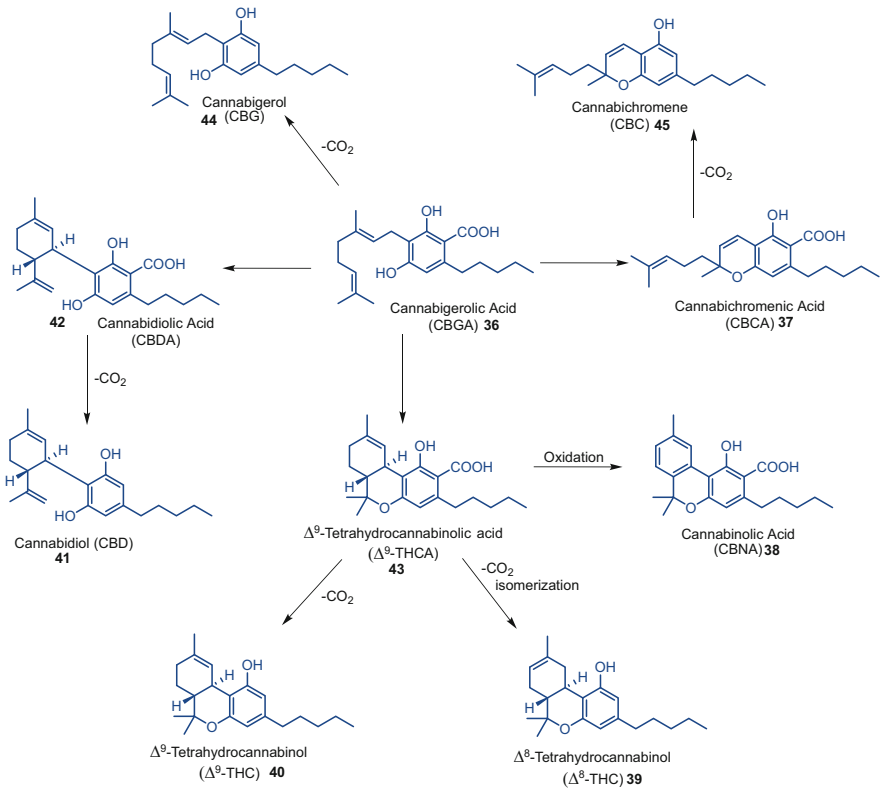


**Fig. 10.9** Chemical structure of the main cannabinoid

**Table 10.3** Cannabidiol profile

| International non-proprietary name (INN) | Cannabidiol |
|---|---|
| Chemical abstract service (CAS) registry number | 13956-29-1 |
| Other chemical names | CBD; 2-[1R-3-methyl-6R-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol |
| IUPAC name | 2-[(6R)-3-methyl-6-prop-1-en-2-ylcyclohex-2-en-1- yl]-5-pentylbenzene-1,3-diol |
| Trade names | Epidiolex® Arvisol® (in development) |
| Physical appearance | Crystalline solid |
| Molecular formula | $C_{21}H_{30}O_2$ |
| Molecular weight | 314.469 g/mol |
| Melting point | 62–63 °C |
| Solubility | Approximately 23.6 mg/mL in DSMO and ethanol |

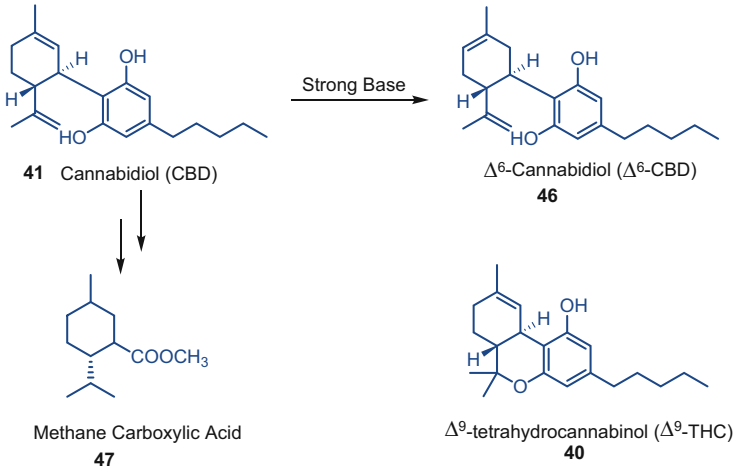


**Fig. 10.10** Transformation of CBD

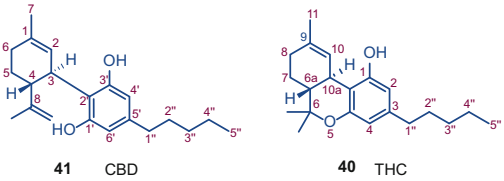


**Fig. 10.11** Nomenclature of CBD and THC

### 10.5.1 Stereoisomers of Cannabidiol

Cannabidiol (CBD) commonly mentions to the naturally occurring (−)-enantiomer and has been taken to importance over the (+)-enantiomer which was first synthesized by Shah et al. (Shah 1988). Unlike (−)-CBD, (+)-CBD has been found to be having moderate affinity for the $CB_1$ and $CB_2$ receptors. In a mouse seizure model, interestingly (+)-enantiomer was more active than (−)-enantiomer, as an anticonvulsant agent (Leite et al. 1982).

### 10.5.2 Synthesis of Cannabidiol

Till date, numerous strategies have been adopted for the synthesis of cannabidiol, and majority of reports has the disadvantage of very low and poor yield. Among them, the most common strategies are by condensation of olivetol and an enantiopure monoterpene (Fig. 10.10). Even after several attempts has been made, a good-yielding route for the synthesis of cannabidiol is still unavailable in the literature (Kinghorn et al. 2017) (Fig. 10.12).

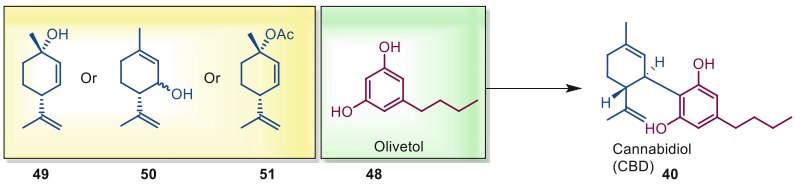


**Fig. 10.12** Synthesis of cannabidiol

### 10.5.3 Natural Homologues of Cannabidiol

Till date, of over a hundred natural cannabinoids, seven have been classified as CBD-type analogues, and among them, four natural side chain homologs of cannabidiol have been found having methyl, n-propyl, n-butyl and n-pentyl groups. Among all side chain homologs, n-pentyl substituted one (CBD itself) has been largely explored (ElSohly and Slade 2005, ElSohly and Gul 2014, Aizpurua-Olaizola et al. 2016). All seven naturally available CBD analogues are having 5′-methyl-2′-(prop-1-en-2-yl)-1′,2′,3′,4′-tetrahydro-[1,1′-biphenyl]-2,6-dioles with retention of trans-(1R,6R) configuration (Fig. 10.13).

Along with side chain homologues, cannabidiolic acid (CBDA) and cannabidivarinic acid (CBDVA-C3), the C3'-carboxylic acid derivatives of CBD and cannabidivarin (CBDV) have also been isolated. Furthermore, a monomethyl analogue of CBD has also been isolated from plant. Having similarity with other non-steroidal anti-inflammatory drugs, selective inhibition of cyclooxygenase-2 has been observed by CBDA (Takeda et al. 2008)**.** CBDA has been demonstrated to have an inhibitory action of cell migration in the highly destructive human breast cancer MDA-MB-231 by modification of RhoGTPase activity (Takeda et al. 2012). The C4'-propyl analogue of CBD, CBDV, demonstrates weak affinity towards $CB_1$ and $CB_2$ receptors (Hill et al. 2013, Rosenthaler et al. 2014), while its inhibition activity to the presumed endogenous ligand LPI in hGPR55-HEK293 cells is established (Anavi-Goffer et al. 2012). Cannabinodiol (CBDND-C5) and cannabinodivarin (CBDND-C3), two aromatic analogues of CBD, have been isolated from the plant Lebanese hashish in 2005 (ElSohly and Slade 2005). CBDND-C5, available in low concentration in the plant's flowers, is likely to be a compound synthesized during photochemical transformation of CBD (Turner et al. 1980). The structure of aromatic homologues of CBD is given in Fig. 10.14.

### 10.5.4 Biosynthesis of THC and CBD (Fig. 10.15)

Cannabidiolic acid (CBDA) and $\Delta^9$-tetrahydrocannabinolic acid (THCA) are the respective precursors for the synthesis of CBD and THC in plants. CBDA and THCA both have been derived from the common cannabigerolic acid (CBGA) via two-electron transfer from substrate to enzyme-bound FAD and further transferring to the molecular oxygen to reoxidize FAD. CBDA and THCA are synthesized from

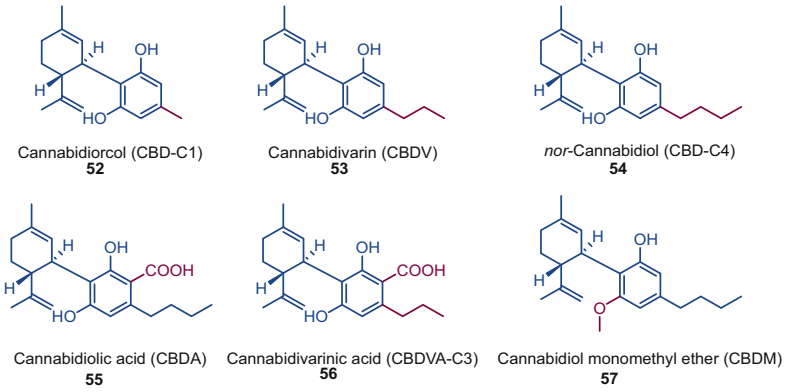


**Fig. 10.13** Natural homologues of cannabidiol

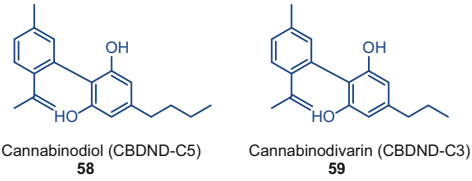


**Fig. 10.14** Aromatic homologues of cannabidiol

the corresponding ionic intermediates via respective enzyme-catalysed stereoselective cyclization. Furthermore, non-enzymatic decarboxylation of CBDA and THCA resulted in the formation of CBD and THC (Taura et al. 2007).

## 10.6 Pharmacological Applications of Cannabidiol (CBD)

Cannabidiol (CBD) is a potential pharmacotherapeutic molecule used for the treatment of numerous diseases including neurodegenerative disorders, vomiting, nausea and anxiety. A number of studies from literature have illustrated the pharmacological applications of CBD. It has great significance for the pain management and presently under preclinical research for various medicinal applications.

### 10.6.1 Neurodegenerative Disorder

#### 10.6.1.1 Parkinson Disease (PD)

PD is primarily linked with the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), with subsequent compact levels of dopamine in the striatum. L-DOPA or levodopa acts as a precursor of dopamine and is mostly used in the

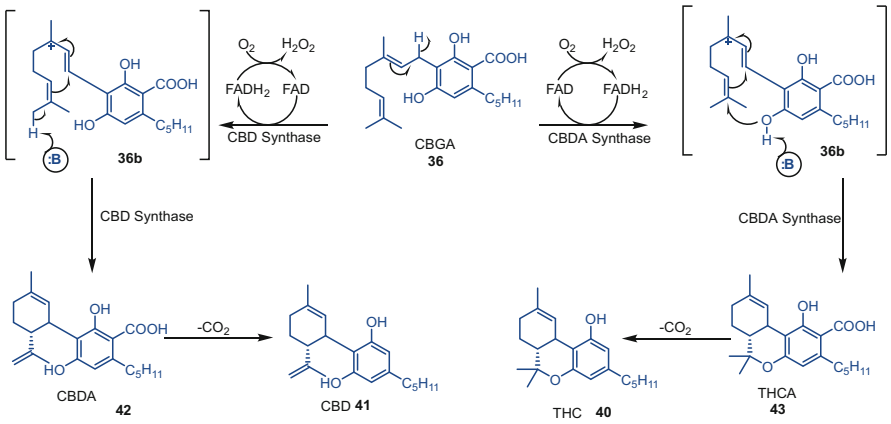


**Fig. 10.15** Biosynthesis of CBD and THC

treatment of PD by increasing the level of dopamine. However, L-DOPA can be unstable after a long-term treatment showing fluctuations in symptom improvement particularly motor symptoms. In order to prove the CBD effect on symptoms of psychosis, studies were carried out on PD patients. For 4 weeks' treatment with CBD, the psychotic symptoms were decreased which is calculated by the techniques like Brief Psychiatric Rating Scale and the Parkinson Psychosis Questionnaire. For 6 week, treatment shows improvement in the quality of life in PD patients (Iuvone et al. 2009). The anxiolytic and antipsychotic-like effects of cannabidiol are proven with animal studies (Zuardi et al. 2006a, b). Further study on the patients suffering from schizophrenia when treated with CBD (Zuardi et al. 2006a, b) and reports of CBD trial with amisulpride (Leweke et al. 2007) suggested CBD as safe and effective alternative prospect to be established as an antipsychotic drug.

A report of CBD in animal models of PD (Lastres-Becker et al. 2005, García-Arencibia et al. 2007) also showed its neuroprotective effects suggesting its antioxidant properties which protect from degeneration of dopaminergic neurons. CBD did not have any effect on motor symptoms but because of its action on 5-HT1a receptor is known for the catalepsy behaviour in rodents (More and Choi 2015). In vivo studies also showed its neuroprotective effects suggesting its antioxidant properties which protect from degeneration of dopaminergic neurons. CBD did not have any effect on motor symptoms but because of its action on 5-HT1a receptor is known for the catalepsy behaviour in rodents. Studied in vivo, CBD increases the RNA level of an antioxidant enzyme Cu and Zn superoxide dismutase in *substantia nigra*, also preventing from dopamine depletion, and is also involved in reduction of tyrosine-mediated activity in caudate-putamen (Zanluca et al. 2015) showing its neuroprotective effect. Synaptogenesis and axonal growth are the basis of its biological mechanism that is usually induced by NGF (Zanluca et al. 2015). CBD enhances synaptophysin expression, Growth Associated Protein 43 (GAP-43) and

synapsin-1 expression, thus inducing neurite formation and elongation besides synaptic vesicle formation (Zanluca et al. 2015).

#### 10.6.1.2 Huntington Disease

HD is a hereditary disorder which occurs from the repetition of CAG in genetic allele which forms a poly Q enlargement in "Huntingtin" (a specific protein) that upsets striatal and cortical neurons generating motor abnormalities like chorea and amentia. CBD has been studied for the removal of hyperkinetic symptoms. It acts as a disease-modifying agent. Treatment of HD was done by Sativex (a formulation containing CBD as active constituent) after preclinical trials (Sagredo et al. 2012).

CBD was found to have neuroprotective effects in rats lesioned with 3-nitropropionic acid, a mitochondrial toxin that replicates the complex II deficiency characteristic of HD patients and that provokes striatal injury by mechanisms that mainly involve the $Ca^{2+}$-regulated protein calpain and generation of ROS (Fernández and Ruiz et al. 2013).

#### 10.6.1.3 Prion Diseases

Prion disease is a neurodegenerative disorder characterized by the accumulation of protease-resistant prion protein (PrPres) in CNS. CBD introverted the neurotoxic effects of PrPres and affected PrPres-induced microglial cell migration in a concentration-dependent manner (Dirikoc et al. 2007). Thus, the neurons can be protected from multiple molecular and cellular factors involved in the different steps of the neurodegenerative process formed during prion infection by CBD. Lack of toxic side effect and specifically targeting the brain make it a promising drug.

#### 10.6.1.4 Multiple Sclerosis (MS)

MS is another central nervous disorder affecting an estimated 2.3 million people globally. The signs of this disorder include muscle pain, tiredness, fatigue and depression. These symptoms also lead to decreased physical activity, adversely affect functional independence and adversely affect patient quality of life having harmful effect on patient's life. Despite substantial improvement in disease-modifying therapy being made in recent years, none of the existing therapies delay MS-related symptoms. CBD is the most concentrated chemical component of *Cannabis* and believed to primarily drive therapeutic benefit. Total 132 original studies explains CBD's profile by pointing out ecstasy is not induced physiological parameters (heart rate, blood pressure, and body temperature) are not altered. High doses (1.5 g/day) of CBD in MS have been shown to be tolerated consistently by humans (Rudroff and Sosnoff 2018).

#### 10.6.1.5 Epilepsy

Epilepsy is a brain disorder affecting around 65 million people worldwide. Anti-seizure properties of CBD do not act through cannabinoid receptors, but it acts through ionic channels, neurotransmitter transporters and multiple seven-transmembrane receptors (Lattanzi et al. 2018).

Dravet syndrome (DS) is the epilepsy of infancy characterized by seizures like myoclonic and partial seizures, psychomotor delay and ataxia. It decreases $Na^+$ ion or may drive numerous types of GABAergic (Mistry et al. 2014). CBD acts as protective agent against convulsions in diverse animal studies (Carlini et al. 1973; Karler et al. 1973). Higher doses of CBD (0.1 g per kg) reduce the occurrence, period and harshness of seizures in DS mice; however, decreasing the doses (10–20 mg/kg) increases the societal behaviours (Kaplan et al. 2017a, b). Such effect reliant on numerous mechanisms depends on the doses of CBD.

LS is a complex, severe childhood-onset epilepsy encephalopathy affecting 2 cases per 100,000 population (Archer et al. 2014)**.** CBD is the approved drug for LS and decreases the rate of seizures. However, the effectiveness of CBD against this disease was confirmed by treating both children and adults at doses 10 or 20 mg/kg daily. The significant decrease in the occurrence of drop seizures was observed. CBD dose of 20 mg/kg and valproate showed an enhancement in liver amino transferase concentrations due to the interaction between CBD and valproate (Devinsky et al. 2018). There is a great demand for the curing adult and children suffering from epileptic seizures with medical cannabis. When children were treated with CBD, there is 89% reduction in seizure (Tzadok et al. 2016). The CBD-based drug has been recently approved by the US FDA for the treatment of seizures that are related to infrequent and severe forms of epilepsy (Wise 2018).

#### 10.6.1.6 Autism

Neurodegenerative disorders characterized by non-verbal communicative behaviours while social interaction, repetitive movements, intellectual disabilities. Worldwide occurrence of autism is approximately 1% (Baron-Cohen et al. 2014). Autism was diagnosed in 1 of 59 children. It commonly occurs in age of 8 years (Christensen et al. 2018). A recent study confirms that there is decrease in the plasma levels of the endocannabinoid anandamide as compared to healthy ones showing the involvement of impaired anandamide signalling in the pathophysiology of autism (Karhson et al. 2018). The interaction between ECD signalling and oxytocin-mediated was studied. Oxytocin drives anandamide-mediated signalling at CB1 receptors which regulate social behaviour. Thus, the impairment in ASD may be due to deficiency in the signalling mechanism of anandamide. Intranasal oxytocin treatment may show therapeutic effects in ASD (Anagnostou et al. 2012).

Epilepsy is predominantly found in individuals with autism-like behaviour, e.g. Angelman syndrome, Rett syndrome, Dup15q syndrome, etc. (Poleg et al. 2019). In another clinical study on Sturge-Weber syndrome patients, CBD improves quality of life (Kaplan et al. 2017a, b). In an online survey on the children whose parents have infantile spasms (IS) and Lennox-Gastaut syndrome (LGS), there is reduction in seizures remarkably and quality of life also improved (Kaplan et al. 2017a, b) after treatment with CBD (Hussain et al. 2015).

#### 10.6.1.7 Anxiolytic

In animal studies, CBD has similar effects to anxiolytic drugs in different paradigms. In human studies, the anxiolytic effects of CBD have been elicited in subjects

submitted to the Simulation Public Speaking Test (SPST). CBD has anxiolytic properties associated with an action on the limbic and paralimbic brain areas as observed using functional neuroimaging in healthy volunteers (Bergamaschi et al. 2011).

#### 10.6.1.8 Alzheimer's Disease

Alzheimer's disease is a neurodegenerative disorder caused by brain cells to waste away (degenerate) and die followed by other psychiatric disorders (Kumar et al. 2018). It is the most common cause of dementia (Premoli et al. 2019). CBD is a non-psychoactive phytocannabinoid that reduces reactive gliosis and the neuro-inflammatory response as well as promotes neurogenesis. It has also been observed in AD rodent models that CBD reverses and prevents the development of cognitive deficits (Watt and Karl 2017). CBD in vitro inhibited the hyperphosphorylation of tau and reduced Aβ production by promoting APP ubiquitination.

CBD dose dependently inhibited glial fibrillary acidic protein (GFAP) mRNA and protein expression in in vivo anti-inflammatory effects in a mouse model of AD where the mice were intrahippocampally injected with human Ab42 and then treated daily with intraperitoneal (i.p.) injections of CBD (2.5 or 10 mg/kg) for 7 days (Esposito et al. 2007). GFAP is the best-known marker of activated astrocytes and thought to be one of the main features of reactive gliosis (Esposito et al. 2007). CBD is able to reduce-induce reactive gliosis and also reduce both iNOS and interleukin-1b (IL-1b) protein expression and the related NO and IL-1b release (Esposito et al. 2007).

#### 10.6.1.9 Depression

*C. sativa* has shown significant effects upon the brain, such as euphoria and mood elevation (de Morais et al. 2018). CBD has antidepressant properties, since its actives 5HT1a receptor (El-Alfy et al. 2010; Linge et al. 2016). Depression requires chronic treatment; in this way, currently CBD has been tested against the results of chronic unpredictable stress, which incorporates anhedonia and anxiety-like behaviour (de Morais et al. 2018). Chronic treatment with CBD had the option to counteract these behavioural changes, an effect that relies upon hippocampal neurogenesis (Schiavon et al. 2016). These studies suggested that CBD can be considered as a potential candidate for the treatment of mood disorders.

#### 10.6.1.10 Post-traumatic Stress Disorders (PTSD)

The essential conditions to develop PTSD can be summarized as follows (Bernardy and Friedman 2015):

- Primary or secondary experience of a strong trauma.
- Frequent instinctive and intrusive recalls of the experienced trauma.
- Persistent escaping and rejection of everything connected to the trauma.
- Negative emotional state with endless feelings of fear.
- Anger, horror, guilt or shame for almost everything.

- Irritable behaviour, hypervigilance, exaggerated startle response, attention problems and nap problems.

Unfortunately, there are no effective pharmacological or psychological treatments for PTSD (Forman-Hoffman et al. 2018). Approved treatments for PTSD are anxiolytics and antidepressants, which have substantial side effects and are unproductive possibly lacking a specific target in the memory process (Forman-Hoffman et al. 2018). Aiming at these reasons, the use of extracts from *C. sativa* in trauma-related disorders has been taken into consideration. Capos et al. in 2012 have demonstrated the effect of CBD in decreasing the defensive behaviours induced by predator experience (Campos et al. 2012a, b). Furthermore, it was found that CBD blocked the trauma-related responses when given before the acquisition phase (Papagianni and Stevenson 2019). Additional exploration revealed that CBD affects learning and memory of aversive events, processes related to PTSD pathophysiology (Uhernik et al. 2018). However, in contrast with the above results, ElBatsh et al. have reported increase of state of fear on repeated administration of CBD (ElBatsh et al. 2012). CBD can enable the extinction of aversive memories also in PTSD-affected humans (Premoli et al. 2019).

### 10.6.2 Diabetes Mellitus

Type 1 diabetes is an autoimmune disorder in which destruction of insulin takes place with the releasing of pancreatic β-cells. When pathogens or nitric oxide (NO) pollution from atmosphere enters in the body, the immune system gets activated via nitric oxide synthase causing damage in tissues via reactive nitrogen species. CBD (5 mg/kg) reduces type 1 diabetes in mice compared to untreated mice shown by Weiss and co-workers. The pro-inflammatory cytokines IFN-γ and TNF were also decreased in plasma by treatment with CBD. Treatment of old female NOD mice with CBD for 11–14 weeks either in the initial symptoms of diabetes or in inactive diabetes stage enhances the appearance of the disease. There is reduction in the level of the cytokine IL-12 and increase in the level of IL-10 (Weiss et al. 2008).

Pathophysiological alterations in the vasculature are the major complication of diabetes mellitus. Retinopathy and nephropathy are microvascular complications, while common macrovascular complication of diabetes is atherosclerosis. Studies with CBD are carried out on rats induced by streptozotocin showing its protective effects. Treatment with CBD barred death of retinal cells and increase the vascular permeability. Its treatment also results in the reduction of oxidative stress and decreased the levels of TNF-α, vascular endothelial growth factor and intercellular adhesion molecule (Weiss et al. 2008). It also shows effectiveness against other complications in diabetes and atherosclerosis (Rajesh et al. 2007).

### 10.6.3 Rheumatoid Arthritis and Ankylosing Spondylitis

RA and AS are prolonged, universal, inflammatory diseases, mainly in the musculoskeletal system (Hendricks et al. 2019). The symptoms of RA and AS are fatigue and pain, cognitive dysfunctions like concentration and memory problem. RA affects smaller as well as larger joints (Aletaha et al. 2010). AS is characterized by back pain and stiffness and affects spinal and sacroiliac joints; this disease involves numerous pain mechanisms, and there is a clinical challenge for the treatment of these diseases.

Nociceptive and non-nociceptive components may be involved in treatment of pain related to RA and AS. It is established on the interaction between central sensitization and peripheral inflammation (Woolf 2011, Ahmed et al. 2014). The immediate pain is initiated by the swelling of the synovial tissue and/or consecutive oedema of the subchondral bone which causes pain leading to a sensitization of the peripheral nociceptors (Goyal et al. 2019). Thus, peripheral nociceptors and central neuropathic pain mechanism at different stages cause chronic. The progress of arthritis is blocked by the administration of CBD orally or i.p. Besides reduction in suppressing T-cell response, CBD has reduced the release of bioactive tumour necrosis factor (TNF) from synovial cells isolated from arthritic knee joints of treated mice. This study shows that CBD has antiarthritic effect which results from the combination of immunosuppressive and anti-inflammatory actions (Kidd et al. 1995; Glintborg et al. 2010; Christensen et al. 2016; Hendricks et al. 2019).

### 10.6.4 Anticancer Activity

CBD is reported to have anticancer activity against breast cancer cell lines and annotated its effects on key neoplastic pathways. CBD binds to specific G-protein-coupled receptors (GPCR). CB1 is found mainly in the brain and nervous system, whereas CB2 is expressed predominantly by immune cells. CBD induced the endoplasmic reticulum stress which inhibits AKT and mTOR signalling as shown by decreased levels of phosphorylated mTOR and 4EBP1 and cyclin D1. Beclin1 plays a central role in the induction of CBD-mediated apoptosis in MDA-MB-231 breast cancer cells. Despite enhancing the interaction between beclin1 and Vps34, it inhibits the connection between beclin1 and Bcl-2 (Shrivastava et al. 2011). CBD was shown to be more effective inhibitors of human breast cancer cells among three groups of cannabinoid compounds tested. The three groups are:

- Phytocannabinoid with affinity for $CBD_1$ and $CBD_2$ receptors.
- Phytocannabinoids with zero empathy for CBD1 and CBD2 receptors.
- Synthesized compounds with affinity for CBD1 and CBD2 receptors.

CBD regulates the genes which are responsible for proliferation of cells and invasion. It decreases the Id-1 expression whose overexpression causes breast cancer. With this property, it is known to reduce tumour aggressiveness.

#### 10.6.4.1 CBD and Leukaemia/Lymphoma

CBD can also cure lymphoblastic diseases first shown by Gallily et al. (Gallily et al. 2003). In the case of human HL-60 cell line leukaemia, CBD mediated apoptosis by activation of caspase-3 activation. It also had no effect on human monocytes from normal individual. CBD exposure led to a substantial reduction in the number of viable cells mediated by the CBD-2 receptor as well as an activation of apoptosis, both in vitro and in vivo both using murine EL-4 lymphoma cell line and the human Jurkat and Molt-4 leukaemia cell lines. The exposure of CBD in Jurkat cells results in the activation of caspase-8, caspase-9 and caspase-3 and polymerase cleavage (ADPribose) polymerase, and full-length bid decrease indicates a possible crosstalk between intrinsic and extrinsic apoptotic pathways (McKallip et al. 2006). CBD may act as effective treatment against leukaemia.

#### 10.6.4.2 Angiogenesis

New blood vessels have been formed in angiogenesis from the pre-existing blood vessels and thus constitute another approach for cancer treatment. Cannabidiols were proven to show anti-angiogenic factors (Freimuth et al. 2010). Cannabidiol repressed the proliferation, migration and invasion of HUVE cells by inducing cytostasis of the endothelial cells without apoptosis (Solinas et al. 2012). In addition to having pro-apoptotic, anti-proliferative and anti-invasive actions, it also shows anti-angiogenic effects and anti-invasive acts.

### 10.6.5 Cannabidiol in Pain Management

Cannabinoids are also known for different types of pain management. *Cannabis* has been utilized historically in certain forms for the treatment of pain including neuropathic pain, inflammatory pain and pain in cancer (Fine and Rosenfeld 2013). CBD also have analgesic effect in hyperalgesic and inflammatory states (Iversen and Chapman 2002). The dissociative effect of THC on the pain was not indicated by peripheral mechanisms alone. It has been shown that the activation of amygdala leads to the inter-individual reaction to cannabinoid analgesia, and there is a dissociative effect of tetrahydrocannabidiol in the brain, which results in pain relief in humans (Lee et al. 2013). THC and CBD were known to have best pain-relieving properties. CBD's anti-inflammatory role was due to its agonist action on CB2 receptors that had both primary and secondary pain influences. THC also has detrimental memory effects that are lessened by CBD. An oral spray consisting of 27 mg THC and 25 mg cannabidiol per ml was licensed for the treatment of multiple sclerosis in Canada, New Zealand, Israel and in several European countries (Morgan and Curran 2008).

#### 10.6.5.1 Neuropathic Pain

ECS is effective through peripheral nervous system and central nervous system in controlling pain at the backbone, supraspinal and peripheral levels. ECS works via integrative pain centres as the grey matter (Walker et al. 1999a, b), thalamus (Martin

et al. 1996) and the spinal cord (Hohmann et al. 1995; Richardson et al. 1998a, b). ECS is an endogenous mediator of stress-induced analgesia and fear-conditioned analgesia suppress pain-related phenomena (Strangman and Walker 1999) and allodynia (Rahn et al. 2007). In the PNS (Ibrahim et al. 2005), the ECS has key role in suppressing both hyperalgesia and allodynia via CB1 (Richardson et al. 1998a, b) and CB2 mechanisms.

#### 10.6.5.2 Cancer Pain

CBD is effective against cancer-related pain by acting on CB1 or CB2 receptors without any side effects. So far, studies of the effectiveness of CBD in cancer pain (as well as in neuropathic pain) have used inadequate doses of cannabidiol (Farquhar-Smith 2009; Johnson et al. 2010) to determine efficacy (Zhornitsky and Potvin 2012). Its less bioavailability reflects its part of ineffectiveness (Russo 2011).

#### 10.6.5.3 Additional Pain-Related Therapeutic Benefits of Cannabinoids

Cannabinoids helps in removing pain, mood disorders and also helps in decreasing the risk of sleep disorders.

### 10.6.6 HIV/AIDS

An investigation conducted in 2005 on 523 HIV-positive individuals established that 143 of them used cannabis as a way of managing HIV/AIDS symptoms. Out of these, 97% reported appetite improvement during the time when they used cannabis. Researchers attributed this to the CBD that is found in cannabis (Woolridge et al. 2005). The world is gradually accommodating CBD as a potential treatment for a number of medical conditions. Plausibly the milestone achievement for CBD has been the approval of a CBD-based drug, Epidiolex, for the treatment of severe forms of childhood epilepsy. Cannabis used in the research area for treating HIV symptoms has also advanced. CBD-based products are preferred for HIV patients to help in easing away their pain, anxiety and other HIV-related symptoms.

CBD is medically important in the viable treatment of HIV/AIDS and also in autoimmune disease. CBD is extensively used in various diseases such as pain, emesis, nausea and fatigue; these all are common side effects of HIV and AIDS. The compounds within cannabis have also been found to alter immune responses in patients with HIV. Some patients suffering from AIDS might possess symptoms such as weight loss. CBD can stimulate direct lipogenic action that improves and changes fat distribution in patients and help in controlling obesity (Rossi et al. 2018). CBD influences immunity. It shows immunosuppressant effects consistently; CBD show positive effects in boosting immunity; this will offer the much-needed hope in the treatment of HIV and AIDS. Few studies investigated the action of CBD on T-cell function. The results were confirmed that CBD suppresses immunity and T-cell function with the involvement of various mechanisms. CBD is a well-known mood elevator compound and most presumably utilized by patients to reduce

symptoms of anxiety, stress and depression. At the point when it's combined with THC and other compounds found in cannabis, the effect formed is more powerful and durable. This is a result of the following effect. Likewise, CBD is able to disguise the intoxicating effects of THC so that marijuana treatment is better tolerated by HIV patients.

Research on this section is little, and according to the examinations, we recognize that CBD may play a significant role in organizing certain symptoms of this disease. Thus, CBD (cannabidiol) may be a viable option to handle specific HIV symptoms without unsafe side effects. Along with all these, the cannabidiol may also diminish the reproduction of HIV. A few investigations demonstrate that CBD shows potential medical advantages for HIV/AIDS. More investigations are in progress to build up the effects of CBD on HIV/AIDS patients (Woolridge et al. 2005). In addition, cannabidiol is a non-psychoactive substance and it will not cause a "high". CBD is additionally legal in the USA.

### 10.6.7 Nausea and Vomiting

The treatment of nausea is difficult with new anti-emetic agents than is vomiting (Andrews and Horn 2006). CBD and its dimethyl-heptyl homologue suppress nausea in a test model with rodents. Cannabidiol may go about as a 5-HT1A autoreceptor agonist to lessen poison actuated nausea and vomiting. CBD controls nausea by diminishing the arrival of serotonin receptors and diminishing the arrival of serotonin, so there'll be less incitement of the vomiting centre in the brain, in which it's a major role of activation of CB2 receptor because of its preventing nausea and vomiting but how it does this is not as well-established compared to the role of the CB1 receptors. Anandamide is an endogenous cannabinoid discharged through our body and furthermore actuates the CB1 receptors to create their anti-emetic impacts. Unfortunately, its action is brief while it's easily degraded (Parker et al. 2002, 2003, 2011).

$CB_1$ agonism suppresses vomiting, and it is switched through $CB_1$ antagonism, and $CB_1$ inverse agonism promotes vomiting. CBD is the first non-psychoactive compound that suppresses nausea and vomiting within an insufficient dose range (Parker et al. 2002, 2003, 2011). CBD alone has a better drug safety profile, is nontoxic and is very well-tolerated even at high doses. THC and CBD are sheltered to use for nausea and vomiting.

### 10.6.8 Antipsychotic Effect of CBD

First antipsychotic properties of CBD were published in 1982 (Zuardi et al. 1982). Initial studies confirmed the interaction between 9-tetrahydrocannabinol and CBD in health volunteers. The coadministration of the two cannabinoids resulted in less anxiety and psychotomimetic symptoms than 9-THC alone. It was believed that it may be due to the dependency of CBD attenuation of 9-tetrahydrocannabinol on a

pharmacodynamic rather than pharmacokinetic interaction. It was shown that the dose of CBD did not have any effect on the level of 9-tetrahydrocannabinol (Consroe et al. 1991). When the patients were treated with the cannabis devoid of CBD, an increase in the psychotic episodes was observed in patients (Rottanburg et al. 1982). These results led to numerous studies which proved that there is a link between psychosis and CBD, when CBD showed greater antipsychotic activity as compared to placebo in patients undergoing treatment of schizophrenia (McGuire et al. 2017).

### 10.6.9 CBD for Recreational Use

CBD has direct neurotoxic action in hippocampus neurons, while THC caused neuron death. Prolonged use of *Cannabis* is related to the decrease of neuronal and axonal integrity in the DLPFC. The results is interesting as the decreased ratio of NAA/tCr in DLPFC and neuropsychological deficits were also reported in schizophrenia (Hermann et al. 2007), breathing-related to CNS as the opioid analgesics have the possibility of combining with other CNS depressants like benzodiazepines which creates problems (Zutler and Holty 2011). There are also reports showing the suppression of apnoea by CBD. This is an interesting area for further research and clinical applications of CBD in sleep and pain medicine (Carley et al. 2002).

## 10.7 Pharmacology of Cannabidiol

Studies on pharmacology of CBD have began during the 1970s, along with its hypnotic and anticonvulsant properties, distributed in 1981. From that point forwards, a number of clinical and preclinical pharmacological studies have been established for CBD (Scuderi et al. 2009).

### 10.7.1 Routes of Administration and Dosage

As indicated by clinical studies, CBD has been used orally via inhalation like smoking and vaporization (Labrecque et al. 1978). Moreover, a capsule or dissolved in oil solution (e.g. olive or sesame oil) has also been used for administration. It is also administered via intranasal or sublingual routes (Fasinu et al. 2016). CBD is viably taken up in the lungs by the circulating blood. Aerosolized CBD has been reported to yield rapid peak plasma concentrations in 5–10 min and higher bioavailability than oral (Labrecque et al. 1978). An extensive scope of oral doses has been reported mostly 100–800 mg/day (Fasinu et al. 2016).

### 10.7.2 Pharmacokinetics

Cannabidiol oral bioavailability is 13–19%, while by inhalation it is 11–45% (Mechoulam et al. 2002, Scuderi et al. 2009). The elimination half-life of CBD is 18–32 h (Devinsky et al. 2014). Bioavailability from oral conveyance was evaluated to be 6% because of critical first-pass metabolism (Hawksworth and McArdle 2004). In healthy male volunteers, the mean ± SD entire blood levels of CBD at 1, 2 and 3 h after organization of 600 mg oral CBD were accounted for to be 0.36 (0.64) ng/mL, 1.62 (2.98) ng/mL and 3.4 (6.42) ng/mL, respectively (Martin-Santos et al. 2012). Cannabidiol is quickly scattered into the tissues with a high volume of distribution of ~32 L/kg. Like THC, CBD is exceptionally lipophilic compound, so it is specially gathered in adipose tissues (Ohlsson et al. 1986; Fasinu et al. 2016). With the help of enzymes like CYP1A1, CYP1A2, CYP2C9, CYP2C19 and CYP3A4,CYP3A5, UGT1A7, UGT1A9 and UGT2B7 isoforms, CBD metabolizedin the liver and intestines. The essential way is hydroxylation to 7-OH-CBD, and further various metabolites are excreted in urine and faeces. The human liver microsome (HLM) study established that cannabidiols was metabolized through pooled HLMs to eight monohydroxylated metabolites (6α-OH-, 6β-OH-, 7-OH-, 1″ -OH-, 2″ -OH-, 3″ -OH-, 4″ -OH- and 5″ -OH-CBDs). Among these considerations, 6α-OH-, 6β-OH-, 7-OH- and 4″ -OH metabolites are significant ones.

### 10.7.3 Pharmacodynamics

Cannabidiols have two main cannabinoid receptors (CB1, CB2). Both are situated in the CNS but CB2 have low densities in the CNS. And both are found in periphery on cells with immune function and in the gastrointestinal tract, and CB1 is only some expression in peripheral tissues. CBD does not bind and act directly at CB1 receptors. Several studies showed its potential agonist effects at CB1 receptors and low affinity for these receptors. It progresses towards becoming from the development that CBD is able to antagonize cannabinoid CB1/CB2 receptor agonists with evident KB value in the low nanomolar array both in membranes prepared from Chinese hamster ovary (CHO) cells transfected and in mouse whole-brain membranes with hCB2 receptors (Thomas et al. 2007).

Some observation represents that CBD bind to sites different from cannabinoid receptors as normal CBD and the (+)-synthetic one both stimulate the type-1 vanilloid receptor (Bisogno et al. 2001). It is also reported that cannabidiols also bind to 5-HT1A and exert anxiolytic effect (Campos and Guimarães 2008) and also during ischaemia reduction of cerebral localized necrosis size (Mishima et al. 2005). Cannabidiols have been showing to act as a serotonin 5-$HT_{1A}$ receptor partial agonist (Russo 2005). It is an allosteric modulator of the μ- and δ-narcotic receptors also (Kathmann et al. 2006). At low concentrations (nanomolar to micromolar), CBD hinders the orphan G-protein-coupled receptor GPR55, the equilibrative nucleoside transporter and the transient receptor capability of the melastatin type

8 (TRPM8) channel (Pertwee 2008), and cannabidiols increase the action of the serotonin 5-HT1a receptor, the a1 and a3 glycine receptors and the transient receptor capability of ankyrin type 1 (TRPA1) channel, with a bidirectional impact on intracellular calcium (Pertwee 2008).

### 10.7.4 Toxicology

A few investigations have been conducted identifying with the safety and side and toxic effects of CBD after its administration in vivo and in vitro, yet this part will summarize such conclusion. Concentrate on toxicity quality and side effects of cannabidiols admission will be talked about, just as the biological factors influenced by cannabidiol interaction with different substances (Machado Bergamaschi et al. 2011; Iffland and Grotenhermen 2017).

- CBD influences the development of tumoral cell lines but did not have any impact in most non-tumour cells. On the other hand, a pro-apoptotic impact has been seen in lymphocytes.
- Cannabidiols affect only the growth of tumoral cell lines and don't influence the non-tumour cells. On another way observed is the pro-apoptotic effect on lymphocytes.
- CBD has no impact on the improvement of an embryo.
- Some studies confirmed its possible effects on hormone changes, and other studies signify no effect, since it relies on the technique utilized for a specific hormone.
- CBD has no impact on diverse physiological and biochemical functions.
- It has no noteworthy effects on animal behaviour unless if exceptionally high dosages are administered.
- Cannabidiols may have impacts on the immune system; however, it isn't clear.
- CBD might show drug interactions through hindrance of some cytochrome P450 catalysts; however, it is not mentioned that these impacts happen at physiological concentrations.

According to toxicity studies, $LD_{50}$ value determines the level of toxicity. For Turkish marijuana smoke, evaluated lethal dose qualities dependent on the substance of cannabinoids were 10 mg/kg, while for CBD it is around 35 mg/kg. This dose-related impact was most extreme in CBD-treated human beings and animals (Rosenkrantz and Hayden 1979).

### 10.7.5 Adverse Effects

According to the literature, CBD doesn't produce the impacts that regularly appeared with other cannabinoids such as THC. It is also not known to deliver significant impacts in a human investigation of abuse potential (Martin-Santos et al. 2012).

Moreover, a psychological and psychomotor function is not adversely affected. Several human studies were carried out to check whether these impacts likewise happen in people. Controlled human examinations in regard to the potential physical reliance impacts (e.g. withdrawal and resistance) of cannabidiol have not been accounted. In animal studies, serious adverse effects such as embryo-fetal toxicity, spermatogenesis reduction and neurotoxicity were observed. However, doses administered in these studies were higher than those used in human trials. No investigations have been distinguished of the physical reliance capability of CBD in animals (Hayakawa et al. 2007).

### 10.7.6 Mechanism of Action of Cannabidiol

CBD is another cannabinoid that is not psychoactive and does not bind to CB receptors but shows anticonvulsant and anti-inflammatory effects, and it also has antipsychotic (indirect antagonist of CB agonists), analgesic and antidepressant effects (mediated via 5HT1a agonism) (Campos et al. 2017) (Table 10.4).

$CB_1$ and $CB_2$ are two key receptors of cannabinoids. $CB_1$ is an endocannabinoid receptor primarily largely found in the central nervous system (but not in the medulla) and acts primarily to inhibit the release of neurotransmitters. CB2 is located in the periphery on immune and nerve cells. The role of the endocannabinoid system in humans is its effects on neurogenesis, short-term memory, appetite stimulation, analgesia, inhibition of immune function and reduction of the HPA axis during stress (Mechoulam et al. 2007). THC is the first psychoactive cannabinoid in *Cannabis* as it binds to $CB_1$ and $CB_2$ receptors with relatively same affinity but mostly its effect on CB1 receptors in the brain (Mechoulam et al. 2007).

At higher concentrations, it stimulates the atomic peroxisome proliferator-activated receptor-c (PPAR-c) and the transient receptor capability of vanilloid types 1 (TRPV1) and 2 (TRPV2) channels (Bisogno et al. 2001). The pharmacological profile of CBD includes PPARγ agonism and intracellular calcium discharge (Campos et al. 2012a, b). This represses the cellular uptake and the unsaturated fat amide hydrolase-catalysed deprivation of N-arachidonoyl-ethanolamide (Bisogno

**Table 10.4** Mechanism of action of CBD

| | Serotonin HT1A | Vanilloid TRPV-1 | GPR55 | Antioxidant |
|---|---|---|---|---|
| Agonist | ✓ | ✓ | | |
| Antagonist | | | ✓ | |
| Receptor independent | | | | ✓ |
| Regulates | Depression<br>Sleep<br>appetite | Pain<br>Inflammation<br>Body temperature | Bone density<br>Blood pressure<br>Cancer cell proliferation | Neuroprotection |

et al. 2001). Cannabidiols also have potent antioxidant properties, possibly due to its polyphenolic nature. The mechanism of action of cannabidiols is given in Fig. 10.16.

Recent studies recognized orphan receptors like GPR3, GPR6 and GPR12 as new molecular targets for the CBD (Brown et al. 2017, Laun and Song 2017). CBD might be an antagonist of GPR55 receptor (Ryberg et al. 2007). It likewise may act as an inverse agonist of GPR3, GPR6 and GPR12. Being novel targets for CBD, GPR3, GPR6 and GPR12 may participate in various functions.

## 10.8 Marketing Approvals of CBD as Medicinal Product

### 10.8.1 Sativex®

Sativex® 1:1 formulation of CBD and THC is present in market by GW Pharmaceuticals in many countries for the treatment of seizures related to Lennox-Gastaut and Dravet syndromes in patients who have not responded effectively to other therapies. Sativex® was demonstrated in relieving spasticity in adult patients with MS in study GWSP0604. æ-9- THC and CBD are the primary active components in Sativex®. THC being a psychotropic agent might produce drug abuse. Both active components, THC and CBD are scheduled under the Controlled Drugs and Substances Act. Due to the lack of establishment of safety and efficacy of Sativex® under 18 years of age, it should not be used in adolescents or children. This combination is also frequently recognized as nabiximols (Portenoy et al. 2012; Romero-Sandoval et al. 2017).

### 10.8.2 Epidiolex®

Epidiolex®, produced by GW Pharmaceuticals (UK), is a liquid oral formulation. It is the first highly purified, plant-derived CBD, with formulation based in a new category of anti-epileptic drugs. It is recently (June 2018) approved by FDA as Orphan Drug designation for the treatment of seizure disorders and Dravet and Lennox-Gastaut syndrome in patients 2 years of age or older (Sekar and Pack 2019).

#### 10.8.2.1 Arvisol®

Echo Pharmaceuticals in the Netherlands has been developing Arvisol® containing pure CBD intending to be registered for the treatment of various neurological disorders including epilepsy and schizophrenia. Currently, Arvisol® is in phase I clinical trials (Babalonis et al. 2017).

### 10.8.3 ZYN002

Zynerba® Pharmaceuticals is developing ZYN002, the first and only pharmaceutically produced CBD formulated as a permeation-enhanced gel for

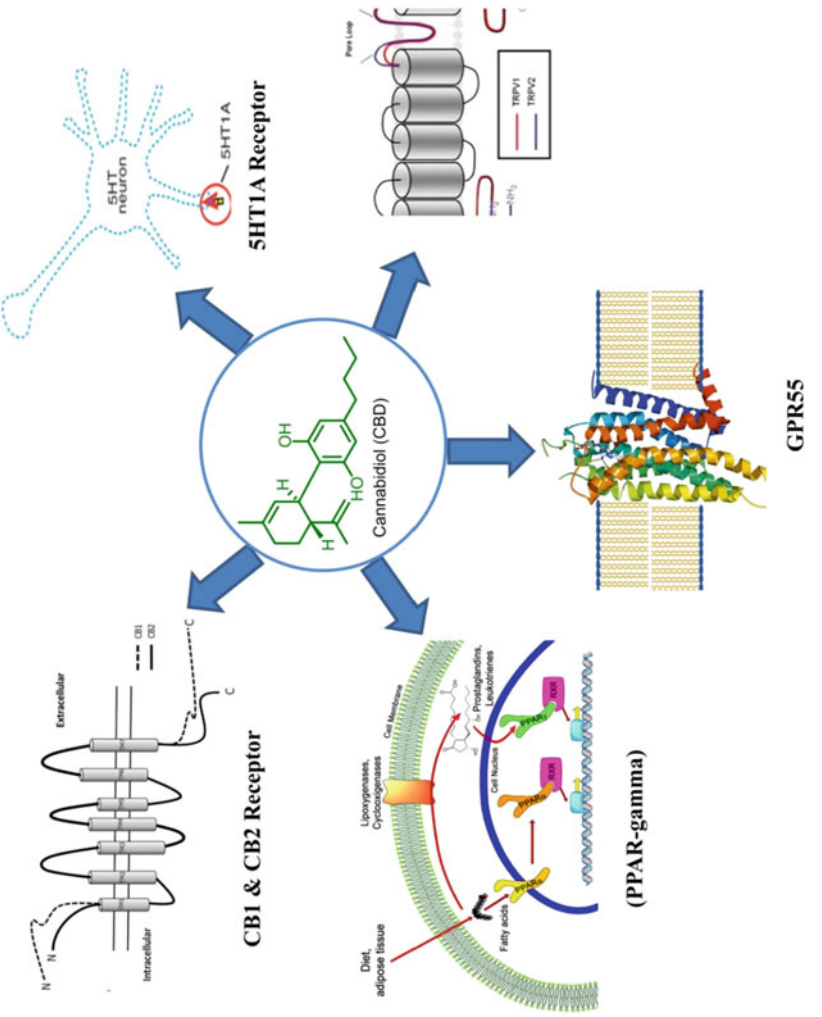


**Fig. 10.16** Mechanism of action of cannabidiol

transdermal delivery and patent protected through 2030, targeting Fragile X syndrome, refractory focal epilepsy and developmental and epileptic encephalopathies. Because CBD is nearly insoluble in water, ethanol and propylene glycol as solubilizing agents and Transcutol® HP as a permeation enhancer are being used in ZYN002. Presence of the permeation enhancer in ZYN002 increases the delivery of CBD through the layers of the skin and into the circulatory system. Transdermal delivery allows the CBD in ZYN002 to avoid stomach acid degradation and the first-pass liver metabolism that occurs with oral or oral mucosal delivery methods. Currently it is in open-label phase II testing for Fragile X syndrome (Heussler et al. 2019).

A pure CBD product has been developed after isolation in a crystalline powder by Bionorica® (Germany). An oral solution of pure CBD has been developed by INSYS Pharmaceuticals (USA) which is presently in clinical trial phase III as add-on therapy with vigabatrin for the treatment of infantile spasm-type seizures and in clinical trial phase II for the treatment of childhood absence epilepsy and Prader-Willi syndrome (Arndt and Wit 2017). Oral formulation PT101, containing purified CBD implanted in gelatin matrix pellets, has been developed by PhytoTech Therapeutics of Tel Aviv, Israel, and currently, it is in clinical trial phase II for the treatment of paediatric intractable epilepsy (Atsmon et al. 2018).

In 2015, GW Pharmaceuticals was granted Fast Track designation by the FDA for intravenous CBD for the treatment of neonatal hypoxic-ischaemic encephalopathy (NHIE) (Bonn-Miller et al. 2017). Orphan designation (EU/3/15/1520) was granted by the European Commission for the use of CBD in the treatment for perinatal asphyxia. (https://www.fda.gov/NewsEvents/PublicHealthFocus/ucm484109.htm.)

## 10.9 Legal Aspects of CBD and Cannabis

The research on this important plant highly suffered because of researcher's interest, stringent regulatory norms and partly lack of patentable intellectual property. To clear this logjam, many research-based programmes have been initiated around the globe (countries mentioned above), and these bring value in terms of not only medical uses but also economy of these regions. Those countries which have legalized its cultivation have started reaping the benefits in terms of structured research and value as raw material. In India, consumption and production of the *Cannabis* is governed by the Narcotic Drugs and Psychotropic Substances Act, 1985, which may lead to a jail of 6 months or a fine of Rs 10,000 for its consumption, while illegal production and cultivation can be punished with a jail term of up to 10 years. However, taking inspiration from the West, India (both politically and industrially) has voiced for legalizing cannabis in the country.

## 10.10 Conclusion and Future Direction

*Cannabis* is having a long history of being used by mankind for its therapeutic properties both in traditional and Western medicine. Isolation and synthesis of cannabinoids, along with more effective synthetic derivatives and the outcome of cannabinoid receptors and their endogenous ligands, have rehabilitated the attention in potential therapeutic uses. THC, the most abundant cannabinoid present in plant *Cannabis*, witness less focus of being psychoactive in nature, while non-psychotropic cannabidiol has shown potent therapeutic effects in numerous clinical and preclinical studies. Undeniably, action of CBD in a non-specific way on multiple receptor systems causes a concert of responses to both central and peripheral therapeutic actions. However, the mechanism of action involved in the biological responses of CBD remains poorly understood. Detailed chemical and pharmacological aspects of cannabidiol described in this chapter open new gateway for future studies on its therapeutic actions with thorough mechanism.

**Acknowledgments** We would like to acknowledge the Council of Scientific and Industrial Research (CSIR), New Delhi, India, and DST-SERB for the financial support through research grants in the form of HCP0008 and GAP2185.

**Conflict of Interest** All authors declare no conflict of interest for this manuscript.

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# Genetic Variability in *Ocimum* L. Germplasm: Medicinal and Economic Potential for Value Addition and Product Development

11

Smita Singh, Raj Kishori Lal, and Bikarma Singh

**Abstract**

Plants are essential for any ecosystem and are considered as the most important source of herbal medicine. They have been used for treatment of different diseases of human beings worldwide since the beginning of human civilization. Among the plant community, one of the genus *Ocimum* L. belonging to the family Lamiaceae is of high value in terms of economic upliftment and product development. Most of the species under this genus are cultivated throughout the tropical and the subtropical agro-climatic zones for medicine and for extraction of essential oils for product development in aroma-based pharmaceutical industries. The growth form of this aromatic genus *Ocimum* can be categorized as herbs, under-shrubs or shrubs that on distillation yield essential oils of various active aroma chemicals, such as eugenol, methyl eugenol, linalool, methyl chavicol, germacrene A and D, elemicin, ß-elmene and (Z)-ocimine, and several other active constituents. These volatile compounds have a tremendous value in pharmaceutical, modern perfumery and food processing industries. Evaluation of biological activities of active ingredients of *Ocimum* indicated great medicinal properties, such as anti-biotic, anti-cancerous, anti-ageing, anti-stress, anti-pyretic, diaphoretic, diuretic, stomachic, anti-microbial and insecticidal, and

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_11

other similar importance in herbal formulations. A survey on 40 genetic stocks (or accessions) of *Ocimum* available at the CSIR-CIMAP belonging to five species, *viz*. *Ocimum basilicum* L. (25: French basil 7, Sweet basil 6, Zanzibar basil 1, Indian basil 10 and Thai basil 1), *Ocimum tenuiflorum* L. (9: Krishna/holy basil 4 and Shyam tulsi 5), *Ocimum kilimandscharicum* Baker ex Gurke (1: Champhor tulsi), *Ocimum africanum* Lour. (2: Hoary basil) and *Ocimum gratissimum* L. (3: African basil/van tulsi/tree basil/clove basil), was undertaken for study. It has been observed that some selected lines of *Ocimum* produce high quantities of essential oils, like *Ocimum tenuiflorum* (*O. sanctum* L.) CIM-Ayu (80% eugenol) and EOH-1 (89.75% methyl chavicol). These active ingredients have high international demand for aroma-based value additions and product development from essential oils. Extension and promotion of these *Ocimum* species will add and contribute to the economic upliftment of the developing countries like India and elsewhere in the world.

**Keywords**

Aromatic plants · Genetic variability · *Ocimum* · Citral · Eugenol · Aroma-based product development

## Abbreviations

| | |
|---|---|
| AP | Arunachal Pradesh |
| BST | Bench Scale Trial |
| CIMAP | Central Institute of Medicinal and Aromatic Plants |
| CSIR | Council of Scientific and Industrial Research |
| J&K | Jammu and Kashmir |
| RBD | Randomized Block Design |
| UP | Uttar Pradesh |
| WB | West Bengal |

## 11.1 Introduction

*Ocimum* L., a versatile aromatic genus of the family Lamiaceae, is represented by 66 species across the World (TPL 2013), and is well known for its medicinal properties and economically important essential oils (Kalita and Khan 2013; Singh 2019a). The genus is very variable and possesses wide range of intra- and inter-specific genetic diversity (Singh et al. 2019). The nomenclature of *Ocimum* species and its varieties is complicated and confusing, and in several instances the oil extracted from morphologically identical plants show different physico-chemical

properties. Species like *Ocimum gratissimum* L., *Ocimum africanum* Lour., *Ocimum basilicum* L., *Ocimum kilimandscharicum* Baker ex Gurke L. and *Ocimum tenuiflorum* L. are examples of well-known important species of the genus that grow in different parts of the World and are known to have curative biological functions (Lal et al. 2004; Barik et al. 2006; Singh 2019b). The plants flourish predominantly as herbs and shrubs and usually survive as annuals or perennial plants in habit. They possess glandular hairs or sessile glands secreting strongly scented volatile secondary metabolites in the form of oils. The dry leaves of *Ocimum* used as leaf herbal tea, essential oils and its chemical derivatives (eugenol, methyl eugenol, linalool, methyl chavicol, germacrene A and D, elemicin, ß-elmene, (Z)-ocimine) are exported to European and Arab countries in sizable quantity every year. The annual export of dry leaves herb, its products, essential oils and derivatives of chemical constituents of *Ocimum* is worth 5000 tons (Bhasin 2012). People know the plant as surasah in sanskrit and tulsi in hindi. Due to antioxidant and anti-ageing effects of tulsi, people use fresh leaves in panchamrut/charanamrut drink after Holi puja (Kumar et al. 2013). In India, tulsi is considered divine and is regarded not merely as a Godsent utility, as most sacred plants are viewed to be, but as an incarnation of the Goddess Herself. The classic Hindu myth, Samudramanthana, or the 'Churning of the Cosmic Ocean', explains that Vishnu spawned tulsi from the turbulent sea as a vital aid for all mankind (Anonymous 1973, Singh 2020). The tulsi leaves, when consumed, can control thirst and so is invaluable to weary travellers.

Apart from religious importance, *Ocimum* has several medicinal properties. Phytochemical investigation reveals that they are rich in carbohydrate, fibre, phosphorous, calcium, protein, iron, beta-carotene, vitamins $B_1$ and $B_2$ and in aromatic oils (Lal et al. 2004). It is effective against cold and cough, indigestion, stomach pain and diarrhoea. Nausea, ulcers, ringworm and asthma can also be effectively treated by using tulsi ingredients or extract. It is also helpful in lowering blood sugar and increasing lactation (Lal et al. 2003; Singh and Bedi 2017). The oil is used as antiperspirant as well as fly and mosquito repellent (Table 11.1). Available genetic stocks at the CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP, Lucknow) include 105 genetic stocks (or accessions) belonging to five *Ocimum* species – *Ocimum kilimandscharicum*, *Ocimum africanum, Ocimum gratissimum, Ocimum tenuiflorum* and *Ocimum basilicum* – and 7 varieties, which include CIM-Ayu, CIM-Angana, CIM-Kanchan, CIM-Saumya, CIM-Surabhi, Kushmohak and Vikarsudha (Figs. 11.1 and 11.2). In future, there will be possibility to develop more varieties of herbs, increase oil yield containing specific chemicals like eugenol, methyl eugenol, germacrene A and D, linalool, elemicin, ß-elmene and (Z)-ocimine, and produce other chemical contents from other herbal products (Ismaile 2006).

**Table 11.1** Medicinal uses of different *Ocimum* species

| *Ocimum* species | Essential oil | Medicinal properties |
|---|---|---|
| *Ocimum tenuiflorum* L. (Synonym: *O. sanctum* L.) | Essential oil obtained after hydro-distillation is rich in high quality essential oil isolate, eugenol, which is of great value in flavouring and used in the synthesis of vanillin. | The plant is pungent, bitter in taste and is reported to possess antituberculosis, antiseptic, antibiotic and anti-cancerous properties. Leaves have diaphoretic, stimulant and expectorant properties. Their juice is applied in cold, cough and chest troubles. |
| *Ocimum basilicum* L. | Essential oil is pale yellow, slightly viscid with a sweet spicy herbal odour, having methyl chavicol as a main constituent. It is used in flavour and perfume industry. | The plant is stomachic, stimulant, carminative, antipyretic, diaphoretic, expectorant, diuretic and also useful in heart, brain and blood diseases, asthma, inflammations and enlarged spleen. |
| *Ocimum gratissimum* L. | Essential oil is pale yellow with high percentage of eugenol. It is used in flavouring of food products. | The plant has bitter sharp taste and is useful in diseases of brain, heart, liver and spleen, strengths the gums and removes foul breath. It is diaphoretic, stomachic and is good for the treatment of fever. |
| *Ocimum kilimandscharicum* Guerk. | Essential oil is light yellow with strong odour of camphor. The oil is widely used in perfume, flavour and pharmaceutical industry. | The plant has carminative, stimulant, antipyretic, antifungal and antibacterial properties. |
| *Ocimum africanum* Sims. | Essential oil is light yellow and is a rich source of citral. It is used in perfume, flavour and cosmetic industries. The oil has been reported to possess potent antibacterial and antifungal activities. | The plant is used to cure fever, dysentery and haemorrhages from nose. Leaves and seeds are used in migraine. |

## 11.2 Methodology

In total, 180 collections were assembled from different States of India including few exotic ones from 6 countries (Tanzania, Thailand, Singapore, Slovak Republic, the USA and South Africa). After removing of duplicates, 40 genetic stocks were examined (Table 11.2) for high herbage content and essential oil yield with type of quality during an initial evaluation trial in field (design RBD, rep-2) (Lal 2012). The fresh 100 gm aerial parts of *Ocimum* spices were collected from plants from the field of CSIR-Central Institute of Medicinal and Aromatic Plants and processed by

**Fig. 11.1** *Ocimum* germplasm for genetic improvement and development of value-added products: (**a**) *O. tenuiflorum* (synonym: *O. sanctum*, CIM-Ayu), (**b**) *O. tenuiflorum* (CIM-Kanchan), (**c**) O. *tenuiflorum* (synonym: *O. sanctum*, CIM-Angana), (**d**) *O. kilimandscharicum,* (**e**) *O. basilicum* (French basil), (**f**) O. *basilicum* (Indian basil)

hydro-distillation for 3–4 h in a clevenger apparatus to obtain the crude essential oils. Identification of the essential oil composition was done by gas chromatography (GC; Clevenger 1928).

## 11.3 Results and Discussion

The morphological and essential oil yields were observed in 40 accessions (or lines of five *Ocimum* species: *Ocimum tenuiflorum*, *O. kilimandscharicum*, *O. africanum*, *O. gratissimum* and *O. basilicum*) and 7 varieties, viz. CIM-Ayu, CIM-Angana, CIM-Kanchan**,** CIM-Saumya, CIM-Surabhi, Kushmohak and Vikarsudha. Essential oil yield, oil content and herb yield were found to vary from 98.98 to 465.00, 0.30 to 1.20 and 28.18 to 53.08, respectively, in different populations of *O. basilicum* (Lal et al. 2008; Verma et al. 2011).

**Fig. 11.2** *Ocimum* germplasm for genetic improvement and development of value-added products: (**a**) O. *basilicum* (Sweet basil), (**b**) *O. gratissimum*, (**c**) *O. africanum* (**d**) African basil

The percentage of methyl chavicol is 89% and linalool is 1.01% in population 1 (EOH-1) chemotype (Table 11.4). Essential oil yield, oil content and herb yield were found to vary from 48.0 to 183.67, 0.29 to 0.54, and 15.83 to 36.17, respectively, in *O. africanum.* The maximum oil yield found in population 1 had high oil content (0.54%), with high citral 76.62% (geranial 46.59% + neral 30.03%) genotype (OC-1) identified (Table 11.4). Literature surveys revealed that the essential oil of *Ocimum basilicum* has been investigated in detail. Chemotypes described so far for this species are methyl chavicol, linalool and β-ocimene (Ozcan and Chalchat 2002). Essential oil compositions of *Ocimum africanum* are citral (geranial + neral) and β-ocimene. The occurrence of huge chemical variations among *Ocimum* populations collected from diverse localities seems to be due to the divergent climatological and geographical conditions as well as different genetic factors (Ojo et al. 2012). The major chemical constituents found in *Ocimum* having industrial importance are given in Fig. 11.3.

**Table 11.2** Total representation of *Ocimum* accessions used in the study

| Code | Genotypes/cultivar | Botanical name | Origin |
|---|---|---|---|
| G1 | French basil | *Ocimum basilicum* | Chennai, AP (India) |
| G2 | Vikarsudha | *Ocimum basilicum* | CSIR-CIMAP, Lucknow, UP (India) |
| G3 | Sweet basil | *Ocimum basilicum* | Gandhi Nagar, Gujarat (India) |
| G4 | French basil | *Ocimum basilicum* | Bangalore, Karnataka (India) |
| G5 | French basil | *Ocimum basilicum* | Mangalore, Karnataka (India) |
| G6 | French basil | *Ocimum basilicum* | Chandigarh |
| G7 | Shyam tulsi (CIM-Angana) | *Ocimum tenuiflorum* | CSIR-CIMAP, Lucknow, UP (India) |
| G8 | Sweet basil | *Ocimum basilicum* | Singapore |
| G9 | Sweet basil | *Ocimum basilicum* | Singapore |
| G10 | Sweet basil (Kushmohak) | *Ocimum basilicum* | CSIR-CIMAP, Lucknow, UP (India) |
| G11 | Sweet basil | *Ocimum basilicum* | Košice, Slovak Republic |
| G12 | Krishna tulsi (CIM-Ayu) | *Ocimum tenuiflorum* | CSIR-CIMAP, Lucknow, UP (India) |
| G13 | French basil | *Ocimum basilicum* | Mangalore, Karnataka (India) |
| G14 | Indian basil | *Ocimum basilicum* | Muzaffarpur, Bihar (India) |
| G15 | Indian basil (CIM-Saumya) | *Ocimum basilicum* | CSIR-CIMAP, Lucknow, UP (India) |
| G16 | Holi Basil | *Ocimum tenuiflorum* | Udaipur, Rajasthan (India) |
| G17 | Zanzibar basil | *Ocimum basilicum* | Tanzania |
| G18 | Indian basil | *Ocimum basilicum* | Bareilly, Uttaranchal (India) |
| G19 | Scare Basil (CIM-Kanchan) | *Ocimum tenuiflorum* | CSIR-CIMAP, Lucknow, UP (India) |
| G20 | Indian basil | *Ocimum basilicum* | Lucknow, UP (India) |
| G21 | Indian basil | *Ocimum basilicum* | Lakhimpur (Kheri), UP (India) |
| G22 | Kapoor/camphor tulsi | *Ocimum kilimandscharicum* | CSIR-CIMAP, Lucknow, UP (India) |
| G23 | Shyam tulsi | *Ocimum tenuiflorum* | Nasik, Maharashtra (India) |
| G24 | Holi Basil | *Ocimum tenuiflorum* | Lucknow, UP (India) |
| G25 | Indian basil (sel-2) | *Ocimum basilicum* | CSIR-CIMAP, Lucknow, UP (India) |
| G26 | Hoary basil (Selection-1) | *Ocimum africanum* | CSIR-CIMAP, Lucknow, UP (India) |
| G27 | Sweet basil | *Ocimum basilicum* | Trivandrum, Kerala (India) |
| G28 | Shyam tulsi | *Ocimum tenuiflorum* | Lucknow, UP (India) |
| G29 | Hoary basil | *Ocimum africanum* | Allahabad, UP (India) |
| G30 | French basil | *Ocimum basilicum* | Haridwar, Uttaranchal (India) |

(continued)

**Table 11.2** (continued)

| Code | Genotypes/cultivar | Botanical name | Origin |
|---|---|---|---|
| G31 | African basil | *Ocimum gratissimum* | CSIR-CIMAP, Lucknow, UP (India) |
| G32 | Thai basil | *Ocimum basilicum* var. *thyrsiflora* | Thailand |
| G33 | Shyam tulsi | *Ocimum tenuiflorum* | Puralia, WB (India) |
| G34 | Hybrid | *O. basilicum* × *O. kilimandscharicum* | CSIR-CIMAP, Lucknow, UP (India) |
| G35 | Tree/van basil | *Ocimum gratissimum* | Jammu, J&K (India) |
| G36 | Indian basil | *Ocimum basilicum* | Phagwara, Punjab (India) |
| G37 | Shyam tulsi | *Ocimum tenuiflorum* | Barabanki, UP (India) |
| G38 | Clove basil | *Ocimum gratissimum* | Shillong, Meghalaya (India) |
| G39 | Indian basil | *Ocimum basilicum* | Razaganj, UP (India) |
| G40 | Indian basil | *Ocimum basilicum* | Rishikesh, Uttaranchal (India) |

**Table 11.3** BST trial of methyl chavicol type *Ocimum* (entries 13, design RBD, replications 3, plot size: 12.25 $m^2$)

| Entries | Herb yield/ plot (kg) | Oil contents (%) | Oil yield (g/plot) | Methyl chavicol (%) | Linalool content (%) |
|---|---|---|---|---|---|
| EOH-1 | 53.08 | 1.20 | 465.00 | 89.75 | 1.01 |
| EOH-2 | 38.59 | 0.63 | 240.90 | 81.95 | 0.735 |
| EOH-3 | 33.50 | 0.39 | 130.63 | 39.32 | 41.12 |
| EOH-4 | 36.17 | 0.30 | 108.51 | 55.31 | 28.80 |
| EOH-5 | 40.59 | 0.31 | 125.84 | 46.89 | 27.27 |
| EOH-6 | 28.18 | 0.35 | 98.98 | 61.97 | 19.63 |
| EOH-7 | 32.60 | 0.43 | 138.65 | 34.12 | 35.23 |
| EOH-8 | 37.85 | 0.43 | 160.95 | 67.89 | 4.44 |
| EOH-9 | 31.29 | 0.50 | 156.45 | 60.83 | 28.54 |
| EOH-10 | 35.77 | 0.65 | 232.09 | 51.87 | 23.91 |
| EOH-11 | 31.00 | 0.68 | 209.00 | 68.30 | 25.45 |
| EOH-12 | 35.00 | 0.67 | 232.83 | 17.78 | 43.50 |
| CIM-Saumya | 32.25 | 0.63 | 201.75 | 73.36 | 19.93 |
| Mean ($\bar{x}$) | 35.80 | 0.52 | 192.43 | – | – |
| Range | 28.18–53.08 | 0.30–0.88 | 98.98–465.00 | – | – |
| CD $_{(5\%)}$ | 2.80 | 0.069 | 38.29 | – | – |
| CD $_{(1\%)}$ | 3.92 | 0.097 | 53.66 | – | – |
| *F* value | 46.81** | 59.89** | 59.02** | – | – |

*BST* Bench Scale Trial, *RBD* Randomized Block Design
**F test indicating analyzing of variance at significant level

**Table 11.4** BST trial of citral type *Ocimum* (entries 12, design RBD, replications 3, plot size: 12.50 $m^2$)

| Entries | Herb yield/plot (kg) | Oil content (%) | Oil yield (g/plot) | Neral (%) 1 | Geranial (%) 2 |
|---|---|---|---|---|---|
| OC-1 | 34.17 | 0.54 | 183.67 | 56.39 | 28.37 |
| OC-2 | 23.17 | 0.32 | 70.00 | 30.03 | 46.59 |
| OC-3 | 20.27 | 0.36 | 73.33 | 25.84 | 36.22 |
| OC-4 | 26.10 | 0.28 | 80.33 | 26.42 | 35.84 |
| OC-5 | 25.67 | 0.40 | 102.67 | 18.47 | 31.45 |
| OC-6 | 15.97 | 0.30 | 48.00 | – | – |
| OC-7 | 25.73 | 0.39 | 100.67 | – | – |
| OC-8 | 17.93 | 0.33 | 59.00 | – | – |
| OC-9 | 24.27 | 0.29 | 69.33 | – | – |
| OC-10 | 15.83 | 0.30 | 49.00 | – | – |
| OC-11 | 18.40 | 0.33 | 60.00 | – | – |
| Local OC (check) | 20.28 | 0.32 | 64.00 | 18.47 | 37.75 |
| Mean ($\bar{x}$) | 22.23 | 0.35 | 80.00 | – | – |
| Range | 15.83–34.17 | 0.28–0.54 | 49.00–183.67 | – | – |
| CD $_{5\%}$ | 3.36 | 0.053 | 18.53 | – | – |
| CD $1_{\%}$ | 4.56 | 0.071 | 25.18 | – | – |
| *F* value | 21.28** | 15.77** | 34.23** | – | – |

**F test indicating analyzing of variance at significant level

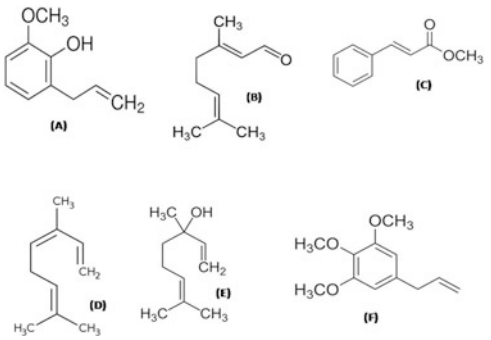


**Fig. 11.3** The major chemical constituents in the genus *Ocimum*: (**a**) Eugenol, (**b**) Citral, (**c**) Methyl chavicol, (**d**) β-Ocimene, (**e**) Linalool, (**f**) Elemicin

## 11.4 Conclusion

In recent few decades, there has been a resurgence of interest in investigating the health-promoting uses of *Ocimum* species across the globe. The nutritional and pharmacological properties of *O. tenuiflorum*, *O. kilimandscharicum*, *O. africanum*, *O. gratissimum* and *O. basilicum*, including several varieties, viz. CIM-Ayu, CIM-Angana, CIM-Kanchan, CIM-Saumya, CIM-Surabhi, Kushmohak

and Vikarsudha in its natural form, have been traditionally used since ancient times, and have provided a new platform for researchers for synergistic interactions of several different active phytochemicals. However, because of its inherent genotypic variations and biochemical complexity, *Ocimum* standardization has eluded modern science. *Ocimum* ingredients are general vitalizers that increase physical endurance in humans, the reason being it contains no caffeine or other stimulant. Chemical characterization of two new species of *Ocimum* was observed in this study. In *O. basilicum* and *O. africanum* population, EOH-1 and OC-1, respectively, obtained maximum essential oil yield. *Ocimum* is traditionally used as a cure-all in many parts of the world, including India. The essential oil compositions of available species of the genus is very much helpful in the pharmaceutical industry and in formulation of drug principles. Preclinical studies in animal models have demonstrated several therapeutic applications of *Ocimum* and recognized them as useful active constituents having anti-diabetic, wound healing, anti-oxidant, anti-microbial, gastroprotective, immunomodulatory, anti-inflammatory, anti-cancerous and several other biological properties, which provide leads for industries in the development of new medicines and drug formulations.

**Acknowledgements** The authors are thankful to the CSIR's Directors, Central Institute of Medicinal and Aromatic Plants, Lucknow and Indian Institute of Integrative Medicine, Jammu for help and extension of these aromatic crops across India under various projects like Aroma Mission.

**Conflict of Interest** The authors declare no conflict of interest.

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# Chemical Constituents and Pharmacological Activities of *Marrubium vulgare* L., an Important Medicinal Herb

12

Shabir A. Dar, Anil Bhushan, and Prasoon Gupta

**Abstract**

*Marrubium vulgare* L. (Lamiaceae), popularly called pahari gandana (Hindi) and truppad (Kashmiri), is a herb indigenous to Asia, Europe, and the Mediterranean region. In India, this species is mainly found in Kashmir at an altitude of 1524–2438 meters. In traditional medicine, *M. vulgare* is used in Europe, Tunisia, Brazil, and Pakistan to cure ailments associated with respiration such as asthma and cough. The phytochemical investigation showed the availability of flavonoids, phenylpropanoid esters, steroids, tannins, saponins, and terpenoids as major metabolites. Its aerial parts mainly contain marrubiin, a furan labdane diterpenoid, considered an important marker compound of *Marrubium* genus. Pharmacological studies have shown that *M. vulgare* exhibits antispasmodic, antinociceptive, antihypertensive, antidiabetic, gastroprotective, antioxidant, anti-inflammatory, and hepatoprotective properties. The present chapter summarizes all the scientific researches so far being done on the plant to make an attempt to unveil its secondary metabolites so that their therapeutic properties could be assessed.

**Keywords**

Angiosperms · *Marrubium vulgare* · Marrubiin · Flavonoids · Phenylpropanoid · Antidiabetic

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_12

## 12.1 Introduction

Plants have been a very important part of human civilization since the ancient times when man was learning to survive. Humans utilized plants and plant products to fulfill all the most important necessities for their nourishment, and their existence is extremely reliant on primary producers, mostly plants. Near about 5000 plant species have been widely utilized by humans as food. Besides fulfilling primary needs, the plant species have been utilized as medicines for various human illnesses (Sullivan and Shealy 1997). Such plants are called traditional medicinal plants and have played the most important roles in our ancient system of medicine. Those plants have been provided hypothesis and become primary source of modern drug discovery by the pharmaceutical industry. The World Health Organization has prepared a list of over 21,000 species of plants that are in use as traditional medicines around the world, with more than 80% of the world's population believing on plant-based medicines for their primary health care (Gurib-Fakim 2006).

Among a total of 21,000 medicinal plant species, the family Lamiaceae (also mint family) is known as the biggest family explored for the discovery of bioactive secondary metabolites (Wink 2003). This family is represented by 7200 species belonging to 236 genera (Brauchler et al. 2010). Many species belonging to this family are greatly aromatic and mainly produce oil that evaporates in low temperature due to the existence of peripheral glandular structures (Giuliani and Bini 2008). Among them, the genus *Marrubium* L. has attracted most of the researchers around the world, due to the presence of active chemical and pharmacological properties. This genus normally consists of approximately 40 species. Among these, *Marrubium vulgare* L. is a perennial herb found along road sides, and in waste areas.

## 12.2 Methodology

Data related to *Marrubium vulgare* were collected from published articles using Google Scholar, SciFinder, PubMed, and Science Direct. Books and journals available in library were also consulted while preparing this manuscript. More than 160 articles were studied of which 105 important references are included in this chapter. The present chapter is formulated in such a way so that it becomes easy for researchers to get up to date information on this particular plant, whether related to its phytochemistry or pharmacology.

## 12.3 Morphological Description

*Marrrubium vulgare* (Fig. 12.1) height varies from 25 to 45 cm. Leaves of the plants vary from 2.0 to 5.0 cm in length along with very dense surface, mostly sheltered in downy hairs. They also possess blunt and rounded tips. The stems mostly contain branched and woody base. These woody stems are quadrangular and very tightly

**Fig. 12.1** Habit and morphology of *Marrubium vulgare* (left side: habit of whole plant; middle: root parts used as medicine; right side: leaves)

covered with hairs. Fruits or nutlets are enclosed by calyx. Seeds are brown or black and vary from 1 to 2.5 mm in length (Halvorson 2003).

## 12.4 Traditional Uses of *Marrubium vulgare*

*Marrubium vulgare* is utilized as traditional medicine to treat different types of ailments. Conventionally, the leaves of the plants have been used to get relief from joint pain, chest infection, inflammation, inflamed eyes, cough and cold, and night-time blindness. This plant is also used as a purgative agent and widely used as a bitter tonic and appetizer, and is also helpful in expulsion of fetus (Kirtikar and Basu 1996). It is also used as a herbal tea due to its stimulating and antispasmodic properties (Yamaguchi et al. 2006). It is also used in curing head-pain, and the chemicals in it contain diterpene labdane and marrubiin (Piccoli and Bottini 2008; Karioti et al. 2003). It is also used to treat liver problems and flu (Balme 1982; Grieve 1984; Chevallier 1996; Lorenzi and Matos 2002; Sahpaz et al. 2002a). The leaves and stems are used as antiseptic, cholagogue, antispasmodic, and as stimulant (Grieve 1984; Chiej 1984; Launert 1981; Lust 1983; Mills 1985; Bown 1995). It is often made into a syrup or candy, though it can be used as a tea (Grieve 1984). Due to bitter nature, this plant species is also helpful in digestive functioning of the stomach (Bown 1995; Chevallier 1996). Traditionally, this herb has been considered helpful for the treatment of persistent fever and cholera (Anonymous 2005). *M. vulgare* extracts are also used as flavoring agents in food industries in the USA (Bradley 1992; Vincenzi et al. 1995). The volatile oil produced by *M. vulgare* has a folk status for its use in calming nervous heart. The tiny amount of marrubiin, a labdane diterpene, has the potential to normalize heartbeats. The hot water-extract is used as sweat-inducer, whereas the cold infusion of bitter taste is used as tonic for digestion. The extract of *M. vulgare* is also useful in curing malaria and reducing fever (McIntyre et al. 1988).

The dry flowering stem is used in treating menstrual irregularities and pain. It is also useful in treating sore wounds. The presence of highly volatile constituents makes it the best stimulant and antihelmintic, as well as useful in amenorrhea, chronic rheumatism, dyspepsia, and hepatitis (Singh and Panda 2005; Haq et al. 2011). *M. vulgare* extract also showed appetite stimulant effects through bitter receptors (Janssen et al. 2011). This plant species is also used by local people for candy in the province of United Kingdom. The Egyptians and Romans used plant extracts as the antidote for snake bites. The extract assists to destroy cankerworms when sprayed on fruiting trees. It is also used to ease heartburn, in digestion, and to raze worms in human intestine.

Traditionally, people slowly chewed fresh chopped leaves of *M. vulgare* along with honey to treat cold and sore throat. A candy formulation that contains fresh leaves (four ounces), crushed seeds of cardamom, one teaspoon anise seed, and 250 mL water is used as a standard care for cough in children aged between 5 and 12 years (Barrett 2009). In the Latin American country Brazil, the plant is employed in treating gastrointestinal disorders, inflammation, and respiratory ailments (Meyre Silva et al. 2005; Culpeper 2006). An infusion of leaves is given as an insecticidal (Benedum et al. 2006).

## 12.5 Phytochemistry

A large number of chemical studies have been carried out on this plant around the world and have confirmed the occurrence of different classes of molecules in *M. vulgare*, which belongs to diterpenes, sesquiterpenes, flavonoids, phenylpropanoid esters, and essential oils (EOs).

### 12.5.1 Diterpenoids

Various terpenoids (Fig. 12.2) are characterized from aerial parts of *Marrubium vulgare*. The first diterpenoid to be isolated is marrubiin, which was first isolated in 1842; since then, a number of research work has been published regarding isolation, structure elucidation, chemical reaction, stereochemistry, and synthesis of this bioactive compounds (Fulkke et al. 1968; Knoss et al. 1997).

In a separate research, the low-pressure-based chromatographic method was developed for the marrubiin isolation (Knoss et al. 1997). In the year 1992, Rey and co-workers established stereochemistry of marrubiin by X-ray crystallography (Rey et al. 1992). It has been reported that marrubiin has two rings. Some other diterpenoids like marrubenol, marrubiol, peregrinin, premarrubiin, marrubinone B, and vulgarol have also been isolated from aerial parts of *Marrubium vulgare* (El Bardai et al. 2003b; Popa et al. 1968; Sahpaz et al. 2002b; Masoodi et al. 2015; Henderson and McCrindle 1969; Popa and Pasechnik 1975). A novel compound, 11-oxomarrubiin, was identified from methanol extract (ME) of whole plant of *M. vulgare* (Shaheen et al. 2014). Additionally, two new labdane diterpenoids,

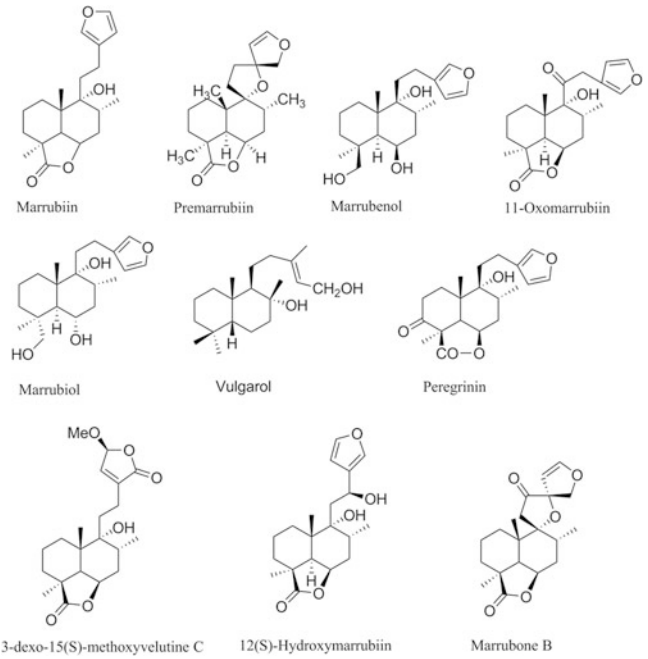


**Fig. 12.2** Diterpenoids of *Marrubium vulgare*

3-deoxo-15-methoxyvelutine C and 12(S)-hydroxymarrubiin, were isolated and identified from the extract of methanol from the collection from Srinagar, Jammu and Kashmir (Masoodi et al. 2015).

## 12.5.2 Flavonoids

Various flavonoids (Fig. 12.3) including aglycones and their glycosides were reported from different plant parts of *M. vulgare*. These include chrysoeriol, vitexin, isoquercitrin, quercetin 3-O-rhamnosyl-glucoside, apigenin 7-lactate, luteolin, apigenin and its 7-(6″-p-coumaroyl)- glucoside, apigenin-7-O-glucoside, luteolin-7-O-β-D-glucoside, and luteolin 7-lactate (Nawwar et al. 1989; Kowalewski and Matlawska 1978; Rahman 2005).

In the year 2014, a flavone derivative 3-hydroxyapigenin-4′-O-(6″-O-p-coumaroyl)-β-D-glucopyranoside was isolated from alcoholic extract of *M. vulgare* (Shaheen et al. 2014). Alkhatib et al. (2010) have reported Ladanein from dichloromethane extract of *M. vulgare*. In another study, a flavone 7-O-β-glucuronyl luteolin was reported for the first time in aerial part of *M. vulgare* along with some well-characterized compounds such as 5,6-dihydroxy-7,4′-dimethoxyflavone (ladanein) and 7-O-β-glucopyranosyl luteolin (Pukalskas et al. 2012).

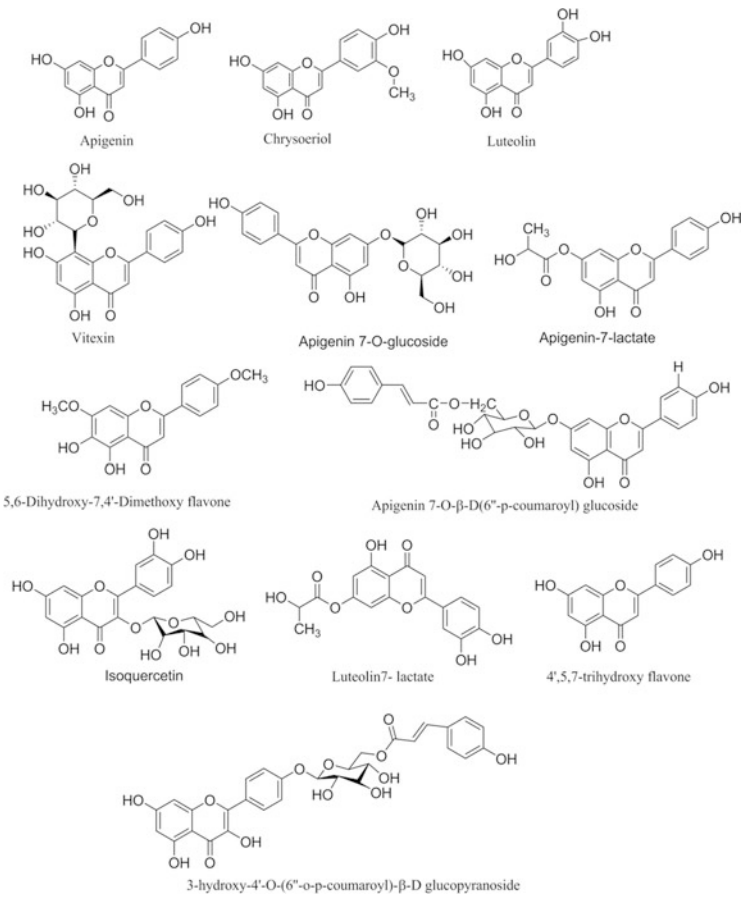


**Fig. 12.3** Flavonoids from *Marrubium vulgare*

### 12.5.3 Phenylpropanoid and Phenylethanoid Glycosides

Several phenylpropanoids (Fig. 12.4) like forsythoside B, (+) (E)-caffeoyl-L-malic acid, ballotetroside, acteoside, and arenarioside were isolated from flowering portion of this species (Sahpaz et al. 2002a).

Two compounds, verbascoside and forsythoside B, were isolated by Pukalskas et al. (2012) from the above-ground portion of this species using gradient solvent of methanol:water:acetic acid (79:20:1). Vulgarcoside A, a diglycoside derivative of the diterpene peregrinol, was isolated from this plant (Pukalskas et al. 2012). Sahpaz and co-workers have reported few phenylethanoid glycosides such as marruboside and acetyl marruboside (Sahpaz et al. 2002a).

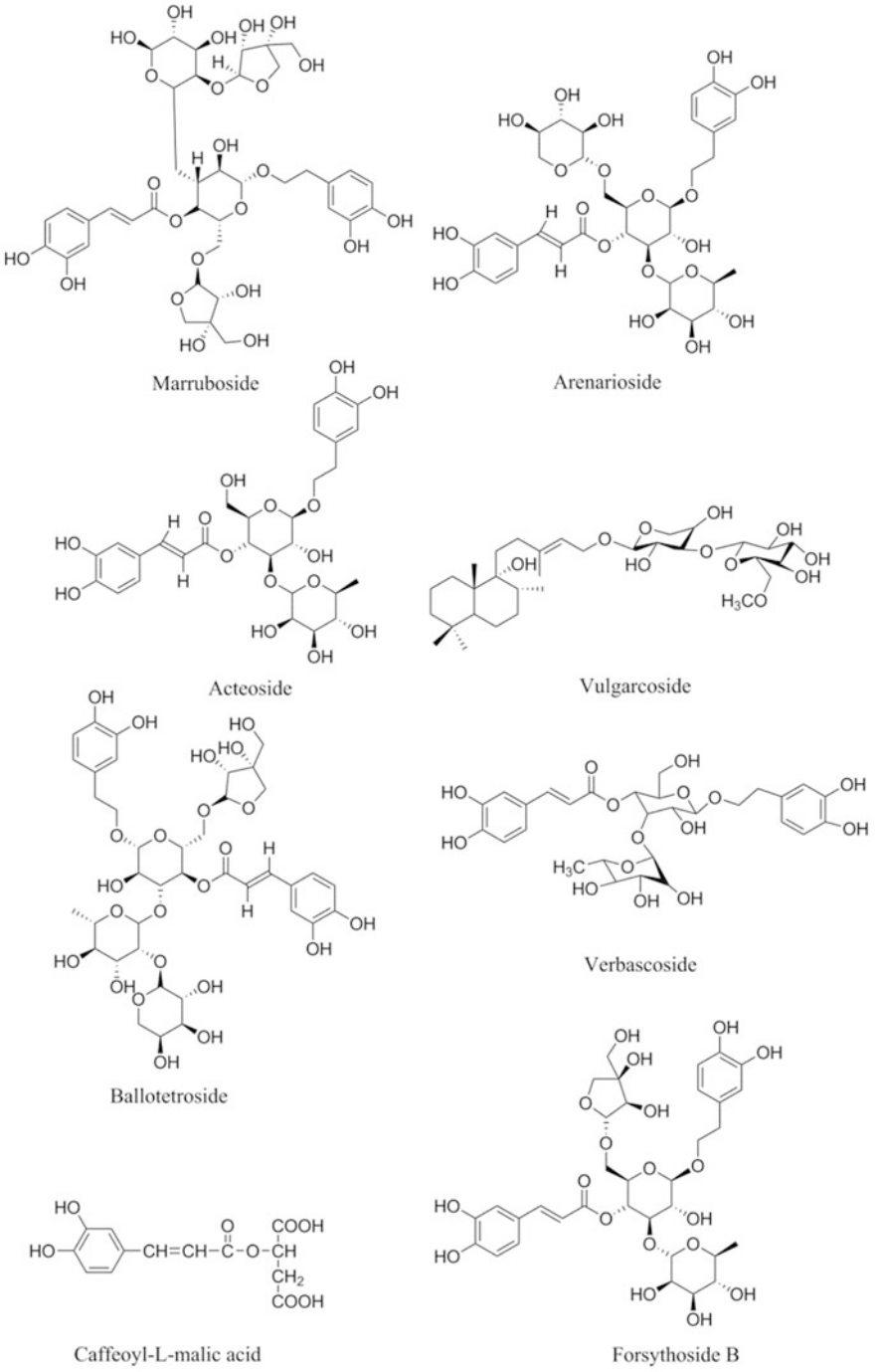


**Fig. 12.4** Phenylpropanoid and phenylethanoid glycosides isolated from *Marrubium vulgare*

### 12.5.4 Active Compounds in Essential Oil of *Marrubium vulgare*

To date, several essential oils present in *M. vulgare* have been studied by different workers across the globe. They include monoterpenes, sesquiterpenes, and esters.

*Monoterpenes*: Monoterpenes (Fig. 12.5) isolated from *Marrubium vulgare* includes: pinene, tricyclene, thymol, carvacrol, α-pinene, piperitone, 1,8-cineole, sabinene, limonene, p-cymene, α-terpinolene, camphene, p-fenchene, geranial, a-thujone, citronellyl acetate, β-citronellol, and p-menthane-5,6-dihydroxy-3-carboxylic acid, commonly known as marrubic acid (Saleh and Glombitza 1989, Ahmad et al. 2010, Salama et al. 2012, Abadi and Hassani 2013, Zawislak 2012; Kadri et al. 2011).

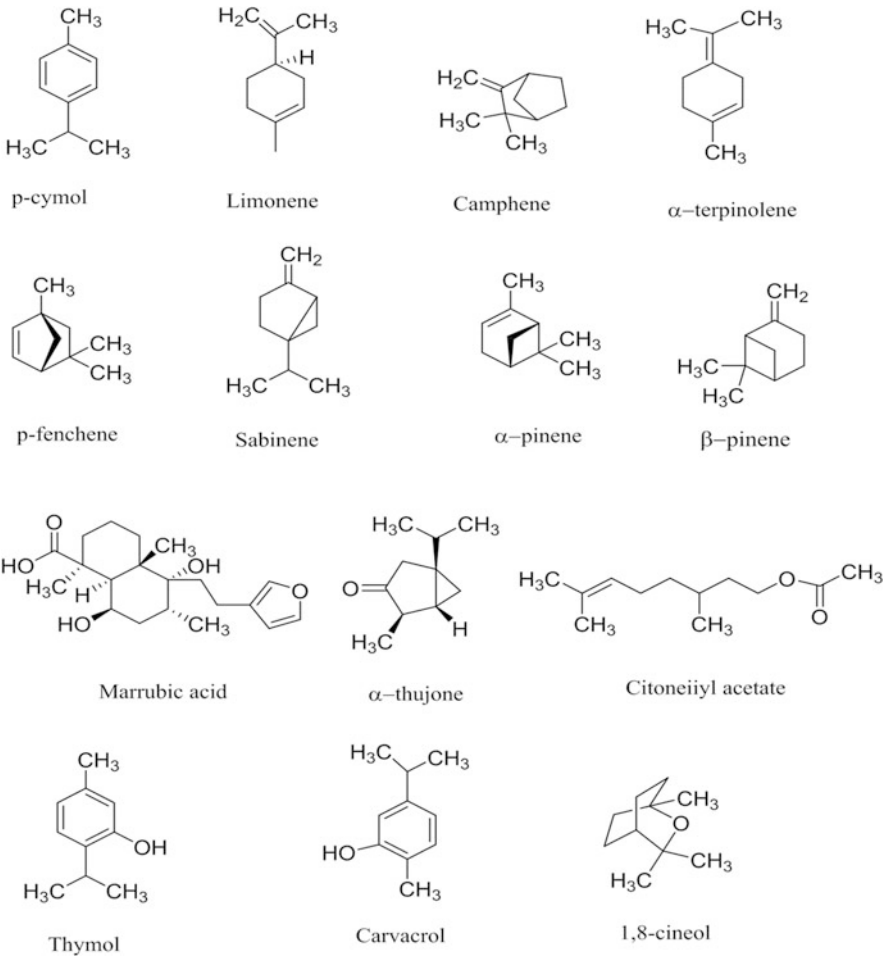


**Fig. 12.5** Active constituents under the class monoterpenes of *Marrubium vulgare*

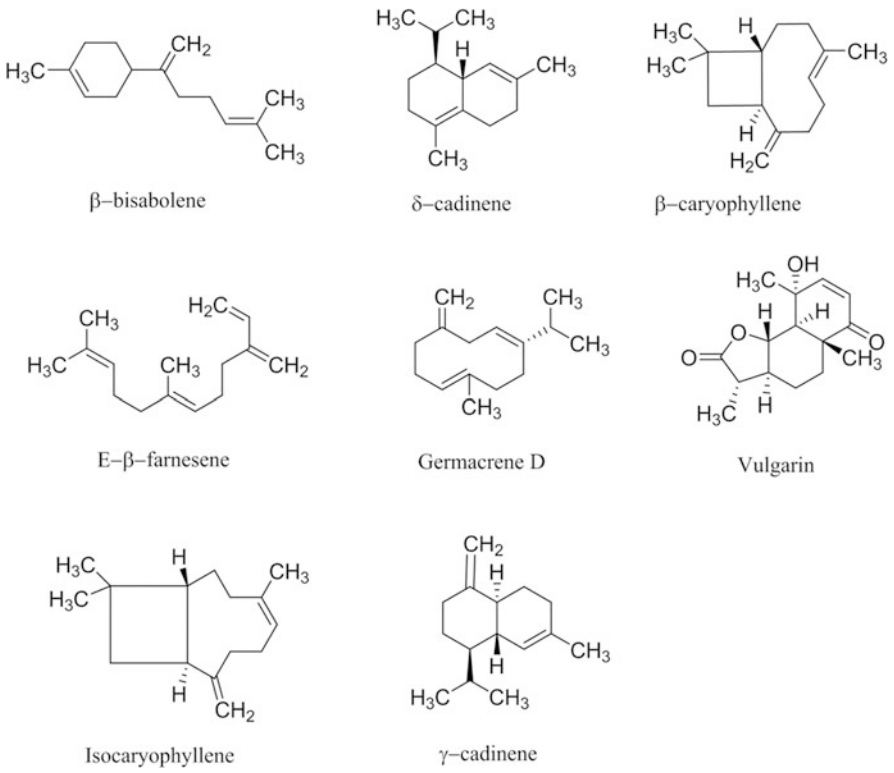


**Fig. 12.6** Sesquiterpene in essential oil of *Marrubium vulgare*

*Sesquiterpene*: From *Marrubium vulgare*, following sesquiterpenes (Fig. 12.6) have been isolated: β-bisabolene, δ-cadinene, isocaryophyllene, γ-cadinene, E-β-farnesene, β-caryophyllene, germacrene D-4-ol, Germacrene D, Vulgarin, γ-eudesmol, ledene, and transcaryophyllene (Salama et al. 2012; Zawislak 2012; Kadri et al. 2011; Al Ahl et al. 2015; Amer 1993).

## 12.5.5 Other Chemical Constituents

Besides pentacyclic triterpene ursolic acid, two steroids including β-sitosterol and stigmasterol, and phenolic acids such as gallic and caffeic acids, have also been isolated from this species (Nawwar et al. 1989; Laonigro et al. 1979). Small amounts of alkaloids, betonicine and turicine have also been isolated in separate studies (Baxter et al. 1998; Daniel 2006). Other compounds such as 2-(omega-1)-dimethylalkanes, 3-methylalkanes, 2-methylalkanes, and 3-(omega-9)-dimethylalkanes have been reported from the above-ground parts of the plant (Meyresilva and Cechinelfilho 2010).

## 12.6 Pharmacological Activities

### 12.6.1 Anti-inflammatory Activity

Genus *Marrubium vulgare* is regularly found in European and Mediterranean countries, hence several researches took place in these regions. In folklore, inflammation and neuro-sedative disorders are treated. The hydro-alcoholic extract (HAE) and methanol extract (ME) were studied separately, which exhibited significant anti-inflammatory activities. The ME at a dosage of 200 mg/kg proved anti-inflammatory function against prostaglandin $E_2$ and carrageenan-induced inflammation in Swiss mice (Kanyonga et al. 2011). The HAE of *M. vulgare* showed both the in vivo and in vitro anti-inflammatory properties (Abbouyi et al. 2013). The study was assessed by investigating its effect on $O_2$ consumption and $O_2$ generation by rat pleural polymorphonuclear leukocytes (PMNs) stimulated with $O_2$. Five major phenylpropanoid esters, (+) (E)-caffeoyl L-malicacid, acteoside, arenarioside, forsythoside B, and ballotetroside, were isolated, characterized, and their anti-inflammatory activity was studied by Sahpaz et al. (2002a). In another research, ME was assessed on isoproterenol (100 mg/kg/day) induced myocardial infarction (MI) in rat model (Yousefi et al. 2014). It was observed that extract administered orally with dosages of 10, 20, and 40 mg/kg/12 h significantly reduces levels of tumor necrosis factor-α (TNF-α) and peripheral neutrophil count. It was also revealed that the anti-inflammatory activity might be attributed to the presence of marrubiin and other glycosidic phenyl propanoid esters that were isolated from methanolic extract.

### 12.6.2 Analgesic and Antinociceptive Activities

*Marrubium vulgare* being utilized in traditional medicine to cure a number of diseases, its hydro-alcoholic extract (aerial parts) showed significant analgesic activity (deSouza et al. 1998). The study clearly proves the analgesic strength with inhibitory dose 50% ($ID_{50}$) at 22.2 and 272.2 mg/kg for the i.p. and p.o., respectively. It was also discovered that these effects may be due to steroids and terpenes. Meyre-Silva et al. (2005) also recorded that *M. vulgare* had shown strong analgesic properties, which was accredited to marrubiin, a furan labdane-type diterpene. Marrubiinic acid, which was synthesized from marrubiin, and two of its ester derivatives, marrubiinic acid benzyl and methyl esters, showed antinociceptive activity to acetic acid-induced abdominal writhing in mice at doses of 10 mg/kg i.p. and 50 mg/kg orally. Marrubiin was also described to have antinociceptive effect in nociception mice models by DeJesus et al. (2000). In this study, marrubiin was shown to have exhibited dose-dependent antinociceptive effects with 90–900 μmol/kg by p.o. route or 3–90 μmol/kg by i.p. route. The study was completed to assess the antinociceptive effects due to acetic acid-induced, formalin-induced, and capsaicin-induced pains. The results clearly showed that marrubiin is very effective in inhibiting writhing responses (acetic acid-induced) in mice with an $ID_{50}$ value of

2.2–90 μmol/kg, i.p. For formalin-induced responses, the $ID_{50}$ values for i.p. and oral routes were found to be 6.6 μmol/kg and 126 μmol/kg, respectively. For capsaicin-induced inflammation, the $ID_{50}$ value was found to be 28.8 μmol/kg. In 2011, Kanyonga et al. (2011) proved that ME at a dose of 200 mg/kg of *M. vulgare* exhibited the analgesic activity analogous to the acetylsalicylic acid.

### 12.6.3 Antiodematogenic Activity

Marrubiin being the main constituent of *Marrubium vulgare*, the researchers always try to discover its biological importance. In a study done by Stulzer and co-workers on mice model, marrubiin obtained from this species showed considerable and dose-dependent antiodematogenic effects on micro-vascular leakage in mice ears for different phlogistic agents (Stulzer et al. 2006). The percentage inhibition for histamine at 13.84 mg/kg was found to be 73.7%, for carrageenan at 13.61 mg/kg it was 63.0%, and for bradykinin it was 70% at 18.82 mg/kg. However, dextran yields slight inhibition of 32% as a phlogistic agent.

### 12.6.4 Antispasmodic Activity

Since *Marrubium vulgare* is used in folk medicine in numerous countries against several diseases, including gastrointestinal disorders, so to prove its biological feature research is being conducted in most of the countries where this species grows naturally. One such study was done in Brazil, where in vitro studies were conducted on aerial parts and roots of *M. vulgare* for antispasmodic effects on numerous smooth muscles, and it was observed that HAE (50% ethanol) exhibits antispasmodic properties in considerable range (Schlemper et al. 1996). Antispasmodic activity of plant extract may be due to the inhibition of neurotransmitters with a reasonable selectivity toward cholinergic contraction. A considerable difference was observed for bradykinin on guinea-pig ileum at 1 mg/mL of extract.

### 12.6.5 Gastroprotective Activity

In Brazil, *M. vulgare* is used traditionally for the management of respiratory and gastrointestinal infections; so on scientific basis, Paula de Oliveira et al. (2011) evaluated a diterpene marrubiin isolated and characterized from ME of this species for gastroprotective activity. It was observed that ME produced a considerable ulcer-protective effect on alcohol-induced mice model at a dose of 50 and 100 mg/kg, and the result was equivalent to the standard drug omeprazole at 30 mg/kg. In case of indomethacin-induced ulcers, methanolic extract exhibited potential activity at a dose of 50 mg/kg and cimetidine showed similar efficacy at a dose of 100 mg/kg. In both ulcer models, marrubiin (at 25 mg/kg) has shown considerable reduction in gastric parameters.

### 12.6.6 Antihypertensive Properties

Water extract of *M. vulgare* is extensively used as antihypertensive drug in folk medicine in Morocco. In order to establish its scientific basis, aqueous extract of *M. vulgare* was evaluated in normotensive Wistar-Kyoto rats/spontaneously hypertensive rats (SHRs) (El Bardai et al. 2001). Oral administration of aqueous extract considerably lowered the observed systolic blood pressure (SBP) in SHRs and inhibited the noradrenaline and potassium chloride (100 mM) induced contractile responses of rat aorta in an in vitro study. These results clearly indicate that hypotensive activity exhibited vascular relaxant activity. Vasorelaxant activities of two secondary metabolites marrubenol and marrubiin were reported in a study from aqueous extract and its cyclohexane fraction (El Bardai et al. 2003a). Marrubenol and marrubiin have inhibited contraction in dose-dependent manner in rat aorta. Marrubenol (inhibitory concentration 50% [$IC_{50}$] values 7.7 ± 1.9 μM) was found somewhat more effective than marrubiin ($IC_{50}$ values 24 ± 2.3 μM). In Mexico, for ethnomedical practices, *M. vulgare* is used as an antihypertensive drug (Jorge et al. 2013).

### 12.6.7 Antidiabetic and Antihyperlipidemic Activities

Knowing that *Marrubium vulgare* is traditionally used for management of diabetes, a scientific study was conducted to investigate the hypoglycemic effect on alloxan-induced diabetic rats, and results clearly demonstrated that the ethanol extract (EE) of aerial parts of *M. vulgare* showed hypoglycemic effect at a dose of 300 mg/kg with percentage inhibition of 30.3% (Novaes et al. 2001). A similar study was done on alloxan albino Wistar rats for the evaluation of antidiabetic and antihyperlipidemic effects of aqueous extract of *M. vulgare* (Boudjelal et al. 2012). A 50% reduced blood glucose level was observed at a dose of 100 mg/kg, and at 200 and 300 mg/kg, >60% reduction in blood glucose level was observed. In addition, a considerable lowering in total cholesterol, triglyceride, and lipid levels was also observed due to the same extract. These results were found comparable to the market drug glibenclamide.

In the year 2015, a study was conducted in streptozotocin-induced diabetic model to evaluate antidiabetic and antidyslipidemic effects of *M. vulgare* (Elberry et al. 2015). It was observed that methanolic extract of *M. vulgare* considerably lowered the glucose level in blood after treatment for 14 days at a single dose of 500 mg/kg/day. Furthermore, methanolic extract also showed a significant increase in tissue glycone and plasma insulin. A rare fatty acid, 6-octadecynoic acid (6-ODA) was identified in methanol extract of *M. vulgare* and reported to function as PPARγ α agonist (Ohtera et al. 2013). In another study, effectiveness of plant extract of *M. vulgare* was investigated in cyclosporine A and streptozotocin-induced diabetes mellitus-type 1 in mice (Maraia 2014). Thus, this species was recommended as useful in curing auto-immune diabetes.

### 12.6.8 Antihepatotoxic Activity

Horehound is used in herbal medicine of Saudi Arabia for the treatment of gastroenteric, respiratory, and inflammatory disorders. In Saudi Arabia, a study was performed to investigate the antihepatotoxic activity of methanolic extract against carbon tetrachloride ($CCl_4$) induced hepatic damage in rats (Elberry et al. 2010). The observation exposed that methanolic extract (500 mg/kg/day) significantly reduced the levels of lactate dehydrogenase (LDH), aspartate transaminase (AST), and alanine transaminase (ALT), suggesting antihepatotoxic effect of *M. vulgare*. This effect may be attributed partially to the antioxidant activity of the extract. $CCl_4$-induced hepatotoxicity in albino mice was also studied by Ibrahim et al. (2014). The effects of aqueous, ethanol, and petroleum ether extracts from aerial parts of *M. vulgare* were also evaluated. The results showed that ethanol extract exhibited strong protection against the damage caused by carbon tetrachloride ($CCl_4$).

In another study, first time 12 active compounds isolated from *M. vulgare* were evaluated for their drug-likeness and hepatoprotective activity in a computer-based in-silico model (Verma et al. 2012). Among the 12 compounds, vulgarin exhibited significant antihepatotoxic activity against $CCl_4$-induced toxicity in Wistar rat model. Akther et al. (2013) also reported hepatoprotective activity of methanolic extract of whole plant of *M. vulgare* against paracetamol-induced toxicity in Wistar rats (Akther et al. 2013).

### 12.6.9 Antioxidant Activity

Majority of the Mexican people make teas from leaflets, while roots of some medicinal plants are used to treat various ailments including infections, arthritis, heart disorders, headaches, fever, asthma, and menstrual pain. So, in a study, over 30 medicinal plants were investigated for evaluation of antioxidants (VanderJagt et al. 2002). Among them, it was reported that aqueous extract of leaves of *M. vulgare* contained about 560 μmol/g Trolox equivalent/g dry weight. In another study methanol extract of leaves showed strong antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH; $IC_{50}$ = 35 μg/mL) radical scavenging (Chedia et al. 2014). Antioxidant activities of acetone extract (AE), deodorized acetone extract (DAE), and deodorized water extract (DWE) from leaves of *M. vulgare* were tested at 80 °C in rapeseed (*Brassica napus*) oil (Weel et al. 1999) and found that acetone extract was better as compared with water extract.

Essential oils (EOs) are considered to be naturally occurring antioxidants, so various researches were performed on the essential oils obtained from *M. vulgare*. In one such research done in Tunisia, it was reported that the antioxidant effects of aerial parts of *M. vulgare* were produced through their essential oils probably due to the hydroxylated and oxygen-containing compounds (Kadri et al. 2011). Based on the results obtained from DPPH, β-carotene bleaching test, and reducing power assay, it was concluded that EOs obtained from *M. vulgare* can be used as a natural

food preservative and to improve human health as a natural antioxidant. Comparing the antioxidant activity with synthetic butylated hydroxyanisole (BHT), it was revealed that EOs exhibited an $IC_{50}$ value of 153.84 μg/mL, which was two times greater than BHT (Abadi and Abdellatif 2013). In a study, the antioxidant activities of five compounds (5–6-dihydroxy,7–4′-dimethoxy flavone, 7-O-β-glucopyranosyl luteolin, 7-O-β-glucuronyl luteolin, verbascoside, and forsythoside B) isolated from aerial parts of *M. vulgare* were examined using DPPH and $ABTS^+$ free radical scavenging assays, and compared with rosmarinic acid (Pukalskas et al. 2012). The effects of forsythoside B and verbascoside were found comparable to that of natural rosmarinic acid in $ABTS^+$ assay. Among the fractions, the *n*-butanol fraction from which four compounds, except 5–6-dihydroxy,7–4′-dimethoxy flavone, were isolated exhibited highest antioxidant activity in the β-carotene bleaching assay and linolenic acid model. There was another study in which antioxidant capacity was again found in methanol leaf extract of *Marrubium vulgare* (Amessis-Ouchemoukh et al. 2014). In this study, antioxidant capacities of methanol and acetone extracts were evaluated using DPPH, $H_2O_2$, total antioxidant capacity, and iron-reducing power assays. The percentage of DPPH radical scavenging activity of methanol extract in the range of 51.90–97.15% suggests its importance as a natural preservative and in the prevention of oxidative stress-related disorders.

### 12.6.10 Antimicrobial Activity

Various studies have been done for the evaluation of antimicrobial activities of *Maruubium vulgare*, the most widely used herb as medicine in Arab counties. Ethanol extracts (EEs) from leaves and flowers of *M. vulgare* were investigated for their antimicrobial potential using rapid colorimetry (XTT) and viable count method (Al-Bakri and Afifi 2007). The promising antimicrobial activity shown by EE of *M. vulgare* was against *Bacillus subtilis* and *Staphylococcus aureus*, while against *Escherichia coli* and *Pseudomonas aeruginosa* it was very weak. Promising activity was also exhibited against *S. aureus*, *Staphylococcus epidermidis*, and *B. subtilis* when methanolic extract of *M. vulgare* whole plant was evaluated for its antimicrobial activity (Masoodi et al. 2008). With regard to other two strains, that is, *P. vulgaris* and *E. coli*, there was a moderate effect. In this study, six bacterial organisms were used and ciprofloxacin was used as a standard drug. In another study, the *M. vulgare* roots were extracted with ethanol and inhibition assay of biofilm formation was done in methicillin-resistant *S. aureus* (MRSA; Quave and Smeltzer 2009). The minimum inhibitory concentration (MIC) was determined after 18 h growth using broth microtiter dilution method. A significant inhibition of biofilm formation ($IC_{50}$ = 32 μg/mL) and adherence ($IC_{50}$ = 8 μg/mL) was established in extract of roots at dose-dependent manner. A remarkable antimicrobial activity against *Staphylococcus aureus* and *S. epidermidis* was also shown by EE of *M. vulgare* and that study was done in Turkey in which 22 plant species were investigated (Kunduhoglu et al. 2011). The study was done on ethanol, acetone, and ether extracts obtained from leaves, flowers, and stems of 22 plant species.

*Helicobacter pylori* bacterium is considered the major cause of infection in gastric disorders; a study was undertaken in which anti-*Helicobacter pylori* activity was investigated (Robles-Zepeda et al. 2011). In the study, methanol extract at a concentration of 10 mg/mL of *M. vulgare* was evaluated using broth micro-dilution method and MIC was observed at 800 μg/mL. In another study, essential oil of *M. vulgare* was evaluated for antimicrobial activity and MIC was determined. (Zarai et al. 2011). In yet another study, essential oil was investigated against series of bacterial and fungal strains by agar disk diffusion method. The results showed a considerable antimicrobial activity against Gram positive bacteria with MIC values in the range of 1.12–2.6 mg/mL by zone inhibition assay. However, Gram negative bacteria exhibited resistance against tested sample. MIC was found in the range of 12.5–25 mg/mL in a study where methanolic extract of *M. vulgare* was evaluated for antibacterial activity against seven pathogenic bacteria (Chedia et al. 2014). In this study, total phenolic content was also determined and it was concluded that antibacterial activity was due to high content of phenolic compounds in the extract. Tuberculosis, one of the serious health problems, has developed resistance to some of the antibiotics, which has enforced researchers to create or discover new drugs.

EOs are considered naturally occurring antioxidants and were evaluated for antibacterial activity against different Gram positive and Gram negative pathogens. In one study, EOs showed varied antibacterial activity against *Listeria monocytogenes*, *P. aeruginosa*, *Agrobacterium tumefaciens*, and *Salmonella enterica* (Abadi and Abdellatif 2013). By Mueller-Hinton broth dilution method, the MIC values were obtained, which were in the range of 0.1–15 μg/mL. In another study, the essential oils and ethanol extract prepared from the leaves of *M. vulgare* were studied for antimicrobial activity against 17 clinical strains of *Staphylococcus aureus* (Bokaeian et al. 2014). Minimum MIC value for the extract was found to be 2.5 mg/mL and maximum MIC value for essential oils was found to be 2.5 mg/mL. This study confirmed the use of essential oils and ethanol extract of *M. vulgare* as antibacterial agents.

### 12.6.11 Anticancer Activity

In recent years, the search for naturally occurring anticancer chemotherapeutic drugs continued at a great speed and research focus on *M. vulgare* in this field is no way behind. Yamaguchi and co-workers (2006) reported antiproliferative effect on colon cancer cells with leaf extract. In this study, methanol extracts upregulated gene (NAG-1) through a trans-activation of the NAG-1 promoter at a dose of 250 μg/mL. This was for the first time that a major compound ladanein isolated from *M. vulgare* was studied for its cytotoxic activity against drug dasatinib-resistant murine leukemia cell line (DA1-3b/M2$^{BCR\text{-}ABL}$). In the study, ladanein showed nonsignificant (20–40 μM) activity against series of cancer cell lines, including K562, K562R, and 697 human leukemia cell lines, but was found inactive to MOLM13 and human peripheral blood mononuclear cells (Alkhatib et al. 2010). In vitro cytotoxicity of *M. vulgare* against cancer cell lines was studied in Morocco,

Tunisia, and Dubai. In one of the studies, essential oils obtained from *M. vulgare* were examined using a modified MTT assay for their cytotoxic activity against HeLa cell lines (Zarai et al. 2011). The results depicted that essential oils destroyed HeLa cells by 27% in a concentration of 0.25 mg/mL and 100% cells died at >0.5 mg/mL concentration. A considerable cytotoxic effect with $IC_{50}$ at 258 mg/mL against tumor cells was also observed. In another study, alcoholic leaves extract and isolated phenolic compounds, viz., acacetin, apigenin, and its glycoside, were tested against U251 (Ehrlich tumor cell lines) and breast cancer line (MCF7). Alcoholic extract and its secondary metabolites showed significant anticancer activity against U251 with effective dose for 50% ($ED_{50}$) < 20 μg/mL whereas extract and acacetin showed moderate activity against MCF7 with $ED_{50}$ > 20 μg/mL (Nawal and Atta 2013). Different extracts including methanol, hexane, dichloromethane, and ethyl acetate obtained from aerial parts of *M. vulgare* were evaluated for cytotoxic activity against tumor cell lines (Belayachi et al. 2013). The results showed that dichloromethane extract of *M. vulgare* was effective against colorectal cancer cell lines (SW620 cells), acute T-cell leukemia, and mantle cell lymphoma cell line with $IC_{50}$ at 30 μg/mL.

### 12.6.12 Antiprotozoal, Molluscicidal, and Mosquitocidal Activities

*Marrubium vulgare* is also used for treating intestinal disorders. In two separate studies, *M. vulgare* was reported for antiprotozoal property. In one study, acetone and methanolic extracts of *M. vulgare* were found to have activity against *Entamoeba histolytica* and *Giardia lamblia* with growth inhibition $IC_{50} = 7$ at a dose of 12 μg/mL and $IC_{50} = 90$ at 34 μg/mL (Ramos-Guerra et al. 2007). Methanolic extracts of this plant species were reported to be effective against *Trypanosoma cruzi* with percentage growth inhibition between 88% and 100% at a concentration of 150 μg/mL (Molina-Garza et al. 2014).

Regarding molluscicidal and mosquitocidal activities, the volatile oils of *M. vulgare* were used to evaluate their activities on eggs of *Biomphalaria alexandrina* and *Culex pipiens* (Salama et al. 2012). The result clearly showed 100% ovicidal activity at 200 ppm/24 h. Another study stated that methanol extract of *M. vulgare* leaves are very effective against fourth instar larvae of the mosquito *C. pipiens* L. (Amel and Selima 2015).

## 12.7 Conclusion and Future Perspectives

The traditional uses, phytochemistry, and pharmacology of *Marrubium vulgare* are presented here in this chapter. Regarding chemical constituents, diterpenes, flavonoids, and phenylpropanoids are the major ones identified. Marrubiin is one of the major labdane diterpenes and exists in higher concentration. The pharmacological activities of *M. vulgare* proved its potential for the development of new efficacious botanicals in future. It is evident from the literature that mostly methanolic extracts of leaves and aerial parts have been studied in detail; however,

no substantial work has been carried out, so there is a need to work over other plant parts (root, stem, flowers, seeds) along with different extracts and their fractions. Though the plant contains different classes of secondary metabolites, but only marrubiin, marrubenol, and ladanein were well studied and hence other compounds need to be investigated. Clinical trials should be initiated for this plant, which is crucial for the diagnosis of herbal toxicity and development of plant-based new drugs.

**Acknowledgment** The authors SA Dar and A Bhusan are thankful for the financial aid received from Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Government of India (EMR/2016/002584), and CSIR LAB Project (MLP 4011).

**Conflict of Interest** All contributors of this manuscript declare that they have no publication-related conflict of interest.

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# 13 Ethnobotany as a Science of Preserving Traditional Knowledge: Traditional Uses of Wild Medicinal Plants from District Reasi, J&K (Northwestern Himalaya), India

Shiekh Marifatul Haq and Bikarma Singh

**Abstract**

Investigating traditional knowledge is a science of preserving age-old practices, and now new drugs and nutraceutical products are formulated based on traditional inventory. From 2017 to 2019, an ethnobotanical documentation of the plants growing in district Reasi (a part of Jammu Himalaya) was carried out to collect information regarding different usages of the plant species growing in the region through questionnaire and interviews. Floristically, a total of 90 species belonging to 80 genera and 48 families were investigated to be used as economic plants for medicine, food, herbal tea, fire and tanning purposes. The species distribution patterns across the families were unequal, with half of the species contributed by 12 families, 5 families with 2–3 members, and 31 families were monotypic. In terms of the functional trait diversity, herbaceous and perennial woody forms were dominant over the other forms. Out of 90 plant species, 10% were used as single usage, 27% double usage and 63% multi-usage. PAST software, a multivariate ecological community analysis software, was used to find the relationship between ethnobotanical usage and plant species. Four plant usages (clusters) were determined at a vertical distance value of 0.5, where the clusters are distinctly separated. The present study will provide a baseline data for the future researchers, policymakers, common public, land managers and other stakeholders to develop scientifically informed strategies for conservation of

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_13

natural resources and sustainable use of plant diversity in hotspot regions like Himalayas and other similar biodiversity-rich sites elsewhere in the world.

**Keywords**

Ethnobotany · Herbal medicine · Functional trait · Reasi district · Himalaya

## Abbreviations

| | |
|---|---|
| FD | Fodder |
| FW | Fuelwood |
| HT | Herbal Tea |
| MD | Medicinal |
| PO | Poisonous |
| RY | Resin Yield |
| TD | Tanning/Dying |
| TW | Timber wood |
| V/EF | Vegetable/Edible Fruit |

## 13.1 Introduction

Floristic investigation of a particular area refers to the study and knowledge of plants—their number, distribution and inter- and intra-specific relationships. The collection and complication of all plants of a particular area/region forms its flora, and the scientific process to inventory this task refers to the floristic study. For documentation purposes, floristic studies are an obligatory measure and a tool for conservation and sustainable utilization of plant diversity (Jayanthi and Rajendran 2013). Floristic diversity, whether wild or cultivated, of a region is a reflection of vegetation and plant resources (Panda et al. 2013). It is only by means of a floristic study that we can achieve the challenging goal of documentation of plant diversity and its conservation and sustainable use. The complication of floristic data are useful in future vegetation studies for reference (Qureshi et al. 2011).

Ethnobotanical information has immense importance in understanding the dynamic relationship existing between the flora of region and the socio-cultural system (Mahmood et al. 2011). Traditional knowledge associated with plants and their uses by local tribal indigenous people is always helpful in the conservation of biodiversity and traditional cultures associated with a particular tribe(s) (Ajaib et al. 2010; Singh et al. 2018a, b; Thakur et al. 2020). Since time immemorial, mankind has cultivated the habit of observing wild plants for thousands of years and had used them for different purposes. The ethnic tribal communities have adopted the utilization of local plants as the traditional means of healing their health care systems and are broadly used by all sections of the community, whether directly as folk remedies or applied as the modern medicine (Singh and Lal 2008; Mahmood et al. 2011). Tribal communities, especially in the developing countries, have always used the local plant diversity to meet their needs for food, medicine, fodder, fuelwood,

vegetable/edible fruit and a variety of other requirements (Pandey 2009). Globally, the wild plants have been always preferred by people due to the cheaper prices, easy availability and minimal side effects (Ekka and Dixit 2007; Jabeen et al. 2015).

North-western part of the Himalayan arc supports a huge number of floristic diversity (Sharma et al. 2010; Singh et al. 2018a; Thakur et al. 2019); however, these areas has been neglected for ethnobotanical information by ecologists and ethnobotanists till now or very less works published from these regions. Jammu Himalaya situated along the North-western boundary of the Western Himalayan biodiversity hotspot is known for its rich biodiversity. Keeping in view the role of the region in the conservation of biodiversity and its varied potential for ecosystem services, the present study was conducted with the specific objectives of quantifying the floristic diversity and ethnobotanical usage of plants. Hopefully, the present study can provide a baseline data for the future researchers, policymakers, common public, land managers and other stakeholders to develop scientifically informed strategies for conservation of natural resources and sustainable use of plant diversity in the Himalayan regions.

## 13.2 Study Area: Geography and Climate

Reasi district (33°4′58.1016″N, 74°49′59.9268″E) falls in the mid-hill zone of the Jammu and Kashmir (J&K) (Fig. 13.1). The district has subtropical, intermediate and temperate climates.

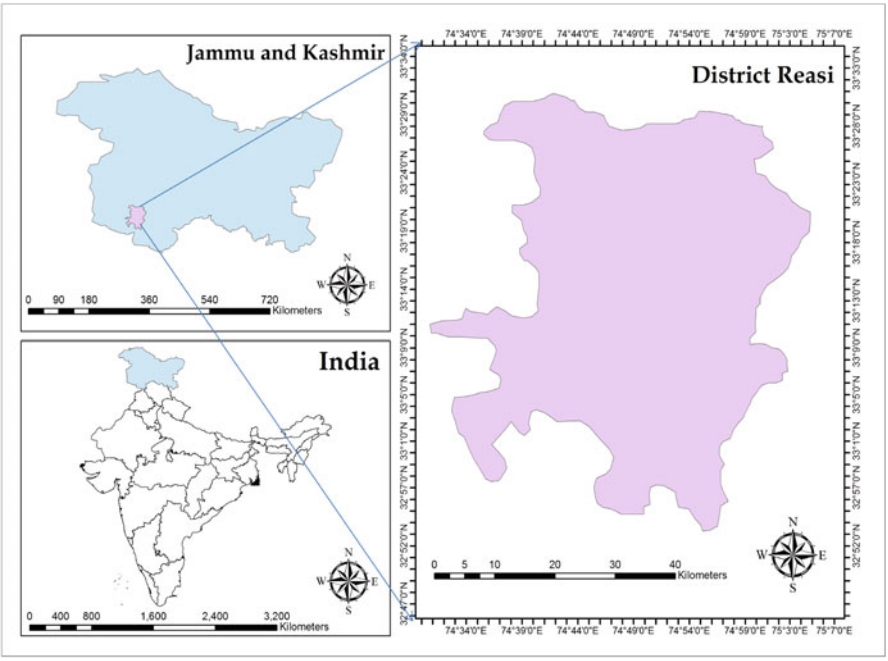


**Fig. 13.1** Location of district Reasi (J&K) in India

The lower hills of Katra, Panthal, Reasi, Pouni and Talwara fall in the subtropical belt. Higher intermediate zone comprises of Arnas, Kanthan, Thanpal, Judda, Dharmari and similar places. Buddan, Mahore, Chasana, Lar and Deval falls in the higher temperate zone. The mean maximum and minimum temperatures range between 35 to 40 °C and 10 to 12 °C, respectively. The annual rainfall of district averaging 1100 mm; rainfall is usually heavy and well distributed from May to September, July being the wettest period. Sometimes dry spell may prevail from December to March. This large variations in the agro-climatic conditions at the micro level is observed due to the mountainous terrains and high peaks in the district. Relative humidity varies from 35 to 90%.

The district Reasi as a whole is rich in flora and fauna, and has a tremendous potential for horticultural fruits, such as citrus, quince, apple, mango, guava and apricot, which are produced in different climatic conditions. The natural vegetation consists of trees, grasses and bushes. The principal coniferous and broad-leaved tree species are *Pinus roxburghii*, *Quercus oblongata*, *Syzygium cumini*, *Ficus palmata*, *Senegallia modesta* and *Albizia lebbeck*. Shrubs include *Lantana camara*, *Justicia adhatoda* and *Vitex negundo*, while herbs and grasses consists of *Ajuga integrifolia*, *Taraxacum officinale*, *Viola biflora*, *Fumaria indica*, *Amaranthus viridis*, *Parthenium hysterophorus*, *Heteropogon contortus*, *Phalaris minor*, *Themeda anathera* and *Saccharum spontaneum.*

## 13.3 Methodology: Survey, Collection and Data Investigation

The reconnaissance field surveys were carried out to get an understanding of the nature of terrain, species composition, accessibility and distribution of various forests of the study area. Forest working plan divisions were consulted for authenticating of the geographical location, administrative jurisdiction and forest vegetation types. The representative forest sites were visited in three trips from 2017 to 2019. During field studies, the detailed relevant data about the plant specimens were recorded. Specimens were collected from field and were dried, preserved, labelled and mounted on herbarium sheets by following standard herbarium techniques (Bridson and Forman 1999). Specimens were identified using the relevant taxonomic literature (Stewart 1972, efloras). The specialized taxonomic database 'The Plant List (www.theplantlist.org)' was used for the updated nomenclature of taxa.

To document the ethnobotanical information of the plant resources of the study area, the questionnaire and semi-interview method was used for collecting indigenous knowledge about the different plant species. The study area was visited on regularly basis and focused was to mainly collect wild plants having economic value. Ethnobotanical knowledge regarding plants was collected from diverse ethnic groups of the area, that is, Gujjars, herbal practitioners, shopkeepers, farmers and wood sellers, by interviewing and filling up the questionnaire. Informants were asked about their common uses of plant species, for example, as medicinal, food, fodder, timber, fuelwood, tanning/dying, vegetable/edible fruit, resin yield, herbal

tea and poisonous plants. Field-based personal findings also added more information to the research work. The respondents were further asked about their species preference if they utilized a species for multipurpose usages—medicinal, fodder, vegetable/edible fruit, fuelwood or timber purposes.

Statistically, the vegetation data was analysed to find out the relationship between ethnobotanical usage and plant species. The presence or absence of plant species data was subjected to classification of different ethnobotanical similarities and differences among the different plant usages via PCORD, Version 5 (McCune and Mefford 1999; Khan et al. 2015; Amjad et al. 2017). Sorensen's (Bray–Curtis) distance was used to identify significant differences among the different plants and ethnobotanical usage similarities (Sorensen 1948; Dalirsefat et al. 2009). The ethnobotanical contribution of different species of plants was developed by using R package software, Version 3.5.1 (R Core Team 2018).

## 13.4 Results

### 13.4.1 Vegetation Composition and Distribution of Plant Species

A total of 90 species belonging to 80 genera and 48 families (Table 13.1, Fig. 13.2) were recorded as plants used in the traditional system of medicine by the local people residing in district Reasi, J&K. The life-spans of plant species were represented by 27 (30.00%) species as annual, 3 (3.33%) species biennial and perennial 60 (66.67%) (Fig. 13.3). The perennial form was predominant over the other forms. Based upon plant habits, the flora of the region can be classified into herbs 48 (53.33%), trees 26 (28.88%), shrubs 11(12.22%) and climbers 5(5.57%) (Fig. 13.4).

### 13.4.2 Species–Family Relationship

The species distribution pattern across 48 families was unequal, with 12 families contributing half of the species, and the remaining were represented by 36 families; a large number of families (i.e. 31) were monotypic (Fig. 13.5).The floristic analysis revealed that the dominant plant families in the study area are Poaceae with 10 (11%) species, followed by Euphorbiaceae 7 (8%) species, Asteraceae 7 (7%) species, Moraceae 5 (6%) species, Fabaceae 4 species and Adiantaceae, Pteridaceae, Lamiaceae and Mimosaceae 3 species each. Amaranthaceae, Rhamnaceae, Meliaceae, Verbenaceae, Bombacaceae, Boraginaceae, Malvaceae and Myrtaceae were represented by 2 species each. The remaining other families such as Acanthaceae, Anacardiaceae, Apocynaceae, Asclepiadaceae, Cannabinaceae, Violaceae and Urticaceae were represented by one species each (Fig. 13.5).

**Table 13.1** Floristic, functional and ethnobotanical usages of flora in district Reasi in Jammu Himalaya, India

| Name of plant species | Family | Growth form | Lifespan | MD | FD | FW | TW | V/EF | PO | TD | RY | HT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Senegallia modesta* (Wall.) P.J.H.Hurter (= *Acacia modesta* Wall.) | Mimosaceae | Tree | Perennial | + | + | + | + | − | − | − | − | − |
| *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. (= *Acacia nilotica* (L.) Delile) | Mimosaceae | Tree | Perennial | + | + | + | + | − | − | − | − | − |
| *Achyranthes aspera* L. | Amaranthaceae | Herb | Perennial | + | + | + | + | − | − | − | − | − |
| *Adiantum capillus-veneris* L. | Pteridaceae | Herb | Perennial | + | + | − | − | − | − | − | − | − |
| *Adiantum raddianum* C.Presl | Pteridaceae | Herb | Perennial | + | + | − | − | − | − | − | − | − |
| *Adiantum venustum* D.Don | Pteridaceae | Herb | Perennial | + | + | − | − | − | − | − | − | − |
| *Ajuga bracteosa* Wall. | Lamiaceae | Herb | Perennial | + | + | − | − | − | − | − | − | + |
| *Albizia lebbek* (L.) Benth | Mimosaceae | Tree | Perennial | + | + | + | − | − | − | − | − | − |
| *Aloe vera* L. | Liliaceae | Herb | Perennial | + | + | − | − | − | − | − | + | + |
| *Amaranthus viridis* L. | Amaranthaceae | Herb | Annual | + | + | − | − | − | − | − | − | − |
| *Lysimachia arvensis* (L.) U.Manns & Anderb. (= *Anagallis arvensis* L.) | Primulaceae | Herb | Annual | + | + | − | − | − | − | − | − | − |
| *Bombax ceiba* L. | Bombacaceae | Tree | Perennial | + | + | + | + | − | − | − | + | − |
| *Urochloa distachya* (L.) T.Q.Nguyen (= *Brachiaria distachya* L.) | Poaceae | Herb | Annual | + | + | − | − | − | − | − | − | − |
| *Broussonetia papyrifera* (L.) L'Her. ex Vent. | Moraceae | Tree | Perennial | + | + | + | + | + | − | − | − | + |
| *Butea monosperma* (Lam.) Kuntze | Fabaceae | Tree | Perennial | + | + | + | + | − | − | + | + | − |
| *Calliandra surinamensis* Benth. | Lamiaceae | Shrub | Perennial | + | + | + | − | + | − | − | − | − |
| *Calotropis procera* (Aiton) W.T. Aiton | Asclepiadaceae | Shrub | Perennial | + | + | + | − | − | − | − | | − |
| *Cannabis sativa* L. | Cannabaceae | Herb | Annual | + | − | − | − | − | + | − | − | − |
| *Carissa spinarum* L. (= *Carissa opaca* Stapf. ex Haines) | Apocynaceae | Shrub | Perennial | + | + | + | − | − | − | − | + | − |
| *Dysphania ambrosioides* (L.) Mosyakin & Clemants. (= *Chenopodium ambrosioides* L.) | Chenopodiaceae | Herb | Annual | + | + | − | − | + | − | − | − | − |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Citrullus colocynthis* (L.) Schrad. | Cucurbitaceae | Climber | Annual | + | + | + | + | + | − | − | + | − |
| *Commelina benghalensis* L. | Commelinaceae | Herb | Annual | + | + | + | − | + | − | + | − | − |
| *Cordia myxa* L. | Boraginaceae | Tree | Perennial | + | + | + | + | + | − | − | + | − |
| *Cynodon dactylon* (L.) Pers. | Poaceae | Herb | Perennial | + | + | − | − | − | − | − | − | + |
| *Cynoglossum lanceolatum* Forrsk. | Boraginaceae | Herb | Biennial | + | + | − | − | − | − | − | − | − |
| *Cyperus rotundus* L. | Cyperaceae | Herb | Perennial | − | + | − | − | − | − | − | − | − |
| *Dalbergia sissoo* Roxb. ex DC. | Fabaceae | Tree | Perennial | + | + | + | + | − | − | − | − | − |
| *Datura innoxia* Mill. | Solanaceae | Herb | Perennial | + | + | + | − | − | + | − | − | − |
| *Dioscorea belophylla* (Prain) Voigt ex Haines | Dioscoreaceae | Climber | Perennial | + | + | + | − | + | − | − | − | − |
| *Dodonaea viscosa* Jacq. | Sapindaceae | Shrub | Perennial | + | + | + | + | − | − | − | − | − |
| *Dryopteris blanfordii* (C.Hope) C.Chr. | Dryopteridaceae | Herb | Perennial | − | + | − | − | − | − | − | − | − |
| *Erigeron bonariensis* L. | Asteraceae | Herb | Annual | + | + | − | − | − | − | − | − | − |
| *Erigeron canadensis* L. | Asteraceae | Herb | Annual | + | + | − | − | − | − | − | − | − |
| *Eucalyptus globulus* Labill. | Myrtaceae | Tree | Perennial | + | + | + | − | − | − | − | + | − |
| *Euphorbia helioscopia* L. | Euphorbiaceae | Herb | Biennial | + | − | − | − | − | + | − | − | − |
| *Euphorbia hirta* L. | Euphorbiaceae | Herb | Annual | + | − | − | − | − | + | − | − | − |
| *Euphorbia obovata* Decne. (= *Euphorbia prolifera* Buch. − Ham. ex D.Don) | Euphorbiaceae | Herb | Perennial | + | − | − | − | − | + | − | − | − |
| *Euphorbia chamaesyce* L. (= *Euphorbia prostrata* Ait.) | Euphorbiaceae | Herb | Annual | + | − | − | − | − | + | − | − | − |
| *Ficus carica* L. | Moraceae | Tree | Perennial | + | + | + | − | + | − | + | − | − |
| *Ficus palmata* Forssk. | Moraceae | Tree | Perennial | + | + | + | − | + | − | + | − | − |
| *Ficus virgata* Reinw. ex Blume. | Moraceae | Tree | Perennial | + | + | + | − | + | − | + | − | − |
| *Fumaria indica* (Hausskn.) Pugsley. | Fumariaceae | Herb | Annual | + | + | + | − | − | − | − | − | − |
| *Geranium rotundifolium* L. | Geraniaceae | Herb | Annual | + | − | − | − | − | − | − | − | − |
| *Grewia villosa* Willd. | Malvaceae | Tree | Perennial | + | + | + | + | − | − | − | − | − |
| *Gymnosporia royleana* Wall. ex M.A. Lawson | Celastraceae | Shrub | Perennial | + | + | + | − | − | − | − | + | − |

(continued)

**Table 13.1** (continued)

| Name of plant species | Family | Growth form | Lifespan | MD | FD | FW | TW | V/EF | PO | TD | RY | HT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Heteropogon contortus* (L.) P.Beauv. ex Roem. & Schult. | Poaceae | Herb | Perennial | + | + | + | − | − | − | − | − | − |
| *Hordeum vulgare* L. | Poaceae | Herb | Annual | + | + | + | − | − | − | − | − | − |
| *Imperata cylindrica* (L.) P.Beauv. | Poaceae | Herb | Perennial | + | + | + | − | − | − | − | − | − |
| *Ipomoea cairica* (L.) Sweet | Convolvulaceae | Climber | Perennial | + | − | − | − | − | − | − | − | − |
| *Justicia adhatoda* L. | Acanthaceae | Shrub | Perennial | + | + | − | − | − | − | − | − | − |
| *Lantana camara* L. | Verbenaceae | Shrub | Perennial | + | + | + | − | − | − | − | − | − |
| *Mallotus philippensis* (Lam.) Muell. − Arg. | Euphorbiaceae | Tree | Perennial | + | + | + | − | − | − | + | − | − |
| *Malva sylvestris* L. | Malvaceae | Herb | Perennial | + | + | − | − | − | − | − | − | − |
| *Malvastrum coromandelianum* L.Garcke. | Malvaceae | Herb | Annual | + | + | + | + | − | − | − | − | − |
| *Mangifera indica* L. | Anacardiaceae | Tree | Perennial | + | + | + | − | − | − | − | + | − |
| *Martynia annua* L. | Martyniaceae | Herb | Annual | + | + | + | − | − | − | − | − | + |
| *Medicago polymorpha* L. | Fabaceae | Herb | Annual | + | + | + | − | − | − | − | − | − |
| *Melia azedarach* L. | Meliaceae | Tree | Perennial | + | + | + | + | − | − | − | − | + |
| *Micromeria biflora* (Buch.-Ham. ex D.Don) Benth. | Lamiaceae | Herb | Perennial | + | + | − | − | − | − | − | − | − |
| *Morus alba* L. | Moraceae | Tree | Perennial | + | + | + | − | − | − | − | − | − |
| *Morus nigra* L. | Moraceae | Tree | Perennial | + | + | + | − | − | − | − | − | − |
| *Debia ovatifolia* (Cav.) Neupane & N.Wikstr. (= *Oldenlandia ovatifolia* L.) | Rubiaceae | Herb | Annual | + | + | + | − | + | − | + | − | − |
| *Osmanthus fragrans* Lour. (= *Olea fragrans* Royle) | Oleaceae | Shrub | Perennial | + | + | + | + | + | − | − | + | − |
| *Oxalis corniculata* L. | Oxalidaceae | Herb | Perennial | + | + | − | − | − | + | − | − | − |
| *Parthenium hystophorus* L. | Asteraceae | Herb | Annual | + | − | − | − | − | + | − | − | − |
| *Phalaris minor* Retz. | Poaceae | Herb | Annual | + | + | − | − | − | − | − | − | − |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Phyllanthus emblica* L. | Euphorbiaceae | Tree | Perennial | + | + | + | + | + | – | – | – | + |
| *Pinus roxburghii* Sargent | Pinaceae | Tree | Perennial | – | + | + | + | – | – | – | + | – |
| *Pisum sativum* L. | Fabaceae | Climber | Annual | + | + | + | – | + | – | – | – | – |
| *Poa annua* L. | Poaceae | Herb | Annual | + | + | – | – | – | – | – | – | – |
| *Psidium guajava* L. | Myrtaceae | Tree | Perennial | + | + | + | – | – | – | – | – | – |
| *Quercus incana* Bartram | Fagaceae | Tree | Perennial | + | + | + | + | + | – | – | – | – |
| *Ranunculus arvensis* L. | Ranunculaceae | Herb | Annual | + | – | – | – | – | + | – | – | – |
| *Ricinus communis* L. | Euphorbiaceae | Shrub | Perennial | + | + | + | + | – | | – | + | + |
| *Rubus fruticosus* L. | Rosaceae | Shrub | Perennial | + | – | – | – | – | – | – | – | – |
| *Saccharum spontaneum* L. | Poaceae | Herb | Perennial | – | – | + | – | – | – | – | – | – |
| *Setaria viridis* (L.) P. Beauv. | Poaceae | Herb | Annual | + | + | + | + | – | – | – | – | – |
| *Sonchus asper* (L.) Hill. | Asteraceae | Herb | Annual | + | – | – | – | – | – | – | – | – |
| *Syzygium cumini* (L.) Skeels | Myrtaceae | Tree | Perennial | + | + | + | + | + | – | – | – | – |
| *Taraxacum officinale* F.H. Wigg | Asteraceae | Herb | Perennial | + | + | – | – | – | – | – | – | – |
| *Themeda anathera* (Nees ex Steud.) Hack. | Poaceae | Herb | Perennial | – | + | – | – | – | – | – | – | – |
| *Toona ciliata* M.Roem. | Meliaceae | Tree | Perennial | + | + | + | + | – | – | – | – | – |
| *Tribulus terrestris* L. | Zygophyllaceae | Climber | Perennial | + | + | + | – | + | – | – | – | – |
| *Trichodesma indicum* (L.) Sm. | Boraginaceae | Herb | Annual | + | + | + | – | + | – | – | – | + |
| *Verbascum thapsus* L. | Scrophulariaceae | Herb | Biennial | + | + | – | – | – | – | – | – | – |
| *Viola biflora* L. | Violaceae | Herb | Perennial | + | + | – | – | – | – | – | – | – |
| *Vitex negundo* L. | Verbenaceae | Shrub | Perennial | + | + | + | + | + | – | – | – | + |
| *Xanthium spinosum* L. | Asteraceae | Herb | Annual | + | + | + | – | – | – | – | + | – |
| *Zanthoxylum armatum* DC. | Rutaceae | Tree | Perennial | + | – | – | – | – | – | – | – | – |
| *Ziziphus jujuba* Mill. | Rhamnaceae | Tree | Perennial | + | + | + | + | + | – | – | – | – |

*MD* medicinal, *FD* fodder, *FW* fuelwood, *V/EF* vegetable/edible fruit, *PO* poisonous, *TD* tanning/dying, *RY* resin yield, *HT* herbal tea, *TW* timber wood

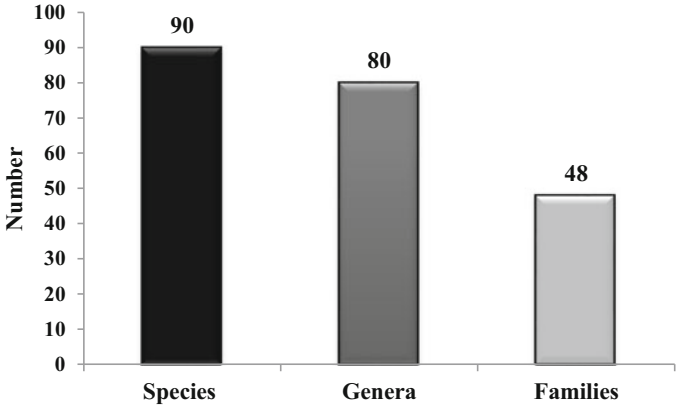


**Fig. 13.2** Taxonomic overview of flora in the Reasi area

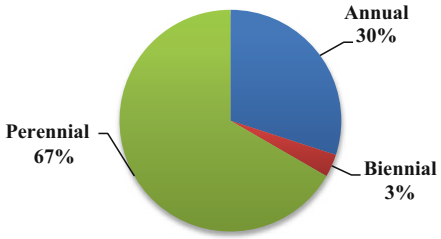


**Fig. 13.3** Contribution of plant's life-spans in terms of percentage

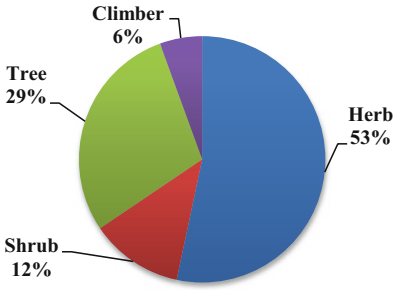


**Fig. 13.4** Contribution of plant's growth forms

### 13.4.3 Ethnobotanical Information

Based on the investigated knowledge, the data are discussed under the subheads single usage, double usage and multi-usages. The pictorial representation is given in Fig. 13.6.

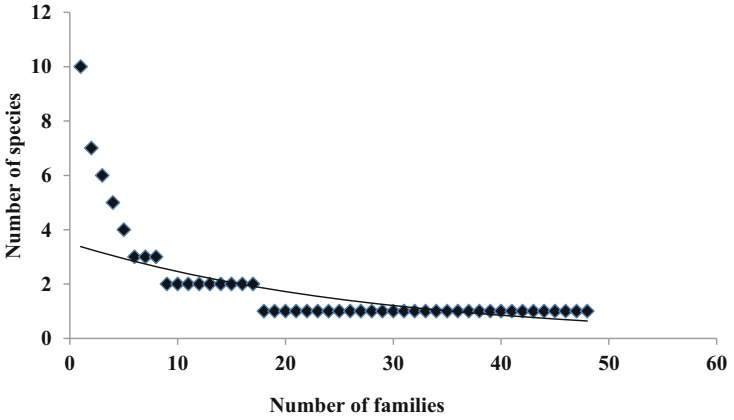


**Fig. 13.5** Species–family relationship of flora in the study area

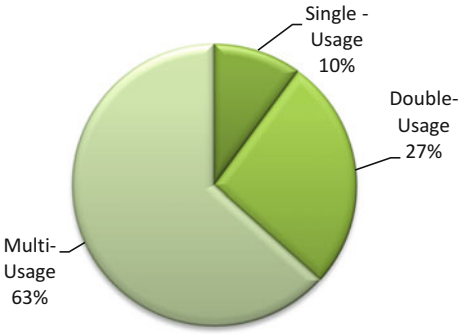


**Fig. 13.6** Proportion of plants in different ethnobotanical-usages

#### 13.4.3.1 Double Usage

The plants used for more than one medicinal purposes, for example, *Adiantum caudatum*, *Amaranthus viridis*, *Lysimachia arvensis*, *Cannabis sativa, Conyza canadensis*, *Euphorbia obovata*, *Euphorbia hirta*, *Justicia adhatoda* and *Malva sylvestris*. About 27% of plants in the study area had double usage.

#### 13.4.3.2 Multi-usage

Plant species such as *Senegallia modesta*, *Achyranthes aspera*, *Aloe vera*, *Bombax ceiba*, *Butea monosperma*, *Carissa spinarum*, *Cordia myxa*, *Ficus carica*, *Ficus palmata*, *Melia azedarach*, *Phyllanthus emblica*, *Pinus roxburghii*, *Ricinus communis*, *Trichodesma indicum*, *Vitex negundo*, *Vachellia nilotica* and *Ziziphus jujuba* were used for more than two purposes and are called multi-usage plants. Maximum (63%) of the plant species reported in the study area had multi-usage.

#### 13.4.3.3 Plant Usage Classification

Four plant usages (clusters) were determined at a vertical distance value of 0.5, where the clusters are distinctly separated (Fig. 13.7). The clusters of plant species grouped in one limb are more similar in usage. However, the cluster limb one and four displayed the maximum dissimilarity with their neighbouring clusters. Moreover, the similarity in usage decreased with the increasing distance between the groups (cluster). The dendrogram generated four distinctly separate clusters based on the plant usage. Medicinal, tanning/dying and vegetable/edible fruit form one arm of the cluster one and other arm is formed by timber wood, fodder and fuelwood plants. Resin yield, herbal tea and poisonous plants formed second, third and fourth clusters of the dendrogram respectively.

The plant usage and the distribution of plant species across different categories were disproportionate, with the maximum 29% species found to be of medicinal use, followed by fodder (26%), fuelwood (18%), timber wood (8%), vegetable/edible fruit (7%), poisonous (3%), tanning/dying (2%), resin yield (4%) and herbal tea (3%) (Fig. 13.8).

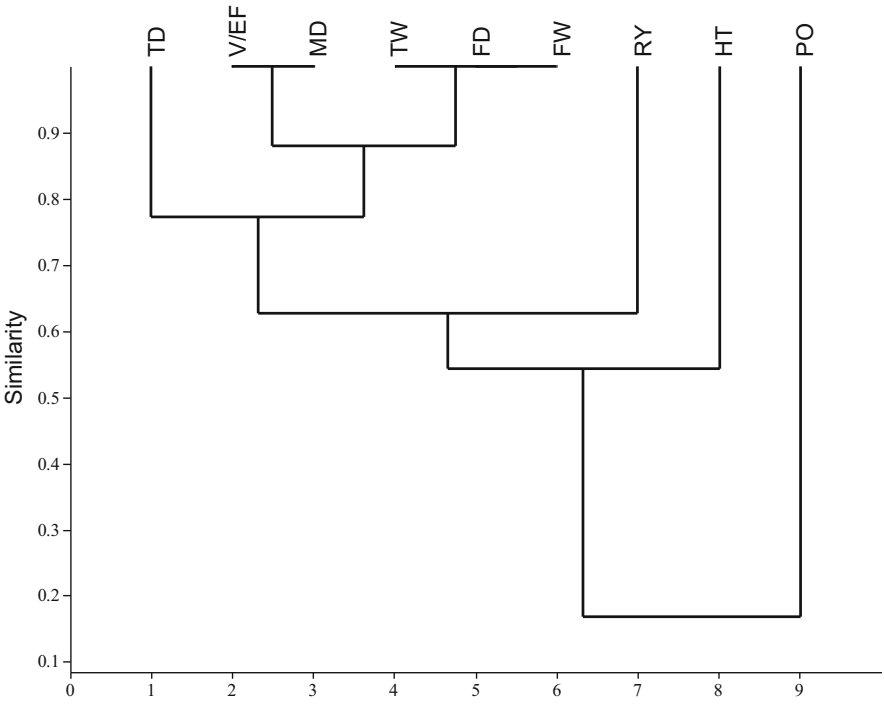


**Fig. 13.7** Cluster diagram of the flora based on ethnobotanical usage in the study area

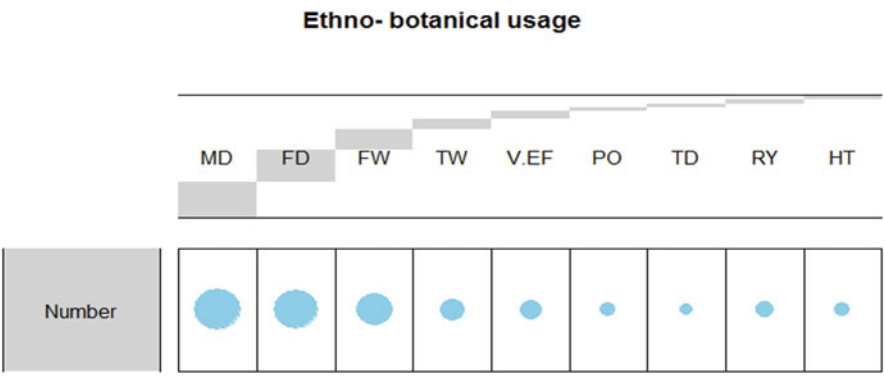


**Fig. 13.8** Proportion of plants in different ethnobotanical usages in the study area

## 13.5 Discussion and Compared Studies

Floristic documentation is recognized as a useful parameter for identifying spatial pattern in plant diversity and composition, and when combined with ethnoecological knowledge, can provide important information on the processes that maintain and sustain such ecosystem (Khan et al. 2013; Mokganya and Tshisikhawe 2019). The present study of ethnobotanical investigation reported 90 plant species belonging to 80 genera and 48 families from district Reasi. The species richness recorded in the present study is more or less similar to those reported by several workers from different regions of Himalaya (Singh et al. 2009, 2010; Qureshi and Bhatti 2010; Singh and Shanpru 2010; Roy et al. 2010; Shaheen et al. 2011; Singh and Borthakur 2011; Singh et al. 2012a, b; Khan et al. 2013; Singh 2015; Ullah 2015; Ajaib et al. 2016; Singh and Bedi 2017; Singh 2019a, b). However, in terms of floristic distribution patterns, these research observations can be compared with those from mountain regions in the Himalayas, where floristic groups such as Poaceae, Euphorbiaceae and Asteraceae were the most dominant representative families. Similar results were obtained by Ajaib et al. (2014) in Upper Kurram Agency, Pakistan. Kabeer and Vyas (2018) in Nilgiri Biosphere Reserve reported Poaceae as the most dominant family, whereas in the other studies by Khan et al. (2014) in Manoor valley, Rahman et al. (2018) in Shahbaz Garhi, district Mardan, Pakistan and Gairola et al. (2010) in Uttarakhand, Garhwal Himalaya, India, reported the family Asteraceae as the most dominant family with respect to similar investigations. The floristic analyses revealed the unequal distribution of species across families, and about 31 families were monotypic in the present study. These values are more or less similar to the previous observed values from different regions of Himalaya (Gairola et al. 2010; Singh et al. 2016; Rahman et al. 2018). From district Reasi, a total of 90 plants were collected. Some plants are medicinal, such as *Senegallia modesta*, *Albizia lebbek, Ajuga integrifolia*, *Aloe vera*, *Ipomoea cairica*, *Justicia adhatoda*, *Cyperus rotundus*, *Datura innoxia*, *Cynodon dactylon*, *Euphorbia*

*hirta*, *Euphorbia helioscopia*, *Taraxacum officinale* and *Oxalis corniculata*, as these plant were also of medicinal usage in other parts of Himalaya (Mahmood et al. 2011; Khan et al. 2013; Shaheen et al. 2014; Singh et al. 2014, 2016; Ajaib et al. 2016; Singh et al. 2018b).

Plant growth form is a measurable trait in comparing geographically separated plant habitats and also regarded as the potential indicator of prevailing environmental conditions than do the taxonomic identity alone (Southwood 1977; Lavorel and Garnier 2002). In the present study, species of trees were in higher proportion (26%) than species of shrubs (11%), which clearly depicts the smooth functioning of a forest ecosystem in the study area (Khan et al. 2015). This trend is contradictory to various studies undertaken from different Himalayan regions (Gairola et al. 2010; Chawla et al. 2012; Qureshi et al. 2014; Choudhary and Nama 2014). However, this finding is in support to the results of Khan et al. (2015). Out of 90 plant species, 10% were used as single usage, 27% double usage and 63% multi-usage. Our results are further supported by Ajaib et al. (2016).

## 13.6 Conclusion

These findings signify the relationship between provisioning ecosystem goods and services of flora for human well-being in the study area. The population of different communites residing in the district Reasi possesses valuable traditional knowledge of plant biodiversity. There was a disparity in traditional knowledge at the personal level depending upon the relation between the individual and the specific plant species or group that he/she prioritizes for confident uses, and it is reported in a number of other studies also (Costanza 2008; Quijas et al. 2010).The study demonstrates that plants are used to sustain a wide range of livelihood activities and predominantly as a resource of traditional medicines (Khan et al. 2013). Furthermore, the plant biodiversity of the study area provides fuelwood, timber wood, food, fodder, vegetable/edible fruit, herbal tea, tanning/dying, resin yield and other services to the native communities. Local inhabitants, particularly the older generation, prefer to live in district Reasi because of the existing provisioning ecosystem benefits and their traditional ethnoecological knowledge. This study going to serve as a base-line information for biodiversity conservation and also for developed of medicinal formulations.

**Acknowledgements** Authors are thankful to Director CSIR-IIIM, Jammu for providing research facilities and moral support. We are also thankful to the Head, Department of Botany, University of Kashmir and Srinagar for providing necessary facilities during the present study. Helping hand rendered by the staff of Centre for Biodiversity and Taxonomy is highly acknowledged. Thanks are also due to the Principal Chief Conservator Forests, Govt. of Jammu and Kashmir, India for permission and logistic support during field work in the study area.

**Conflict of Interest** No conflict of interest between the authors, particularly with respect to this manuscript.

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# Medicinal Value of High-Altitude Plants of Indian Himalaya

14

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**Abstract**

Plants are the primary source of the therapeutic needs for mankind since ancient times and capable of growing under extreme conditions. The diversity in ecological growing conditions and also variability in altitude that ranges from 100 to 7500 m above the sea level introduce diverse kinds of medicinal plants in the higher altitude. In Indian Himalayan region, there are a number of medicinal plants growing wildly such as *Aconitum heterophyllum*, *Hippophae rhamnoides*, *Inula racemosa, Rhodiola rosea,* and *Sinopodophyllum hexandrum*. *Aconitum heterophyllum* has active alkaloids such as lycoctonine which shows significant activity against *Pseudomonas aeruginosa* and *Salmonella typhi*. Analgesic and anti-inflammatory activities are significantly shown by some other alkaloids such as aconitine and mesaconitine derivatives. *Inula racemosa* contains a large amount of sesquiterpene lactones such as alantolactone and isoalantolactone and a large number of biological activities such as antimalarial, antifungal and hypoglcaemic and *Sinopodophyllum hexandrum* contains a variety of bioactive molecules such as flavonoids and lignans which possess antioxidant and antiapoptotic potential and hence help in radio-protection. The root of *Rhodiola rosea* contains more than 140 active constituents, and among them the two most potent are rosavin and salidroside. These constituents help in decreasing stress, improve brain function, reduce symptoms of depression etc. The adverse conditions in higher altitude is due to the presence of ultraviolet (UV) filters, potent antioxidants, free radical quenchers and antifreeze carbohydrates, and the plants growing in such habitat adapt themself to different mechanisms of metabolite synthesis. This is the major reason for the availability of diverse and unique

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_14

chemical entities in the high-altitude plants. On the other hand, these processes are not shown by plants growing in low altitude. Their biochemical machinery has been able to bear the aggressive climatic conditions by the way of new biosynthetic twists leading to new molecular skeletal, which are absent in the lower region plants. High-altitude plants gain a lot of economic and medicinal importance. The amount and variety of chemical constituents present in high-altitude plants differ from plants growing in lower region, and this attracts researchers to investigate and explore their medicinal applications for human health care.

**Keywords**

Medicine · High altitude · Himalaya · *Hippophae* · *Aconitum* · *Inula* · *Sinopodophyllum* · *Rhodiola*

## Abbreviations

| | |
|---|---|
| ALT | Alanine Amino Transferase |
| cAMP | Cyclic Adenosine Monophosphate |
| GSH | Glutathione |
| IHR | Indian Himalayan Region |
| $LD_{50}$ | Lethal Dose |
| NMR | Nuclear Magnetic Resonance |
| SBT | Sea Buck Thorn |
| TPA | Tumour Promoting Agent |
| UV | Ultra Violet |
| WHO | World Health Organization |

## 14.1 Introduction

For the daily requirements of human beings throughout the ages, they have been dependent on nature for the sources of food stuffs, clothing, fertilizers, fragrances, shelters, medicines and transportation. In developing countries, a large proportion of population depends on plants for their health care systems. The medicinal plants are in major use since long time because of their lesser side effects. The allopathic medicines take lesser time for cure of disease, however, associated with adverse effects on the health of individual. Medicinal plants are used traditionally for the common day-to-day ailments like cold, cough, fever and headache. Even their role is proved in coping with harmful diseases, including cancer, hepatitis, AIDS. Industrialists also make use of plant materials for preparing fine chemicals, cosmetics and pharmaceuticals. Medicinal plants play a prominent role in the discovery of new drugs (Blumthaler et al. 1994; Philipson 1990). The various pharmacologically and biologically active agents obtained from natural resources

help to detect many pharmaceutically valuable medicines that play a major role in the treatment of human diseases. Because of the devoted attempts of scientists, a variety of potent drugs and a good amount of therapeutic leads and various pharmacologically active compounds were created from herbal drugs. Considering the medicinal importance of high-altitude Himalayan plants, this communication provide description of important plants that belong to high altitude region. World Health Organization (WHO) has also recognized the traditional use of these medicinal plants and created guidelines, strategies and standards for botanical medicines.

## 14.2 Medicinal Plants from High-Altitude Himalayan Region

India has a wealthy and ancient traditional medicine legacy. Ayurveda, an ancient Indian system of medicine, is recognized as Atharavaveda-upaveda portion (1000 BC). All the formulations listed in Indian Ayurveda are still used in India and these formulations have lately started to spread throughout the world. In India there are various varieties of many medicinal and economically important plants. It is a land of great biodiversity being the home for a great variety of flora and fauna. According to a report of WHO, about 80% of the world population depends upon the plants for medicinal uses. About 20,000 of the plant species found in India are medicinal and mainly about 100 of the species are commercially cultivated. India has a long history of traditional use of medicinal plants, which are used for the treatment of various diseases. The rich biodiversity of Himalayan region has always been the botanist's pride. The suitable environment conditions, topography and the diversified landforms sustain different species of plants which are of great medicinal importance to mankind. The Indian Himalayan region harbours notable plant diversity derived from both elevation and precipitation steep gradients and has traditionally been a significant source of medicinal plants. Medicinal plants of high altitude are of great interest throughout the Himalayan region, as they are essential for traditional health care and for trade in large-scale collection. These include significant species of medicinal plants (e.g. *Aconitum heterophyllum, Hippophae rhamnoides, Inula racemosa, Rhodiola rosea* and *Sinopodophyllum hexandrum*). These plants have many medicinal uses in curing people suffering from different diseases. Different parts of plants have been used for the treatment of diseases. The medicinal importance of these plants includes antimicrobial and antitumour effects, antistress, anti-inflammatory, useful in malaria and jaundice, dermatological effects, cardio-protective effects, antioxidant properties, antiulcer, anticancer, antiasthmatic activity, antiallergic and antifungal properties.

## 14.3 Plants Growing in High-Altitude Region

### 14.3.1 *Aconitum heterophyllum*

#### 14.3.1.1 General Note on *A. heterophyllum*

*Aconitum heterophyllum* wall is a medicinal plant which belongs to the family *Ranunculaceae* and it is commonly known as Patris or Atis. It is mainly found in mild and alpine regions of Himalayas which range between 2400 and 4500 m. There are about 300 species of *Aconitum* found all over the world, out of which 24 species are found in India. It consists of dried, tuberous roots of *A. heterophyllum*, a perennial herb indigenous to the western Himalayas and found in Uttarakhand, Sikkim, Kashmir and Nepal at altitudes between 2500 and 4000 m. It is known as the Queen of all poisons due to the fact that most of the species are highly noxious in nature, with several species still being used on the tips of hunting apex. Thus, this plant must be handled carefully (Beigh et al. 2008; Singh et al. 2015a, b). The roots of the plant are biennial, paired, and tuberous. The colour of the root is white or grey and the stem is rigidly upright, simple or branched. The plant is 15–20 cm in height and globular from below and finely crispo-pubescent in the upper part. Leaves of plants are multifold and glabrous and lower parts of the leaves have long petioles (13 cm). The blades are orbicular-cordate or ovate-cordate in outline with a usually narrow sinus (1–1.5 cm deep). They are usually 5-lobed to the middle and upper parts of the leaves having lobes that completely surround the stem (Rajakrishnan et al. 2016). Inflorescence of the plant is slender raceme or a lax, leafy panicle, crispo-pubescent. Sepals are blue or violet (rarely white) in colour: navicular obliquely erect, shortly or obscurely beaked, 18–20 mm high, 8–9 mm wide. Carpels are 5 in number and elliptic-oblong. Follicles are contagious, linear-oblong, straight, 16–18 mm long. Seeds are pyramidal in shape, 3–4 mm long and blackish brown in colour. *A. heterophyllum* cultivation requires the suitable altitude of about 2400–3600 masl. The plant is cultivated in moist soil, and mostly found in sub-alpine and alpine region of the Himalayas. The cultivation of natural and transplanted population of *A. heterophyllum* requires the rainfall about 600–1500 mm. *A. heterophyllum* plant is cultivated from seeds and tuberous roots: Only in early spring, March–April, seeds are collected and germinated. Generally, by the end of growing season one to two daughter tubers are generated. Daughter tubers are gathered in the autumn after senescent aerial shoot is replanted in the spring. In the first year of development, the fresh crops generate leafy shoots while the cultivated crops generate flowers in the second year of development (Beigh et al. 2008). The trade has led to a drastic decrease in the population of *A. heterophyllum* due to its overexploitation and now this plant is categorized as endangered species. The cultivation of this plant is required due to its huge demand in herbal market and to preserve its wild habitat for future.

#### 14.3.1.2 Chemical Constituents of *Aconitum heterophyllum*

The phytochemicals contents found in extracts of *A. heterophyllum* in three different parts, including leaf, root and stem, consist of alkaloids (Fig. 14.1), carbohydrate,

protein and amino acids, saponins, glycosides (Fig. 14.2), quinones, flavonoids and terpenoids (Figs. 14.3 and 14.4) by the different type of test. Aconitum toxicity is mainly derived from diesterditerpene alkaloids including aconitine, hypaconitine and mesaconitine. They can be converted into less or nontoxic derivatives through different processing methods (Fig. 14.5).

### 14.3.1.3 Molecular Structure of *Aconitum heterophyllum*

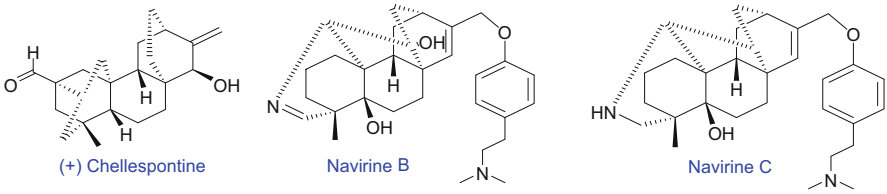


**Fig. 14.1** Structures of alkaloids present in *Aconitum heterophyllum*

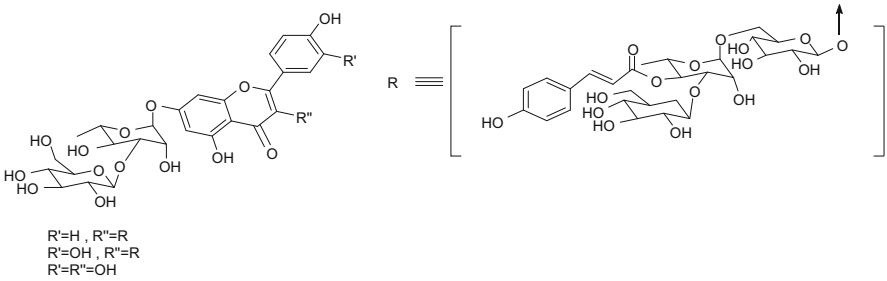


**Fig. 14.2** Structures of flavonoid glycosides present in *Aconitum heterophyllum*

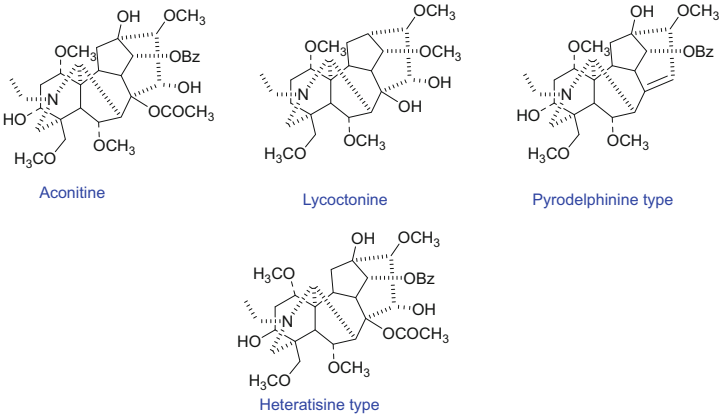


**Fig. 14.3** Structures of C-19 diterpenoid alkaloids present in *Aconitum heterophyllum*

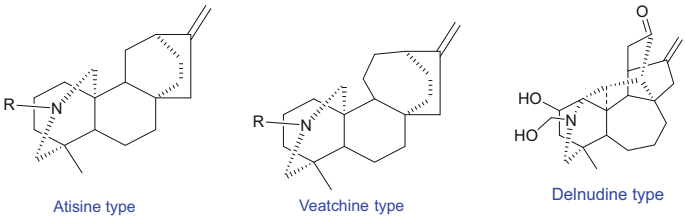


**Fig. 14.4** Structures of C-20 diterpenoid alkaloids present in *Aconitum heterophyllum*

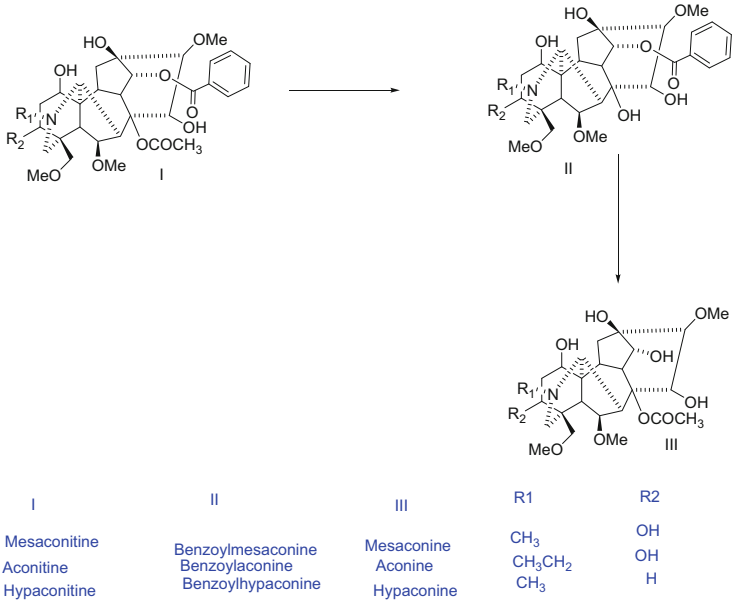


**Fig. 14.5** Detoxification of poisonous alkaloids of *Aconitum*

### 14.3.1.4 Medicinal Uses of *A. heterophyllum*

*A. heterophyllum* consists of many phytoconstituents showing different therapeutic activities. Traditional medicines have been considerably promoted in European countries due to the increasing awareness about side effects of allopathic medicines (Kala 2000; Kala 2005a, b; Olsen and Larsen 2003). In recent years, consumption of herbal medicine is increasing across the world. Nearly 80% of the people living in developing countries reckon on traditional medicine for primary health care (Farnsworth et al. 1985). Lycoctonine showed significant activity against *Pseudomonas aeruginosa* and *Salmonella typhi*. Analgesic and anti-inflammatory activities are significantly shown by some aconitine and mesaconitine derivatives.

#### 14.3.1.4.1 Digestive Disease

In a disease of diarrhoea, fine root powder with dry ginger, beet fruits (bellpetra), nutmeg (jaiphal) and *A. heterophyllum* (atvika) mixed together in equal amount and two pinches with water three times a day are recommended (Singh et al. 2015a, b).

#### 14.3.1.4.2 Respiratory Disease

In patients with cervical lymphadenitis, the juice of *A. heterophyllum* along with milk is beneficial.

#### 14.3.1.4.3 Urinary System

*A. heterophyllum* seeds have diuretic properties that boost the quantity of urine and decrease the burning of urinary tract.

#### 14.3.1.4.4 Reproductive System

The powder of root of *A. heterophyllum* has been used for the treatment of vagina burning and in spermatorrhoea.

### 14.3.1.5 Pharmacological Uses

#### 14.3.1.5.1 Hepatoprotective Activity

It is due to the presence of antioxidants in roots of this plant (Xie et al. 2005). The ethanolic extract of *A. heterophyllum* roots shows hepatoprotective activity in paracetamol-induced hepatic damage in Wistar albino rats (Konda et al. 2013).

#### 14.3.1.5.2 Miscellaneous Activities

The root powder of Ativisha with honey is prescribed for cough irritations and bronchitis; it is also effective against guinea worms and in high blood pressure. The origin of this plant is alexipharmic, antiatrabilious, antiperiodic, antiphlegmatic and carminative.

### 14.3.1.6 Safety and Toxicity Studies

*Aconitum* toxicity derives primarily from diesterditerpene alkaloids, including aconitine, mesaconitine and hypaconitine. Through distinct handling methods, they can be converted into less or less toxic derivatives. *Aconitum* tubers were used only after processing as herbal drug. The standardized technique for evaluating the concentrations of toxic alkaloids in aconite roots is required to guarantee secure use as medicinal herbs of these plants. The processed tubers usually have lower levels of poisonous alkaloids than unprocessed tubers (Csupor et al. 2009). Samaskaras processes crude aconite in the herbal medication scheme of Ayurveda before it is used therapeutically (Thorat and Dahanukar 1991). The plant root is boiled for two successive days with two components of cow urine for 7 h a day. Then it is boiled for the same duration with two components of cow milk. Then the root

processed in such a fashion is washed with lukewarm water, cut into pieces, dried and ground. The research demonstrates that aconite becomes secure after Samaskaras. Crude aconite is seen to be considerably toxic to mice (100% mortality at a dose of 2.6 mg/mouse), whereas the fully processed aconite is completely non-toxic (no dose mortality even eight times higher than that of crude aconite).

## 14.3.2 *Hippophae rhamnoides*

### 14.3.2.1 General Note on *Hippophae rhamnoides*

*H. rhamnoides* is commonly known as sea buckthorn or Leh berry and belongs to family Elaeagnaceae. The name 'sea buckthorn' has been taken from the Greek words Hippo, which means horse, and Phoas, which means to shine. This was due to the fact that its young branches and leaves were used as fodder for horses, which led to a rapid gain in weight in horses and their coat became more shiny. It is obtained from native Eurasian plant communities ranging within 27–69°N latitude (from Russia to Pakistan) and 7°W–122°E longitude (from Spain to Mongolia) located with a centre of origin on the Qinghai-Tibet Plateau (Rousi 1971; Zeb 2004). Being a unique plant, it has gained worldwide attention due to its medicinal and nutritional properties and domesticated in different parts of the world (Rousi 1971). It is distributed naturally in dry temperate and cold desert areas of North-West Himalayas (2590–4175 meter above mean sea level [masl]). Natural distribution areas of sea buckthorn include China, India, Nepal, Pakistan, Romania, France, Germany, Finland, Denmark, the Netherlands, Norway, Canada, Poland, Norway, Latvia, Mongolia, Great Britain and Sweden. From the eight subspecies, the most common subspecies reported is *H. rhamnoides* L. in China and *H. rhamnoides* L. ssp. in Finland (Zubarev 2008). In India, sea buckthorn grows in elevated and cold conditions of Ladakh (Leh and Kargil), Lahaul Spiti parts of Chamba (Pangi), upper Kinaur districts of Himachal Pradesh and Chamoli and districts in Uttarakhand. Sea buckthorn is also found in Dibang valley of Uttarakhand and Sikkim. The production areas of naturally grown sea buckthorn in India are available only for Leh (115 $km^2$) (Stobdan et al. 2008) and Uttarakhand (38 $km^2$) (Yadav et al. 2016). Sea buckthorn is a shrubby, deciduous plant with numerous flowers. Berries of sea buckthorn are most useful part from which the juice is extracted. The majuration of berries of *H. rhamnoides* is divided into three phases. The first phase consists of accelerating seed growth, second phase is the declining transition seed growth and the final phase is called berry maturation phase. Berries are ripened in almost all parts of the world at the start of September. The berries are attached to the undisturbed branches in the whole winter. The amount of tannins (proanthocyanins) present in berry seems to be related with the colour of berry of *H. rhamnoides* (Yang et al. 2016). Sea buckthorn berries are used to prepare various kinds of products involving cosmetics (mainly in China and Russia) and food (mainly in Europe). The value of by-products from berries and leaves accounts approximately for 42 million Euros, in a sea buckthorn business assessment in

Russia, Europe, China and New independent States countries. Sea buckthorn has developed a modified root system which helps in fixing a large quantity of atmospheric nitrogen. There is a symbiotic relationship between fungus and sea buckthorn that led to a nodule formation that helps in fixing atmospheric nitrogen. Sea buckthorn root has a symbiotic mycorrhizal fungus, which is identified as Frankia (Actinomycetes) (Jike and Xiaoming 1992). Sea buckthorn roots have a double capacity of fixing atmospheric nitrogen compared to soybean (an 8- to 10-year-old sea buckthorn forest can fix 180 kg of nitrogen/ha/year) (Kato et al. 2007) and are known to improve the soil structure, which makes it an ideal species for land replenishment and wildlife habitat improvement (Enescu 2014).

#### 14.3.2.2 Chemical Constituents of *Hippophae rhamnoides*

The chemical composition of *H. rhamnoides* depends on the origin, climate and method of extraction and varies according to change in one of these factors. *H. rhamnoides* consists of vitamin C (ascorbic acid), vitamin E, carotenoids, fatty acids, flavonoids, minerals and sugar alcohols. The major unsaturated fatty acids were linolenic acid (omega-3) (20–23%), linoleic acid (omega-6) (40–43%), oleic acid (omega-9) (19–22%) and palmitoleic acid (1–3%) and saturated fatty acids present in lower amount are palmitic acid (7–9%) and stearic acid (3–4%) in seed oil. The two major and essential fatty acids, namely linolenic acid (omega-3) (20–23%) and linoleic acid (omega-6) (40–43%), are present in a large amount in seed oil extracted from the subspecies. The main flavonoids in *H. rhamnoide*s are isorhamnetin, quercitin, myricetin and kaempferol (Zhao and Wu 1997). A large number of bioactive substances likes vitamins (A, C, E, K, riboflavin, folic acid), carotenoids (α, β, δ carotene, and lycopene), phytosterols (ergosterol, lansterol, stigmasterol, amyrins), organic acids (malic acid, oxalic acid), polyunsaturated fatty acids and some essential amino acids are known to be present in a good amount in all parts of sea buckthorn (Beveridge et al. 1999; Yang et al. 2001; Pintea et al. 2005).

#### 14.3.2.3 Molecular Structures of *H. rhamnoides*

The main flavonoids present in sea buckthorn are shown in Fig. 14.6.

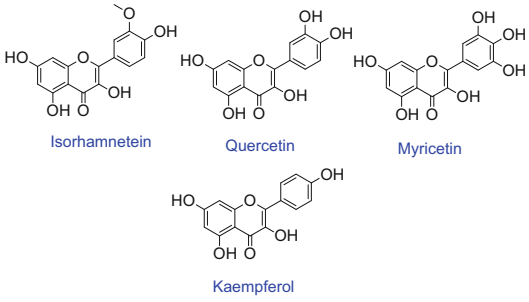


**Fig. 14.6** Structures of flavonoids

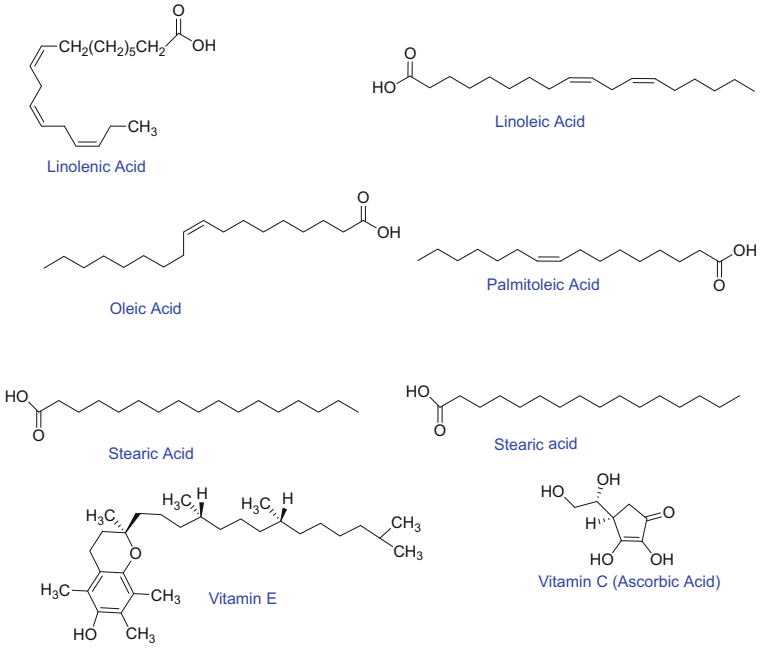


**Fig. 14.7** Structures of unsaturated, saturated fatty acids, and vitamins

**Table 14.1** Structures of different amino acids

| Amino acids | Conc. (mg/100 g) | Amino acids | Conc. (mg/100 g) | Amino acids | Conc. (mg/100 g) |
|---|---|---|---|---|---|
| Aspartic acid | 426.6 | Arginine | 11.3 | Tyrosine | 13.4 |
| Serine | 28.1 | Valine | 21.8 | Isoleucine | 17.4 |
| Glutamine | 19.4 | Cysteine | 3.3 | Methionine | 2.3 |
| Glycine | 16.7 | Alanine | 21.2 | Proline | 45.2 |
| Phenylalanine | 20.0 | Threonine | 36.8 | | |
| Histidine | 13.7 | Lysine | 27.2 | | |

The major and minor unsaturated and saturated fatty acids are presented in Fig. 14.7.

Different types of amino acids present in sea buckthorn are shown in Table 14.1.

#### 14.3.2.4 Medicinal Uses of *Hippophae rhamnoides*

Sea buckthorn is used in traditional medicine system in China, India and Tibet. This plant was used as a medicinal plant in Tibet since 900 AD. The art of using sea buckthorn resources has been well developed by the people living in the trans-Himalayan region. All parts of sea buckthorn plant are used for different purposes that include nutritional supplement, firewood, tree guard, medicine (cough), religious rites, fencing, land replenishment, agricultural implements, improvement in

soil fertility. In traditional system of medicine, this plant has been used for treatment of asthma, gastric ulcers, skin diseases, cough and lung disorders. This plant got attraction due to its medicinal and cosmetic uses (Tom et al. 2009). In ancient times, the juice of *H. rhamnoides* was the common medicine. The leaves and fruits were used in Turkish folk medications as antiseptic and wound healing as well as in the therapy of ulcers (Cakir 2004). A large number of pharmacological effects of sea buckthorn have been reported that includes antioxidant, immunomodulatory, antiatherogenic, antistress, hepatoprotective, radioprotective and tissue repair (Suleyman et al. 2001;Geetha et al. 2002a, b; Xing et al. 2002; Gao et al. 2003; Basu et al. 2007; Chawla et al. 2007;Saggu and Kumar 2007a, b; Upadhyay et al. 2009). Some of the important medicinal uses of sea buckthorn are discussed below.

#### 14.3.2.5 Pharmacological Uses of *Hippophae rhamnoides*

##### 14.3.2.5.1 Antimicrobial and Antitumoral Effects

*H. rhamnoides* have shown the inhibitory effects towards gram-positive bacteria. The inhibitory effects are due to the presence of phenolic compounds (Guliyev et al. 2004). The activity against tumour-promoting agent (TPA)-induced tumour has been proven from the 70% *H. rhamnoides* branches extract. Such an activity is due to the presence of three phenolic compounds such as catechin, gallocatechin and epigallocatechin (Ken et al. 2009).

##### 14.3.2.5.2 Antibacterial and Antiviral Effects

A new phytochemical drug hiporamin, having a broad spectrum of antimicrobial and antiviral activities, has been isolated from leaves of *H. rhamnoides*. Hiporamin is basically a purified fraction of polyphenol fraction, containing monomeric hydrolysable gallo-ellagi-tannins (preferably strictinin, isostrictinin, casuarinin, casuarictin pedunculagin and stachyurin according to the Nuclear Magnetic Resonance [NMR] spectra).

##### 14.3.2.5.3 Antiulcer Effects

Hexane extract of *H. rhamnoides* shows activity against indomethacin: gastric ulcer caused by ethanol and stress (Guliyev et al. 2004).

##### 14.3.2.5.4 Antiradiation Effects

*H. rhamnoides* leaf extracts, both aqueous and alcoholic, have been reported to survive in mice at deadly doses of more than 80%. When screened under in vitro condition, the extract showed elevated antioxidant capacity. The leaf extract countered harm to the hemopoietic system by radiation and restored plasma reduction in ferric activity (Kumar et al. 2002; Goel et al. 2002).

#### 14.3.2.5.5 Liver Diseases

*H. rhamnoides* could be used to safeguard the liver from harm caused by calcium tetrachloride. According to Zhao et al., *H. rhamnoides* juice can be combined with antivirus to shorten alanine amino transferase's (ALT's) normalization moment.

#### 14.3.2.5.6 Dermatological Effects

Atopic dermatitis is a condition that makes our skin red and itchy. *H. rhamnoides* has shown great advantageous effects against dermatological disorder (Guliyev et al. 2004).

### 14.3.2.6 Safety and Toxicity Studies of *Hippophae rhamnoides*

Sea buckthorn fruit has an important protective function against oxidative injury caused by arsenic. However, the capacity to remove arsenic from the binding location is lacking, indicating that the herbal extract could be co-administered with a chelating agent of known efficacy during arsenic therapy to obtain the optimal impact of chelation treatments (Gupta and Flora 2006). Ruan et al. (2003) proposed the protective impacts of sea buckthorn seed oil against injury caused by inhalation of sulphur dioxide (Ruan et al. 2003). The administration of sea buckthorn extracts protected from sulphur mustard lethality helps in reducing oxidative damage caused by sulphur mustard (Vijayaraghavan et al. 2006). Animal toxicity tests were performed using formulations and extracts based on sea buckthorn.

After administration of sea buckthorn leave aqueous extract, all biochemical parameters linked to fuel metabolism, liver function, kidney function and haematological parameters stayed within ordinary boundaries. While studying the toxicity, the maximum efficient dose of 10–20 times and 14-day administration cause body weight gain; biochemical parameters namely serum bilirubin, creatinine were found to be unchanged and similar to controls (Saggu et al. 2007). At a dose of 100 mg $kg^{-1}$ body weight, no adverse effects of sea buckthorn leaf extract were found in 90 days in rats (Tulsawani 2010).

## 14.3.3 *Inula racemosa*

### 14.3.3.1 General Note of *Inula racemosa*

*I. racemosa* also known as Pushkaramula belongs to family Asteraceae. It was introduced by Acharya Charak as hikka, swas and parswa shula hara. It is considered as endangered species because of delicate nature of its habitat and its overexploitation due to its various medicinal properties (Parvaiz et al. 2006). It is a well-documented Indian medicinal plant. It is a herb that plays an important role to pacify water (Kapha), air (Vata) and humours ( Doshas) in body. Due to various benefits, it is known by various names like Kasari, an enemy of cough; Sulahara, a pain killer; Hikkanigrahana, stops hiccup; sughandhika, fragrant. There are about 20 species of *inula* occurring in India. Out of which five species of *Inula* are considered to be economically valuable. Racemosa is considered to be most important medicinal as well as aromatic plant in Lahaul valley in northwestern Himalaya. It is also found in

western Himalayas at an altitude of 5000–14,000 ft. from Kashmir to kumaon, Ladakh (Leh) Kashmir region and Afghanistan to Nepal. It has stalwart shrub, bearing large leaves that are arranged in a racemose pattern. Abaxial lamila faces are densely hairy. The leaves are broad and not divided into parts. The leaves that are present on the node of the stem are small in size, elongated and semi-amplexicaule. The lower leaves are large in size with shady yellow colour flowers that blossom in mid to late summer. They are in the form of clusters and grow on apical spike. Basal leaves are 20 − 45 long, 12.5 cm wide. Flowers are large in size, that is, 3.8–5 cm in diameter with triangular tips and bent back. The outer bracts are broad whereas inner bract is linear. The fruits are cylindrical in shape with 8-mm-long pappus, 4-mm-long achene and it has red colour. The stock of the root is branched; fresh roots are irregular and spindle-shaped. The outer portion of roots has dull brownish colour whereas inner portion has yellow colour. They possess sweet, camphoraceous odour and bitter taste.

#### 14.3.3.2 Chemical Constituents of *Inula racemosa*

*Inula racemosa* contains a large amount of sesquiterpene lactones such as Alantolactone and Isoalantolactone (Arora et al. 1980). A lactone is a class of cyclic ester. Sesquiterpene is mainly found in Asteraceae family (Schmidt et al. 2001; Spring et al. 2001). It has a large group of compounds that have challenged the intelligence and technical skills of various chemists and biochemists who are interested in the study of structures, chemistry, synthesis and biological origin. The plant contains eudesmanolide groups such as alantolactone and isoalantolactone which are known to have a wide range of biological activities such as antispasmodic, hypoglycaemic, antimalarial (Lokhande et al. 2007) and antifungal activities (Xie et al. 1995). Daucosterol and beta-sitosterol have been extracted from the roots of plant. The other constituents that are present in *I. racemosa* are dihydroalantolactone, dihydro isoalantolactone, inunolide, dihydroinunolide, isoalloalantolactone (Ravindranath et al. 1978), alloalantolactone (Bhandari and Rastogi 1983), inunal, isoinunal (Kalsi et al. 1988, 1989), alantodiene, etc. It is also a source of essential oil. In Kashmir, about 1.3–2.6% of oil has been obtained from *I. racemosa* (Bokadia et al. 1986).

#### 14.3.3.3 Molecular Structures of *Inula racemosa*

The active constituents present in *I. racemosa* are as follows (Fig. 14.8).

#### 14.3.3.4 Medicinal Uses of *Inula racemosa*

Inula is a large genus in the tribe. Several *Inula* species have been used as traditional medicine throughout the world. But they are most commonly used in China, East

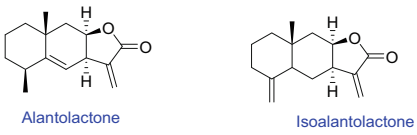


**Fig. 14.8** Major sesquiterpene lactones of *I. racemosa*

Asia and Europe as traditional medicine (Han et al. 2010). In China it has been used against acute enteritis, abdominal pain etc. The roots are commonly used as indigenous medicine, and also used to treat cough and is used as a tonic in veterinary medicine. In Ayurvedic medicines *Inula* is used to treat diseases like tuberculosis and various skin diseases (Shishodia et al. 2008). Ayurveda Acharyas described it as rasayan (immunomodulator) and it is used by Ayurveda physicians for various purposes . The root powder of this plant can be used for the treatment of asthma (Vadnere et al. 2009). It is also used to reduce cholesterol (Mahmood et al. 2010). It can also be used for proper functioning of heart and control of diabetes (Miller 1998). Its leathery leaves can also act as sinks for particulate pollutants mainly carbon particles. *I. racemosa,* also known as 'manu' in Chinese folk medicines, can be used for a long time for proper functioning of stomach and reduce throat pain. It can also act as an antimicrobial agent (Xu and Shi 2011). It is also used to mitigate liver problems, to minimize the pain between neck and shoulder and to prevent abortion. Alantodiene has displayed various biological activities such as plant growth regulator and also increase the nitrate reductase activities in the plant.

#### 14.3.3.5 Pharmacological Uses of *Inula racemosa*

*I. racemosa* is considered to be the most important medicinal plant and it has many pharmacological and clinical uses. Some of them are as follows.

##### 14.3.3.5.1 Anti-inflammatory Activity

Some studies have shown that the aqueous extract of the roots when administered in rats shows maximum inhibition up to 60% with a dose of 400 mg $kg^{-1}$ body weight after a period of 8 h, whereas the standard drug indomethacin shows 69% of inhibition when administered with a dose of 20 mg $kg^{-1}$ body weight (Fallahzadeh and Mohammadi 2016).

##### 14.3.3.5.2 Analgesic Effect

The ethanolic extract of roots of *I. racemosa* is examined on albino rats of both sexes using hot plate. At a dose of 200 mg $kg^{-1}$ body weight after 2 h of administration, its ethanol extract shows latency in percentage protection (42.99% ), whereas the standard drug, that is, aspirin shows (65.47%) latency of percentage protection at a dose of 100 mg $kg^{-1}$ (Arumugam et al. 2012).

##### 14.3.3.5.3 Antibacterial Activity

Disc diffusion method was performed to determine the antibacterial activity of ethanol solution of extract of *Inula racemose* against *Escherichia coli* and *Staphylococcus aureus*. The aqueous extract shows very important antimicrobial activities for these two microorganisms which are tested. The minimum inhibitory concentration (MIC) values are 6.25 mg $mL^{-1}$ and 12.5 mg $mL^{-1}$ whereas ethanol extract has potent activity with an MIC value of 15.625 mg $mL^{-1}$.

#### 14.3.3.5.4 Antioxidant Activity

The ethanolic (70%) extract of the roots of *I. racemosa* was performed in albino rats. The alcoholic extract (in 1% gum acacia) of the roots of *I. racemosa* was administered orally to rats for 21 days for lipid peroxide formation and reduced glutathione content. It has been found that the level of glutathione (GSH) in blood and liver is very high in treated animals. This indicates the antioxidant properties of *I. racemosa* because the increase in level of GSH in the cell results in the destruction of harmful hydrogen peroxide and lipid peroxide by glutathione peroxidise.

#### 14.3.3.5.5 Cardioprotective Activity

*I. racemosa* has been known for its benefits in cardiorespiratory and cardiovascular diseases mainly in angina pectoris (Tripathi et al. 1984). In this study, myocardial ischemia was induced in rats with the help of isoproterenol administration via subcutaneous route dose of 20 mg–100 g twice at an interval of 24 h. The petroleum ether extract of the roots of the plant and alantolactone can be further used for the study of their cardioprotective activity in myocardial ischemia. Alantolactone and petroleum ether extract decreases the lipid peroxide level in the ischemic rats and results in normal level of glutathione content.

#### 14.3.3.5.6 Adaptgenicity Potential

The forced swim test model was performed with 90% ethanol root extract in albino mice. When a dose of 100 mg $kg^{-1}$ and 200 mg $kg^{-1}$ of ethanol extract of roots of *I. racemosa* was given to animals, there is a large decrease in the immobility period with an increase in the level of antioxidant makers, serotonin and adrenaline (Gnanasekaran et al. 2012).

### 14.3.3.6 Toxicology and Dosage of *I. racemosa*

However, the benefits of hydroethanolic extract of the roots *I. racemosa* display the therapeutic effects and it was confirmed by this study. The hydroethanolic extract was not harmful for rats and it is given by intraperitoneal route and its $LD_{50}$ is 2100 mg $kg^{-1}$. The hydroethanolic extract was used in clinical studies and it is evaluated by chronic toxicity studies (Srivastava et al. 1999).

## 14.3.4 *Rhodiola rosea*

### 14.3.4.1 General Notes of *Rhodiola rosea*

*Rhodiola rosea* is an angiosperm belonging to class eudicots and family Crassulaceae. It is also known as king's crown, orpin rose, Arctic root, Aaron's rod and lignum rhodium. *R. rosea* propagates as the groundcover. *Rhodiola rosea* is a perennial herb with thick rhizome and, when it is cut, gives a rose-like fragrance. It is 5–40 cm tall in size, fleshy and tender and has stems of varying lengths growing from short, scaly rootstock. Preparations made from *Rhodiola rosea* are also used as Altai folk medicine as a tonic and treatment of tinctures and infusions (Bykov et al. 1999). The colour of the flower is greenish yellow having red tips with four sepals

and four petals. Flowers are 1–3.5 mm long. They bloom during summer. *R. rosea* is dioecian and perennial plant having separate male and female plants. *Rhodiola* genus have originated in the mountainous regions of Himalayas and Southwest China (Darbinyan et al. 2000). There are about 90 species of *Rhodiola* reported in different regions of the world and 73 species are found in China (Booker et al. 2016). *R. rosea* is widely distributed at high-altitude regions of the world with the perfect climatic conditions for its growth and development. It is found in Arctic and mountainous regions throughout Asia and Europe. There are about more than 200 different species of *R.* rosea that have been explored, among which 20 species are used in traditional medicinal systems in different regions. In Asia, different species of *Rhodiola* as *R. crenulata, R. quadrifida, R. sacra, R. brevipetiolata, R. kirilowii, R. sachalinensis* and *R. quadrifida* are used in traditional medicinal systems. In Northern and Central Asia, distribution of the genus extends from Altai mountainous region across Mongolia into many parts of Siberia. Various botanists established that the different species of *Rhodiola* grow naturally in mountainous regions in higher latitudes showing the circumpolar distribution even to the elevations of Northern Hemisphere. Different varieties of *Rhodiola* species have also been reported across Alaska, northern mountains of the continental United States.

In Canada, the extract is standardized to contain 0.8% salidroside or 1–6% rosavins, whereas in European Union (EU) as a tincture, dried root rhizome or dry extract (ethanol with 67–70% extraction solvent). Most of *R. rosea* raw material is collected by wild crafters of China who sell their produce at the regional collection sites. During the summertime the plant material (roots) is collected from minimum of 4-year plant by digging under the plant and removing the most of rhizome/root part. A part of rhizome is left for regeneration over the next years. Wild crafters are able to distinguish *R. rosea* from *R. crenulata* during the summer season as *R. rosea* has the yellow flowers with reddish buds while *R. crenulata* has purple flowers. The Xinjiang region of Leh district to the south is one of the most abundant producers of *R. rosea* with 4–5 collecting sites and selling about 500 tons of the dry rhizomes annually. Different regions of China, Kazakhstan, Russia and Mongolia have limited supply of *R. rosea* giving less contribution in the US market. Maximum of the Mongolian and Kazakhstani *R. rosea* ends up at higher price in Russian markets. The cultivation experiments have been performed in Poland, Sweden, Finland, Russia, and Germany and found that *R. rosea* can be cultivated in moist and cool climates and varying precipitation regions (Adamczak et al. 2014). *R. rosea* does not prefer the shaded areas. *R. rosea* prefers slightly acidic soils with pH 6–7, which is well drained. It can even grow in the harsh conditions like in sandy loam soils and also on rocks. However, the root size of *R. rosea* is small in these soil conditions as the roots are not able to flourish well because of insufficient nutrients in these soils (Platikanov and Evstatieva 2008). *R. rosea* can be propagated by root division, seedlings and also by the seed germination. For the large-scale cultivation of *R. rosea,* propagation by seedlings is the most useful method (Platikanov and Evstatieva 2008).

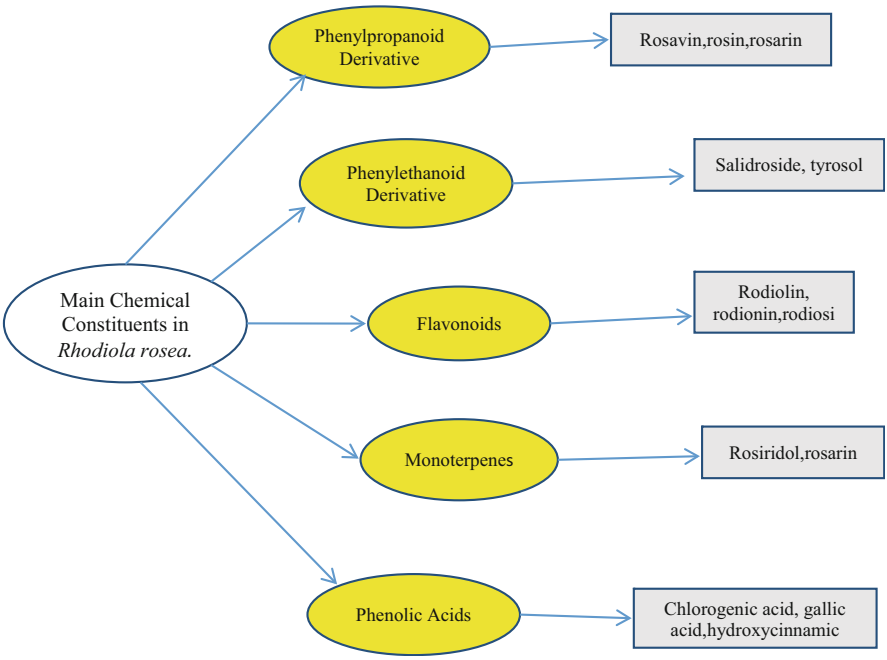


**Fig. 14.9** Chemical constituents of *Rhodiola rosea*

### 14.3.4.2 Chemical Constituents of *Rhodiola rosea*

About 140 compounds are investigated from the rhizomes of *R. rosea* plant that mainly comprises the monoterpenes alcohols, glycosides, aryl glycosides, phenylethanoids, phenylpropnoids, proanthocyanidins, rosarin, rosin, rosavin, salidroside as well as gallic acid derivatives (Panossian et al. 2010). Rhodiosin and rhodionin are the two flavonoids which show the antioxidant (Kwon et al. 2009) and in mice showed the lipase inhibition (Kobayashi et al. 2008). Several flavonoids are also known to show the antiviral effects through neuraminidase inhibition. For the quality assessment of extract containing *R. rosea* quantification of salidroside, rosavin, rosarin and rosin are required (Bykov et al. 1999). Its rhizome extracts are prepared with different concentrations of ethanol. Maceration with different concentrations of ethanol represents the different preparation methods for *R. rosea* that have been used in traditional medicine for a long time (Bykovet al. 1999). The main chemical constituents of *R. rosea* is presented in Fig. 14.9 and Fig. 14.10.

### 14.3.4.3 Molecular Structures of *Rhodiola rosea* Chemical Constituents

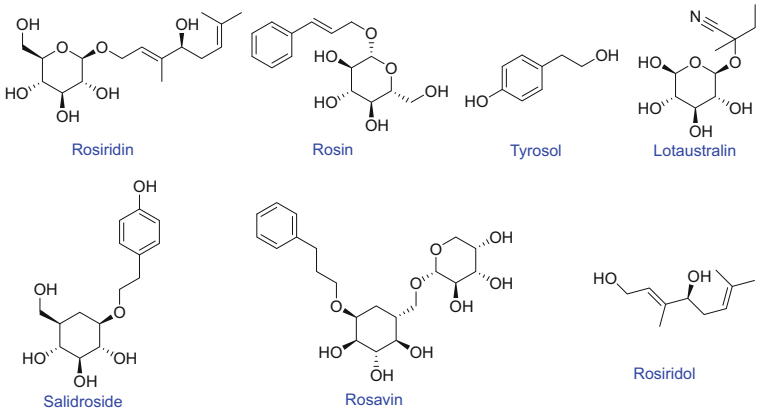


**Fig. 14.10** Structure of *Rhodiola rosea* constituents

### 14.3.4.4 Medicinal Importance of *Rhodiola rosea*

The use of *Rhodiola rosea* as a medicinal plant has a long history in many European countries (Panossian et al. 2010). Between 1748 and 1961, varieties of the medicinal properties of *R. rosea* have been reported in scientific literature of Norway, Sweden, Germany, France, Iceland, and the Soviet Union and it is mainly considered as the adaptogen or physiological stabilizing agent. It promotes homeostasis with various health-promoting effects. In European countries it is used as the traditional herbal product as is used in temporary relief from stress symptoms, for example, sensation of weakness and fatigue. In New Zealand and Australia, it is used as supportive medicine for mental stamina and mental focus. It also has immune function enhancing constituents present in it (Panossian et al. 2010). *R. rosea* is used for the treatment of cold and flu in Central Asia. *R. rosea* is traditionally used for lung inflammation, treatment of cold, fever, strengthening of body as well as for bad mouth breath. It is mainly used as adaptogen and for improving the performance of athletes after prolonged exercise to reduce the recovery time (Booker et al. 2016). *R. rosea* is used as traditional medicine for many centuries in order to increase the work productivity, physical endurance and longevity and to treat depression, anaemia, gastrointestinal ailments, infections and nervous system disorders and for resistance to high-altitude sickness. In the high mountainous regions of Siberia, the villagers still give bouquet of roots to married couples before marriage to enhance fertility and for a healthy birth of children. As written by Carlous Linnaeus, *R. rosea* is used for the treatment of leucorrhoea (vaginal discharge in females), hernia in males, headache and hysteria.

#### 14.3.4.5 Pharmacological and Clinical Studies of *Rhodiola rosea*

The cell cultures of *Rhodiola rosea* on animals and humans have shown antifatigue, antistress, anticancer, immune-enhancing and sexual-stimulating effects (Darbinyan et al. 2000).

##### 14.3.4.5.1 Effects on Central Nervous System of *Rhodiola rosea*

Pharmacological effects of *Rhodiola rosea* were studied long back in 1965 and it was found that low and medium doses had stimulating effects but the large amount of doses have sedative effects. The bioelectrical activity of the brain is increased by the small doses mainly the direct effects on brain bloodstream mall and medium doses of *R. rosea*-stimulated dopamine (DA), norepinephrine (NE), narcotic cholinergic effects, and serotonin (5-HT) effects on the central nervous system (CNS). It increased the permeability of the blood–brain barrier of the brain to precursors of DA and 5-HT as due to its effects on neurotransmitters of the brain (Petkov et al. 1990; Lazarova et al. 1986). These agents from the extract of *R. rosea* enhance the 5-HT levels in the frontal cerebral cortex, which increases the analysing, memory, thinking, calculating, evaluating and planning functions of the cerebral cortex. *R. rosea* also helps in increasing memory.

##### 14.3.4.5.2 Antioxidant Properties of *Rhodiola rosea*

The extracts from *R. rosea* protect the nervous system from oxidative damage caused by free radicals. Roots of *R. rosea* have several active compounds, including phenols, flavonoids. Oligomeric proanthocyanidin is a kind of phenolics that have antioxidant properties (Hernández et al. 2014).

##### 14.3.4.5.3 Against Neuro-Inflammation of *Rhodiola rosea*

The neuroprotective effect of *R. rosea* extract constitutes rosarin and salidroside as the chemical constituents which suppress the generation of NO.

##### 14.3.4.5.4 Effects on Physical Work Capacity of *Rhodiola rosea*

*R. rosea* enhances the physical work capacity and shortens the recovery time after high-intensity exercise. The central nervous system stimulants used by the athletes rapidly damage the catecholamines and decrease the conditioned reflexes. But on the other part *R. rosea* extracts increase the work capacity with lesser mutation.

##### 14.3.4.5.5 Cardioprotective Effects of *Rhodiola rosea*

*R. rosea* prevents the cardiac damage which is stress-induced less myocardial catecholamines and cAMP levels and releasing the less adrenal catecholamine. The antiarrhythmic effect of *R. rosea* is due to the presence of mu-opiate receptors in the myocardial (heart) muscle. Sympathic and parasympathic inputs results in the increased immunity under greater stress conditions without any damage to the heart.

##### 14.3.4.5.6 Effect on Endocrine and Reproductive Activity of *Rhodiola rosea*

The increased effect of *Rhodiola rosea* on the thyroid functioning not even causing the hyperthyroidism is similar to the adaptogen. It improves the thyroid functions by

helping the body to utilize energy properly and keep proper working of brain, muscles and other organs. Estrogenic effect of *R. rosea* mainly depends on the specific hormonal milieu. The effect of *R. rosea* extract (either 100 mg extract orally twice a day for 2 weeks, or 1 mL rhodosin intramuscularly for 10 days) on women suffering from amenorrhea (loss of menstrual cycles) restored the normal menses.

#### 14.3.4.5.7 Effect on Oncogenic Kinase PAK of *Rhodiola rosea*

The major ingredient of *R. rosea* is salidroside which is shown to activate 5′AMP-activated kinase (AMPK). Salidroside is shown to attenuate the tumour-induced angiogenesis dependent on both PK1 and AMPK (Skopińska et al. 2008).

#### 14.3.4.5.8 Antiaddiction Effect of *Rhodiola rosea*

Salidroside, when given to mice, prevented relapse to nicotine (Titomanlio et al. 2014). So, *R. rosea* can be used as the drug for withdrawal from nicotine addiction.

#### 14.3.4.5.9 Antistroke Effect of *Rhodiola rosea*

Strokes are the most common cause of morality and the most common cause of disability worldwide, as reported in 2010.

### 14.3.4.6 Toxicity of *Rhodiola rosea*

According to present scientific reports, there is no adverse effect of *R. rosea* (Ming et al. 2005). But if taken in large amount, it is lethal. As reported, the median lethal dose ($LD_{50}$) of *R. rosea* extract is 3360 mg/kg body weight in rat. Also the consumption of *R. rosea* is associated with hyperactivity, agitation and jittery. Regular consumption of *R. rosea* for few weeks interferes with sleep and induces extra-vivid dreams among consumers. Patients suffering from bipolar disorder are not recommended to consume *R. rosea*. Antidepressant activities trigger the manic episode.

## 14.3.5 *Sinopodophyllum hexandrum*

### 14.3.5.1 General Note of *Sinopodophyllum hexandrum*

*Sinopodophyllum* belongs to family Berberidaceae. It is commonly known as Himalayan mayapple and is present in Himalayan region at an altitude of 2700–4500 masl (Chatterjee 1952). It looks like an ornamental plant with less height ranging from 15 to 45 cm and glossy green foot-shaped leaves. The flowers of this fruit are pink in colour whereas its fruit looks like a bright orange-coloured bulb. In Indian Ayurveda, *Sinopodophyllum* is known by various names such as Bantrapushi and Giriparpat. Among local folks it is famous by the name Ban kakdi (Kala 2005a, b). Rhizome of this plant is responsible for its perennation as other organs are grown from it in winter. The erect and un-branched stem of this plant contains only two large lobed leaves at the top encircling the single flower bud which blossoms during the May season. Light pink-coloured bowl-shaped flower has six petals, six stamens, one pistil of short length and one ovary with 50–150 ovules.

There is no nectar present in it. Its fruit is scarlet or red-coloured berry of about 2.5–5 cm with seeds embedded in pulp. Fruit matures in august (Rajkumar and Ahuja 2010). *Sinopodophyllum* mainly propagates through seeds and cutting of rhizomes. It mainly follows autogamy, that is, self-pollination as very less pollinators visited nectar less flowers. However, solitary bee and honeybees could be the potential pollinators. Self-pollination within a flower is achieved by inclining the pistil and pushing the stigma into contact with anthers after which the pistil comes back to its normal position at the centre of the flower (Xu et al. 1997). *Sinopodophyllum* is mainly distributed across the Himalayan region, east to Afghanistan and north to southwest China (Kala 2005a, b).

#### 14.3.5.2 Chemical Constituents of *Sinopodophyllum hexandrum*

The dried ripe fruit of *Sinopodophyllum* is used as a drug by Tibetan people. It is known as *Sinopodophylli fructus* and is used as traditional Tibetan drug to regulate menstruation, difficult labour and retention of dead foetus or placenta (Shang et al. 1994). Figure 14.11 represents the main constituents of Sinopodophylli fructus.

Roots and rhizomes of *Sinopodophyllum* are used in traditional medicines. Their major constituents are aryltetralin lignans that are known for their antineoplastic, antifungal and immunomodulatory properties (Kharkwal et al. 2008). Nine compounds that were isolated from roots and rhizomes of *Sinopodophyllum* are as follows (Fig. 14.12).

#### 14.3.5.3 Medicinal Uses of *Sinopodophyllum hexandrum*

This plant has been used for several years in traditional systems of medicine. Rhizomes are used for typhoid fever, jaundice, dysentery, chronic hepatitis, scrofula, rheumatism, skin diseases, tumorous growth, kidney and bladder problems. Kaempferol, a polyphenol antioxidant, found in the fruit of this plant helps in the treatment of various diseases such as cancer. It is also used to prevent the oxidative damage of our cells, DNA and lipids. Another constituent of this fruit quercetin, which is a flavonoid with antioxidant properties, exhibits various health benefits. It is also used for the treatment of cardiovascular disease, lung cancer and osteoporosis. Podophyllotoxin, an aryl tetralin lignan, is also a potent antiviral and antitumour agent along with anti-inflammatory and immunosuppressive properties (Mounia et al. 2012). Beta-sitosterol, which is present in rhizomes of *Sinopodophyllum*, is a phytosterol with chemical structure similar to that of cholesterol. It lowers blood cholesterol levels (Rudkowska et al. 2008) and is also known for its potential to reduce benign prostatic hyperplasia (BPH). Himalayan mayapple provides us with many important medicaments and is used as a folk medicine in both Indian Ayurveda and traditional Chinese medicine. Human disturbances have destroyed the natural habitat of these plants, which bring them to the list of endangered species. It is described as endangered species in the list of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Lata et al. 2010). Thus, immediate action is required to protect this important species.

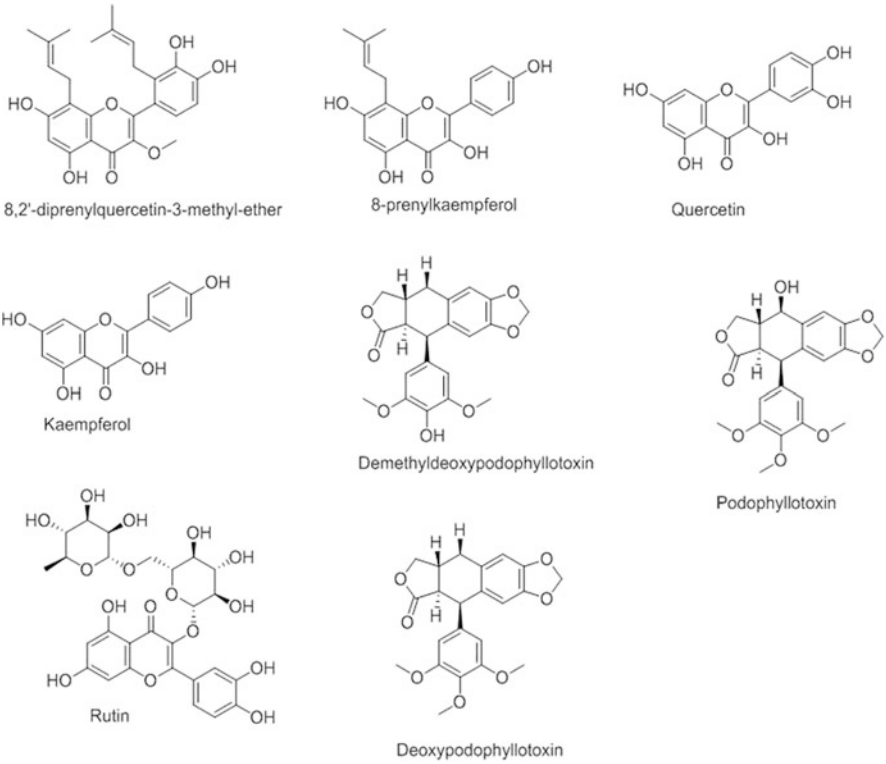


**Fig. 14.11** Main chemical constituents of *Sinopodophylli fructus*

### 14.3.5.4 Pharmacological Applications of *Sinopodophyllum hexandrum*

#### 14.3.5.4.1 Radioprotection of *Sinopodophyllum hexandrum*

*Sinopodophyllum hexandrum* has been known for its radioprotective activity which includes radical scavenging and cell cycle arrest-related activities such as in vitro and in vivo models. It contains a large number of bioactive molecules such as flavonoid and lignans which have antioxidant and antiapoptotic potential and hence helps in radioprotection. It has been observed that it gives approximately 70–95% radioprotection when administered in mice for 1–2 h before lethal whole body 10 Gy radiation.

#### 14.3.5.4.2 Anti-inflammatory Activity of *Sinopodophyllum hexandrum*

The aqueous abstract of *Sinopodophyllum* can be used for various therapeutic purposes and it shows various anti-inflammatory properties (Prakash et al. 2005).

#### 14.3.5.4.3 Insecticidal Activity of *Sinopodophyllum hexandrum*

When dichloromethane extracts of *Sinopodophyllum* is administered in larvae of *D. melanogaster*. It has been found to give LC50 value 0.24 micromol/mL, while in

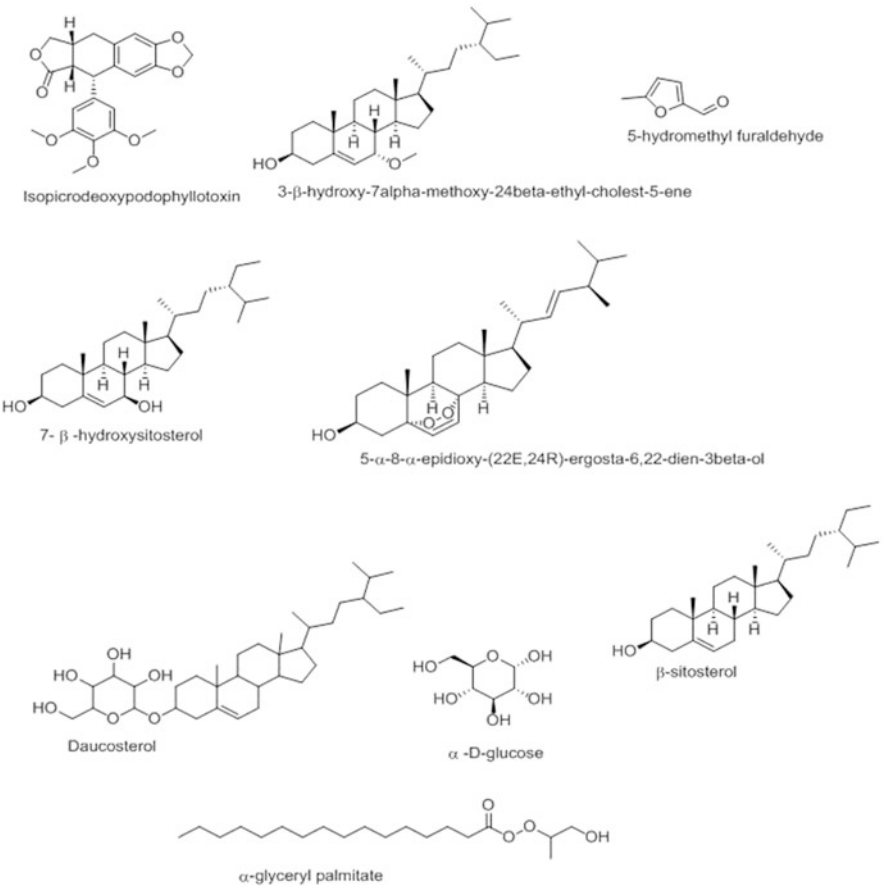


**Fig. 14.12** Main constituents of roots and rhizomes of *Sinopodophyllum*

adults it gives LD50 value of 22 micrograms/adult whereas acetyl podophyllum shows less insecticidal activity, which indicates that 4-hydroxyl group was an important entity in order to increase the activities (Miyazawa et al. 1999).

#### 14.3.5.4.4 Anticancer Activity of *Sinopodophyllum hexandrum*

The precursor for the synthesis of various anticancer drugs such as etoposide, teniposide and etopophose can be prepared from podophyllotoxin (ptox), which is a therapeutically important ligand obtained from *Podophyllum hexandrum* (Bhattacharyya et al. 2016). The demand of podophyllum has been increasing day by day due to its various anticancer properties. Manufacturing of this drug is very expensive. The major issue for the manufacturing of this drug faced by the pharmaceutical companies is the availability of compound from natural resources (Kumar and Dhillon 2015). The root of sinopodophyllum contains various lignans such as podophyllotoxin, podophyllin and bebeerine which shows various activities such as

inhibition of microtubule assembly and treatment of hepatoma, lung cancer, testicular cancer and other tumour (Chattopadhyay et al. 2001).

#### 14.3.5.4.5 Antifungal Activity of *Sinopodophyllum hexandrum*

It also shows antifungal activity against *Aspergillus niger* and *Candida albicans,* which can be determined by using disc diffusion method with minimum inhibitory concentration (MIC) of 16.66 mg/mL in *Aspergillus* and 25 mg/mL for *Candida albicans* (Wani et al. 2013).

### 14.3.5.5 Toxicological Data of *Sinopodophyllum hexandrum*

Sinopodophyllum is highly poisonous and should be taken only under the supervision of a doctor. If consumed without the supervision of a qualified practitioner, it causes various side effects such as severe stomach pain, hallucinations, low blood pressure, kidney failure. It also leads to abortion in women. For the cure of genital warts caused by human papilloma virus (HPV), 0.5% podophyllotoxin gel is applied twice a day for 3 days in a row and repeated for two to four cycles.

## 14.4 Why High-Altitude Plants Are Different from Low Elevation Plants?

The plants growing in high-altitude regions have many physical and chemical adaptations for their growth in high-altitude regions as compared to plants of low-altitude regions with no such adaptations. High-altitude plants have several properties due to which they withstand harsh climatic conditions like the direct sunlight, snowfall, and even very low temperature conditions. The main primary atmospheric changes associated with high-altitude plants are decrease in partial pressure of all atmospheric gases which includes the most important, that is, oxygen and carbon dioxide gases and also there is a decrease in total atmospheric pressure, atmospheric temperature reduction with humidity. There is an increase in radiations under the cloudless sky and higher UV-B radiation fraction under total solar radiation. Larcher and Körner have made a large number of surveys on the adaptive strategies expressed by the plants of mountainous regions. UV-rays concentration is higher at the higher altitudes which destroy the auxin content at the apical shoots, which results in small and bushy plants at high altitudes. The metabolic adaptations are studied by Streb et al. (1997). Wildi and Lütz studied antioxidant properties and pigments of plants of high-altitude regions. The plants of high-altitude region also have needle-shaped leaves for less rate of transpiration from their surface, whereas the plants of low-altitude regions have no such adaptations for their development and growth. They occupy the large surface area for their growth with normal size of stomata and no other adaptations as present in high-altitude plants.

## 14.5 Conclusion and Future Direction

A major geographical area of the Jammu and Kashmir state of India, that is, 70% is covered by Ladakh, which is situated in northernmost part of the country in outer Himalayas. Ladakh is also known as cold arid desert. Although it has adverse climatic conditions, it is the home of various plant species, some of which have great medicinal importance. These plants species have been traditionally used by the local residents for various diseases and gradually they are used as medicinal plants. In this chapter we have made an attempt to gather all the information on five important medicinal plants of high-altitude Indian Himalayan region, which are *Aconitum heterophyllum*, *Hippophae rhamnoides*, *Inula racemosa*, *Rhodiola rosea* and *Sinopodophyllum,* which are used in Indian medicine system, Ayurvedic system. They show many pharmacological properties such as anti-inflammatory, antifungal, anticancer, and antidepression. The maximum yield of medicinal plants can be harvested from the areas of high-altitude regions. Some of the plants species are categorized as endangered. So in order to protect and conserve these plant species, we have to take the following measures, that is, in situ conservation by the establishment of nature reserves or biospheres, huge cultivation of medicinal plants, the various uses of medicinal plants must be spread among the farmers, so they are commercially cultivated. The listed measures and sustainable utilization of these resources will provide a powerful tool towards conservation of medicinal plants. As these plants are of great medicinal and economic value, so their conservation is also important.

**Acknowledgments** The authors would like to acknowledge CSIR and DST-SERB for financial support through research grants HCP0007, HCP0008 and GAP2185.

**Conflict of Interest** The authors declare no conflict of interest.

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# Medicinal Plants of District Kupwara Used in the Treatment of Human Diseases and Their Associated Biological Functions

15

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**Abstract**

Exploration and documentation of plants for novel active ingredients as a means of resource mapping and introduction of new species of plants in new environments are among one of the oldest activities of mankind. Since the beginning of human civilization, researchers have collected much new useful information on plants far away from different geographical locations. With respect to the total land cover, the Kashmir Valleys in Jammu and Kashmir (J&K) region of Himalaya are floristically little less explored due to international boarder problems, especially along the LoC regions of India, China, and Pakistan. There is no doubt that the Himalayan ecosystem contains rich resources of unique medicinal and otherwise economically valued plants, and more than 50% of India's documented biodiversity is from these regions. District Kupwara is one of the twenty-two districts of the J&K Union Territory and is situated in the

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_15

Northwestern part of the Kashmir Himalayas. Review of literatures and search for deposited herbarium samples in Janaki Ammal Herbarium (RRLH) at the CSIR-Indian Institute of Integrative Medicine (Jammu) indicated that the Kupwara region is taxonomically very less explored and there is no evidence of plant collection deposited at RRLH. However, since the district is part of the Himalaya, we can predict that it is also a rich repository of biodiversity and has very unique natural resources in the form of food, medicine and otherwise economically beneficial plants. While studying the phytodiversity composition of Kupwara, a total of 159 species of plants with unique constituents of chemical compounds were documented. These medicinal-value species include 17.61% tree species, 5.03% shrub, 75.47% herbs and 1.8% climbers/or lianas. The dominant families include Asteraceae, Rosaceae, Lamiaceae, Ranunculaceae, Brassicaceae, Solanaceae, Amaranthaceae and Plantaginaceae. Some of the common high-value medicinal plants growing in the region are *Artemisia absinthium, Euphorbia wallichii, Rheum australe*, *Sinopodophyllum hexandrum, Saussurea costus, Taraxacum officinale, Urtica dioica,* and several other typical high-altitude Himalayan plant species. These medicinal plants are used in the treatment of obesity, liver infection, diabetes, intestinal infections, rheumatism, tumors, stomach-ache, insomnia, nerve troubles, skin infection, aphrodisiac, memory-related disorders, and asthma. Several studies have highlighted the pharmacological activities of these plants as anti-microbial, anti-inflammatory, anti-oxidant, anti-cancerous, and other properties that have immense importance in drug research. Therefore, there is an urgent need for proper documentation and research on valuable plants growing in hotspots like Kupwara in the Himalaya and elsewhere across the globe for conservation of biodiversity and for knowledge enrichment through value addition and product development from plants.

**Keywords**

Plant diversity · Medicinal wealth · Kupwara district · Kashmir Himalayas · India

## Abbreviations

| | |
|---|---|
| Lv | Leaves |
| B | Bulbs |
| Ae | Aerial parts |
| Wp | Whole plant |
| R | Roots |
| Rz | Rhizomes |
| Fl | Flowers |
| Fr | Fruits |
| Se | Seeds |
| St | Stolons |
| If | Inflorescences |
| Tu | Tubers |
| R&D | Research and developmental activities |

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## 15.1 Introduction

Humans' long interactions with the environment have witnessed the use of herbal medicine (Singh and Bedi 2017; Singh 2019a), and written accounts testify that most of the Himalayan herbs are useful as medicines (Singh et al. 2016; Singh 2019a). Through constant associations with forests, different ethnic groups residing in Valleys and Hills of Himalaya have accumulated immense knowledge of medicinal plants, which passes from one generation to the next (Rao and Shanpru 1981; Chhetri 1994; Singh et al. 2010; Singh and Borthakur 2011; Singh 2019b). A lot of knowledge about traditional medicinal wealth is documented in literature like the Vedas (6500–400 BC). The system of medicine called Ayurveda has mentioned the use of plants for their therapeutic potential and provides use of 290 herbal drugs (Manandhar 1980). Plants have been used as folklore medicine all over the world for centuries and indigenous communities have developed their own specific knowledge on plant resources, uses, management, and conservation (Ajaib et al. 2010; Singh and Shanpru 2010; Singh et al. 2012). Ethnobotanical studies are tradition which deals with the interaction of humans with the environment. It follows several approaches involving biological and social aspects (Jain 1987; Waller 1993; Martin 1995). Ethnomedicinal treatment is not merely a medical system but part of human culture. The collaborative efforts of many research institutions and companies have used the indigenous knowledge of the people related to the medicinal plants as an assumption in the field of drug discovery (Farnsworth 1993). The use of plants as indigenous herbs is rapidly increasing due to the minimal side-effects and accessibility, and because they sometimes are the only source of healthcare easily available to the poor communities (Cotton 1996). However, the key issue in the current era is the loss of indigenous knowledge associated with medicinal plants, and documented research of traditional knowledge may serve as a guideline to the plant-based therapeutic research for many scientists in India as well as around the world.

In India, traditional healers possess the rich medicinal knowledge of approximately 2500 plant species (WWF1990) and more than hundreds of plant species are used as regular sources of medicine. Knowledge of ethnomedicine is considered a main source of regional economic development and new drug development (Zheng 1997; Xiao 1999; Singh et al. 2018; Thakur et al. 2019; Singh 2020). Rich medicinal wealth is found in the northern regions. To date, 8644 plant species have been reported from the Indian Himalaya, of which 1784 plant species are important sources of phytomedicine (Samant et al. 1998; Singh 2015). The Tibetan Amchi system of medicine so called 'Sowa-Rigpa' reported from Ladakh is one of the well known medical tradition very much similar to Ayurveda and serving as a lifeline for indigenous communities living in high altitude mountain of Himalaya (Raghunathan 1976; Srivastava and Gupta 1982, Visvanath and Mankad 1984; Singh and Hajra 1996; Nawchoo and Buth 1989; Kaul et al. 1995). Indigenous people have an age-old association with forests and have rich knowledge of medicinal plants (Ticktin 2004; Edidiones et al. 1980; Singh et al. 2014). This ethnomedicinal knowledge passes from one generation to the future generation. In select pockets of forests, the trade of few potentially endangered medicinal plants such as *Trillium*

*govanianum* Wall. ex D.Don (Nag-Chatri), *Lilium polyphyllum* D.Don ex Royle (Kakoli or Himalayan white Lily), *Habenaria dentata* (Sw.) Schltr., *Saussurea obvallata* (DC.) Edgew. (Brahma Kamal) and several other Himalayan plant species has become the source of income for the local people (Kala 2005). Now efforts are being made to enact laws to prevent the trade of these plants.

## 15.2 Material and Methods

### 15.2.1 Study Area

District Kupwara (latitude 34°31′15″N, longitude 74°15′37″E) is a remote district of the Kashmir division (J&K), with a geographical area of 2379 $km^2$. The study area is located at an elevation of 1589 m, approximately 114 km away from the summer capital of Srinagar. The district is surrounded by lofty mountains with diverse vegetation and rich diversity of potential medicinal plants. The famous river Kishanganga flows from East to West in this area, serving as the main source of irrigation to the areas under cultivation. The district is home to local Himalayan tribes, which keep moving along with their livestock to different grazing sites in higher-altitude regions. The population of the area can be categorized as (a) *Dards*: this group is found in high-altitude regions and some of them have migrated to the main valley, (b) *Kashmiris*: they occupy the main valley and they are in majority, (c) *Gujjars*: they live in the surrounding areas of the main valley, (d) *Pathans*: they are interspersed in some areas and are the smallest minority, (e) *Bakarwals*: they occupy the high-altitude regions where sufficient grass is available. The luxuriant biodiversity of the study area is characterized by subtropical to alpine climate and by a severe winter season (almost 4 to 5 months), followed by summer and monsoon seasons. The average annual temperature is 14.1 °C. The months of March and April receives the highest rainfall. The climatic variables determine that the local vegetation is composed of subtropical, temperate, and alpine species. The vegetation is dominated by semi-evergreen to coniferous forests at higher altitudes; forests are interspersed with scrubs. Common tree elements of this region are *Cedrus deodara, Morus alba, Platanus orientalis,* and *Salix disperma*. The dominant shrubby species are *Berberis lycium* and *Indigofera heterantha.*

### 15.2.2 Data Analysis

Botanical exploration field studies were carried out to explore the association and dependence of the local people with the environment. Interviews, questionnaires and field discussions were carried out to document the indigenous knowledge of the local people belongs to different community groups. A total of 151 local individuals between the age group of 18-95 were interviewed. Among these individuals, female informants were few in number because of the social setup in the communities. The numbers of male individuals was 120 and the female informants were

**Table 15.1** Cartographic details of informants of District Kupwara

| Categories based on the age of informants | | | | |
|---|---|---|---|---|
| | Informants | | | |
| Age group* | Spearman ranking | Male informants (%) | Spearman ranking | Female informants (%) |
| 15–25 | 4 | 9.16 | 5 | 9.60 |
| 25–35 | 3.5 | 10.83 | 3 | 16.10 |
| 35–55 | 2.5 | 25.00 | 1 | 29.03 |
| 55–65 | 2.5 | 25.00 | 2 | 22.50 |
| 65–75 | 3 | 15.83 | 4 | 12.90 |
| 75–85 | 3.5 | 10.83 | 6 | 6.45 |
| 85–95 | 1 | 33.00 | 7 | 3.22 |

*lower limit in age group was not considered for data analysis

31 (Table 15.1). Field visits were performed during different seasons. Information was also collected to know the time of flowering. Plants were collected and identified by taking the help of different web-sites like The Plant List (http://www.theplantlist.org/), IPNI (http://www.ipni.org), Tropicos (https://www.tropicos.org/) and Index Kewensis 2.0 (1997) for botanical nomenclature of species, species citation, and matching with online floras, and deposited vouchers of the Janaki Ammal Herbarium (acronmy RRLH) of CSIR-Indian Institute of Integrative Medicine, Jammu. Around 159 plant specimens were collected with photographs, identified and later deposited at the RRLH herbarium. The usages, plant parts used, diseases and the mode of administration of the medicinally important plants were recorded from the people. Meetings were arranged with village heads and councillors in order to seek permission as well as guidance to visit the study area. The ethnic communities such as *Dards*, *Gujjars*, *Bakerwals*, and *Pathans* living in the valleys of Kupwara district. *Bakerwals* are the most important tribe concentrated in the upper region of the valley, doing cultivation in the rain-fed areas. They also possess rich knowledge of important high-altitude medicinal plants.

## 15.3 Results and Discussions

The present research comprises a total of 159 species of high-value medicinal plants placed under 132 genera and 59 families, having ethnomedicinal value in District Kupwara (Table 15.2). In term of growth-forms, herbs were the dominant group of plant communities, represented by 120 species (75%), followed by trees twenty-eight species (18%), shrubs eight species (5%), and climbers three species (2%) in the study area (Fig.15.1). In terms of family composition, Asteraceae holds the most dominant family status, representing most of the plant species of medicinal importance, and Orchidaceae is the least dominant family. The top five dominant families recorded during the study are Asteraceae, Rosaceae, Ranunculaceae, Brassicaceae, and Solanaceae (Table 15.3). The local people from different studied localities were

**Table 15.2** Medicinally important plants and their pharmacological applications in District Kupwara, Jammu and Kashmir Himalayas

| Botanical name | Family | Common name | Growth-form | Occurrence | Part Used | Indication |
|---|---|---|---|---|---|---|
| *Abies pindrow* (Royle ex D.Don) Royle | Pinaceae | Pindrow fir | Tree | Wild | Lv | Anti-bacterial, anti-inflammatory, anti-septic and anti-diabetic |
| *Acer caesium* Wall. ex Brandis | Sapindaceae | Indian maple | Tree | Wild | B, Lv | Muscular swelling, boils, and pimples |
| *Achillea millefolium* L. | Asetraceae | Devil's nettle | Herb | Wild | Ae | Gum, inflammation, fever, bloating, gas, and toothache |
| *Aconitum heterophyllum* Wall. ex Royle | Ranunculaceae | Atees | Herb | Wild | R | Diarrhea, high fever, swelling of throat, vomiting, cough, pain in stomach, and abdomen |
| *Aconitum chasmanthum* Stapf ex Holmes | Ranunculaceae | Mohund | Herb | Wild | R | Back pain, throat infection, and chest pain |
| A*corus calamus* L. | Acoraceae | Nagruss | Herb | Wild | R | Pain in stomach, diarrhea, fever, anti-rheumatic, hepatic, and kidney trouble and insecticide |
| *Actaea spicata* var. *acuminata* (Wall. ex Royle) H.Hara | Ranunculaceae | Beneberry | Herb | Wild | R | Nerve sedative and rheumatism |
| *Adiantum venustum* D.Don | Pteridaceae | Maiden hair | Herb | Wild | Wp | Diarrhea, digestive stomach pain, diuretic and expectorant |
| *Ailanthus altissima* (Mill.) Swingle | Simoroubaceae | Tree of heaven | Tree | Wild & Cultivated | R, B | Diarrhea, asthma, cramps, epilepsy, and fast |
| *Ajuga integrifolia* Buch.-Ham. ex D.Don (= *Ajuga bracteosa* Wall. ex. Benth) | Lamiaceae | Jainadum | Herb | Wild | Wp | Blood purifying agent, fever, anti-rheumatic, hair tonic, pain in abdomen, and wound healing |
| *Ajuga parviflora* Benth. | Lamiaceae | Neelkanthi | Herb | Wild | Wp | Antimicrobial, astringent, hypertension, and hepatitis |
| *Althea rosa* L. | Malvaceae | Sazeposh | Herb | Wild | Fl, R | Throat swelling, infection, asthma, cough, urinary infection |
| *Amaranthus caudatus* L. | Amaranthaceae | Lissa | Herb | Cultivated | Lv, Inf | Expectorant and high fever |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Amaranthus viridis* L. | Amaranthaceae | Gunhar/ wasthalk | Herb | Cultivated | Wp | Analgesic and anti-pyretic |
| *Lysimachia arvensis* (L.) U.Manns & Anderb. (= *Anagallis arvensis* L.) | Primulaceae | Scarlet-pimpernel | Herb | Wild | Wp | Allergy and killing of lice |
| *Anaphalis nubigena* DC. | Asteraceae | Daderi-dawa | Herb | Wild | Wp | Skin eruption and wounds |
| *Anaphalis royleana* DC. | Asteraceae | Pearly everlasting | | Wild | | Bruises, burns, cuts, best used for head ache, anodyne, diuretic, and laxative |
| *Anemone obtusiloba* D.Don | Ranunculaceae | Ratanjog | Herb | Wild | Fl | Warming body, wound healing and chronic bronchitis, diuresis, detumescence and enriching blood |
| *Aquilegia vulgaris* L. | Asteraceae | Dadue- jaid | Herb | Wild | Wp | General weakness in livestock and enhancing milk production |
| *Arctium lappa* L. | Asteraceae | Burdock | Herb | Wild | Wp | Breast cancer, diabetes, wrinkled skin, fluid retention, stomach conditions, gout, and acne |
| *Arisaema jacquemontii* Blume | Araceae | Hapat makei | Herb | Wild | R | Boils |
| *Arnebia benthamii* (Wall. ex G. Don) I.M.Johnst | Boraginaceae | Kahazaban | Herb | Wild | R | Disease of tongue, throat, heart, and fever |
| *Artemisia absinthium* L. | Asteraceae | Teethwen | Herb | Wild | Lv | Abdominal pain and chronic fever |
| *Artemisia moorcroftiana* Wall. | Asteraceae | Jangle Teethwen | Herb | Wild | Lv | Abdominal pain, high fever, and vermicide |
| *Asplenium falcatum* Lam. | Aspleniaceae | Birds Nest Fern | Herb | Wild | Wp | Burn, expectorant, head ache, and sterility in women |
| *Atropa acuminata* Royle ex Lindl. | Solanaceae | Indian belladona | Herb | Wild | Wp | Asthma and rheumatic, pain, eye disease, and cough |
| *Avena fatua* L. | Poaceae | common oat | Herb | Wild | R | Diuretic, emollient, diuretic, and refrigerant |
| *Barbarea intermedia* Boreau | Brassicaceae | Winter cress | | Wild | | Pain, wounds, indigestion, scurvy, and lung cancer |

(continued)

**Table 15.2** (continued)

| Botanical name | Family | Common name | Growth-form | Occurrence | Part Used | Indication |
|---|---|---|---|---|---|---|
| *Barbarea vulgaris* R.Br. | Brassicaceae | Bittercress | Herb | Wild | Se | Lung cancer, indigestion, scurvy, and blood impurity |
| *Berberis aristata* DC. | Berberidaceae | Dandledar | Herb | Wild | Fr, Lv | Jaundice, back pain, weakness, and fractures |
| *Berberis lycium* Royle | Berberidaceae | Kawdach | Herb | Wild | Fr, Lv | Antidiabetic, abdominal disorders, and eye diseases |
| *Bergenia ciliata* (Haw.) Sternb. | Saxifragaceae | Winter bigonia | Herb | Wild | R | Fever, asthma, cut and burns, diarrhea, inflammation, ear ailments, and anti-septic |
| *Betula utilis* D.Don | Betulaceae | Burza | Shrub | Wild | Wp | Kidney disorders, anti-inflammatory, and cleaning the wounds |
| *Brassica campestris* L. | Brassicaceae | Field Mustard | Herb | Wild | Rz | Vaginal discharge, ant periodic, inflammation of urethra general body weakness, and internal pain |
| *Brassica oleracea* L. | Brassicaceae | Haakh | Tree | Cultivated | B, Lv | Gout and rheumatism, diuretic, laxative, anti-helminthic, and stomachic |
| *Cannabis sativa* L. | Cannabaceae | Bang | Herb | W | Se | Diarrhea, cholera, rheumatism, wormicide, and skin disease and narcotic |
| *Capsella bursa-pastoris* (L.) Medik. | Brassicaceae | Shepherd's purse | Herb | wild | L | Anti-ascorbutic, astringent, diuretic, emmenagogue, haemostatic, hypertensive, oxytocic, stimulant, vasoconstrictor, vasodilator, and vulnerary |
| *Cardamine impatiens* L. | Brassicaceae | Narrow leaf bittercress | Herb | wild | Lv, S | Antirheumatic, diuretic, and stimulant |
| *Cassiope fastigiata* (Wall.) D.Don | Ericaceae | Himalayan heather | Herb | wild | Wp | Flatulence, indigestion, diabetes, chest pain, and ring worm |
| *Cedrus deodara* (Roxb. ex D. Don) G.Don | Pinaceae | Deodar | Herb | wild | Lv, S | Antiviral, antispasmodic, astringent, insecticide, diuretic, and diarrhea |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Celtis australis* L. | Cannabaceae | Lote tree | Sub-shrub | cultivated | R, S | Leaves and fruits are astringent, lenitive, and stomachic |
| *Chenopodium album* L. | Amaranthaceae | Buthwa | Tree | wild | Lv | Anti-rheumatic, anti-phlogistic, laxative, and contraceptive |
| *Chrysanthemum pyrethroides* (Karelin & Kir. | Asteraceae | Chrysanths | Tree | wild | Lv, Fr | Insecticide, and scabies |
| *Cichorium intybus* L. | Asteraceae | Common chichory | Herb | wild | Lv, S | Rheumatic pain, high fever, internal ulcer, stomach disease, and blood purifier |
| *Cirsium arvense* (L.) Scop | Asteraceae | Creeping thistle | Herb | wild | Fl | Tonic, diuretic, astringent, and hepatic, anti-inflammation |
| *Clinopodium vulgare* L. | Lamiaceae | Wild basil | Herb | wild | Wp | Astringent, cardiac stimulant, and reduces flatulence |
| *Codonopsis rotundifolia* Benth. | Campanulaceae | Posh-hand | Herb | wild | R | Livestock medicine used against asthma, and general weakness |
| *Convolvulus arvensis* L. | Convolvulacaeae | Hiranpudi | Herb | wild | Wp | Fever, urinary tract problem, laxative, increasing bile production, and laxative |
| *Coriandrum sativum* L. | Apiaceae | Dhaniwal | Herb | Cultivated | R | Loss of appetite, diarrhea, hernia, and nausea |
| *Corydalis govaniana* Wall. | Papaveraceae | Govans corydalis | Herb | Cultivated | R, Lv, Fl | Appetizer, diuretic, skin infection, and anti-inflammatory |
| *Cotoneaster affinis* Lindl. | Rosaceae | Purpleberry | Herb | Cultivated | Lv, S | Astringent |
| *Crataegus songarica* K.Koch | Rosaceae | Asian hawthorn | Herb | Wild | Wp | Congestive heart failure, coronary circulation problem diarrhea, and abdominal pain |
| *Cynodon dactylon* (L.) Pers. | Poaceae | Duub | Shrub | Wild | St | Anti-cancer, anti-diabetic, anti-diarrheal, eye disease, and nose bleeding |
| *Dactylorhiza hatagirea* (D.Don) Soó | Orchidaceae | Salam panja | Tree | Wild | Fr | Inflammation of gum and teeth |
| *Datura strmonium* L. | Solanaceae | Jimsonweed | Herb | Wild | Wp | Intoxicating, asthma, teeth pain, asthma, dandruff, antiseptic, and narcotic |

(continued)

**Table 15.2** (continued)

| Botanical name | Family | Common name | Growth-form | Occurrence | Part Used | Indication |
|---|---|---|---|---|---|---|
| *Daucus carota* L. | Apiaceae | Carrot | Herb | Cultivated | Lv | Bladder problem, gout diarrhea, and kidney problems |
| *Delphinium roylei* Munz. | Ranunculaceae | Larkspur | Herb | W | Wp | Intestinal worms, poor appetite, and insomnia |
| *Dioscorea deltoides* Wall. | Dioscoridaceae | Nepal yam | Herb | Cultivated & wild | R | Asthma, indigestion, arthritis, and ape worms |
| *Dryopteris barbigera* (T.Moore ex Hook.) Kuntze | Dryopteridaceae | Wood fern, malefern | Herb | Wild | Se, R | Worm expellant |
| *Duchesnea indica* (Jacks.) Focke. | Rosaceae | Mock strawberry | Herb | Wild | Tu | Anti-coagulant, anti-septic, and febrifuge |
| *Elsholtzia densa* Benth. | Lamiaceae | Dense Himalayan mint | Herb | Wild | Rz | Headaches, colds, diarrhea, and arthritis |
| *Equisetum arvense* L. | Equistaceae | Gandumgud | Herb | Wild | Lv, Fl | Acidity, weakness, kidney infection, scabies, and tooth aches |
| *Erigeron canadensis* L. | Asteraceae | Canadian horseweed | Herb | Wild | Wp | Anti-microbial, anti-oxidant, anti-coagulant, anti-inflammatory, and anticancer |
| *Euphorbia helioscopia* L. | Euphoribaceae | Gurisochal | Herb | Wild | Ae | Vermifuge, anti-cancer, and cholera |
| *Fagopyrum esculentum* Moench | Polygonaceae | Buck wheat | | Wild | | Intestinal worms, hypertension, and diabetes |
| *Ficus carica* L. | Moraceae | Common fig | Herb | Wild | Wp | Metabolic, cardiovascular, respiratory, anti-spasmodic, and anti-inflammatory remedy |
| *Foeniculum vulgare* Mill. | Apiaceae | Badiyan | | Cultivated | | Disease of chest, spleen, and kidney and vermicide |
| *Fragaria nubicola* (Lindl. ex Hook.f.) Lacaita | Rosaceae | Strawberry | Herb | Wild& cultivated | Lv, Se | Treatment of profuse menstruation and to treat blemishes on the tongue |
| *Fritillaria roylei* Hook. | Lilliaceae | Sheethkar | Herb | Wild | Lv | Asthma, rheumatic, and ophthalmic |

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|---|---|---|---|---|---|---|
| *Galium palustre* L. | Fabaceae | Common marsh bedstraw | Tree | Wild | Fr | Diuretics, astringents, stomachic, gout, and epilepsy |
| *Gentiana kurroo* Royle. | Gentiananaceae | Nilkanth | Herb | Wild | Se | Anti-inflammatory, anti-helminthic, refrigerant, and stomachic |
| *Geranium pratense* L. | Geraniaceae | Meadow cranes bill | Herb | Wild | Fr | Healing pain, relieving stress, boosting the immune system and improving the digestive system |
| *Geranium robertianum* L. | Geraniaceae | stork's Bill | Herb | Cultivated & wild | B | Antirheumatic, astringent, mildly diuretic, vulnerary, bleeding, stomach ailments, kidney infections, and jaundice |
| *Geranium wallichianum* D.Don ex Sweet | Geraniaceae | Rattanjot | Herb | Cultivated & Wild | Lv, Fr | General body weakness, anti-rheumatic, diarrhea, throat infection, toothache, and removing of dark circles |
| *Hackelia uncinata* C.E.C.Fisc | Boraginaceae | Hooked stick Se | Herb | Wild | R | Wound healing, expectorant, treatment of coughs, and sores |
| *Hedera helix* L. | Araliaceae | European evy | Herb | Wild | R | Bronchitis, lever disorders, spleen disorders, joint pain, and swelling, ulcers, and parasites |
| *Hedera nepalensis* K.Koch | Araliaceae | Himalayan evy | Herb | Wild | Lv | Anticancer and cytotoxic |
| *Helianthus annus* L. | Asteraceae | Gulaftab | Herb | Cultivated | R | Diuretic, expectorant, snake bites, and high fever |
| *Heracleum candicans* Wall. ex DC. | Apiaceae | Hodge weed | Herb | Wild | Fl | Aphrodisiac, tonic, colic, digestive, anti-pyretic, diaphoretic, itching, skin disorders, and menstrual disorders |
| *Hyoscyamus niger* L. | Solanaceae | Black Henbane | Herb | Cultivated & wild | Lv | Rheumatism, toothache, asthma, cough, nervous diseases, and stomach pain |
| *Hypericum perforatum* L. | Hypericaceae | ST john's wort | Herb | Wild | Lv | General weakness, rheumatism, and urinary irritation |
| *Impatiens brachycentra* Kar. & Kir | Balsiminaceae | Touch-me-not | Herb | Wild | Lv, Fl, R | Cathartic, diuretic, and emetic |

(continued)

**Table 15.2** (continued)

| Botanical name | Family | Common name | Growth-form | Occurrence | Part Used | Indication |
|---|---|---|---|---|---|---|
| *Iris hookeriana* Foster | Iridaceae | Hooker's Iris | Herb | Cultivated | R | Speeding defecation, urination, and against gall bladder disease |
| *Iris napalensis* Wall. ex Lindl | Iridaceae | Mazermund | Herb | Cultivated | Lv | Rheumatic pain, expectorant, and swelling in throat |
| *Juglans regia* L. | Juglandaceae | Doon | Herb | Cultivated | Wp | Anti-bacterial, brain tonic, and sexual weakness |
| *Junierus squamata* Buch.-Ham. ex D.Don | Cupressaceae | Flaky juniper | Herb | Cultivated | Fr, Lv | Skin diseases |
| *Lamium album* L. | Lamiaceae | white nettle | Herb | Wild | R | Anti-spasmodic, astringent, depurative, diuretic, expectorant, hemostatic, sedative, styptic, tonic, and vasoconstrictor |
| *Lavatera cashemiriana* Cambess | Malvaceae | Kashmir mallow | Herb | Wild | R | Demulcent, pectoral, and purgative |
| *Leucanthemum vulgare* (Vaill.) Lam. | Asteraceae | Oxe-eye | Tree | Wild | B, Fr | Wounds, ulcers, and some cutaneous diseases |
| *Linum usitatissimum* L. | Lamiaceae | Flax | Shrub | Wild | WP | Controlling cholesterol, blood sugar level, and diarrhea |
| *Lonicera quinquelocularis* Hard | Caprifoliaceae | Honey Suckle | Herb | Wild | Fr, Lv | Treatment of boils |
| *Malva neglecta* Wallr. | Malvaceae | Sochal | Herb | Wild | Lv | Stomach cramps, nerve tonic wounds, swelling, cough, and ulcer |
| *Malva sylvestris* L. | Malvaceae | Gurisochal | Herb | Wild | Ae | Stomach cramps, diarrhea, and dysentery |
| *Marrubium vulgare* L. | Lamiaceae | White hore hound | Tree | Wild | Se | Liver and gallbladder problems, constipation, loss of appetite, indigestion, coughs, colds, skin damage, ulcers, wounds |
| *Matricaria chamomilla* L. | Asteraceae | Fuckgrass | Tree | Wild | Fr. | Insecticide and fungicide |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Mentha piperita* L. | Lamiaceae | Watermint | Herb | Cultivated | Lv | Irritable bowel syndrome, heart burn, migraine headache, and tension head ache |
| *Mentha arvensis* L. | Lamiaceae | Corn mint or Field mint | Herb | Cultivated | Wp | Appetizer, useful in gastric troubles, treat flatulence, digestive problems, gall bladder problems, and coughs |
| *Mentha longifolia* (L.) L. | Lamiaceae | Jangle pudina | Herb | Wild and cultivated | Wp | Asthma, indigestion, and appetizer |
| *Morus alba* L. | Moraceae | White mulberry | Herb | Cultivated | Wp | Antidiabetic, cardiovascular health, anticancer effects |
| *Morus nigra* L. | Moraceae | Mulberry. | Herb | Cultivated | Lv | Laxative and runny nose |
| *Myosotis arvensis* (L.) Hill | Boraginaceae | Forget me not | Herb | Wild | Lv | Astringent and ophthalmic |
| *Origanum vulgare* L. | Lamiaceae | Baber | Herb | Wild | Lv | Promotes menstrual flow, also used in summer for cooling effect and diuretic fever |
| *Oxalis acetosella* L. | Oxalidaceae | Wood sorrel | Tree | Wild | Fr, R | Anodyne, antiscorbutic, astringent, diuretic, emmenagogue, expectorant, febrifuge, irritant, and stomachic |
| *Oxalis corniculata* L. | Oxalidaceae | Creeping wood sorrel | Tree | Wild | Lv | Anti-malarial, hepatitis B, abdominal pain, and blood purifier |
| *Parrotiopsis Jacquemontiana* (Decne.) Rehder | Hamamelidaceae | Poush/poh | Herb | Wild | Wp | Skin infection and eruption and general body pain |
| *Persicaria amplexicaulis* (D.Don) Ronse Decr. | Polygonaceae | Red bestort | Herb | Wild | Wp | Treatment of fever, joint pain, and flu |
| *Pinus wallichiana* A.B.Jacks | Pinnaceae | Himalayan pine | Herb | Cultivated & wild | Lv | Skin infection, wounds, sores, burns, and treatment of cough |
| *Plantago lanceolata* L. | Plantaginaceae | Gul | Herb | Wild | Wp | Cough, asthma, urinary infection, and boils and wound healing |
| *Plantago major* L. | Plantaginaceae | Budgull | Shrub | Wild | S | Fever, dysentery, back pain, anti-rheumatic, cough, and urinary infection |

(continued)

**Table 15.2** (continued)

| Botanical name | Family | Common name | Growth-form | Occurrence | Part Used | Indication |
|---|---|---|---|---|---|---|
| *Platanus orientalis* L. | Platanaceae | Oriental plane | Herb | Cultivated & wild | R | Anti-oxidant and anti-inflammatory agent |
| *Polygonatum acuminatifolium* Kom. | Asparagaceae | King solomon's seal or solomon's seal | Tree | Wild | Res. | Pain, fever, inflammation, allergy, and weakness |
| *Populus ciliata* Wall. ex Royle. | Salicaceae | Himalayan poplar | Herb | Cultivated | Lv | Blood purifier and tonic stimulant, muscular swelling in cow, rheumatism and muscular swelling due to menstruation |
| *Populus nigra* L. | Salicaceae | Black poplar | Herb | Cultivated | Lv | Curing scurvy, disinfectant, diaphoretic, diuretic, expectorant, blood purifier, stimulant, tonic vulnerary, astringent, diuretic, and tonic |
| *Portulaca oleraceae* L. | Portulaceae | Nunner | Tree | Wild | Lv, B | Ulcer liver, heart, kidney, bladder diseases and cough |
| *Potentilla nepalensis* Hook. | Rosaceae | Nepal cinquifoil | Herb | Wild | R | Burns |
| *Prunus avium* (L.) L. | Rosaceae | Wild cherry | Tree | Cultivated | B | Astringent, diuretic, and tonic |
| *Prunus cerasus* L. | Rosaceae | Sour cherry | Tree | Cultivated | Bd, B | Astringent, bitter and febrifuge, fevers, coughs and colds |
| *Prunus cornuta* (Wall. ex Royle) Steud. | Rosaceae | Himalayan bird cherry | Herb | Cultivated | Lv | Stimulates respiration, improves digestion and gives a sense of well-being |
| *Prunus persica* (L.) Batsch | Rosaceae | Peach | Herb | Cultivated | R | Insect killing, vermicide, and wound healing |
| *Prunus tomentosa* Thunb | Rosaceae | Nanking cherry | Tree | Cultivated | Fr | Stimulates respiration, digestive problems and gives a feeling of well-being |
| *Pteris cretica* L. | Pteridaceae | Cretan brake | Tree | Wild | R | Anti-bacterial |
| *Pyrus communis* L. | Rosaceae | European pear or common pear | Tree | Wild | Fr | Diuretic, obesity, hypertension, Analgesic, suitable for diabetes |
| *Pyrus pashia* Buch.-Ham. ex D. Don | Rosaceae | Wild Himalayan pear | Tree | Wild | Lv | Conjunctivitis and diarrhea |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Quercus baloot* Grif | Fagaceae | Holam oak | Tree | Wild | Fr | Astringent, anti-bacterial, anti-fungal, anti-septic, styptic and hemostatic, acute diarrhea, dysentery and hemorrhages, toothache or gum problem and injury due to cuts |
| *Ranunculus arvensis* L. | Ranunculaceae | Corn butter cup | Herb | Wild | Lv | Stomach cramps, cough, and appetizer |
| *Ranunculus hirtellus* Royle | Ranunculaceae | softly hairy butter cup | Tree | Wild | B | Anti-swelling |
| *Ranunculus laetus* Wall. ex Hook. f. & J.W.Thomson | Ranunculaceae | Cheerfull buttercup | Tree | Wild | Fr | Wound healing |
| *Ranunculus palmatifidus* Riedl | Ranunculaceae | Reidl | Tree | Wild | Wp | Anodyne, anti-spasmodic, diaphoretic, and rubefacient |
| *Raphanus sativus* L. | Brassicaceae | Mug | Herb | Wild | Wp | Cold coughs, digestive disorders caused by bile duct, fever, gall stone, loss of appetite, and anti-inflammatory |
| *Rheum australe* D.Don (= *Rheum emodi* Wall.) | Polygonaceae | Pambchalan | Herb | Wild | R | Rheumatic pain and wounds |
| *Rheum webbianum* Royle | Polygonaceae | Rhubarb | Herb | Wild | Lv | Indigestion, astringent, wounds, flatulence and diuretic |
| *Robinia pseudoacacia* L. | Fabaceae | Kikar | Tree | Cultivated | Wp | Wounds and digestive disorders |
| *Rubia cordifolia* L. | Rubiaceae | Indian madder | Herb | Wild | R | Blood disorders, excess heat in the lungs, kidneys, and intestines and reduce swelling |
| *Rubus niveus* Thunb. | Rosaceae | Mysore raspberry | Herb | Wild | R | Dysentery |
| *Rubus ulmifolius* Schott | Rosaceae | Elm leaf black berry | Herb | Wild | R | Treatment of ulcers, redness of eyes, vaginal disorders, anti-inflammatory, diarrhea, hemorrhoids, anti-pyretic and carminative agent |
| *Rumex acetosa* L. | Polygonaceae | Abji | Tree | Wild | Lv | Stomachic, anti-helminthic, and laxative |

(continued)

**Table 15.2** (continued)

| Botanical name | Family | Common name | Growth-form | Occurrence | Part Used | Indication |
|---|---|---|---|---|---|---|
| *Rumex dentatus* L. | Polygonaceae | Toothed Dock | Herb | Wild | R | Chest involvement, asthma, and skin disease |
| *Salix alba* L. | Salicaceae | Veer | Shrub | Cultivated | Lv | Joint pain and osteoarthritis |
| *Salix wallichii* Wimm. ex Andersson | Salicaceae | Neeruvanji | Shrub | Cultivated | Fr | Anodyne and febrifuge. |
| *Saussurea lappa* (Falc.) Lipsch | Asteraceae | Kuth | Herb | Wild | Wp | Cough and asthma |
| *Scrophularia decomposita* Royle ex Benth. | Scrophulariaceae | Fern leaf fig wort | Herb | Wild | Wp | Anti-inflammatory, mildly purgative, stimulant, and treatment chronic skin disease |
| *Sinopodophyllum hexandrum* (Royle) T.S.Ying | Berberidaceae | Wanwangun | Tree | Cultivated & wild | Lv | Acidity, diarrhea, tumor, heart abnormalities, chronic, and constipation |
| *Solanum nigrum* L. | Solanaceae | Black night shade | Tree | Wild | B | Abdominal pain, diuretic, narcotic, heart disease, antiperiodic, and hepatitis |
| *Solanum tuberosum* L. | Solanaceae | Alu | Shrub | Cultivated | R | Peptic ulcers, pain acidity, rheumatic, joint pain, and skin rashes |
| *Sorbaria tomentosa* (Lindl.) Rehder | Rosaceae | Kashmir false spirea | Herb | Wild | Lv, S | Asthma |
| *Sorghum halepense* (L.) Pers. | Poaceae | Durham | herb | Wild | Fr | Antidote, boils, and skin infection |
| *Spergularia rubra* (L.) J.Presl & C.Presl | Caryophyllaceae | Sandwort | Herb | Wild | Lv, Se | Diuretic, treatment of kidney stones, acute and chronic cystitis urinary troubles |
| *Stellaria media* (L.) Vill. | Caryophyllaceae | Nick hack | Herb | Wild | Tu | High fever, stomach ache, pain, chest involvement, and diuretic |
| *Taraxacum officinale* (L.) Weber ex F.H.Wigg | Asteraceae | Madan hand | Shrub | Wild | Fr, Se | Chronic cough, asthma, infection, internal ulcer, abdominal swelling, stomach cramps, and acidity urine irritation |
| *Taxus wallichiana* Zucc. | Taxaceae | Himalayan yew | Herb | Wild | R | Anti-cancer, sedative, and aphrodisiac |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Thymus linearis* Benth. | Lamiaceae | Jangli ajwain or Himalayan thyme | Herb | Wild | Lv | Anti-periodic, diuretic, purgative, and anti-microbial |
| *Trachyspermum ammi* (L.) Sprague | Apiaceae | Jawand | Herb | Wild | Wp | Abdominal pains, bronchial problems, and asthma |
| *Urtica dioica* L. | Utricaceae | Soi | Herb | Wild | Wp | Stomach pain, skin infection, and paralyzed limbs |
| *Viburnum grandiflorum* Wall. ex DC. | Adoxaceae | Kilmish | Tree | Wild | Lv, Fr, B | Alternative, anti-ascorbutic, very mildly diuretic, emmenagogue, febrifuge, and leaves are used in the treatment of scurvy, impurity of the blood |
| *Viola odorata* L. | Violaceae | Banapsa | Herb | Wild | R | Chest disease, cough, cold, diaphoretic, disinfectant, and anti-pyretic |

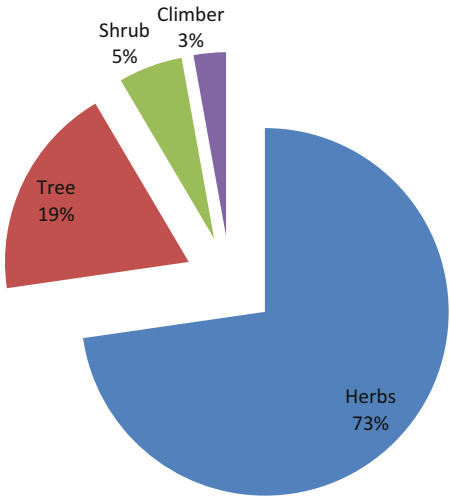


**Fig. 15.1** Life-forms of medicinal plants in District Kupwara

**Table 15.3** Top five medicinally important plant families used by indigenous local communities of Kupwara in Kashmir Himalayas

| Families | No. of species | Percentage contribution | Species under the family |
|---|---|---|---|
| Asteraceae | 15 | 9.37% | *Achillea millefolium, Anaphalis royleana, Aquilegia vulgaris, Arctium lappa, Artemisia absinthium, A. moorcroftiana, Atropa acuminata, Chrysanthemum pyrethroides, Cichorium intybus, Cirsium arvense, Erigeron canadensis, Helianthus annus, Saussurea lappa, Matricaria chamomilla,* and *Leucanthemum vulgare* |
| Rosaceae | 15 | 9.37% | *Cotoneaster affinis, Crataegus songarica, Fragaria nubicola, Potentilla nepalensis, Prunus avium, P. cerasus, P. cornuta, P. persica, P. tomentosa, Pyrus communis, P. pashia, Rubus niveus, R. ulmifolius,* and *Sorbaria tomentosa* |
| Lamiaceae | 9 | 5.66% | *Ajuga integrifolia, A. parviflora, Clinopodium vulgare*, *Elsholtzia densa*, *Lamium album, Marrubium vulgare, Mentha piperita, M. arvensis*, *M. longifolia*, *Origanum vulgare,* and *Thymus linearis* |
| Ranunculaceae | 7 | 4.40% | *Actaea spicata, Aconitum heterophyllum, A. chasmanthum, Anemone obtusiloba, Delphinium roylei, Ranunculus arvensis, R. hirtellus, R. laetus,* and *R. palmatifidus* |
| Brassicaceae | 7 | 4.40% | *Barbarea intermedia, Brassica campestris, B. oleracea, B. vulgaris, Capsella bursa-pastoris, Cardamine impatiens,* and *Raphanus sativus* |

found to have their own specific knowledge on the traditional use of wild plants used as medicines and food. This study also focused on certain threats related to the disappearance of traditional knowledge from the mountainous people, which is due to modernization and migration towards the cities for quality of life. Medicinal plants represent a significant contribution to human health and it has been suggested that their use is one of the most significant ways in which humans directly reap the benefits provided by plant diversity. In the present study, 109 species of plants were wild, 40 species were cultivated and 10 species were both cultivated and wild. It has been observed that the use of wild medicinal plants for primary healthcare needs in the study area might be due to the lack of proper health facilities and interior location in the.

### 15.3.1 Plant Parts Used as Medicine and Mode of Utilization

The local people use different parts of the plant. Leaves are the most used plant part for medicinal preparations, followed by root, bark, rhizome, whole plant, and fruit. The diversity of the medicinal plants is illustrated by their usage diversity. The different parts of the plant—including root, rhizome tuber, bulb, bark, stem, leaves, flower, fruit, seed, whole plant or aerial part, resin, bud, inflorescence, and stolon—are either used in fresh or dried condition. In some species, multiple plant parts are used as medicine. The details of different plant parts used as medicine recorded during the study are presented in Fig. 15.2. The plants collected from the study area were used in different ways, either orally or applied externally to the affected body area. The type of ailments define the method of application as a medicine, which are used mostly in the form of decoction, infusion, vegetable, resins, extract, paste,

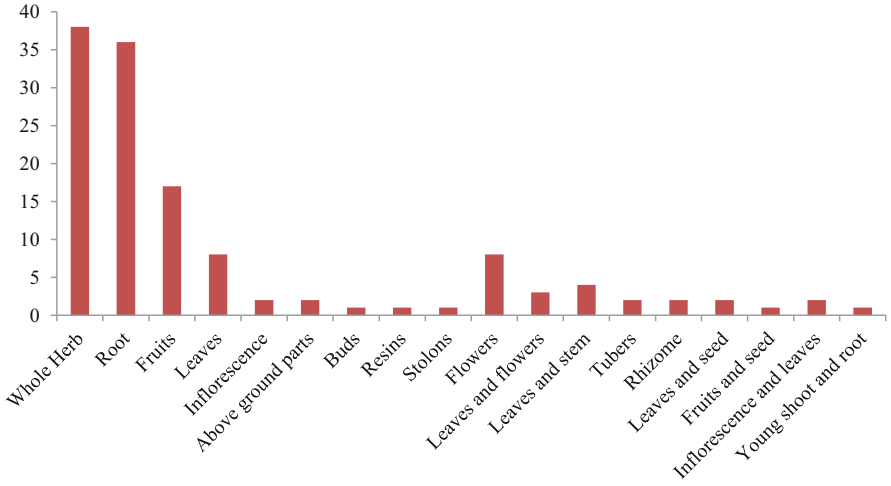


**Fig. 15.2** Plant parts used in treating different types of diseases

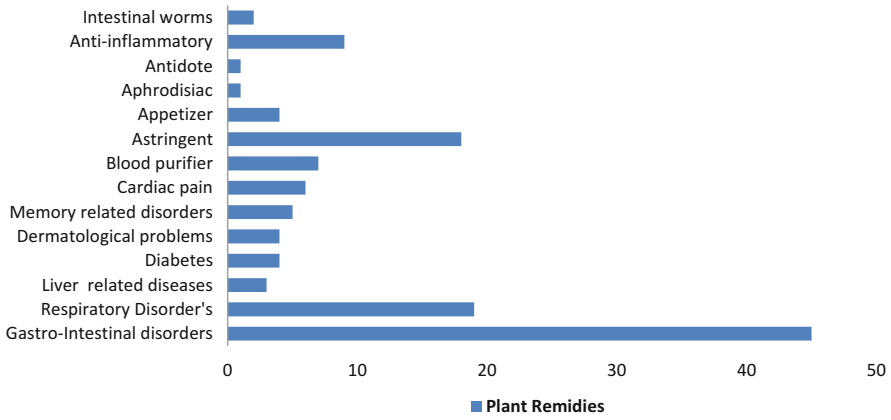


**Fig. 15.3** Major disease category and number of remedies treated in the study area

crushed, tincture, ointment, or some whole plant organs. The higher use of herbs for medicinal purposes in the study area may be due to their ease of collection, greater abundance and high effectiveness in the treatment of ailments in comparison to other life-forms (Singh and Shanpru 2010; Adnan et al. 2012), while in other regions it may also be due to seasonal variability or differences in socio-cultural beliefs and practices of healers. With regard to trees, their extensive use in the preparation of ethnomedicines might be linked to their ability to withstand long dry seasons, thus resulting in their availability throughout the year. The major disease category and number of remedies treated in the study area are presented in Fig. 15.3.

### 15.3.2 Previous Research Studies and Comparison with Present Outcomes

It has been observed that identification and documentation of species is a useful parameter for identifying and studying the spatial patterns in plant diversity and composition (Barik et al. 2006). Ethnic knowledge can provide important information on the processes that maintain and sustain the diversity of the given ecosystem (Singh and Borthakur 2011; Thakur et al. 2020). The present study reports the ethnobotanical investigation of 159 plant species from District Kupwara in Kashmir Himalaya, and the data presented herewith is more or less similar to the earlier documented studies in other regions of the Himalaya (Kumar et al. 2009; Mahmood et al. 2011, 2012; Singh et al. 2016). In terms of family composition and distribution, this research is also in-line with reported dominant families of Himalaya such as Asteraceae, Rosaceae, Lamiaceae, Ranunculaceae and Brassicaceae (Dar and Sundarapandian 2016). In Uttarakhand, Garhwal Himalayas, India reported Asteraceae as a dominant family. In terms of diversity in growth-forms (or life-forms), herbaceous group of species were the dominant plant

community, followed by trees, shrubs, and lianas. Drying the live plants and making them into powder, boiling for tea, extracting juice, and pounding into a paste are the common preparation methods of utilizing medicinal plants observed during the present study. Grinding or crushing and boiling are the most common and effective methods of active ingredient extraction in a major part of the world where herbs are used as medicines (Singh et al. 2019), and this is also common practice among the local people of the study area.

## 15.4 Conclusion

Traditional herbal medicines are an integral source of livelihood in many regions of the Himalayas. The investigated study listed out plenty of wild medicinal plants to treat a wide range of human ailments, and the local healers are experts in the preparation of various herbal formulations. Moreover, the use of specific plant parts, similar uses of same plants in different regions and multiple uses of single plants for the preparation of medicinal remedies suggest the prevalence of biologically active compounds across a range of medicinal plant species. Despite being a backward region of Kashmir Himalaya, District Kupwara has a rich diversity of plants and the local people of different communities such as *Gujjars, Paharis, Pathans*, and *Kashmiris* have long associations with forests and have heavily depended on natural resources. The indigenous knowledge possessed by ancestors gets passed on to the next generation by way of the use of the same traditional techniques. Some high-value medicinal plants such as *Aconitum heterophyllum, Saussurea costus, Bergenia ciliata,* and *Gentiana kurro* have lots of traditional importance in drug formulation. From the present study, it was found that Asteraceae is a dominant family in most parts of the Himalayan belts and several herbal formulations are developed by using members of this family. The plants documented were used as whole plants or different parts of the plant, that is, roots, fruits, leaves, inflorescence, aboveground parts, bulbs, resins, and stolons. We can conclude that the study area is home to various medicinal plants and ethnic communities, and in future, R&D on traditionally used herbal formulations is required to explore lesser-known medicinal plants growing in the high Himalayan regions.

**Acknowledgement** First, the authors would like to thanks Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, Uttar Pradesh India for Ph.D registration, and the research funding agency Council of Scientific and Industrial Research (CSIR) for Doctorate Fellowship and also to Director, Indian Institute of Integrative Medicine, Jammu for the necessary facilities and encouragement. Further, the authors would like to extend sincere thanks to the local people for sharing their valuable knowledge on the indigenous use of herbal medicinal plants of District Kupwara.

**Conflict of Interest** The authors declare no conflict of interest related to the publication of this manuscript.

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# *Capsicum chinense* Jacq.: Ethnobotany, Bioactivity and Future Prospects

16

Joyashree Baruah and Mohan Lal

**Abstract**

India is home to many exotic and endemic species and a vast range of variability is found in a single plant species. Naga King Chilli or Bhut Jolokia (*Capsicum chinense* Jacq.) is one such plant native to Assam and considered as a naturally developed hybrid of *C. chinense* and *C. frutescens*. This plant has found a special place in Assamese culture for making pickles, adding hotness to different food-stuff and curing various ailments such as arthritis, asthma, cough and sore throat, headache, gastritis, toothache and muscle pain, while capsaicin has found many pharmaceutical applications, including anti-arthritis, weight loss, thermoregulation, anti-cancer, anti-oxidant, anti-microbial, anti-inflammatory and cardiovascular activity. Moreover, the hotness and sweet aroma of Bhut Jolokia are currently gaining huge commercial importance in national and international markets. So to fulfil the high commercial demand of capsicum or capsaicinoid in the world market, preference should be given on large-scale cultivation of Bhut Jolokia variety with high capsaicin content.

**Keywords**

*Capsicum chinense* · Medicinal value · Biological activity · Agrotechnology

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_16

## Abbreviations

| | |
|---|---|
| CSIR-NEIST | Council of Scientific and Industrial Research-North East Institute of Science and Technology |
| DRDO | Defence Research and Development Organization |
| IBPGR | International Board for Plant Genetic Resources |
| OC | Oleoresin capsicum |
| RAPD | Randomly Amplified Polymorphic DNA |
| SHU | Scoville Heat Unit |
| WHO | World Health Organization |

## 16.1 Introduction

India possesses one of the richest floras across the globe and is regarded as a rich conservatory of medicinal plants. It is a home to many exotic and endemic species. Such bountiful vegetation can be attributed to a vast range of soil, climate, topography and ecology present therein. According to reports, around 49,000 species of plants are revealed, which is about 12% of the total plant varieties known to mankind (Sujatha 2017). Of these, 17,500 species are flowering plants (Saikia and Khan 2017) and about 3000 plant species have medicinal properties and were used in traditional medicines (Sujatha 2017). Since prehistoric times, different plant species have been used for various medicinal purposes. There has been record in ancient Unani manuscript, Chinese writings and Egyptian papyrus about the use of herbs in the traditional medicine, while indigenous culture such as Rome, Iran, Egypt, America and Africa used herbs for healing purposes. With the increase in population growth, followed by inadequate drug supply, forbidding cost of treatments, side effects of synthetic drugs and resistance of pathogens to drugs, emphasis has been laid on the application of plant materials as an alternative source of medicine. According to WHO (World Health Organization), approximately 80% people around the globe rely on herbal medicine and around 21,000 plant species are likely to be used as medicinal plants. As per records, about three-fourth of the world population depends on plant-based products for their health care needs (Khan 2016).

North East (NE) India, situated between latitude of 21°34′ to 29°50′N and longitude 87°32′ to 97°52′E, harbours one of the richest reservoirs of plant diversity in India. The mysterious, unexplored terrains of NE India are undoubtedly "centres of attraction for biologist". It covers an area of 262,230 sq. km and contributes about 50% of India's total diversity. Of the total flowering plants found in India, around 8000 species are found in this region (Saikia and Khan 2017) and about 40% are endemic (Mao and Hynniewta 2000). A wide variety of plant species are used as food and medicine by the ethnic tribal communities of this region. The vast range of climate, topography and edaphic factors has resulted in a great range of ecological habitats. Agriculture is the mainstay of the people of North East and the crops commonly grown include rice, ginger, turmeric, brinjal, sweet potato, cucurbits, yams and so on. Chilli is one such crop plant that is cultivated by the people of North

East regardless of ethnicity and place. North East India harbours rich genetic diversity of *Capsicum* species, which are known by different vernacular names, such as Naga King Chilli or Bhut Jolokia (*Capsicum chinense* Jacq.), Jati Jolokia (*C. annum*), Dhan or Mem Jolokia (*C. frutescens*), Ohm Jolokia (*C. baccatum*) and Bhikue Jolokia (*C. pubescens*) (Sarwa et al. 2013). Of these, *C. chinense* is extremely popular among the people for its high pungency and pleasant aroma. North East India, with its unique ecological conditions and high humidity, acting as speciation hub gave rise to world's hottest chilli variety – Bhut Jolokia (Guinness World Book Records 2006; Purkayastha et al. 2012a; Sarpras et al. 2016). In Assam, it is commonly called as Bhut Jolokia or Bih Jolokia (Poison Chilli). In Nagaland, it is known as Raja Mircha/Raja Chilli, while in Manipur, it is called oo-morok (Baruah et al. 2014; Bhutia et al. 2019).

## 16.2 Taxonomic Position

- Kingdom – Plantae
- Division – Magnoliophyta
- Class – Magnoliopsida
- Order – Solanales
- Family – Solanaceae
- Genus – *Capsicum*
- Species – *chinense* Jacq.

## 16.3 Botanical Enumeration

*Capsicum chinense* Jacq., a semi-perennial crop, self-pollinated species, also known as Bih Jolokia, Naga Jolokia and Raja Mircha, is an indigenous capsicum variety of North East India. It is a profusely branched shrub that reaches a height of about 45–150 cm. Stem is green due to anthocyanin pigments on the nodes, up to 1–4 cm girth; stem of young plant moderately soft and weak, while mature plants are slightly woody, with older stem slightly greyish in colour. Leaf surface possesses a crinkle look as other *C. chinense* species. Leaves are green to pale green in colour, opposite, ovate to ovate-lanceolate, apex acute-subacute or shortly acuminate, margins entire but undulate, base attenuate, variable in size from 10 to 14 cm length, 5.5–7.5 cm breadth, venation unicostate reticulate. Petiole is concave on upper side, up to 1–2 cm long. Flowers are 2–2.5 cm long, pendant with white to creamy white corollas, sometimes a touch of light green colour; calyx 5, 3 mm long, gamosepalous and persistent; corolla 5, 8 mm long; stamens 5, 4 mm long, epipetalous; anthers pale blue in colour, basifixed, filaments purple with fleshy hair; carpel 1, 6 mm long, ovary ellipsoidal with 3 locules, style 2–3 mm, stigma diffuse. Fruit is 4–9.5 cm long, conical to subconical, with colour ranging from light green, green and yellow when young to bright red, chocolate and orange at maturity. Fruit possesses 4–5 hollow locules with central placentation and nearly about 25–35 wrinkled seeds.

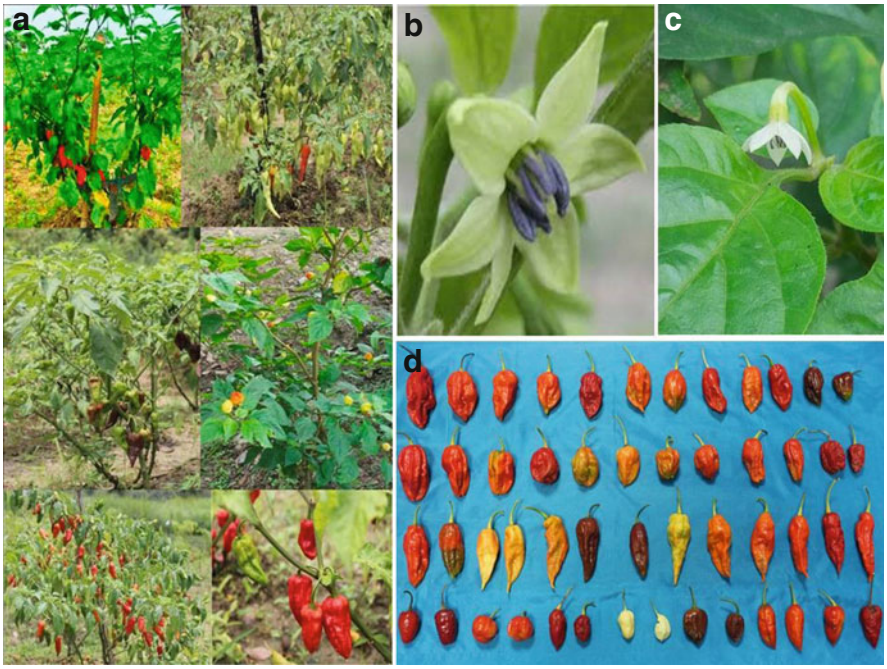


**Fig. 16.1** Variations or trends observed in plant habitat (**a**), flower colour (**b**, **c**), fruit colour and shape (**d**) of *Capsicum chinense* (Bhut Jolokia)

Each chilli fruit weighs about 5–12 g under field condition, with a single plant producing around 20–140 fruits depending on the fruit size (Borgohain and Devi 2007; Bhagowati and Changkija 2009; Purkayastha et al. 2012b; Malangmeih and Rahman 2016; Gogoi 2017) (Fig. 16.1).

## 16.4 History

Chilli is considered as one of the most powerful spices starting from an ancient created myth to the most powerful weapon for self-defence. From the archaeological data, it was confirmed that human civilization has been cultivating chilli species since 6000 years (Perry 2007; Baruah et al. 2014). The genus is indigenous to South America and was introduced to India towards the end of the seventeenth century by Portuguese explorers (Basu and De 2003; Meghvansi et al. 2010; Dhaliwal et al. 2014) and to the North East India by Christian missionaries (Yumnam et al. 2012; Bhutia et al. 2019). India is considered as the secondary centre of diversity of the chilli plant (IBPGR 1983), especially *C. annum* species (Dhaliwal et al. 2014), and among all the domesticated species, Brazil is considered as the centre of origin of *C. chinense* (Casali 2016). The geomorphological distribution of chilli in Asia and their contributing factors are not properly documented. However, it was believed

that chilli exchange took place along the Ganges and the coasts by the Asian, Arab and European traders. In course of time, natural selection has played a pivotal role in producing several chilli varieties differing in morphology and pungency (Meghvansi et al. 2010).

*C. chinense* has a long-standing relation with the people of North East India. Archaeological data showed that the villages of Assam have been cultivating Bhut Jolokia for 7000 years (Basu and De 2003). Its use in traditional medicines for curing different ailments has been described in Vedic and several ancient literature (Saikia 2018). Its rise to glory as the number one hottest chilli (currently placed seventh among the hottest chillies in the world) has been an interesting one. Its saga of hotness remained unnoticed for many years to the world beyond North East India until it was discovered by Mathur (2000) from Defence Research Development Organization (DRDO), Tezpur, Assam, India. It rose to fame only when Dr. Paul Boshland of Chile Pepper Institute reported the hotness rating of 10,01,304 SHU (Scoville Heat Unit), thus officially registering the name in the Guinness Book of World Records as the world's hottest chilli pepper.

The plant has been called by different names in North East India. In Assam, it is called 'Bhut Jolokia', the probable reason being its introduction to Assam by the people of Bhutan (Saikia 2018), whereas some people believe that it got the name due to its capacity to repel ghost (Bhut means Ghost, Jolokia means Chilli). Its hotness causes extreme sensation in the throat like that of poison, which is called 'bih' in assamese and thus got the name Bih Jolokia (Bhagowati and Changkija 2009). The people of Assam also refer it as Naga King Chilli, as it resembles the ferocious Naga warriors of Nagaland. In Manipur, it is called oo-morok (oo = tree, morok = chilli), while in other parts of North East India, it is known as Raja Mirchi (Raja means King, i.e. King chilli) (Baruah et al. 2014; Bhutia et al. 2019).

At the time of its discovery, it was reported that Bhut Jolokia belongs to the species *C. frutescens*. However, many controversies arose regarding its classification since many researchers believe that members of *C. frutescens* cannot produce such high pungent chilli. Finally, it was confirmed based on morphological features that the particular plant belongs to *C. chinense* (Bosland and Baral 2007). Later, based on the molecular marker analysis (RAPD (Randomly Amplified Polymorphic DNA)), Bosland and Baral concluded that there is a possibility of the incorporation of *C. frutescens* genes into Naga King Chilli (*C. chinense*), and it is hence regarded as an interspecific hybrid of *C. frutescens* and *C. chinense*. Purkayastha et al. (2012b) described the plant as a separate species and named it *C. assamicum*, thus creating again a taxonomic enigma among the research community.

## 16.5 Ethnobotanical Usages

Since time immemorial, this indigenous variety has been conventionally used by the people of North East for treating various ailments. The most common among them is to heal gastrointestinal disorders. Regular consumption in low amounts is very useful against the disorder. It secrets saliva and gastric juice, due to high pungency,

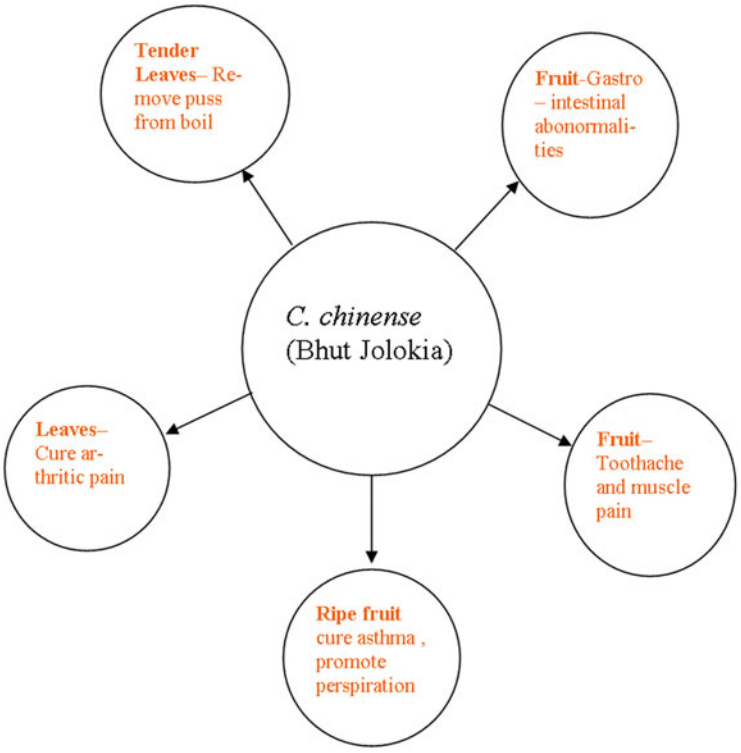


**Fig. 16.2** Ethnobotanical use of the plant *C. chinense* Jacq

when consumed, thus influencing gastrointestinal activity in a positive way (Roy 2015; Gogoi 2017). The pungent principle of the plant is also responsible to cure asthmatic problems. Patients suffering from asthma get relief by regular consumption of the fruit in low amounts. Boshland (1996) reported that Maya people has a long tradition of using *Capsicum* fruits to get relief from cough, sore throat and asthma problems. Traditionally, hot infusion of the fruit is used to cure toothache and muscle pain, digestive diseases and chronic congestion (Bhagowati and Changkija 2009; Sarwa et al. 2014). It is also used to restore muscles after heavy workouts. The grind leaves of the plant are also applied along with coconut oil to remove pus from boils (Deorani and Sarma 2007; Bhagowati and Changkija 2009). It is also used as the summer heat remedy as it increases body perspiration (Roy 2015) (Fig. 16.2).

## 16.6 Bioactivity of the Plant

Different bioactive compounds were reported from Bhut Jolokia fruits, such as phenolics, flavonoids, ascorbic acid, total protein and carbohydrate and reducing sugar (Kundu et al. 2014). Of these, the main active compounds of the plant are the

strong capsaicinoids, which give hot sensation on intake. The capsaicinoids include group of five characteristic pungent compounds, namely, capsaicin, dihydrocapsaicin, homocapsaicin, nordihydrocapsaicin and homohydrocapsaicin. Capsaicin and dihydrocapsaicin constitute about 80% or more of the total capsaicinoid content of the fruit (Sarwa et al. 2013). Sarpras et al. (2018), however, during their work reported only four components of capsaicinoids, namely, capsaicin, dihydrocapsaicin, nordihydrocapsaicin and nonivamide. These capsaicinoids have found many applications in medicine and biological activities and are described in brief in the following subsections.

### 16.6.1 Effect of Chilli on Weight Loss

Obesity will develop when food consumption (energy intake) exceeds the energy required by the body. Study by Whiting et al. (2014) provides strong evidence on the positive effect of chilli consumption in increasing energy metabolism (Varghese et al. 2016; Parvez-Masud 2016).

### 16.6.2 In Antipain Therapy

Capsaicin formulation is used in tropical creams for postherapeutic neuralgia pain treatment, diabetic neuropathy and different types of arthritic problems (Reynolds 1999; Anon 2003; Meghvansi et al. 2010; Ashwini et al. 2015). The tropical formulation prepared with Bhut Jolokia extracts has been found to be superior in action than 'Thermagel' (a marketed product of capsaicin) in reducing of joint pain and swelling (Sarwa et al. 2014; Roy 2015). It has also been shown to be effective against pain from psoriasis, pruritus, bladder disorder and cluster headache (Reynolds 1999).

### 16.6.3 Regulation of Body Temperature

The cuisines of tropical countries contain more capsaicin than those of the northern part because it is believed that hot peppers bring about warmth and not burning or hot sensation, thereby playing a role in thermoregulation (Lee 1954; Meghvansi et al. 2010; Abdel-Salem 2016).

### 16.6.4 Cancer Prevention

Several studies have shown that capsaicin can reduce malignant cell growth by arresting cell cycle, apoptosis and autophagy and inhibiting cellular metabolic activation (Surh 2002; Zhang et al. 2003; Ghosh and Basu 2010; Oh et al. 2010; Arora et al. 2011). Capsaicin showed positive results in the breast cancer treatment

and prostate cancer cells, while dihydrocapsaicin induces autophagy in HCT116 human colon cancer cells (Oh et al. 2010; Thoennissen et al. 2010; Yang et al. 2010; Arora et al. 2011). Amruthraj et al. (2014) found that capsaicinoids extracts from Bhut Jolokia (*Capsicum chinense*) reduce cell viability when HepG2 cells are exposed to acetonitrile extract. It was further concluded that in a dose-dependent manner the extract suppressed the release of nitric oxide (NO), lactate dehydrogenase (LDH) and lipid peroxidation (LPO) production.

### 16.6.5 Source of Antioxidant and Anti-inflammatory

Chillies are considered as a good source of the antioxidant activity. Antioxidant properties of the capsaicinoid found in Bhut Jolokia have a positive effect in the prevention of many oxidative stress-related diseases (Liu and Nair 2010). Several studies have confirmed that the incidence of Alzheimer's disease among Indian village people is comparatively reduced compared to the Western part (Ganguli et al. 2000; Lim et al. 2001) because their dietary supplement regularly includes different spices rich in neuroprotective and antioxidant agents (Shobana and Naidu 2000; Rao 2003). Capsaicin's antioxidant property can even prevent cardiovascular diseases (Baruah et al. 2014). Hazarika et al. (2014) concluded that capsaicinoids extracted from *C. chinense* can be used as antioxidant drug for inhibiting the activity of nitric oxide synthase (iNOS). Liu and Nair (2010) determined the health-beneficial COX enzyme inhibitory activity of capsaicin and dihydrocapsaicin extracted from Bhut Jolokia, thereby describing the potential of this plant as the anti-inflammatory source. In their study, it was found that pure capsaicin and dihydrocapsaicin inhibited the COX-1 enzyme activity by 50% at 42 and 45 μg/mL, respectively, while the COX-2 enzyme inhibition by 50% at 75 and 125 μg/mL, respectively.

### 16.6.6 As Antimicrobial Agent

Bioautographic tests done by Soetarno and Sukandar-Yulinah (1997) revealed that capsaicin is the major antimicrobial component reported to disrupt the microorganism's membrane (Cowan 1999). Molina-Torres et al. (1999) reported that high capsaicin concentrations reduce the growth of *E. coli* and *P. solanacearum*, whereas they strongly inhibit the growth of *B. subtilis*. The study suggested that capsaicin has a wide effect on a variety of microorganisms. Amruthraj and Raj-Preetam (2013), in their study, showed that fruits of *C. chinense* have wide antibacterial activities against the human pathogens.

### 16.6.7 Remedy for Gastric Ulcer

Das et al. (2008) for the first time studied the ulcer protective activity of the ethanolic extract of chilli (*C. frutescens*) and Ranitidine (commercially available drug used to prevent ulcers in stomach and intestines) on albino mice. The study showed that the ethanolic extract of the plant reduced gastric ulcer by lowering pepsin and increasing mucus secretion.

### 16.6.8 Cardiovascular Activity

Capsaicin has the potential to reduce the occurrence of cardiovascular diseases by inhibiting the aggregation of platelet and the activity of VIII and IX clotting factors. Capsaicin has the ability to pass through platelet plasma membrane, thereby altering membrane fluidity (Adams et al. 2009; Zhou et al. 2010). It was found that capsaicinoids have potent beneficial effects on the cardiovascular system that include various cardiovascular diseases, such as coronary heart disease, myocardial infarction, hypertension and atherosclerosis (Harada and Okajima 2009; Peng and Li 2010).

### 16.6.9 Other Non-pharmacological Applications

Chilli having the high pungency level is mostly suitable for the preparation of 'oleoresin capsicum' as well as the extraction of capsaicin. The people living near the forests use the powder of the pepper or their smoke to keep elephants away from their crops. Oleoresin capsicum has gained the notice of law enforcement agencies as the non-lethal agent for riot control and for criminal incapacitation. The Government of India has taken initiatives to find an alternative to pellet guns, that is, chilli-filled grenades, which are basically shotguns that have dispersed hundreds of protesters over the last few weeks. The grenades, when fired, temporarily stun and immobilise the targets more effectively than tear gas or pepper guns. However, its use as the future riot controlling weapon has not received green signal as it might raise concerns regarding health and safety issues.

## 16.7 Business Potential and Future Prospects

The spice sector in India is growing at a great pace. India produces more than four million tonnes of spice and exports nearly about 180 spice products to 150 nations. India holds the record as the largest producer and exporter of chillies in the world and Indian chilli is exported to different countries, such as the USA, the UK, Canada, Saudi Arabia, Singapore and Malaysia. Out of the total area allotted for spice cultivation, around 30% of the area is occupied by chilli plantation (Indian Horticulture Database 2011). North East alone contributes 51.72% of the total production,

although it has only a marginal area (8%) under chilli cultivation (Spice Statistics, Spice Board 2004; Yumnam et al. 2012). Among the NE states, Assam holds the maximum area under chilli cultivation, whereas Nagaland tops the chart in terms of production (Bhutia et al. 2019).

With an increase in the global demand of chilli and the need to maintain India's position as the number one producer and exporter, emphasis should be given on large-scale chilli production with intact quality. Nowadays, many countries of the world, including India, are putting a ban on the use of artificial colours and approve the use of natural colours, such as those obtained from the capsicum species. The red colour obtained from the fruit can be used as capsicum oleoresin. The present market demand of chilli oleoresin is not known, but it is expected to be around 100–200 tonnes/annum. Since chilli colour is a new product, its exact demand is not documented till now. As artificial colours are banned and being replaced, the red colour can became a good replacement for synthetic colours in the food industry (De 2003).

Bhut Jolokia can certainly become the ideal chilli variety of India for large-scale cultivation, natural red dye, oleoresin capsicum and capsaicin as it has very high capsaicin (3–5% or more) content (Baruah et al. 2014). The Indian chilli varieties available till date are not suitable for this purpose as they contain less capsaicin (<1%), a standard needed for the commercial extraction of capsaicin. An increased cost of capsaicin extraction with low content is not favourable for commercial cultivation. So the cultivation of *C. chinense* having high capsaicin content variety can be taken up extensively to increase the production (Baruah et al. 2019). The oleoresin and capsaicin have high demand at both domestic and international markets and are very costly products (Borgohain and Devi 2007). Moreover, the hotness and sweet aroma of Bhut Jolokia are currently gaining huge popularity (Sarwa et al. 2013). It has become a hot favourite of the Asian people and even restaurants have several items incorporated with red hot chillies in their menus. Bhut Jolokia with high pungency, vitamin C content and medicinal properties is sure to make a place in the world market. However, till date not much importance has been given regarding the successful commercial cultivation of Bhut Jolokia, which is an important spice crop of the North East India. Those produced from local farms only a small portion enters the national market. It is because the various cultivation aspects of this important crop are not standardized or there is lack of superior varieties available in public domain. Currently CSIR-NEIST, Jorhat, Assam has developed two superior and high yielding, disease-resistant varieties of Bhut Jolokia (Jor Lab BJ-2 red colour and Jor Lab BJ-3 brown colour fruits) which is expected to show fruitful results in days to come. In addition to this, several products can be made from chillies that can attract national and international market, such as whole dried chilli, powdered or ground form, in the form of crushed or flakes, paste or mashed form, distilled oil for flavouring industry, as ingredients in sauce, pickles in oil or brine and pepper spray or chilli grenade.

## 16.8 Conclusion

Local initiatives have already been undertaken in Assam for exporting value-added products made from Bhut Jolokia to European markets. Some of the local products, such as whole dried chilli and crushed or flakes forms, are already being sold at Nagaland and Dimapur markets, which find a channel to enter the Asian markets, particularly Myanmar and Thailand. To capture the national and international markets, commercial cultivation of Bhut Jolokia appears to be an attractive option for Indian farmers, particularly the North East India. The government and private organizations are putting efforts on the commercial cultivation Bhut Jolokia in the region. The Department of Agriculture under Government of Assam has taken up the scheme 'Technology Mission for Development of Horticulture in North Eastern Region including Sikkim (Mini Mission-II)' under which 500 ha of land is allotted for the cultivation of this important plant. Farmers are provided an incentive of Rs. 18750.99/ha for purchasing seeds and necessary equipments. With the advent of scientific package added with the modern technology, Bhut Jolokia farming is expected to become popular among farmers and contribute to the economy of the region. It is also expected that the region generates women employment opportunities through this chilli led development process.

**Acknowledgements** The authors are grateful to CSIR for providing CSIR-SRF fellowship (ACK. No. 113500/2 K17/1) and also to the Director, CSIR-NEIST, Jorhat, for his encouragement and guidance throughout the work.

**Conflict of Interest** The authors of the manuscript declare that there is no conflict of interest with any other organization or entity with financial support.

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# Indigenous Plant Knowledge for Human Health Care from Jasrota Wildlife Sanctuary (Western Himalaya), India

17

Bishander Singh, Anand Kishor, and Bikarma Singh

**Abstract**

Wild plants and unseen microbes of undisturbed ecosystems are considered as rich repository sites of active ingredients, and these natural constituents often provide new leads and opportunity to characterized new natural product compounds having wide applications in drug-discovery programmes. Government recognized natural biomes and protected areas such as national parks, and wildlife sanctuaries often provide opportunity for new discovery or chances to get un-described plants, microbes or their derivatives natural products. Several far-flung regions in Himalayas are still less explored, and protected areas in these regions are hub of medicinal and aromatic plants. Considering importance of natural resources, a research study on documentation of medicinal plants of Jasrota Wildlife Sanctuary (JWS) and adjoining areas in Jammu and Kashmir (J&K) union territory was carried out as this area is floristically and ethnobotanically less explored. A total of 125 species belonging to 57 families of medicinal plants were documented subjected to preliminary investigation, which includes 41.60% herbs, 32.80% trees, 20% shrubs and 5.60% lianas. Dominant members belong to family Fabaceae (11 genera and 12 species), Lamiaceae (7 genera and 8 species), Asteraceae (6 genera and 7 species), Caesalpiniaceae (6 genera and 6 species), Euphorbiaceae

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_17

(4 genera and 5 species), Moraceae (2 genera and 5 species), Rosaceae (5 genera and 5 species), Apocynaceae (4 genera and 4 species), Brassicaceae (4 genera and 4 species) and Convolvulaceae (4 genera and 4 species). While studying and analysing ethnobotanical information, high frequency index (FI) of medicinal plants recorded was of *Boerhavia diffusa* (93.83%), *Mallotus philippensis* (82.72%), *Viola odorata* (70.37%), *Tridax procumbens* (65.43%), *Cissampelos pareira* (65.43%), *Tinospora cordifolia* (62.96%), *Grewia optiva* (62.96%), *Morus alba* (59.26%), *Justicia adhatoda* (58.02%), *Cannabis sativa* (58.02%), *Butea monosperma* (56.79%) and *Solanum nigrum* (52.09%). Lesser used plants among local populace were *Cyperus rotundus* (9.88%), *Robinia pseudoacacia* (11.11%), *Commelina benghalensis* (13.81%), *Ficus racemosa* (14.81%), *Amaranthus spinosus* (14.81%), *Dioscorea deltoidea* (14.81%), *Xanthium strumarium* (16.05%), *Senna occidentalis* (16.05%), *Albizia lebbeck* (16.05%), *Syzygium cumini* (18.52%) and *Senegalia catechu* (18.52%) in the study area. These medicinal plants are used in treatment of obesity, liver infection, diabetes, intestinal infections, rheumatism, tumours, stomach ache, insomnia, nerve troubles, skin infection, eye problem, dental care, memory related disorders, skin infection, joint pain and bone fracture. Species with high frequency index should be analysed for pharmacological research and for discovering new medicines.

**Keywords**

Medicinal plants · Ethnobotany · Jasrota Wildlife Sanctuary · Himalaya · Conservation

## 17.1 Introduction

Plant-derived natural products provide new leads in the drug-discovery process (Rao et al. 2015). Different plant parts possess several bioactive molecules in the form of glycosides, large quantities of flavonoids, tannins and minerals (Friedman et al. 1986; Duke 2002; Ahmad et al. 2009; Ragupathy and Newmaster 2009; Bolson et al. 2015). Flavonoids are known to exert antioxidant, anti-inflammatory and lipid-lowering effects, while the content of glycosides in plants can act as cardio-tonic agent (Singh 2020). Drug discovery starts with the documentation of traditionally used plants to curing aliments associated with humans and animals (Virjee et al. 1984; Zent 2001; Yineger et al. 2008; Ugulu et al. 2009; Badwaik et al. 2011). Indigenous medicines have attracted the interests of both private and public sections of society due to the adverse effects of modern medicine (Dubey et al. 2004). India has a rich tradition of plant-based knowledge on health care (Kumar et al. 2007). The knowledge of indigenous medicinal use of plants is getting depleted and are being lost because of no proper documentation and conservation planning (Singh and Shanpru 2010; Bhatia et al. 2014; Thakur et al. 2020). For the conservation of

such knowledge, documentation of medicinal plant is required which will facilitate the scientific research for the validation of traditional use, safety and efficacy of potential plants (Basumatary et al. 2004; Bunalema et al. 2014) as well as help in preservation of traditional knowledge and culture associated with particular tribe (Khoshbakht and Hammer 2005).

Protected regions in the form of forests and wetland ecosystems are recognized areas and managed through legal or other effective means to achieve the long-term nature conservation and cultural ethics (Dudley 2008; Singh et al. 2012, 2018a). In India, a total of 733 protected regions are recognized in the form of national parks, wildlife sanctuaries, conservation reserves and community reserves to conserve and protect the unique biodiversity of the country. District Kathua in Jammu and Kashmir Union Territory is a part of Indo-Malayan Biogeographic Realms and known for ethnic tribes such as *Gujjars, Dugars, Paharis* and local *Punjabis*. The inhabitants residing in far-flung regions are inaccessible to modern health care facilities and devoid of roads and modern transport facilities. Therefore, they had to depend mostly on local floras to meet their daily needs such as fuel, food, timber, fodder and to cure various types of ailments by direct or indirect usage of these plants. Due to rich biodiversity and traditional knowledge, researchers have worked on various aspects of ethnobotany, particularly in district Kathua (Sarin and Kapur 1989; Kirn et al. 1999; Kant and Sharma 2001; Tantray et al. 2009; Khan et al. 2009; Kumar et al. 2009a, b, 2012; Kumar and Bhagat 2012; Sharma et al. 2012; Kaul et al. 1990; Siddique et al. 1995; Bhat et al. 2012; Lone et al. 2014; Jeelani et al. 2013; Hassan et al. 2013; Bhushan and Kumar 2013; Bhatia et al. 2015; Singh and Bedi 2017; Singh et al. 2018b). Taking into consideration the value of rich traditional knowledge and to fill the gap of unexplored regions of Western Himalaya, the present research work was planned to document floristic wealth and ethnomedicinal plants of Jasrota wildlife Sanctuary (JSW) in Western Himalaya.

## 17.2 Materials and Methods

### 17.2.1 Study Area

Jasrota Wildlife Sanctuary (Kathua district) is located on the banks of the river Ujh in Jammu and Kashmir, within Himalayas of Indo-Malaya Biogeographic Realm. Jasrota village lies to north of this sanctuary and adjoining area has peculiar types of Shivalik vegetation composition. It is lying between the latitude of 32°27′ to 32°31′ N and longitude of 75°22′ to 75°26′ E, and the elevations vary from 356 to 650 m above mean sea level. This sanctuary and adjoining areas are compact mass of natural forest and spreads in total area of 10.04 $km^2$ with core area totally virgin and undisturbed. Since the sanctuary is located at the junction of two biotic provinces, this sanctuary shows the agro-ecological link of Indian geographical region such as trans-Himalaya and Himalaya; this region has very unique and interesting biodiversity harbouring several threatened, endemic and otherwise economic value plants and also represents a centre of origin for a significant number of cultivated crop

plants and their wild relatives (Sharma et al. 2012; Singh 2019). Kathua region as a whole harbours a very rich flora comprising 1567 species of vascular plants which represent 35.3% of the total flora of J&K and 8.6% that of India including species of medicinal and aromatic plant (Singh et al. 2012). Forest cover of district Kathua spread in 1152 $km^2$ (FSI 2009) whose elevation varies from 253 to 4162 m above sea level (Ashutosh et al. 2010). About 85% of the JWS is covered by natural forests, and the major vegetation type in this sanctuary is typical Himalayan subtropical Shivalik forest. The subtropical vegetation covers include semi-evergreen and deciduous forests. Peculiar Shivalik hill forests can be seen in the JWS where tree species such as *Senegalia catechu*, *Butea monosperma*, *Dalbergia sissoo*, *Cassia fistula*, *Ficus benghalensis*, *Ficus palmata*, *Mallotus philippensis*, *Dalbergia sissoo*, *Ziziphus mauritiana*, coupled with shrubby layers of *Carissa spinarum*, *Lantana camara*, *Justicia adhatoda*, *Cannabis sativa*, *Tinospora cordifolia* and *Calotropis procera*, dominate the forest cover.

The climate in Kathua district and particularly in Jasrota Wildlife Sanctuary varies according to altitude and the typical Shivalik land-form. The subtropical climate in the study area can be divided into three distinct seasons (summer, rainy and winter) prevail throughout the Jasrota and surrounding belts of Kathua, however, the high altitude regions of the district experiences snow and occasionally frost during winter. The mean annual temperature varies from 8 to 42 °C. The months March to June are the summer season, and average temperature ranges between 36 and 42 °C. July to October is the rainy season (temperature varies from 25 to 35 °C), whereas November to February is winter season (temperature varies from 8 to 25 °C). Rainfall in Kathua and particularly in Jasrota is little less as compared to the northernmost and the southern regions of Jammu province, varying from 200 to 300 mm in plain, while in some parts of high altitude it varies from 500 to 1000 mm. The other regions has almost same type of climate throughout the year. The extensive and densely populated regions of the Kathua are the home of many ethnic groups such as *Duggar, Paharis, Punjabi, Gujjars* and other community. Most of the local peoples are distributed throughout large parts of J&K and across political boundaries. These groups of inhabitants practices primitive culture, having unique tradition and follows peculiar rituals. The main occupation is collection of forest resources and traditional farming. Duggars and Paharis people belongs to Indo-Aryan (ethno-linguistic) group living in Himalaya, whereas Gujjars are ethnic pastoral group of people that used to be nomadic and living in Himalaya. There is no village inside the Jasrota Wildlife Sanctuary; however, there are several villages around and nearby Jasrota territory and on the bank of river Ujh.

### 17.2.2 Medicinal Plant Survey and Data Collection

Extensive field surveys were carried out in different locations of JWS during 2018–2019 for collection of indigenous information on medicinal plant species being used by the local people of the study area. The study was carried out by conducting interviews and group discussions among the local people on the

indigenous use of plant species as medicine and food. The people selected for interview were based on their knowledge, their interests and skills in identification of plants. Discussion with the informants was carried out in local Dogri language. The objective of present study was elaborated to the informants prior to study. The interviews were conducted face to face with selected informants like community aged person and youth. All participants signed their consent forms to show their willingness to participate in the study. Plant specimens of species recorded during the interviews were collected and identified. The final list of the plants was prepared by consulting The PlantList database (http://www.theplantlist.org), the International Plant Names index (http://www.ipni.org) and Tropicos (https://www.tropicos.org) for the updated botanical nomenclatures of species. The information collected included common diseases curable by plants, local name of plant species, habit, wild/cultivated, plant-part used, ethnomedicinal use, method of crude drug preparation and mode of administration.

### 17.2.3 Data Analysis

The data collected through interviews from the informants were analysed using different quantitative indices. The frequency index was calculated using a mathematical formula adapted by Madikizela et al. (2012). The formula FI = FC/N × 100 was followed, where 'FI' is the frequency index, which expresses the percentage of frequency of listing a plant species by participants, 'FC' is the number of participants who listed a particular plant species and 'N' is the total number of participants.

## 17.3 Results and Discussion

### 17.3.1 Demography of Informants

A total of 81 informants (49 male and 32 female) between the ages of 21 and 70 years were participated in a questionnaire: 26.53% male and 28.13% female informants never attended school; 22.45% males and 40.63% females attended school for classes 1–5; 30.612% males and 21.88% females attended school for classes 6–10; 12.24% males and 9.38% females attended classes for intermediate; 6.12% males attended classes in graduation and 2.04% males in post-graduation (Table 17.1). Older or the aged informants mentioned more number of plants species than younger participants. The less percentage of female informants (36.28%) is because of the social setup which does not allow ladies to move out of the houses and talk with the strangers.

Education of the informant was negatively correlated with the knowledge of medicinal plant, that is, as the educational level increases the knowledge of medicinal plant increases, especially in males. The reasons for this may be that: (i) they have to move away from their residence to acquire higher education, (ii) after getting

**Table 17.1** Informants' age class and literacy rate of the study area

| | Male | Female | Total |
|---|---|---|---|
| | 49(67.72%) | 32(36.28%) | 81(100%) |
| Age class | Male | Female | |
| 21–30 | 11 (22.45%) | 3 (9.38%) | 14(17.28%) |
| 31–40 | 13(26.53%) | 8 (25%) | 21(25.93%) |
| 41–50 | 17 (34.69%) | 6 (18.75%) | 23(28.40%) |
| 51–60 | 5 (10.20%) | 13 (40.63%) | 18(22.22%) |
| 61–70 | 3 (6.12%) | 2 (6.25%) | 5(6.17%) |
| Total | 49(100%) | 32(100%) | 81(100%) |
| Literacy level | Male | Female | |
| Never attended a school | 13 (26.53%) | 9 (28.13%) | 22(27.16%) |
| Attended school for classes 1–5 | 11 (22.45%) | 13 (40.63%) | 24(29.63%) |
| Attended school for classes 6–10 | 15 (30.612%) | 7 (21.88%) | 22(27.16%) |
| Intermediate (class 12) | 6 (12.24%) | 3 (9.38%) | 9(11.11%) |
| Graduate | 3 (6.12%) | 0 (−) | 3(3.70%) |
| Post-graduate | 1 (2.04%) | 0 (−) | 1(1.23%) |
| Total | 49(100%) | 32 (100%) | 81 (100%) |

education they move to cities for employment, and (iii) being educated they are exposed to modernization (education, occupation and consumerism). Heinrich et al. (1998) and Bhatia et al. (2014) stated that the declining indigenous medicinal plant use knowledge among the younger generations may be attributed to improving status of education in the community and the low interest of the younger generation to inherit and use ethnomedicinal knowledge, whereas Emmanuel and Dider (2011) held modernization (education, occupation and consumerism) responsible for this erosion of medicinal plant knowledge.

### 17.3.2 Floristic Description

The preliminary study lists a total of 125 species used by people residing nearby Jasrota Wildlife Sanctuary. Table 17.2 depicts native, naturalized and few cultivated medicinal plant species along with their local name, frequency index and mode of utilization. Of the total 125 documented species, 32.80% were trees, shrubs (20%), lianas (5.60%) and herbs (41.60%) (Fig. 17.1). No epiphytic species of medicinal value were recorded. Total 57 families and 112 genera of plants were used by the local people for the ethnomedicinal purpose (Table 17.2). Most dominant families were Fabaceae (11 genera and 12 species), Lamiaceae (7 genera and 8 species), Asteraceae (6 genera and 7 species), Caesalpinaceae (6 genera and 6 species), Euphorbiaceae (6 genera and 6 species), Moraceae (2 genera and 5 species), Rosaceae (5 genera and 5 species), Apocynaceae (4 genera and 4 species), Brassicaceae (4 genera and 4 species) and Convolvulaceae (4 genera and 4 species). Leaves were the most used (43.48%), followed by whole plants (12.80%), roots (10.40%), seeds (6.40%), fruits (10.40%), flowers/inflorescence (4.80%), barks

**Table 17.2** Medicinal plants in Jasrota Wildlife Sanctuary in Western Himalaya

| Vernicular name | Botanical name | Family | Habit | People cited | Frequency index (FI) | Part used | Ailment treated | Preparation |
|---|---|---|---|---|---|---|---|---|
| Rati | *Abrus precatorius* L. | Fabaceae | Lianas | 12 | 10.62 | Leave | Used to treat bones fracture | Paste |
| Khair | *Senegalia catechu* (L.) Link (= *Acacia catechu* (L.f.) Willd.) | Fabaceae | Tree | 6 | 5.31 | Wood | Used to cure mouth ulcer | Decoction |
| Kikar | *Vachellia nilotica* (L.) P. J.H.Hurter (= *Acacia nilotica* (L.) Delile) | Fabaceae | Tree | 19 | 16.81 | Bark | Used to treat skin disease and piles | Powder |
| Ghandhni | *Achillea millefolium* L. | Asteraceae | Herb | 12 | 10.62 | Leave | Used in skin disease | Paste |
| Puthkanda | *Achyranthes aspera* L. | Amaranthaceae | Herb | 37 | 32.74 | Root | Used in snakebite | Chew |
| Breen | *Acorus calamus* L. | Acoraceae | Herb | 19 | 16.81 | Rhizome | Treatment of intestinal worms, cough and chest infection | Powder |
| Hansraj | *Adiantum capillus-veneris* L. | Adiantaceae | Herb | 17 | 15.04 | Rhizome | Used to curing herpes | Paste |
| Bill | *Aegle marmelos* (L) Corr. | Rutaceae | Tree | 29 | 25.66 | Leave | Used for treatment of jaundice and asthma | Extract |
| Ban khodi | *Aesculus indica* L. | Sapinaceae | Tree | 15 | 13.27 | Seed | Used to remove dandruff | Powder |
| Neeli jadi | *Ageratum conyzoides* L. | Asteraceae | Herb | 59 | 52.21 | Leeave | Used to stop bleeding of cut or wound | Juice |
| Neelkanthi | *Ajuga integrifolia* Buch.-Ham. | Lamiaceae | Herb | 78 | 69.03 | Leave | Used as anti-diabetic and hair-tonic | Juice |
| Sirin | *Albizia lebbeck* (L.) Benth. | Caesalpinaceae | Tree | 13 | 11.50 | Bark | Used for curing impotency | Powder |
| Kuar gandal | *Aloe barbadensis* L. | Xanthorrhoeaceae | Herb | 89 | 78.76 | Latex | Used as headache and burns | Decoction |
| Jadi | *Alternanthera sessilis* (L.) R.Br. ex DC. | Amaranthaceae | Herb | 17 | 15.04 | Shoot | Used as tonic | Juice |

(continued)

**Table 17.2** (continued)

| Vernicular name | Botanical name | Family | Habit | People cited | Frequency index (Fl) | Part used | Ailment treated | Preparation |
|---|---|---|---|---|---|---|---|---|
| Kandiari | *Amaranthus spinosus* L. | Amaranthaceae | Herb | 12 | 10.62 | Leave | Used as laxative properties | Juice |
| Kokoon | *Lysimachia arvensis* (L.) U.Manns (=*Anagallis arvensis* L.) | Primulaceae | Herb | 32 | 28.32 | Whole plant | Used as dermatitis | Extract |
| Peelikandiari | *Argemone mexicana* L. | Asteraceae | Herb | 18 | 15.93 | Leave | Used to cure ringworm | Extract |
| Sanspod | *Asparagus adscedens* Roxb. | Asparagaceae | Lianas | 77 | 68.14 | Seed | Remove urinary stones | Decoction |
| Neem | *Azadirachta indica* A. Juss. | Meliaceae | Tree | 71 | 62.83 | Leave | Used for stomach ache | Chew |
| Jhinti, | *Barleria cristata* L. | Acanthaceae | Shrub | 54 | 47.79 | Leave | Reduce swellings and cough | Powder |
| Kared | *Bauhinia variegata* L. | Caesalpinaceae | Tree | 34 | 30.09 | Flower | Used for curing dysentery, diarrhea and piles | Decoction |
| Lit-sit | *Boerhavia diffusa* L. | Nyctaginaceae | Herb | 76 | 67.26 | Root | Used in jaundice and nocturnal emission | Decoction |
| Simbal | *Bombax ceiba* L. | Malvaceae | Tree | 24 | 21.24 | Root | Used to treat diarrhea | Decoction |
| Sarsoon | *Brassica compestris* L. | Brassicaceae | Herb | 37 | 32.74 | Seed | Used to detoxify the effect of poisonous insect or weed intake | Extract |
| Gonglu | *Brassica rapa* L. | Brassicaceae | Herb | 32 | 28.32 | Whole plant | Used as galactagogue | Decoction |
| Palah,Dhak | *Butea monosperma* (Lam.) Taub. | Fabaceae | Tree | 56 | 49.56 | Flower | Used for removing urine blockages | Powder |
| Desi ak | *Calotropis procera* R. Br. | Ascelpiadaceae | Shrub | 13 | 11.50 | Root | Used externally on skin diseases | Extract |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Lit-sit | *Cannabis sativa* L. | Cannabaceae | Shrub | 47 | 41.59 | Leave | Used as anti-venom | Paste |
| Kralmond | *Capsella bursa-pastoris* Medik | Brassicaceae | Herb | 24 | 21.24 | Whole plant | Used for treatment of bloody urine diarrhea | Decoction |
| Kralmondi | *Cardamine impatiens* L. | Brassicaceae | Herb | 19 | 16.81 | Whole plant | Used as stimulant, diaphoretic, stomachic, carminative and diuretic | Decoction |
| Garna | *Carissa spinarum* L. | Apocynaceae | Shrub | 20 | 17.70 | Fruit | Used in curing pile | Powder |
| Karangal | *Cassia fistula* L. | Caesalpinaceae | Tree | 89 | 78.76 | Leave | Used for curing pneumonia | Extract |
| Kasundi | *Senna sophera* (L.) Roxb. (= *Cassia occidentalis* L.) | Caesalpinaceae | Shrub | 13 | 11.50 | Seed | Used as aperients | Decoction |
| Lohki | *Senna tora* (L.) Roxb. (= *Cassia tora* L.) | Caesalpinaceae | Herb | 16 | 14.16 | Leave | Used in curing stomachic and high fever | Decoction |
| Sadabahar | *Catharanthus rosea* (L.) G.Don | Apocynaceae | Herb | 38 | 33.63 | Leave | Used for curing dyspepsia and skin disease | Decoction |
| ABrahmi | *Centella asiatica* (L.) Urb. | Apiaceae | Herb | 58 | 51.33 | Leave | Used as antidiabetic | Chew |
| Battal bel | *Cissampelos pareira* L. | Menispermaceae | Lianas | 53 | 46.90 | Leave | Tonic for improving memory power | Chew |
| Gargal | *Citrus medica* L. | Rutaceae | Tree | 37 | 32.74 | Leave | Used to cure diarrhea | Decoction |
| Duss | *Colebrookia oppositifolia* Sm. | Lamiaceae | Shrub | 38 | 33.63 | Leave | Applied on wounds and bruises | Paste |
| Churra | *Commelina benghalensis* L. | Commelinaceae | Herb | 5 | 4.42 | Whole plant | Used as laxative | Extract |
| Hiran padi | *Convolvulus arvensis* L. | Convolvulaceae | Herb | 12 | 10.62 | Leave | Applied on acne | Paste |
| Andal | *Cuscutta cassytoides* Nees ex Engelm (= *Cuscuta reflexa* Roxb.) | Convolvulaceae | Liana | 33 | 29.20 | Whole plant | Used in treatment of skin itching | Paste |

(continued)

**Table 17.2** (continued)

| Vernicular name | Botanical name | Family | Habit | People cited | Frequency index (FI) | Part used | Ailment treated | Preparation |
|---|---|---|---|---|---|---|---|---|
| Khabbal | *Cynodon dactylon* (L.) pers | Poaceae | Herb | 18 | 15.93 | Root | Used to cure piles | Decoction |
| Dila | *Cyperus rotundus* L. | Cyperaceae | Herb | 8 | 7.08 | Root | Used as antidote to all poisons | Decoction |
| Taail | *Dalbergia sissoo* Roxb. | Fabaceae | Tree | 19 | 16.81 | Leave | Used for menstrual disorder. | Decoction |
| Kochhad | *Daphne oleoides* Schreb | Thymelaeaceae | Shrub | 24 | 21.24 | Leave | Used to cure abscess | Paste |
| Dhatura | *Datura stramonium* L. | Solanaceae | Shrub | 42 | 37.17 | Leave | Used in inflammation | Extract |
| Sansaaru | *Debregeasia seaeneb* (Forssk.) Hepper & J.R.I.Wood (*Debregeasia salicifolia* (D.Don) Rendle) | Urticaceae | Shrub | 17 | 15.04 | Twig | Applied as ear drop | Juice |
| Baans | *Dendrocalamus strictus* Blume | Poaceae | Tree | 9 | 7.96 | Shoot | Used as tonic | Powder |
| Kins | *Dioscorea deltoidea* Wall. ex Kunth. | Dioscoreaceae | Lianas | 12 | 10.62 | Leave | Applied on wounds, burns and swellings | Paste |
| Bhangra | *Eclipta prostrata* L. | Asteraceae | Herb | 23 | 20.35 | Whole plant | Used as hair tonic | Decoction |
| Safeda | *Eucalyptus globulus* Labill. | Myrtaceae | Tree | 13 | 11.50 | Leave | Used to treat nose blockade | Decoction |
| Dudhal-patal | *Euphorbia helioscopia* L. | Euphorbiaceae | Herb | 15 | 13.27 | Latex | Applied on abscess | Decoction |
| Dudii | *Euphorbia hirta* L. | Euphorbiaceae | Herb | 36 | 31.86 | Whole plant | Used to cure piles | Paste |
| Neeli Santh | *Evolvulus alsinoides* L. | Convolvulaceae | Herb | 44 | 38.94 | Whole plant | Used as brain tonic | Decoction |

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|---|---|---|---|---|---|---|---|---|
| Barh | *Ficus benghalensis* L. | Moraceae | Tree | 33 | 29.20 | Latex | Used to expel out the thorns which are broken down inside the body | Powder |
| Kharkhumbal | *Ficus hispida* L. f. | Moraceae | Tree | 29 | 25.66 | Fruit | Used as galactagouge | Powder |
| Phagara | *Ficus palmata* Forssk. | Moraceae | Tree | 33 | 29.20 | Fruit | Used for curing constipation | Powder |
| Rumbal | *Ficus racemosa* L. | Moraceae | Tree | 12 | 10.62 | Fruit | Used for treatment of kidney problems | Powder |
| Bar,peepal | *Ficus religiosa* L. | Moraceae | Tree | 23 | 20.35 | Seed | Used for curing piles | Powder |
| Khorti | *Galium aparine* L. | Rubiaceae | Herb | 31 | 27.43 | Whole plant | Used as laxative | Juice |
| Laal jehari | *Geranium wallichianum* Sweet. | Geraniaceae | Herb | 42 | 37.17 | Root | Used for curing chronic diarrhea and dysentery | Extract |
| Dhaman | *Grewia optiva* J.R. Drumm.ex Burrett | Malvaceae | Tree | 51 | 45.13 | Leave | Used to recover from general weakness | Extract |
| Karera | *Hedera napalensis* C. Koch. | Araliaceae | Tree | 23 | 20.35 | Leave | Used to remove hair lice | Decoction |
| Gudaal | *Hibiscus rosa-sinensis* L. | Malvaceae | Shrub | 13 | 11.50 | Flower | Used as diuretic | Decoction |
| Kagar | *Holarrhena pubescens* Wall. ex G.Don) (= *Holarrhena antidysenterica* Wall. ex A.DC.) | Apocynaceae | Tree | 41 | 36.28 | Bark | Used as antispasmodic | Decoction |
| Basantalu | *Hypericum perforatum* L. | Hyperiaceae | Herb | 29 | 25.66 | Leave | Used as diuretic | Decoction |
| Teera | *Impatiens balsamina* L. | Balsaminaceae | Herb | 31 | 27.43 | Leave | Used as antiphalogistic | Paste |
| Bilaitti Aak | *Ipomoea carnea* Jacq. | Convolvulaceae | Shrub | 43 | 38.05 | Leave | Used for curing abscess and joint to relieve pain | Extract |

(continued)

**Table 17.2** (continued)

| Vernicular name | Botanical name | Family | Habit | People cited | Frequency index (FI) | Part used | Ailment treated | Preparation |
|---|---|---|---|---|---|---|---|---|
| Sanairad | *Jasminum humile* L. | Oleaceae | Lianas | 38 | 33.63 | Root | Used for treatment of ringworm | Paste |
| Brenkar | *Justicia adhatoda* L. | Acanthaceae | Shrub | 47 | 41.59 | Root | Used in curing cough, asthma, chronic bronchitis | Powder |
| Kamble | *Lannea coromandelica* (Houtt.) Merr. | Anacardaiceae | Tree | 39 | 34.51 | Bark | Used for treatment of toothache | Paste |
| Panjphulli | *Lantana camara* L. var. *aculeata* (L.) Mould | Verbenaceae | Shrub | 79 | 69.91 | Latex | Used to promote hair growth | Decoction |
| Mahendi | *Lawsonia inermis* L. | Lythraceae | Shrub | 39 | 34.51 | Leave | Used to cure vitiligo | Decoction |
| Alsi | *Linum usitatissimum* L. | Linaceae | Herb | 17 | 15.04 | Seed | Used for treatment of abscess | Powder |
| Sadai | *Lotus corniculatus* L. | Fabaceae | Herb | 33 | 29.20 | Whole plant | Used as anti-spasmodic | Extract |
| Kamilla | *Mallotus philippensis* Muell.Arg. | Euphorbiaceae | Tree | 67 | 59.29 | Fruit | Used for killing worms in the stomach | Powder |
| Baddi Bareaar | *Malvastrum cormandelianum* (L.) Garcke | Malvaceae | Herb | 42 | 37.17 | Leave | Used as styptic | Paste |
| Amb | *Mangifera indica* L. | Anacardiaceae | Tree | 37 | 32.74 | Leave | Used for curing dysentery | Chew |
| Sareri | *Medicago lupulina* L. | Fabaceae | Herb | 56 | 49.56 | Leave | Used in constipation | Extract |
| Daraink | *Melia azedarach* L. | Meliaceae | Tree | 28 | 24.78 | Bark | Used to cure skin itching, rashes and eczema | Decoction |
| Pili senji | *Azadirachta indica* A.Juss. (= *Melilotus indica* (A.Juss.) Brandis) | Fabaceae | Herb | 41 | 36.28 | Leave | Used as aperients | Extract |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Pootna | *Mentha arvensis* L. | Lamiaceae | Herb | 73 | 64.60 | Leave | Used as constipation | Decoction |
| Jangali Pootna | *Mentha longifolia* L. s | Lamiaceae | Herb | 20 | 17.70 | Leave | Used as appetizer | Decoction |
| Chui mui | *Mimosa pudica* L. | Fabaceae | Shrub | 44 | 38.94 | Leave | Used for curing diabetes | Decoction |
| Toot | *Morus alba* L. | Moraceae | Tree | 48 | 42.48 | Fruit | Used for curing jaundice | Juice |
| Lal kaneer | *Nerium oleander* L. (= *Nerium indicum* Mill.) | Apocynaceae | Shrub | 27 | 23.89 | Leave | Used for treatment of heart disease | Decoction |
| Desi Tamakoo | *Nicotiana plumbaginifolia* Viv. | Solanaceae | Herb | 18 | 15.93 | Whole plant | Used externally on animal's body to get rid of external parasites | Paste |
| Haar-shringaar | *Nyctanthes arbor-tristis* L. | Oleaceae | Shrub | 36 | 31.86 | Leave | Used to kill intestinal worms | Extract |
| Naazposh | *Ocimum basilicum* L. | Lamiaceae | Shrub | 64 | 56.64 | Seed | Used for treatment of digestive problems | Decoction |
| Tulsi | *Ocimum tenuiflorum* L. | Lamiaceae | Shrub | 77 | 68.14 | Leave | Used for curing cough and cold | Decoction |
| Darraati | *Oenothera rosea* Soland | Onagraceae | Herb | 13 | 11.50 | Leave | Used for treatment of renal colic | Decoction |
| Tarappar sula | *Opuntia humifusa* (Raf.) Raf. (= *Opuntia vulgaris* Mill.) | Cactaceae | Shrub | 32 | 28.32 | Whole plant | Used as eardrop | Juice |
| Tantu | *Oroxylum indicum* (L.) Kuntze. | Bignoniaceae | Tree | 28 | 24.78 | Leave | Used in stomach ache | Decoction |
| Khattibooti | *Oxalis corniculata* L. | Oxalidaceae | Herb | 16 | 14.16 | Leave | Used to check toothache and halitosis | Chew |
| Aamla | *Phyllanthus emblica* L. | Euphorbiaceae | Tree | 63 | 55.75 | Bark | Used for treatment of constipation and hair care | Powder |

(continued)

**Table 17.2** (continued)

| Vernicular name | Botanical name | Family | Habit | People cited | Frequency index (FI) | Part used | Ailment treated | Preparation |
|---|---|---|---|---|---|---|---|---|
| Pataka | *Physalis angulata* L. (= *Physalis minima* L.) | Solanaceae | Herb | 37 | 32.74 | Leave | Used as ear-drop | Juice |
| Chir-pine | *Pinus roxburghii* Roxb. | Pinaceae | Tree | 39 | 34.51 | Resin | Used for curing boils, cuts and wound | Decoction |
| Gobba | *Plantago major* L. | Plantaginaceae | Herb | 49 | 43.36 | Seed | Used for treatment of gastric and peptic ulcers, irritable bowel syndrome, diarrhea and dysentery | Decoction |
| Kali Suaali | *Pogostemon benghalensis* (Burm. f.) Kuntze. | Lamiaceae | Shrub | 24 | 21.24 | Leave | Used for curing dyspepsia, cold and cough | Decoction |
| Masloon | *Bistorta amplexicaule* D.Don (= *Polygonum amplexicaule* D.Don) | Polygonaceae | Herb | 17 | 15.04 | Root | Used for curing cold and cough | Decoction |
| Pipli | *Persicaria hydropiper* (L.) Delarbre (= *Polygonum hydropiper* L.) | Polygonaceae | Herb | 26 | 23.01 | Whole plant | Used for treatment of diarrhea, bleeding piles and painful menstruation | Extract |
| Vanaclin | *Potentilla atrosanguinea* L. | Rosaceae | Herb | 42 | 37.17 | Root | Used to treatment of toothache | Chew |
| Aru | *Prunus persica* (L.) Batsch | Rosaceae | Tree | 27 | 23.89 | Leave | Used to curing cuts, wounds, burns and boil to soothe inflammation | Paste |
| Amrood | *Psidium guajava* L. | Myrtaceae | Tree | 19 | 16.81 | Leave | Used for curing oral ulcers | Chew |
| Darunni | *Punica granatum* L. | Punicaceae | Tree | 31 | 27.43 | Fruit | Used as tonic for anaemic individuals | Juice |
| Batangi | *Pyrus pashia* Buch.-Ham.ex D.Don | Rosaceae | Tree | 19 | 16.81 | Fruit | Used as insect repellent | Extract |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Charmula | *Ranunculus arvensis* L. | Ranunculaceae | Herb | 36 | 31.86 | Leave | Used for treatment of fever and asthma | Decoction |
| Darrili | *Ranunculus laetus* Wall. | Ranunculaceae | Herb | 53 | 46.90 | Latex | Promotes hair growth | Decoction |
| Korkhand | *Ranunculus muricatus* L. | Ranunculaceae | Herb | 37 | 32.74 | Whole plant | Used for treatment of periodic fever, asthma and arthralgia | Decoction |
| Arandi | *Ricinus communis* L. | Euphorbiaceae | Shrub | 18 | 15.93 | Leave | Relieve from headache | Paste |
| Kikkar | *Robinia pseudoacacia* L. | Fabaceae | Tree | 9 | 7.96 | Leave | Used as anti-spasmodic | Decoction |
| Gulab | *Rosa indica* L. | Rosaceae | Shrub | 11 | 9.73 | Flower | Used as aperients | Decoction |
| Aakhey | *Rubus ellipticus* Sm. | Rosaceae | Shrub | 17 | 15.04 | Fruit | Used as aperients | Powder |
| Baddi ammi | *Rumex hastatus* D. Don | Polygonaceae | Herb | 39 | 34.51 | Leave | Used as antiphlogistic | Paste |
| Kali jadi | *Salvia moorcroftiana* Wall. ex Benth. | Lamiaceae | Herb | 13 | 11.50 | Root | Used for treatment of cough and cold, stomach ache, dysentery and fever | Powder |
| Vandhamni | *Sida cordifolia* L. | Malvaceae | Herb | 42 | 37.17 | Whole plant | Used for treatment of stomach ache | Decoction |
| Takala | *Silene vulgaris* (Moench) Garcke | Caryophyllaceae | Herb | 8 | 7.08 | Leave | Used as demulcent | Paste |
| Kaayankothi | *Solanum nigrum* L. | Solanaceae | Herb | 43 | 38.05 | Leave | Used as antiphlogistic | Paste |
| Marmiri | *Stellaria media* (L.) Vill. | Caryophyllaceae | Herb | 24 | 21.24 | Whole plant | Used for treatment of broken bones | Paste |
| Jari | *Synedrella nodiflora* (L.) Garetn. | Asteraceae | Herb | 19 | 16.81 | Leave | Used as styptic | Paste |
| Jaamnoo | *Syzygium cumini* (L.) Skeels | Myrtaceae | Tree | 15 | 13.27 | Leave | Used for curing oral ulcers | Chew |
| Lmli | *Tamarindus indica* L. | Caesalpinaceae | Tree | 68 | 60.18 | Fruit | Used to cure anorexia and detoxify the effect of poisonous weed intake | Extract |
| Bathur | *Taraxacum officinale* Weber ex Wigg. | Asteraceae | Herb | 28 | 24.78 | Leave | Used as galactagogue | Decoction |

(continued)

**Table 17.2** (continued)

| Vernicular name | Botanical name | Family | Habit | People cited | Frequency index (FI) | Part used | Ailment treated | Preparation |
|---|---|---|---|---|---|---|---|---|
| Sarphank | *Tephrosia purpurea* (L.) Pers. | Fabaceae | Herb | 14 | 12.39 | Root | Used for treatment of typhoid | Decoction |
| Bhara | *Terminalia bellerica* Roxb. | Combretaceae | Tree | 38 | 33.63 | Fruit | Used for curing constipation | Powder |
| Gloe | *Tinospora cordifolia* (Willd.) Hook.f. & Thomson | Menispermaceae | Lianas | 91 | 80.53 | Stem | Used to cure hyperpyrexia | Extract |
| Pakhra, | *Tribulus terrestris* L. | Zygophyllaceae | Herb | 17 | 15.04 | Fruit | Used for treatment of impotency | Powder |
| Kumra | *Tridax procumbens* (L.) L. | Asteraceae | Herb | 53 | 46.90 | Leave | Used as styptic | Extract |
| Gagar luth | *Trifolium pratense* L. | Fabaceae | Herb | 21 | 18.58 | Whole plant | Used as anti-spasmodic | Extract |
| Saddar bot-soi | *Urtica dioica* L. | Urticaceae | Herb | 11 | 9.73 | Leave | Used to remove dandruff | Paste |
| Soottamakoo | *Verbascum thapsus* L. | Scrophulariaceae | Herb | 17 | 15.04 | Flower | Used to cure cough, asthma, bronchitis and pneumonia | Powder |
| Banapsha | *Viola odorata* L. | Violaceae | Herb | 57 | 50.44 | Root | Used for treatment of cough, cold and bronchitis | Decoction |
| Bana | *Vitex negundo* L. | Verbenaceae | Shrub | 32 | 28.32 | Flower | Used for curing diarrhea | Extract |
| Jojra | *Xanthium strumarium* L. | Asteraceae | Shrub | 13 | 11.50 | Leave | Used for curing headache | Paste |
| Adrak | *Zingiber officinale* Roscoe. | Zingiberaceae | Herb | 79 | 69.91 | Leave | Used for treatment of cold, cough and pyrexia | Juice |
| Bair | *Ziziphus mauritiana* Lam. | Rhamnaceae | Tree | 11 | 9.73 | Seed | Used to cure diabetes | Powder |

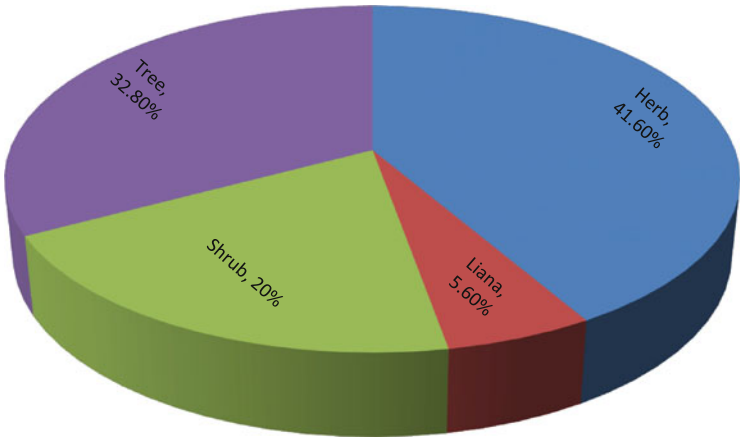


**Fig. 17.1** Life form of the studied plants from JWS

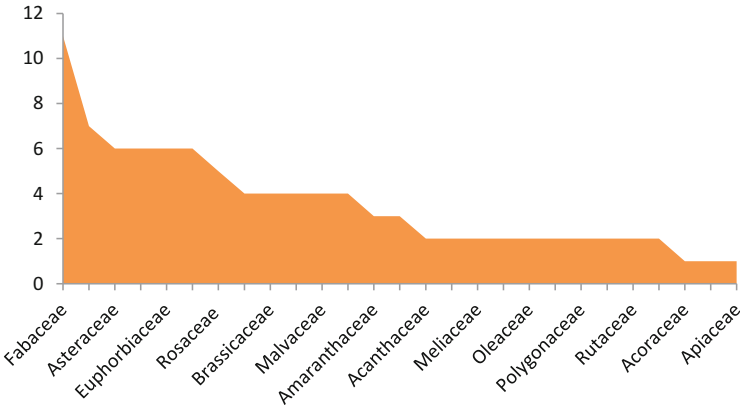


**Fig. 17.2** Dominant families based on species composition in JWS

(5.60%), latex (3.20%), rhizomes (1.60%), shoost (1.60%), stems (0.80%), resins (0.80%) and woods (0.80%) (Figs. 17.3 and 17.4). The informants generally collect plants from the nearby location or cultivate or purchase them from the local market.

According to Rao et al. (2015), Fabaceae, Asteraceae, Lamiaceae, Moraceae, Caesalpinaceae, Rosaceae, Euphorbiaceae, Solanaceae, Malvaceae and Ranunculaceae are the most commonly used medicinal plant families. There seems to be a tendency for a few plant families to stand out in any pharmacopoeia (Gazzaneo et al. 2005). Kumar and Bhagat (2012) have reported the dominance of these families in the local medicinal flora of Jasrota. The most dominant plant families of medicinal species were Fabaceae, Asteraceae, Lamiaceae, Moraceae, Caesalpinaceae, Rosaceae, Euphorbiaceae, Solanaceae, Malvaceae and

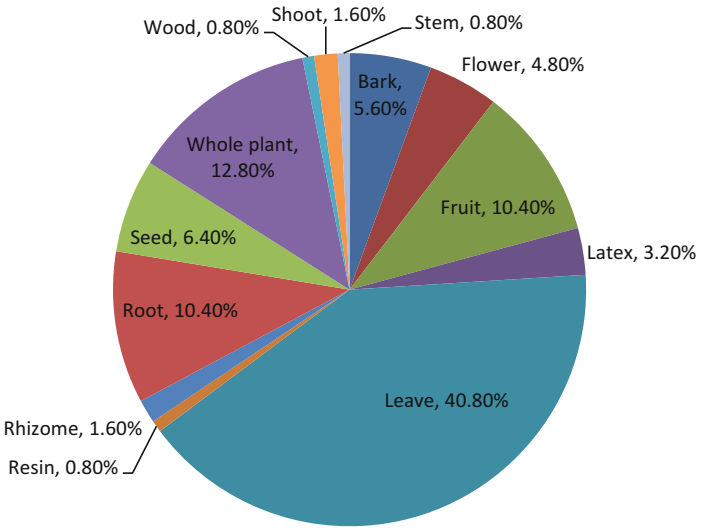


**Fig. 17.3** Plant parts used as medicine in JSW

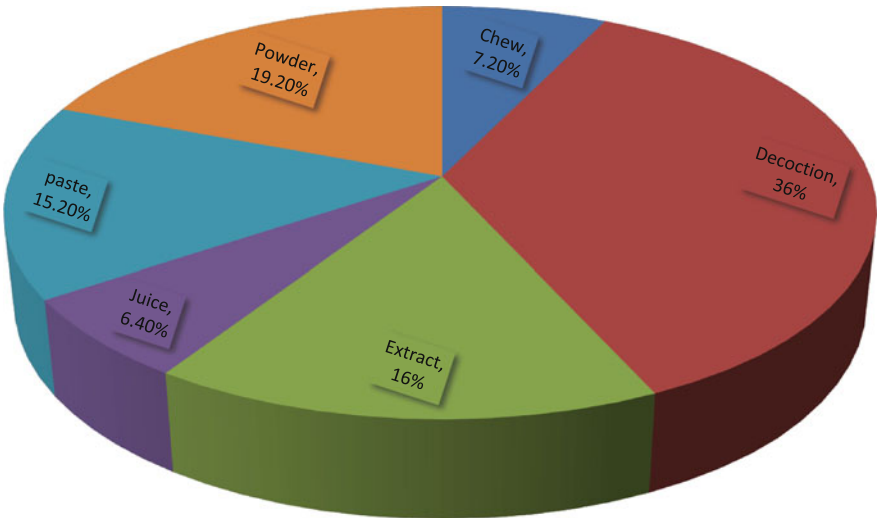


**Fig. 17.4** Application mode of studied medicinal plants from JSW

Ranunculaceae (Fig. 17.2) and could be attributed to their wider distribution, abundance in the study area and predominant herbaceous habit (Bhatia et al. 2014). Families Lamiaceae and Rosaceae are plants rich in essential oils intended for industries to produce medicine perfumes and cosmetics (Morales and Simon 2009). Asteraceae and Euphorbiaceae are the families with a large number of species and consequently with a higher probability of being used by the human population (Pereira et al. 2009).

Herbs were the most used medicinal plants observed during the investigation. The finding agrees with the general pattern of dominance of herbaceous species seen in most medicinal plants inventories in India and other countries (Godoy 1994; Tuomilehto et al. 2001; Tabuti et al. 2003; Balunas and Kinghorn 2005; Muthu et al. 2006; Estomba et al. 2006; Nair et al. 2007; Quinlan and Quinlan 2007; Ssegawa and Kasenene 2007; Giday et al. 2010; Musa et al. 2011; Bhatia et al. 2014; Rahaman and Karmakar 2014; Rao et al. 2015). The dominance of herbs in medicinal plant could be related to the fact that they are more easily accessible in the nearby areas than trees and shrubs (Lulekal et al. 2013).The leaves were the most used plant part followed by whole plant, root and fruits. Kumar and Bhagat (2012) have reported the other important plant parts, and root and whole plant as the important plant parts used in the local ethnomedicinal preparation of Jasrota.

Destructive harvesting kills the matured plant and could be a severe threat for survival of the often rare and slowly reproducing medicinal plants (Phillips and Gentry 1993; Philips et al. 1994; Singh et al. 2010, 2014, 2016). Another threat for the local medicinal system is the high contribution of wild plants to the main pool of the medicinal plants. According to Rao et al. (2015) both of these problems are the matter of great concern for the sustainability of the traditional practices and there is an urgent need to create awareness among the inhabitants of the study area about rationing, that is, sustainable collection, conservation, domestication, as well as small-scale (home garden for personal use) and large-scale (for trade) cultivation of medicinal plants. Species such as *Boerhavia diffusa*, *Mallotus philippensis*, *Viola odorata*, *Tridax procumbens*, *Cissampelos pareira*, *Tinospora cordifolia* and *Grewia optiva* with high frequency value are some of the most used medicinal plants in Jasrota (Kumar and Bhagat 2012) as well as other mountainous regions of the state (Rao et al. 2015). *M. longifolia* and *M. arvensis* are used as a carminative, anti-spasmodic, anti-peptic ulcer agent, cooling medicine, anti-diarrhoea and anti-gut spasmodic and to treat indigestion and flatulence (Akram et al. 2011). *O. tenuiflorum* possesses antibacterial, antiasthmatic, antioxidant and diuretic properties (Ebrahimzadeh et al. 2010) and *Justicia adhatoda* is used to treat fever, cold, asthma and respiratory condition (Mills and Bone 2000).

### 17.3.3 Frequency Index (FI)

The most important plants of the study sites on the basis of use value include *Boerhavia diffusa* (93.83%), *Mallotus philippensis* (82.72%), *Viola odorata* (70.37%), *Tridax procumbens* (65.43%), *Cissampelos pareira* (65.43%), *Tinospora cordifolia* (62.96%), *Grewia optiva* (62.96%), *Morus alba* (59.26%), *Justicia adhatoda* (58.02%), *Cannabis sativa* (58.02%), *Butea monosperma* (56.79%), *Evolvulus alsinoides* (54.32%), *Abrus precatorius* (53.09%) and *Solanum nigrum* (52.09%). Lesser used plants are *Emblica officinalis* (6.17%), *Cyperus rotundus* (9.88%), *Robinia pseudoacacia* (11.11%), *Commelina benghalensis* (13.81%), *Ficus racemosa* (14.81%), *Amaranthus spinosus* (14.81%), *Dioscorea deltoidea* (14.81%), *Xanthium strumarium* (16.05%), *Cassia occidentalis* (16.05%), *Albizia lebbeck* (16.05%), *Syzygium cumini* (18.52%) and *Acacia catechu* (18.52%).

**Table 17.3** Aliments-cum-disease category and plant species used for cure and treatment

| Aliments category | Disease categories | Plant used |
|---|---|---|
| Circulatory system/ cardiovascular disease | Blood purification, heart strength and memory power | *E. alsinoides, N. oleander* and *C. pareira* |
| Dermatological disorders | Abscess, itching, blemishes, bloats, vitiligo, boils, burns, cuts and wounds, dermatitis, eczema, herpes, ringworms, wound in lips, scabies and pimples | *C. arvensis, A. barbadensis, A. calamus, A. capillus-veneris, A. conyzoides, L. coromandelica, M. cormandelianum, D. deltoidea, E. helioscopia, J. humile, L. inermis, P. persica, T. procumbens, C. procera, A. mexicana, V. nilotica, S. nigrum, S. nodiflora, S. occidentalis, D. oleoides, C. oppositifolia, C. reflexa, C. rosea, P. roxburghii* and *L. usitatissimum* |
| Dental care | Foul odour, teeth strength, toothache worms in gums and teeth | *C. arvensis, L. coromandelica, V. nilotica, J. humile, S. occidentalis, C. procera, A. conyzoides, C. oppositifolia, P. roxburghii, S. nigrum, A. calamus, A. capillus-veneris, A. barbadensis, O. corniculata* and *P. atrosanguinea* |
| Endocrinal disorder | Diabetes | *C. asiatica, M. pudica* and *Z. mauritiana* |
| Ear, eyes, nose, mouth and throat problems | Conjunctivitis, nose blockade, oral ulcer, eardrop, mouth ulcer, eye sight and opthalmia | *S. cumini, S. catechu, E. globulus, O. humifusa, P. angulata* and *P. guajava* |
| Fevers | Fever | *S. tora, R. arvensis, R. muricatus, A. cadamba, S. nigrum* and *T. cordifolia* |
| Gastro-intestinal disorders | Constipation, dysentery, diarrhea, gastric complaints, vomiting, indigestion, intestinal ulcer, kidney problem, intestinal worms pile and stomach ache | *O. indicum, V. nilotica, B. variegata, E. hirta, C. dactylon, M. philippensis, A. calamus, H. pubescens, V. negundo, P. hydropiper, M. alba, S. occidentalis, P. emblica, L. inermis, P. sylvestris, A. spnosus, A. indica, B. ceiba, C. impatiens, C. spinarum, C. rosea, C. medica, C. benghalensis, F. palmata, F. religiosa, M. indica, M. lupulina, M. indica, M. longifolia, N. arbor-tristis, O. basilicum, R. indica, S. moorcroftiana, S. cordifolia, T. bellerica, V. negundo* and *F. racemosa* |
| Liver disorders | Hepatitis, jaundice, liver enlargement | *A. marmelos, M. alba* and *C. benghalensis* |

(continued)

**Table 17.3** (continued)

| Aliments category | Disease categories | Plant used |
|---|---|---|
| General health | Body refreshment, weakness, body strength, disease resistance and weight loss | *C. pareira, P. granatum, E. alsinoides, E. prostrata, D. strictus, A. sessilis, A. cadamba, D. stramonium, G. optiva* and *P. granatum* |
| Hair care | Dandruff, graying of hair, hair growth and hair loss | *P. emblica, A. indica, E. prostrata, H. nepalensis* and *L. camara* |
| Poisoning | Poison bites, scorpion sting and snake bites | *A. aspera, B. compestris* and *C. rotundus* |
| Respiratory systems diseases | Asthma, bronchitis, chest infection, cold, cough, halitosis, influenza, pharyngitis and whooping cough | *J. adhatoda, B. cristata, V. odorata, A. marmelos, N. oleander, A. calamus, R. arvensis, P. plectranthoides, C. fistula, O. tenuiflorum, P. amplexicaule, S. moorcroftiana, T. procumbens* and *Z. officinale* |
| Muscular-skeleton system disorders | Body pain, headache, joint pain, muscle pain, bone fracture rheumatism and swelling | *A. precatorius, S. media, A. precatorius, I. carnea* and *R. communis* |
| Genito-urinary ailments | Abortion, breast pain, delivery pain, lactation, male fertility, over bleeding, sexual power, impotency, urine blockages, sperm production and venereal diseases | *A. lebbeck, A. adscedens, B. rapa, B. monosperma, D. sissoo, F. hispida, O. rosea* and *T. terrestris* |
| Liver problems | Jaundice | *A. marmelos* and *M. alba* |

High Frequency index (FI) in the present study could be considered as an indicator for the high healing potential of those plants used against the corresponding diseases and therefore could be targeted for further photochemical investigation to indentify the bioactive compounds that are responsible for their high healing potential (Rao et al. 2015). Some of the most widely used species are *Boerhavia diffusa, Achyranthes aspera, Tinospora cordifolia, Psidium guajava, Justicia adhatoda, Phyllanthus emblica, Azadirachta indica*. The herb juice is used as eye drop in conjunctivitis for curing eye inflammation. *Ocimum tenuiflorum* and *Zingiber officinale* recorded for respiratory disorders possess numerous pharmacological properties (Khare 2007) and also find mention in some other studies (Thakur et al. 2020). *Butea monosperma* recorded for urological disorders is a very important medico-religious tree of Shivalik region of Jasrota (Manhas and Rao 2012). Table 17.3 presents aliments-cum-disease category and plant species used for cure and treatment.

## 17.4 Conclusion

The preliminary investigation reveals that inhabitants of Jasrota are rich in ethnomedicinal knowledge, and they are using 125 plants species for curing different diseases. The knowledge of medicinal plant increased significantly with age of the people. But, this knowledge is depleting with the increase in educational level, and reason may be modernization. Realizing this fact, the present study was conducted with aim to prevent the loss of ethnomedicine knowledge in our study area. Knowledge transfer is becoming reduced or limited between parents and the younger generation. However, elders still utilize the native species as part of their dietary supplements. The documentation of these native fruits will revive the awareness of their utilization among the youth age category. The other two concerns for the sustainability of the traditional practices are firstly the contribution of wild plant to the medicinal flora and secondly the medicinal plants harvested through destructive means. There is a high level of consensus among the informants about the usage of medicinal plants in the study area. We emphasize an urgent need to create awareness among the inhabitants of the study area about rationing (i.e. sustainable collection, conservation and domestication) of medicinal plants.

**Acknowledgement** The first author is highly thankful to the Head, Department of Botany, Veer Kunwar Singh University and Director CSIR-IIIM, Jammu for research facilities and moral support. This research documentry is a part of research work of the first author (BS).

**Conflict of Interest** No conflict of interest between the authors for this manuscript.

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# Ethanobotany and Phytochemistry of *Lantana camara* L. (Verbenaceae)

18

Satyendra Kumar, Bikarma Singh, and Anand Yadav

**Abstract**

Genus *Lantana* L. comprises of 129 species under the family Verbenaceae and *Lantana camara* L. is a significant invasive weed species whose growth form is bushy scandent shrub. Inspite of being one of the World's worse invader, this plant species is used in Indian traditional system of medicine to treat ulcers, skin rashes, leprosy, asthma, and various viral disease such as rabies, chicken pox, and measles. Extract of *L. camara* has cytotoxic effect against certain cancer cell lines. Leaves of *L. camara* show insecticidal, antibacterial, and fungicidal properties. This species possesses essential oils and different type of phytochemicals such as phenolic compounds, proteins, alkaloids, and carbohydrates (glycosides, oligosaccharides, iridoid glycosides, phenylethanoid, quinine, saponins, steroids, triterpenes, sesquiterpenoids, and tannin) present in different parts of the plant. In addition to these phytochemicals, *L. camara* also contains flavonoids that have reductive ability indicating antioxidant and anticancerous activities. This communication deals with ethnobotany and

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_18

phytochemistry of *L. camara* and its scope in future research for medicine and herbal formulation development.

**Keywords**

Ethnobotany · Phytochemistry · *Lantana camara* · Cytotoxic · Antioxidant · Drug development

## Abbreviations

DPPH 2,2-diphenyl-1-picrylhydrazyl
MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide)]

## 18.1 Introduction

*Lantana camara* L. belonging to family Verbenaceae is recognized as a valuable medicinal plant reported to be growing all across the world (Kumarasamyraja et al. 2012). Common name of *L. camara* is wild sage and its growth-form is evergreen terrestrial scandent shrub having aromatic properties. The plant grows throughout the tropical region as a decorative plant (Sastri 1962; Das et al. 2003). Historically, the word *Lantana* comes from Latin word "lento" which means 'to bend' (Ghisalberti 2000), and in Sanskrit, it is populalrly known by the name of 'chaturangi' and 'vanacchedi' mentioned in Brahmayurved. In 1753, genus *Lantana* was first described by Carolus Linnaeus and given its binomial name (Munir 1996). There are 126 species of this genus found worldwide and common distribution reported from Central America and Caribbean Islands as the native place for *Lantana* species (Baars 2002). Some species also reported to be originated from Africa and India (Day and McAndrew 2003; Heshula 2009; Thakur et al. 2019, 2020). *Lantana camara* considered as invasive species in many countries (Sharma et al. 1988). However, it is naturalized in most parts of India (Kannan et al. 2008; Aravind et al. 2006; Kimothi and Dasari 2010; Surampalli 2010; Patel 2011; Dobhal et al. 2011). *Lantana camara* extensively use as medicinal herbs (Verma 2016a, b; Sharma et al. 1988; Sharma and Sharma 1989) and its leaves show antibacterial, fungicidal, and insecticidal properties (Chavan and Nikam 1982). Traditionally this species used for treating a variety of diseases including cancer, asthma, skin rashes, leprosy, and various viral disease such as rabies, chicken pox, and measles (Barik et al. 2006; Singh 2019a, b). Some previously reported studies indicated its use in reduction of ulcer development in rats and mice (Sathish et al. 2011). *Lantana camara* possesses different types of essential oil compounds and has repository of phytochemical such as phenolic constitutes proteins, alkaloids, carbohydrates (glycosides and oligosaccharides), iridoid glycosides, phenylethanoid, quinine, saponins, steroids, triterpenes, sesquiterpenoids, and other secondary metabolites. *Lantana camara* also contains flavonoids, which have reductive ability,

and indicated that this species possesses antioxidant as well as anticancerous activities, which have an important role in medicinal research programs (Venkatachalam et al. 2011; Kensa 2011; Kalita et al. 2011).

## 18.2 Taxonomic Classification

- Kingdom: Plantae
- Phylum: Spermatophyta
- Class: Dicotyledonae
- Order: Lamiales
- Family: Verbenaceae
- Genus: *Lantana*
- Species: *Lantana camara* L.

## 18.3 Plant Morphology

*Lantana camara* (Fig. 18.1) is an evergreen scandent shrub with strong aroma. Leaves are opposite, ovate, acute or sub-acute, crenate, and serrate along the margin (Thamotharan et al. 2010). It is a multi-stemmed and deciduous perennial plant that grows up to 180 cm in height. When plant is green, stems are covered with stiff hairs, square, and rough. Leaves with long petioles and have blunt toothed margins. Its flowers are dense, stalked, small in cluster, and multi-colored which include yellow-orange-red mix or white-pink-lavender. Its flower color changes subsequently. Berries especially seeds are very poisonous; however, insects and birds usually like them; berries are in round shape, fleshy, initially green in color, later turns to purple when matured or become blue-black after losing maturity (Priyanka and Joshi 2013; Ved et al. 2018).


**Fig. 18.1** General morphology of *Lantana camara*

**Table 18.1** Ecological requirements of *Lantana camara*

| Habitat | Requirement |
|---|---|
| Light range | Sun to full Sun |
| pH range | 4.5–8.5 |
| Temperature | Intolerant of frequent or prolonged freezing |
| Annual rainfall | 1000–4000 mm |
| Soil range | Mostly sandy to clay loam |
| Water range | Semi-arid to normal |
| Altitude | Up to 2000 m above sea level |
| Light conditions | Unshaded habitat |

Source: Priyanka and Joshi (2013)

## 18.4 Ecology and Habitat

*Lantana camara* naturalized in a wide ecological niche as it has ability of tolerance to the wide range of climate. Wide ecological adaptation results them in widespread and diverse distribution. *Lantana camara* grows in different habitats such as near beachfronts, rainforest edges, wastelands, and forests disturbed by fire or logging (Day and McAndrew 2003). It can also grow well in disturbed habitats such as railway tracks, roadside, and canals (Thakur et al. 1992; Sharma et al. 2005; Kohli et al. 2006). It is said that the invasion and spread of this species to the other habitat are enhanced by anthropogenic activities (Sahu and Singh 2008) (Table 18.1).

## 18.5 Geographical Distribution

*Lantana camara* reported to have naturalized in countries and islands between 35°N and 35°S latitudes (Priyanka and Joshi 2013). It is a native species of Central America and Caribbean Islands (Baars 2002). The presence of this species reported from Florida (USA), Jamaica, Brazil, Mexico, and Trinidad. It also spreads to tropical regions of America and the entire South American temperate zones (Day and McAndrew 2003; Sanders 2006; Taylor et al. 2012; Ved et al. 2018). Some species of the genus *Lantana* are also believed to be originated from Africa and India (Baars 2002; Day and McAndrew 2003; Heshula 2009). In many countries, this species is categorized as invasive or alien species where this species is introduced as an ornamental plant or secondary forest species (Sharma et al. 1988; Singh et al. 2018a, b) (Fig. 18.2).

Historically this species was introduced during 1809 in Calcutta Botanical Garden by the Britishers as an ornamental plant (Brandis 1882; Aravind and Rao 2001; Nanjappa et al. 2005). After that this species as time passes gets acclimatized and naturalized in India (Ved et al. 2018). At present this species is found in almost all geographical regions of India except the Thar Desert and its surroundings of very hot climate (Kannan et al. 2008; Aravind et al. 2006; Kimothi and Dasari 2010; Surampalli 2010; Patel 2011; Dobhal et al. 2011). Researchers point out that *L. camara* is very rapidly expanding in many regions of India, thus it is considered as a sample species for

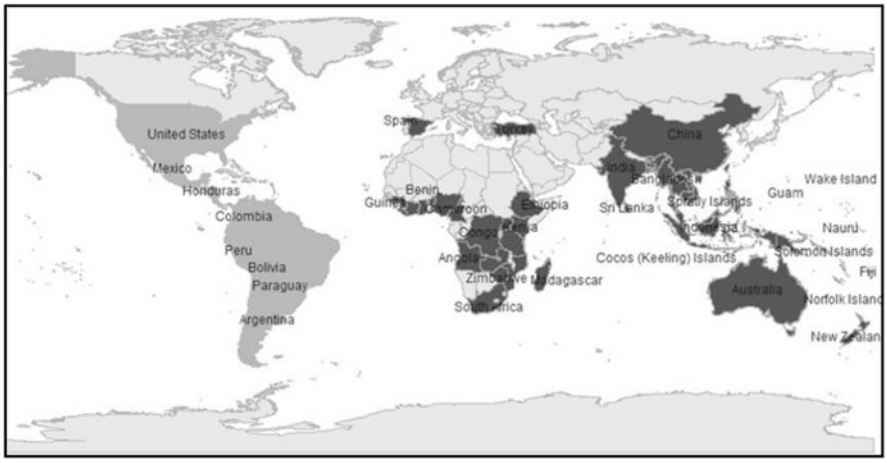


**Fig. 18.2** *Lantana camara* native (light gray) and introduced or naturalized (dark gray) regions (Priyanka and Joshi 2013)

future threat to forest ecosystems (Roy et al. 2002; Day and McAndrew 2003; Sharma et al. 2005; Kohli et al. 2006; Dogra et al. 2009) (Table 18.2).

## 18.6 Ethnobotany

Several plants as a medicine have been used for several years to treat deadly disease occurring throughout the world (Hiremath and Sundaram 2005; Verma 2016a). Crude drugs developed from the extract of various part of medicinal plant, which possess different types of pharmacological properties (Verma 2016b). Based on the experiences, knowledge of indigenous plants which are used as medicine by tribal population passes from generation to generation (Pathak and Mishra 2011). Plants with food and medicinal properties have been used by various communities recognized as tribal, rural and urban for the treatment of various diseases. Effect of medicinal plant on diseases mainly depends on the active phytochemical constitutes present in plant. Chemical constitutes are responsible for the antioxidant activity, which may prevent oxidative damage. Therefore, these medicinal plants may be useful for treatment of cancer and neurodegenerative diseases (Visconti and Grieco 2009; Rodrigo et al. 2013; Paul et al. 2016).

Several studies of *L. camara* have pointed out various therapeutic uses mainly as herbal medicine (Sharma et al. 1988; Sharma and Sharma 1989). In many parts of the world, this species has been used to treat a wide variety of disorders (Ross 1999). The leaves of *L. camara* in Central and South America were made into a paste and applied to treat sores, chicken pox and measles. Preparations in the form of crude drug from the plant used to cure fever, cold, rheumatism, asthma and high blood pressure in many parts of the world (Irvine 1961; Chavan and Nikam 1982; Sharma

**Table 18.2** General characteristic of *Lantana camara*

| Characteristics | Description | References |
|---|---|---|
| Native | Tropical area of Central and South America | Day and McAndrew (2003), Sanders (2006), and Taylor et al. (2012) |
| Distribution | Countries between 35° N and 35° S latitudes | Priyanka and Joshi (2013) |
| Plant category | Annuals or biennials or perennial scandent shrubs, ground covers | Sundufu and Shoushan (2004) |
| Local name | Wild or red sage | Saxena et al. (2012), Singh and Bedi 2017, and Ved et al. (2018) |
| Flower characteristics | Axillary, long lasting, showy, unusual | Priyanka and Joshi (2013) |
| Flower color | Pink, yellow, orange | Saxena et al. (2012) and Sharma et al. (1988) |
| Tolerances | Drought, heat and humidity, pollution, slope, wind | Priyanka and Joshi (2013) |
| Propagation methods | Budding | Priyanka and Joshi (2013) |
| Foliage characters | Fragrant, evergreen, poisonous | Priyanka and Joshi (2013) |
| Leaves | Opposite or whorled, simple, oral or oval-oblong with pointed tips, toothed edges and many veins giving a wrinkled appearance | Verma (2018) |
| Biological properties | Antiseptic, antiproliferative, antimicrobial, fungicidal, insecticidal, nematicidal activity, antioxidant activity | Basu and Hazra (2006), Begum et al. (1995), Sharma et al. (1999), and Saxena et al. (1992) |
| Medicinal use | Fever, cough, asthma, rheumatism, cold, dysentery | Oliver-Bever (1982) |

and Sharma 1989; Begum et al. 2003; Sharma et al. 2007; Barreto et al. 2010; Kalita et al. 2012; Ved et al. 2018). Water extract of the whole plant used to cure bronchitis and the root in powdered form mix with milk given to children for stomach ache in Ghana (Irvine 1961). Extracted *L. camara* oil is used as an antiseptic and this helps in the treatment of rough skin, itches, and wounds. Externally decoctions were applied to cure leprosy and scabies. In Asian countries, leaves of *L. camara* used to treat small cuts, ulcer, autoimmune diseases like rheumatism and intestinal worms. Active phytochemical lancamarone, a steroid, extracted from *L. camara* believed to have cardiotonic properties, which pushes heart to supply enough blood to other organ of the body. In India, young leaves are boiled with water to use as herbal tea which helps to cure cough. Lotion of pounded leaves applied on cuts and swellings (Verma and Verma 2006). Leaves of *L. camara* are also used as a repellent, and help in protecting human from the bites of insects, pests and mosquitoes (Kalyanasundaram 1991; Novak 1985). The native inhabitants of African continent (Ethiopia, South Africa, Nigeria, Kenya and Tanzania) traditionally

use *L. camara* as aromatic plants to prevent insects or act as insect repellent (Pavela and Benelli 2016). In Ibadan and Nigeria, inhabitants also use *L. camara* as mosquito repellents (Egunyomi et al. 2010). In Vanuatu, juice from the leaves of *L. camara* used to cure cuts and wounds (Bradacs et al. 2011). In Lwamondo area (Limpopo province, South Africa), smoke of dried roots and leaves is used by women in case of painful menstruation (dysmenorrhoea). Leaves and roots are boiled with water and the mixture extract drink is used for treating protein deficient malnutrition called kwashiorkor (Mahwasane et al. 2013). In Vha-Venda (South Africa), juice extracted from the fresh leaves is also used to cure eye injuries or painful eyes (Mabogo 1990). Water extract and physical mixture of *L. camara* are used for pest control in South Uganda (Mwine et al. 2011).

In India, the Ayurvedic system of medicine have been existing since Vedic period (1500–500 BC) (Singh et al. 2014, 2016). Charaka and Sushruta, two of the earliest distinguished Indian scholars who compiled Charak Samhita and Sushruta Samhita book, respectively, have provided sufficient knowledge of the medicinal properties of Indian plants (Singh et al. 2009; Singh 2015; Kumar et al. 2020). Vedas have information of Ayurveda, although Atharva Ved is explained Ayurveda in much broader ways as a rich material of herbal medicine (Alice and Asha 2007). The herbal drugs are important for the people of the third-world countries to cure diseases because these drugs are economically cheaper and have little or no side effects. India is native habitat of several indigenous biodiversity. In Theni district (Tamil Nadu), fresh flower of *L. camara* is used for the treatment of headache (Ignacimuthu et al. 2008). Assam in Northeast India, smoke of dried aerial parts of *L. camara* is used as a repellent for mosquitoes. Polishing of house floor with leaf extracts of *L. camara* plant is routinely done to drive away mosquitoes and other insects (Dua et al. 1996; Namsa et al. 2011). Bark decoction of *L. camara* is taken once in a day for treatment of malaria and smoke of leaves used as a larvicidal and repellent activity in human (Dua et al. 1996; Mir et al. 2019; Dwivedi and Karwasara 2003). Crushed seeds of this species in Khasi and Garo Hills (Meghalaya) are used to intoxicate fishes (Neogi et al. 1989). Decoction of leaves of *L. camara* is drunk for the treatment of Tetanus in the Dibru-Saikhowa biosphere reserve of Northeast India (Purkayastha et al. 2005). In Kadamguda village, Malkangiri district of Orissa, India, *L. camara* stem is used as toothbrush for bright tooth (Prusti and Behera 2007).

## 18.7 Phytochemistry

Major chemical constituents in *L. camara* mostly belong to the group of glycosides, oligosaccharides, triterpenoids, iridoid, naphthoquinones, flavonoids, phenylpropanoid, lantadene A, lantadene B, lantadene C, and lantadene (Sharma et al. 1988; Sharma et al. 1990; Sharma and Dawra 1991). The major phytochemical constituents in leaves and stems of *L. camara* are oleanonic acid and ursonic acid (Hart et al. 1976). The characteristic of triterpenoids in the leaves of this species is different from that in the roots. Pentacyclic triterpenoid oleanolic acid and oleanonic acid are the major constituents of the roots of *L. camara* (Hart et al. 1976). Major

triterpenoids isolated from the roots of *L. camara* are lantanolic acid β-O-angeloyl oleanolic acid, 22β-O-senecioyl-oleanolic acid, 22β-hydroxy oleanonic acid, α-hydroxy-ursolic acid, and 3β-isovaleryl-19α-hydroxy-ursolic acid (Pan et al. 1993). Major flavonoids have been reported from *L. camara* plant are camaraside (3,5-dihydroxy-4,6-dimethoxyflavanol-7-O-glucopyranoside), linaroside (7-O-(β-D-glucopyranosyl)-6,4-dimethoxy-5-hydroxy flavone), lantanoside (7-O-(6-O-acetyl-β-D-glucopyranosyl)-6,4-dimethoxy-5-hydroxy flavone), camaroside (4,5-dihydroxy-3,7-dimethoxyflavone-4-O-β-D-glucopyranoside), 3-methoxy quercetin.

The major iridoid glycosides are theveside, theviridoside, geniposide, 8-epiloganin, shanzhiside methyl ester, lamiridoside. Some oligosaccharides recorded from *L. camara* include ajugose, stachyose, verbascotetracose, verbascose, lantanose A, lantanose B. Essential oil extracted from the stem, leaves, flowers, or aerial parts of *L. camara* plant has also been investigated and major oil constituents recorded includes β-cymene, α-phellandrene, α-pinene, dipentene, γ-terpinene, caryophyllene, cadinene, cineol, linalool, geraniol, and eugenol. The chemical structure of various chemical constituents recorded from *L. camara* is illustrated in Figs. 18.3a, 18.3a, and 18.3c (Table 18.3).

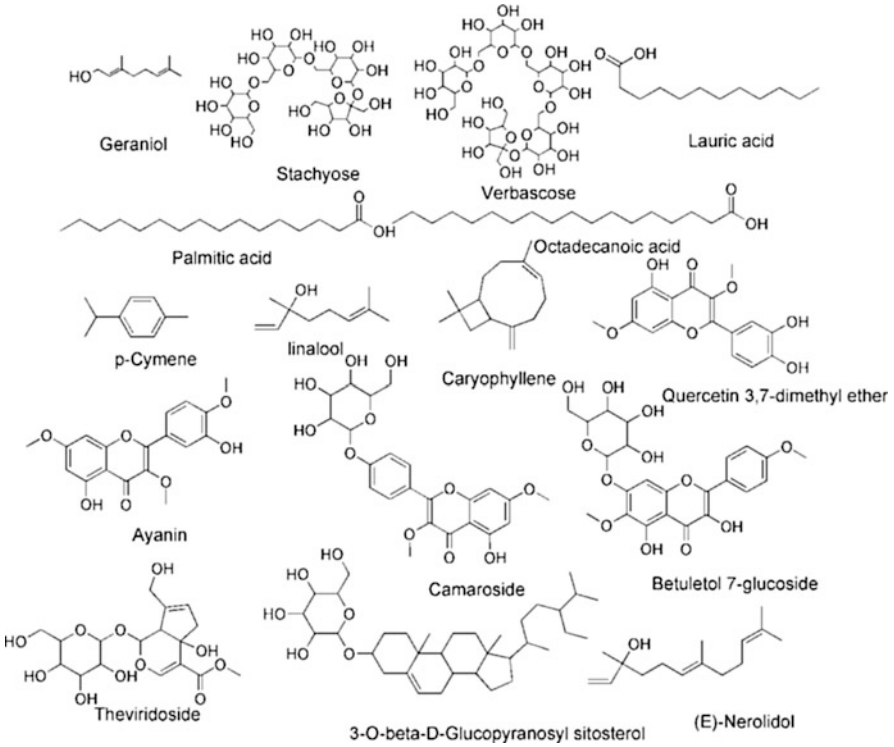


**Fig. 18.3a** Chemical structure of compound present in *Lantana camara*

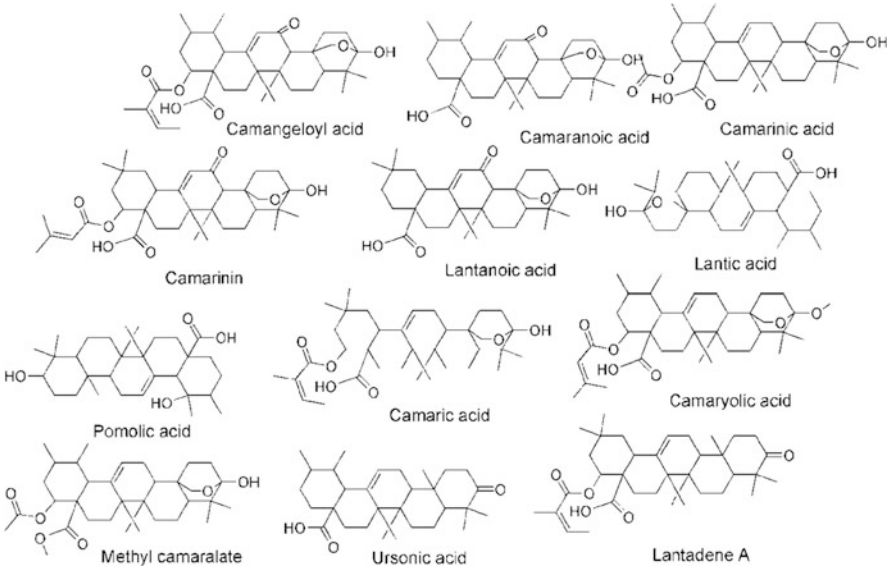


**Fig. 18.3b** Chemical structure of compound present in *Lantana camara*

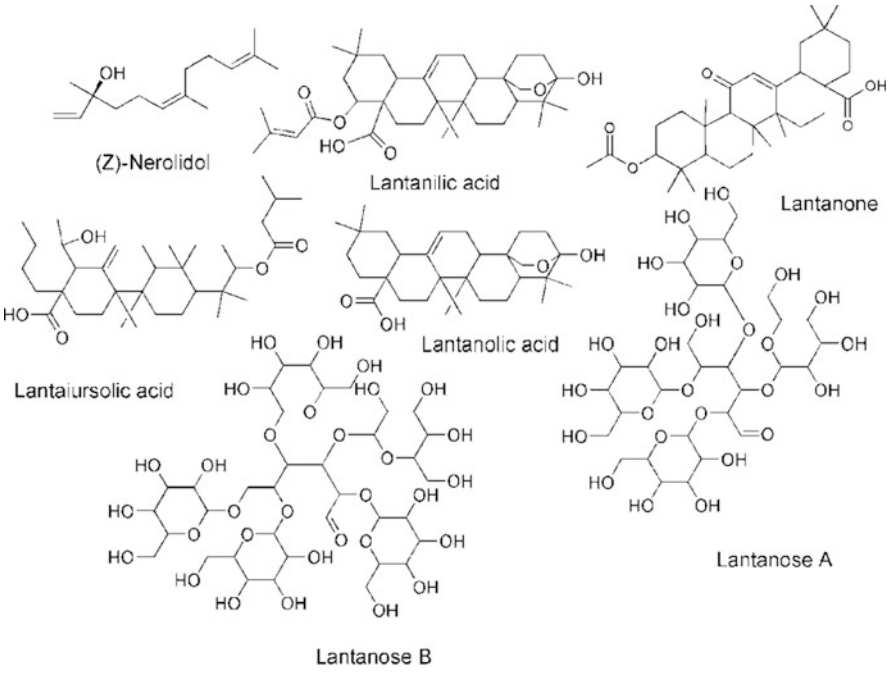


**Fig. 18.3c** Chemical structure of compound present in *Lantana camara*

**Table 18.3** Major active chemical constituents of *Lantana camara*

| Compound name | Molecular formula | Mass | References |
|---|---|---|---|
| Geraniol | $C_{10}H_{18}O$ | 154 | Ahmed et al. (1972) |
| Stachyose | $C_{24}H_{42}O_{21}$ | 666 | Inada et al. (1997) |
| Verbascose | $C_{30}H_{52}O_{26}$ | 828 | Barros et al. (2016) |
| Lauric acid | $C_{12}H_{24}O_2$ | 200 | Sundufu and Shoushan (2004) |
| Palmitic acid | $C_{16}H_{32}O_2$ | 256 | Begum et al. (2003) and Sundufu and Shoushan (2004) |
| Octadecanoic acid | $C_{18}H_{36}O_2$ | 284 | Begum et al. (2003) |
| p-Cymene | $C_{10}H_{14}$ | 134 | Ngassoum et al. (1999) |
| Linalool | $C_{10}H_{18}O$ | 154 | Sundufu and Shoushan (2004) |
| Caryophyllene | $C_{15}H_{24}$ | 204 | Sundufu and Shoushan (2004) |
| Quercetin 3,7-dimethyl ether | $C_{17}H_{14}O_7$ | 330 | Ghisalberti (2000) |
| Ayanin | $C_{18}H_{16}O_7$ | 344 | Barros et al. (2016) |
| Camaroside | $C_{23}H_{24}O_{11}$ | 476 | Barre et al. (1997) |
| Betuletol 7-glucoside | $C_{23}H_{24}O_{12}$ | 492 | Begum et al. (2008) |
| Theviridoside | $C_{17}H_{24}O_{11}$ | 404 | Ford and Bendall (1980) |
| 3-O-beta-D-Glucopyranosyl sitosterol | $C_{35}H_{60}O_6$ | 576 | Basu et al. (2017) |
| (E)-Nerolidol | $C_{15}H_{26}O$ | 222 | Sonibare and Effiong (2008) |
| Camangeloyl acid | $C_{35}H_{50}O_7$ | 582 | Begum et al. (2003) |
| Camaranoic acid | $C_{30}H_{44}O_5$ | 484 | Begum et al. (2008) |
| Camarinic acid | $C_{32}H_{48}O_6$ | 528 | Siddiqui et al. (1995) |
| Camarinin | $C_{35}H_{50}O_7$ | 582 | Begum et al. (2008) |
| Lantanoic acid | $C_{30}H_{44}O_5$ | 484 | Begum et al. (2008) |
| Lantic acid | $C_{30}H_{46}O_4$ | 470 | Begum et al. (2008) |
| Pomolic acid | $C_{30}H_{48}O_4$ | 472 | Begum et al. (2008) |
| Camaric acid | $C_{35}H_{52}O_6$ | 568 | Begum et al. (2003) |
| Camaryolic acid | $C_{36}H_{54}O_6$ | 582 | Begum et al. (2003) |
| Methyl camaralate | $C_{33}H_{50}O_6$ | 542 | Sharma et al. (2007) |
| Ursonic acid | $C_{30}H_{46}O_3$ | 454 | Sharma et al. (2007) |
| Lantadene A | $C_{35}H_{52}O_5$ | 552 | Sharma et al. (2007) |
| Lantadene B | $C_{35}H_{52}O_5$ | 552 | Sharma et al. (2007) |
| (Z)-Nerolidol | $C_{15}H_{26}O$ | 222 | Singh et al. (2010) and Zoubiri and Baaliouamer (2012) |
| Lantanilic acid | $C_{35}H_{52}O_6$ | 568 | Sharma et al. (2007) |
| Lantanone | $C_{32}H_{48}O_5$ | 512 | Sharma et al. (2007) |
| Lantaiursolic acid | $C_{35}H_{56}O_5$ | 556 | Pan et al. (1993) |
| Lantanolic acid | $C_{30}H_{46}O_4$ | 470 | Sharma et al. (2007) |
| Lantanose A | $C_{30}H_{52}O_{26}$ | 828 | Sharma et al. (2007) |
| Lantanose B | $C_{36}H_{62}O_{31}$ | 990 | Sharma et al. (2007) |

## 18.8 Conclusion

*Lantana camara* has unique biological properties and can be considered as potential medicinal plant for research. It is strongly believed that the details provided on the phytochemical, ethnobotany and various biological properties will help a lot in the use of this plant in different field of biological research that may help in drug discovery. This species has been in use to health problems in different parts of the globe. *Lantana camara* exhibits antimicrobial activity, and screens for anticancer activity against human cancer cell lines that help a lot in drug development. MTT assay confirmed that the leaves of *L. camara* have cytotoxic effect on various cancer cell lines. In vitro studies show that crude extract of *L. camara* shows antioxidant properties in DPPH radical scavenging assay and nitric oxide free radical scavenging assay. This species also reported to have anti-inflammatory activities and this may help in controlling stroke, cancer and other neurodegenerative diseases. Traditionally, *L. camara* has been used as a herbal medicine and used in many parts of the world to treat a wide variety of human disorders such as sores, chicken pox, measles, kwashiorkor fever, cold, rheumatism, asthma, high blood pressure, bronchitis, and stomach ache. Essential oil of this species is used in the treatment of skin, itches, leprosy, and scabies. Water extract and physical mixture of *L. camara* are used for pest control. In the twenty-first century, the human health care is the main concern worldwide. Therefore, this species may act as potent plant for drug discovery program. For using crude plant as a drug, more clinical trial and drug development is needed. Current clinical evidence of *L. camara* is limited. Research should be carried out to find out correlation between chemical constitute and mode of action. Molecular modeling may provide the way for drug discovery. Phytochemical and other constituents of *L. camara* may useful in the future as a source of herbal drug which may useful for mankind.

**Acknowledgments** The first author would like to thanks his PhD supervisor, parents and wife for this motivation and help during the course of this study.

**Conflict of Interest** The authors declare no conflict of interest for this manuscript.

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# *Cymbopogon winterianus* Jowitt ex Bor, a Hub for Various Industrial and Pharmaceutical Applications

19

Sunita Munda and Mohan Lal

**Abstract**

*Cymbopogon winterianus* Jowitt ex Bor is an aromatic grass belonging to the family Poaceae. The essential oil produced from this crop is of immense significance due to its use in perfume, soap, cosmetic, detergent, and flavour industries at low concentration and in pharmaceutical industry. The ethnopharmacological evidence as well as the scientific community has also proved its different biological activities like anti-inflammatory, antimicrobial, and anticonvulsant activities which is of great help to the pharmaceutical industries. New herbal substitutes without side effects may be formulated with proper dose and concentration. Besides this, it is of great demand in biopesticide industry because of its ability to ward off many insects, ticks, and mosquitoes and destroy weeds. The global production of citronella oil is about 5000 tonnes which is equal to 20 million USD, while in India 350 tonnes of essential oil is produced. Several researches are being carried out in this crop; therefore, we have tried to compile the data altogether and provide the up-to-date information on the different activities of *C. winterianus* which will be useful to the scientific community for proper exploitation of the resources available. Researchers are also ongoing on the premises of CSIR-NEIST, Jorhat, for the development of high herbage and

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_19

essential oil-yielding variety of this crop. Moreover, the institute is also maintaining more than 100 number of germplasm collected from different parts of India.

**Keywords**

Aromatic grass · *Cymbopogon* · Essential oil · Bioactive compounds · Biological activities · Industrial application

## Abbreviations

| | |
|---|---|
| AQ | Anthraquinone |
| CL | Confidence level |
| EO | Essential oil |
| FID | Flame ionization detector |
| GC | Gas chromatography |
| L | Lethal concentration |
| LEO-CD | Leaf essential oil complexed with $\beta$-cyclodextrin |
| MS | Mass spectroscopy |
| RF | Resistance factor |
| SP | Synthetic pyrethroids |

## 19.1 Introduction

*Cymbopogon winterianus* Jowitt ex Bor, also known as Java citronella, belongs to an industrially important genus *Cymbopogon* that has the potential of essential oil production. The name *Cymbopogon* was first of all given by Sprengel (1815) that comprises 140 species worldwide, out of which 45 species are known to occur in India (Lal et al. 2018). Globally it is grown in the tropical and subtropical areas of America, Africa and Asia (Sangwan et al. 2001), while Tamil Nadu, Uttar Pradesh, Karnataka, West Bengal and northeast states are the major contributing states in India (Lal et al. 2016). *C. winterianus* can acclimatize different environmental conditions (Wany et al. 2014), but its cultivation is best suited in warm and humid climate with adequate rainfall. It is grown on sloping lands, terraces and even land (Munda et al. 2020). The citronella essential oil is produced from the stems and leaves of this plant. Citronella essential oil is of two types: Ceylon citronella oil and Java citronella oil produced from two different chemotypes *C. nardus* Rendle (inferior type) and *C. winterianus* Jowitt (superior type), respectively. The term *C. winterianus* was named after an oil distiller of Sri Lanka (formerly Ceylon) who differentiated *C. nardus* into Maha Pengiri and Lena Batu and named the former as *C. winterianus* after his name Winter (Chang 2007; Shasany et al. 2000). Morphologically they can be differentiated based on their leaf length and shape and chemically through their chemical constituents (Wany et al. 2014). The Ceylon chemotype is rich in geraniol (18–20%) and limonene (9–11%), while the Java

chemotype is rich in citronellal (32–45%) and geraniol (11–13%) (Katiyar et al. 2011). According to the world trade record of important essential oils, citronella essential oil ranks 20th (Lawrence 1993). This oil is of immense value due to its application in different industrial sectors in the field of food as flavour additives in low quantities (45 ppm) (Katz et al. 2008), perfumery in aroma industries, and soap, cosmetics and pharmaceutical industries (Wany et al. 2013). This is also registered since 1948 in the United States as "McKesson's oil of citronella", a plant-based insect repellent for human application. Moreover, it was also considered as nontoxic biopesticide by the United States Environment Protection Agency (EPA) (Trongtokit et al. 2005; Anonymous 1997, 2001, 2004, 2006, 2007).

## 19.2 Botanical Description

Java citronella is a tufted, perennial and aromatic grass sprouting from rhizomes with several erect culms attaining a height of 110–122 cm. The culms and leaf sheaths are glabrous and smooth in nature. The culms are arranged closely with purplish or yellow sheaths depending on the germplasm variety. The leaves are broad, green and sword-shaped, drooping at around two-thirds of the leaf length. Leaf blades are linear with long filiform tip; the upper surface of the leaf is green in colour, while the lower portion glaucous in nature, with midrib smooth and margins serrulately scabrid (chromosome number, $2n = 20 = 40$).

## 19.3 High-Yielding Varieties

- Manjusha: Suitable for North Indian plains
- Mandakini: Suitable for foothills of Himalayas
- Bio-13: High citronellal and geraniol content
- Jor Lab C-5: More herbage and essential oil yield

## 19.4 Extraction of Essential Oil

The extraction of essential oil is carried out in different processes depending upon the plant species as follows: lavender and patchouli oils are extracted, Java citronella oil is carried out by distillation process, citrus peel oils are produced through cold pressing also known as expression and the solvent extraction is carried out for flowers/leaves having very less essential oil content. Since the solvents denature easily, supercritical fluid extraction using hexane is carried out. Though the florasol extraction is banned due to its ozone-friendly nature, the essential oil produced from this method is pure because it causes no degradation through extreme high temperatures. In case of *C. winterianus*, essential oil is usually extracted using Clevenger apparatus that is hydro-distilled for 3.5 hours (Guenther 1950). This method is the easiest, most reliable and most cost-effective method for extraction of essential oil as it requires no solvents/chemicals (Fig. 19.1).

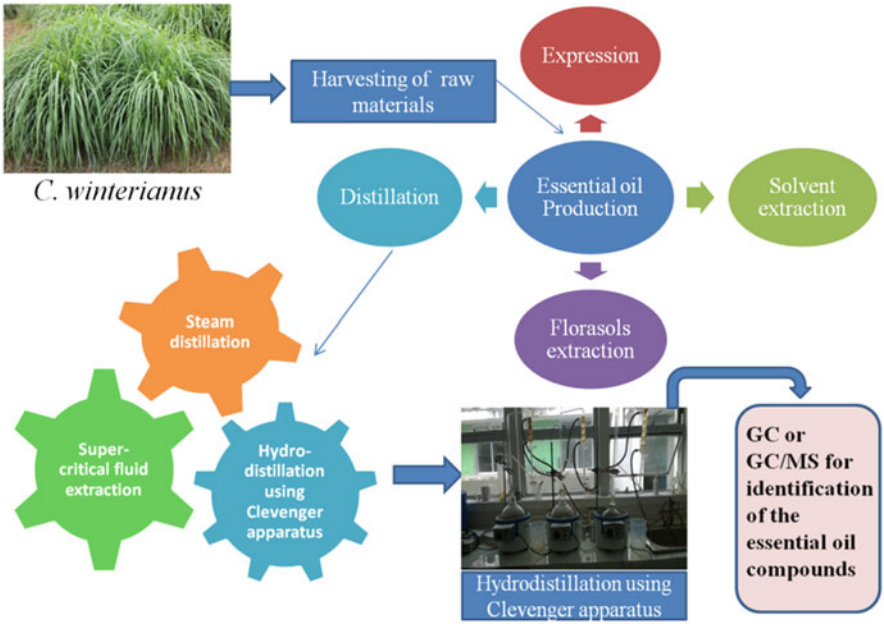


**Fig. 19.1** Extraction of essential oil along with its different extraction procedures

## 19.5 Chemical Composition of Essential Oil

The chemical constituents of essential oil are analysed using GC-FID and GC-MS (Silva et al. 2011; Setiawati et al. 2011; Vargas et al. 2010). Thin-layer chromatography (TLC) is also used for the qualitative analysis of the essential oil, but high-performance thin-layer chromatography is the most improved, advanced and reliable technique for the purity check of the samples (Lehri et al. 2011). Sometimes the useful unknown metabolites cannot be identified in GC-MS analysis; therefore to overcome this constraint, LC-MS analysis is the best among all since it uses electrospray ionization which can identify even the small traces of the constituent (Catharino et al. 2005) (Figs. 19.2 and 19.3) (Table 19.1).

## 19.6 Biological Activities and Pharmaceutical Importance

### 19.6.1 Anticonvulsant Activity

Brazilian folk medicine has been using citronella essential oil as anticonvulsant and anxiolytic and in relieving pain (Sharma et al. 2009). In justification to this, a study on rodents also showed that essential oil obtained from the leaf of this crop possesses

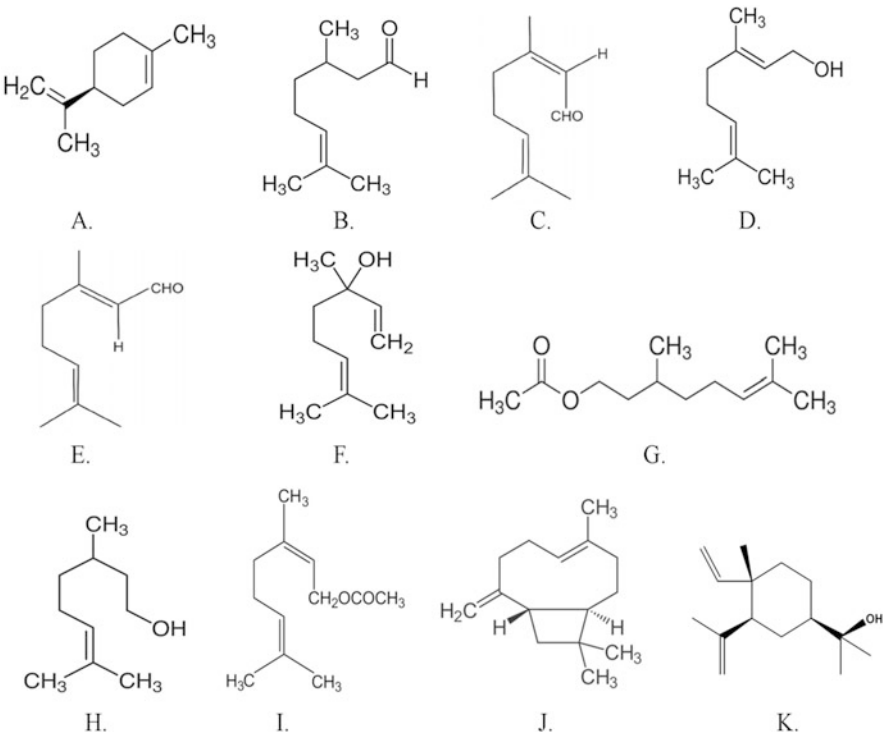


**Fig. 19.2** Major chemical constituents present in the leaf essential oil of *C. winterianus*, (**a**) limonene, (**b**) citronellal, (**c**) neral, (**d**) geraniol, (**e**) geranial, (**f**) linalool, (**g**) citronellyl acetate, (**h**) citronellol, (**i**) geranyl acetate, (**j**) β-caryophyllene, (**k**) elemol

anticonvulsant and CNS (central nervous system) depressant activity (Quintans-Junior et al. 2008; Silva et al. 2010).

## 19.6.2 Antimicrobial Activity

The essential oil of *C. winterianus* possesses significant antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhimurium, Bacillus subtilis, Candida albicans, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Timung et al. 2016; Munda et al. 2019). Geraniol and citronellal found in the essential oil of the crop showed antifungal activity against *A. fumigatus, Aspergillus flavus, C. krusei and Candida parapsilosis* (Mesa Arango et al. 2009). The essential oil at a concentration of 625 μg/mL and 1250 μg/mL inhibited the growth of *Candida albicans* which affects people with immune deficiency (Oliveira et al. 2011). Dermatophytosis is a disease caused by *Trichophyton rubrum* that affects both man and animals and can be treated using *C. winterianus* essential oil (Pereira et al. 2011).

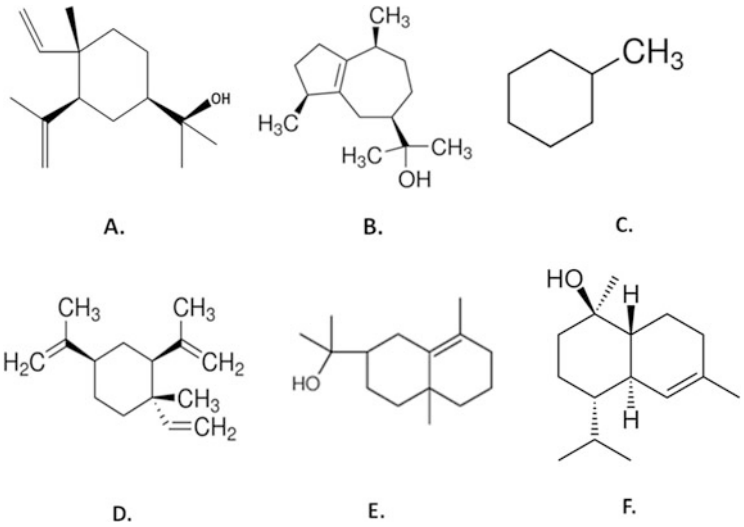


**Fig. 19.3** Major chemical constituents present in the root essential oil of *C. winterianus*, (**a**) α-elemol, (**b**) guaiol, (**c**) methyl cyclohexane, (**d**) β-elemene, (**e**) γ-eudesmol, (**f**) τ-muurolol

### 19.6.3 Antinociceptive and Anti-inflammatory Activity

*C. winterianus* leaf essential oil complexed with *β*-cyclodextrin (LEO-CD) was effective against orofacial pain. It works by activating the central nervous system without any change in the coordination of locomotor activity. Therefore this natural drug can be implemented as a substitute for the antinociceptive synthetic drugs after different biotechnological modifications (Santos et al. 2015). LEO has good antioxidant potential along with anti-inflammatory and antinociceptive properties that works by inhibiting the synthesis of prostaglandins (Leite et al. 2010).

### 19.6.4 Cardiovascular Effect

In Brazil, *C. winterianus* leaves are used to prepare tea for the hypertensive patients. Therefore the cardiovascular effect of *C. winterianus* was investigated in rats and was found that it induces vaso-relaxation and hypotension which was mediated by blocking of $Ca^{2+}$ channel. Arrhythmias and transient bradycardia were induced due to high dose (20 mg/kg) of the essential oil due to activation of cardiac muscarinic receptors (de Menezesa et al. 2010).

**Table 19.1** Major chemical compounds identified by GC/GC-MS from the leaves and root essential oil of *Cymbopogon winterianus*

| Major chemical constituents | Source | Reported KI[a] | Area % | References |
|---|---|---|---|---|
| Methylcyclohexane | Root | 770 | 9.24 | Kumar and Shukla (2014) |
| Limonene | Leaves | 1029 | 2.25 | Silva et al. (2010) |
| | | | 1.27 | Singh and Kumar (2017) |
| Citronellal | Leaves | 1153 | 28.8 | Naqvi et al. (2002) |
| | | | 27.44 | Quintans-Junior et al. (2008) |
| | | | 60.96 | Silva et al. (2010) |
| | | | 27.09 | Santos et al. (2015) |
| | | | 29.15 | Singh and Kumar (2017) |
| Neral | Leaves | 1238 | 6.02 | Quintans-Junior et al. (2008) |
| | | | 7.32 | Santos et al. (2015) |
| | | | 6.52 | Singh and Kumar (2017) |
| Geraniol | Leaves | 1252 | 17.6 | Naqvi et al. (2002) |
| | | | 40.06 | Quintans-Junior et al. (2008) |
| | | | 19.03 | Silva et al. (2010) |
| | | | 37.57 | Santos et al. (2015) |
| | | | 22.52 | Singh and Kumar (2017) |
| Geranial | Leaves | 1267 | 8.05 | Quintans-Junior et al. (2008) |
| | | | 9.63 | Santos et al. (2015) |
| | | | 5.20 | Singh and Kumar (2017) |
| Linalool | Leaves | 1275 | 1.62 | Singh and Kumar (2017) |
| Citronellyl acetate | Leaves | 1352 | 4.5 | Naqvi et al. (2002) |
| | | | 0.79 | Quintans-Junior et al. (2008) |
| | | | 0.54 | Santos et al. (2015) |
| Citronellol | Leaves | 1362 | 9.4 | Naqvi et al. (2002) |
| | | | 10.45 | Quintans-Junior et al. (2008) |
| | | | 11.52 | Silva et al. (2010) |
| | | | 9.53 | Santos et al. (2015) |
| | | | 7.43 | Singh and Kumar (2017) |
| Geranyl acetate | Leaves | 1381 | 6.3 | Naqvi et al. (2002) |
| | | | 1.77 | Quintans-Junior et al. (2008) |
| | | | 1.45 | Santos et al. (2015) |
| | | | 2.63 | Singh and Kumar (2017) |
| β-Elemene | Root | 1390 | 6.37 | Kumar and Shukla (2014) |
| β-Caryophyllene | Leaves | 1419 | 1.0 | Naqvi et al. (2002) |

(continued)

**Table 19.1** (continued)

| Major chemical constituents | Source | Reported KI[a] | Area % | References |
|---|---|---|---|---|
| | | | 0.55 | Quintans-Junior et al. (2008) |
| | | | 0.57 | Kumar (2017) |
| Elemol | Leaves | 1549 | 12.3 | Naqvi et al. (2002) |
| | | | 1.92 | Singh and Kumar (2017) |
| α-Elemol | Root | 1549 | 32.26 | Kumar and Shukla (2014) |
| Guaiol | Root | 1600 | 18.81 | Kumar and Shukla (2014) |
| γ-Eudesmol | Root | 1632 | 8.53 | Kumar and Shukla (2014) |
| τ-Muurolol | Root | 1642 | 6.83 | Kumar and Shukla (2014) |

[a]Source: Adam (2017)

### 19.6.5 Weedicides

Citronella essential oil also possesses weedicidal activity and inhibits the growth of several weeds *Ageratum conyzoides*, *Cassia occidentalis*, *Chenopodium album*, *Parthenium hysterophorus*, *Phalaris minor* and *Malvastrum coromandelianum*. Its weedicidal effect is due to the presence of citronellal in the essential oil that affects the physiology and biochemistry of the weeds by damaging the respiratory and photosynthetic process, clogs stomata, disrupts cuticular wax, causes rapid electrolyte leakage and shrinks epidermal cells. Early administration also leads to necrosis, chlorosis and wilting of the weeds (Singh et al. 2006).

### 19.6.6 Molluscicidal Activity

The concentration of 25, 50, 75 and 100 mg/L of *C. winterianus* EO was lethal and toxic to the snails between 24 and 72 h of exposure. This finding may be helpful in controlling schistosomiasis when oil is administered at appropriate concentration that is not toxic to the other coexisting organisms (Rodrigues et al. 2013). However it is necessary to confirm the results due to the presence of geraniol, citronellol and citronellal (monoterpenes). Ictiotoxicity should also be checked to verify the safety of the oil.

### 19.6.7 Insecticidal Activity

The essential oil of *C. winterianus* was found to control *Acanthoscelides obtectus*, the bean weevil that damages the stored common bean (*Phaseolus vulgaris* L.) at doses >60 or 120 μL/sample (Rodriguez-Gonzalez et al. 2019). It also hinders the larvae growth of *Spodoptera frugiperda* because of its allelochemical property (Labinas and Crocomo 2002). Besides this, the extract of this crop is also effective

in controlling and repelling insects like *Anopheles gambiae* (Insecta: Culicidae) (Deletre et al. 2015), *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) (Silva et al. 2017) and *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae) (Gusmao et al. 2013). Beetles are prevented from damaging the wheat germ and muesli carton (Khanuja et al. 2005), while damage in stored cowpea grains by *C. maculatus* is avoided by the use of citronella essential oil, thereby making it a preservative for stored products (Gusmao et al. 2013).

### 19.6.8 Acaricidal Activity

The ethanolic extract of *C. winterianus* essential oil possesses acaricidal characteristic with 95% confidence level (CL). The $LC_{50}$ value of 0.46% (0.35–0.59%) was observed in the extract, thereby making it useful to control *Rhipicephalus microplus* that is resistant to synthetic pyrethroids (SPs) like deltamethrin and cypermethrin (Singh et al. 2014a, b). Same experiment was also performed on *Hyalomma anatolicum* to check the resistance level against deltamethrin and cypermethrin. Resistance level I was observed only against deltamethrin (RF = 2.81); acaricidal activity was performed only against deltamethrin-resistant *H. anatolicum* larvae. The *C. winterianus* ethanolic extract showed a mortality rate of 93.7 ± 0.66% at the concentration of 5% (Singh et al. 2014a, b). Ethanolic extract of *C. winterianus* leads to high mortality of *T. urticae* by reducing the egg number in in vitro conditions; however field trial along with toxicology test must be performed for its safe and proper use. It is recommended to use efficient ways in controlling the ticks in strawberry and other economical crops (Vicentini et al. 2015). The essential oil formulations of *C. winterianus* and *Syzygium aromaticum* have shown significant efficiency of 92.47–100% for the control of *Rhipicephalus microplus* (cattle tick) after the 20$^{th}$ day of treatment. This tick caused serious harm to the bovines by reducing the production of meat and milk, affecting the leather quality and most importantly transmitting the disease (Mello et al. 2014).

### 19.6.9 Aromatherapy

Citronella essential oil is used in aromatherapy to remove negative energies like stress, anxiety, sadness and aggression from the mind and relaxes the body by reducing headaches, neuralgia, migraines and muscle spasms and boosting positive energies. It also purifies the surrounding environment by repelling insects and preventing the growth of harmful microorganisms. The sweet fragrance along with the cleansing ability makes it an appropriate ingredient to be used in room fresheners and deodorants.

### 19.6.10 Perfumery and Cosmetic Industry

Citronella oil is known for its good fragrance due to the presence of citronellal and geraniol. Further it can also blend with other essential oils like bergamot, lemon, clary sage, geranium, cedarwood, eucalyptus, pine, lavender, tea tree, sandalwood, etc. due to its stable and olfactory properties which can be an advantage to perfume industry (Akhila 2010). In cosmetic industry, it is used in deodorants, bath blends and body sprays to remove the foul body odour by inhibition of odour-causing bacteria. It is known for its healing properties against eczema, acne and dermatitis, acts as sunscreen by reducing the skin damage caused by UV radiation exposure, acts as ideal ingredient for anti-ageing and is useful in sores, swelling, bug bites and fungal infections. It is also a useful ingredient added in shampoos to cleanse the hair scalp from dirt, dead skin and dandruff by regulating the sebum production (newdirectionsaromatics.com).

### 19.6.11 Bioresource from Waste Biomass

The extraction of essential oil produces a large quantity of waste biomass that can be used for conversion of biomass into vermin compost rich in essential elements using *E. eugeniae*. Cow dung addition influences positively the quality by increasing the Na, K, N, Mg and P content. Moreover, there was a decrease in aromatic as well as aliphatic compounds as well as an increase in compounds containing nitrogen (Deka et al. 2011). In addition to this, the waste biomass is also used directly as manure and mulch (Dutta 1982). The solid residue can serve as the raw materials for pulp and paper making through conventional pulping procedure of soda-AQ (soda-anthraquinone) and kraft pulping followed by ECF (elemental chlorine free) bleaching which is ecofriendly in nature. Soda-AQ pulping was more effective than kraft pulping because it produced the handsheets with tensile index (31.5 N m/g), tear index (5.02 m $Nm^2/g$) and burst index (2.20 $kPam^2/g$) (Sharma et al. 2018).

### 19.6.12 Other Uses

Citronella essential oil is used in many industries in manufacturing herbal mosquito repellent incense sticks, candles and polishes (Kakaraparthi et al. 2014). CSIR-North East Institute of Science and Technology, Jorhat, has developed many technologies (animal antifungal cream, herbal room freshener, candles and incense sticks) using citronella oil. Moreover high essential oil-yielding variety of *C. winterianus* (Jor Lab C-5) was also developed using mutagenic agents (Lal et al. 2016). This variety was highly appreciated by the entrepreneurs and technology was transferred to large number of parties.

## 19.7 Global Market and Demand

The citronella oil production globally is estimated to be about 5000 tonnes worth 20 million USD. Indonesia, China, Sri Lanka, Taiwan, Brazil, Argentina and India are the exporters of citronella oil (Lawrence 1985) with China and Indonesia contributing to 40% of the world production. Indonesia is the major exporter of Java-type citronella oil (Robbins 1983), while Sri Lanka is the supplier of Ceylon-type oil with production of 200 tonnes approximately (Oyen and Nguyen 1999). According to Singh et al. (2000), India was an importer 60 years ago and produced about 600 tonnes during 2000. Presently, 350 tonnes of essential oil is produced from India in the states of Assam, Arunachal Pradesh, Tamil Nadu, Karnataka, West Bengal and Uttar Pradesh for the last 6–8 years (Lal et al. 2016). Due to its high use in cosmetic and aroma industries and also with its ability to control stress and anxiety and repel mosquitoes, leeches and bugs, it is of great demand worldwide. It also has unique properties of technical stability and distinctive olfactory that help in compounding and blending of appropriate essences, thereby helping in driving the citronella essential oil market. Citronella oil market may be restrained by the use of synthetic substitutes obtained from turpentine oil and *Eucalyptus citriodora* (Anonymous 1986) due to its cheaper value (Table 19.2).

**Table 19.2** Major global industries that are indulged in citronella oil marketing

| Name of the enterprise | Location |
|---|---|
| Van Aroma | Indonesia |
| Reho Natural Ingredients | Coimbatore, Tamil Nadu |
| Manohar Botanical Extracts Pvt. Ltd. | Kochi, Kerala |
| KIC Chemicals, Inc. | New Paltz, USA |
| Wuhan Dahua Weiye Pharmaceutical Chemical Co. Ltd. | Wuhan City, China |
| Fujian Gaoke Daily Chemical Co. Ltd. | Fujian, China |
| AOS Products Pvt. Ltd. | Ghaziabad, Uttar Pradesh |
| Gramme Products | Budaun, Uttar Pradesh |
| Aromacare Herbals | Kolkata, West Bengal |
| Meena Perfumery | Kannauj, Uttar Pradesh |
| K. K. Enterprise | Udhna, Surat |

## 19.8 Conclusion

As citronella essential oil is used for various purposes like insect repellent, perfumery, flavour, aromatherapy, non-alcoholic beverages and cosmetics, it is of great demand in the industrial sector. Moreover, it also has many biological activities like antimicrobial, anti-inflammatory, anticonvulsant, antinociceptive, etc. which are of high pharmaceutical value. Because of its high economical value, *C. winterianus* could be used as a cheap new industrial raw material. Moreover the extraction procedure of the essential oil is also very simple and cost-effective as it does not require any kind of solvents or chemicals. Even after the extraction of the essential oil, the residual biomass is used as manure and vermicompost and in mulching and paperboard making. This study will definitely help in developing the biological formulation for controlling ticks without degrading the environment. In-depth study of the bioactive constituent present in the plant responsible for various biological activities should be carried out for its proper utilization of the findings.

**Acknowledgement** The authors are thankful to Director of CSIR-NEIST, Jorhat, for providing the necessary facilities and encouraging us throughout the work. Also, deep sense of gratitude is due to CSIR, New Delhi, for granting the fund in the form of CSIR Network Project HCP0007 (Aroma Mission).

**Conflict of Interest** There is no conflict of interest.

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# Botanical Sources, Chemistry Aspects and Biological Functions of Berberine: An Updated Critical Review

20

Bikarma Singh and Anil Kumar Katare

**Abstract**

Alkaloid berberine is chemically represented as quaternary nitrogen structure, first isolated from *Xanthoxylon cava* Wall. long ago in the eighteenth century. Currently, this alkaloid is regarded as the most bioactive compound used by pharma industries for research and development of drugs and herbal formulations, and it is extensively employed for hundreds of years in curing numerous infectious diseases and in traditional Ayurvedic and Chinese medicine for curing diarrhoea as a detoxifying agent. Since long time, this compound is detected, isolated, and quantified from different families of plants such as Annonaceae (e.g. *Xylopia* L.), Berberidaceae (e.g. *Berberis* L.), Menispermaceae (e.g. *Tinospora* Miers), Papaveraceae (e.g. *Argemone* L.), Ranunculaceae (e.g. *Coptis* Salisb.) and Rutaceae (e.g. *Zanthoxylum* L.); most of these plants are growing in high-altitude regions of the Himalaya. Reported studies indicate that berberine possesses several pharmacological activities and cure inflammation, diabetes, cancer etc., thereby multiple of mechanisms, as the case may be halting cycle of the cell progression or triggers apoptosis. This chemical constituent shows significant activities such as antimicrobial (bacterial, fungal, protozoans, viral, helminthes), antidiarrhoeal, antitumor and apoptosis, anticarcinogenic, immunomodulatory, antihyperglycaemic, antioxidant,

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B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4_20

hepatoprotective, cardiovascular and several miscellaneous biological functions associated with human healthcare. This communication reviews and provided various undated information on the botanical sources, berberine extraction techniques and quantification methods, chemistry and several biological functions coupled with different clinical studies undertaken associated with berberine and allied research. This one place data will serve as future baseline data for researchers interested to work more on berberine and pharma industry for drug discovery and medicine development.

**Keywords**

Berberine botanical sources · Chemistry · Biology · Pharmacology · Clinical studies · Drug discovery

## Abbreviations

| | |
|---|---|
| MAP | Medicinal and aromatic plants (MAPs) |
| QPA | Quaternary protoberberine alkaloid |
| R&D | Research and development |
| TM | Traditional medicinal |
| WHO | World Health Organization |
| w/w | Weight/weight |
| mL | Millilitre |
| USE | Ultrasound-assisted solvent extraction |
| MAE | Microwave-assisted solvent extraction |
| SFE | Supercritical fluid extractions |
| PLE | Pressurized liquid extraction |
| v/v | Volume/volume |
| rpm | Rotation per minute |
| ES | Extraction solvents |
| h | Hour |
| $^{0}$C | Degree Celsius |
| g | Gram |
| % | Percent |
| MPa | Mega Pascal |
| m/z | Mega Hertz |
| LC-MS | Liquid chromatography-mass spectrophotometer |
| Mg | Milligram |
| NYHA | New York Heart Association |
| LVEF | Left ventricular ejection fraction |
| ACEI | Angiotensin-converting enzyme inhibitors |
| AMPK | AMP-activated kinase |
| AKT | Protein kinase |
| JAK/STAT | Janus kinase/signal transducers and activators of transcription |
| GSK3β | Glycogen synthase kinase 3β |

| | |
|---|---|
| TLR4 | Toll-like receptor 4 |
| $CCl_4$ | Carbon tetrachloride |
| iNOS | Inducible nitric oxide synthase |
| Th1 | T helper type 1 |
| Th2 | T helper type 2 |
| CD4 | Cluster of differentiation 4 |
| IL-6 | Interleukin 6 |
| TNF α | Tumour necrosis factor-alpha |
| MMP | Matrix metalloproteinase |
| MMP2 | Matrix metalloproteinase-2 |
| MMP9 | Matrix metalloproteinase-9 |
| BBB | Blood-brain barrier |
| 5-ASA | 5-Aminosalicylic acid |
| DSS | Dextran sulphate sodium |
| COX2 | Cyclooxygenase-2 |
| IL6 | Interleukin 6 |
| IL23 | Interleukin 23 |
| mRNA | Messenger Ribonucleic acid |
| NF-kB | Nuclear factor kappa-light-chain-enhancer of activated B cells |
| JAK2 | Janus Kinase 2 gene |
| PGPSN | Postganglionic parasympathetic nerve |
| GBS | Guillain-Barre syndrome |
| AIEPM | Autoimmune encephalomyelitis |
| CCSM | Corpus cavernosum smooth muscle |
| $H_2O_2$ | Hydrogen peroxide |
| NO | Nitric oxide |
| SOD | Superoxide dismutase |
| LDH | Lactate dehydrogenase |
| CV | Cell viability |
| iNOS | Inducible nitric oxide synthase |
| HPW | Hydraulic permeability of water |
| TCM | Transport cell model |
| CMS | Cell membrane stabilizing |
| SCC | Squamous cell carcinoma |
| HCT | Human colon tumour |
| VT | Ventricular tachyarrhythmias |
| CLQR | Chloroquine-resistant |

## 20.1 Introduction

Approximately 65–80% of the human population relies on the use of different plant species and their products (medicine, extracts, nutraceuticals, fruits, vegetables, etc.), for primary health needs and management (Singh 2019a, Zaman et al. 2020). For several years, the use of traditional medicinal (TM) has become one of the main

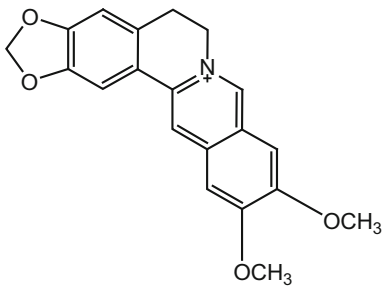


**Fig. 20.1** Chemical structure of berberine

products of the industries and the pharma companies to advanced concepts such as plant therapy and animal welfare medicine, aroma-based therapy, nutrient-based and cosmeceuticals has widened the range of uses (Farnsworth et al. 1985). Medicinal plants maintain the significance for new and valuable sources of drugs and lead molecules or compounds (Singh et al. 2012a, b, 2014, Singh 2019a, Mir et al. 2019). Global herbal trade of medicinal plants grows exponentially, 15% annual growth rate, and is expected to reach 5 trillion US$ by 2050 (Abbasi et al. 2005, Singh et al. 2018, Zaman et al. 2020). Out of over 35,000 herb plants traded globally, at least 20,000 species were known to have medicinal and edible properties (Singh and Shanpru 2010, Singh and Borthakur 2011, Singh et al. 2019, Sharma and Singh 2020). For the globally accepted botanical products, quality assurance and control in crude samples and the preparation of raw herbal extracts to cure ailments or remedies are considered as the most important for human healthcare (Singh et al. 2016, Singh and Bedi 2017, Singh 2019b, Sharma and Singh 2020).

The medicinally valued, yellow alkaloid berberine, chemically known by 5,6-dihydro-9,10-dimethoxybenzo(g)-1,3-benzodioxolo(5,6-a) quinolizinium (Neag et al. 2018), was first isolated from *Xanthoxylon cava* in 1826 by Chevallier and Pelletan (Hahn et al. 1975), and after that, this alkaloid (berberine) was reported from numerous plants (Fig. 20.1). This active compound exists as a mixture of three tautomeric forms, which are equilibrium in nature, and the chemical represents quaternary nitrogen (Neag et al. 2018) and usually forms the salts with mineral acids (hydrogen-chloride, HCI; sulphuric acids, $H_2SO_4$) by taking away one molecule of $H_2O$ (Hahn et al. 1975, Neag et al. 2018). The salt form can be correctly designated as berberinium compound such as berberinium nitrate (nitrate ion, $NO_3$) (Hahn et al. 1975). The berberinium nitrate is not soluble, and nitric acid ($HNO_3$) addition to the solutions (aqueous) of alkaloid usually easily precipitates the $NO_3$ (nitrate), and this led to the quaternary base to have extraordinary high pKα of 15.23 in $H_2O$ (Neag et al. 2018). The extracts of berberine (from *Berberis aristata* L.) were used in several traditional folklore formulations, especially in Ayurvedic system of medicine in India (Chopra and Vishwakarma 2018). Another plant, *Hydrastis canadensis* L., contains the berberine whose extract is famous in the USA as curing remedies (Neag et al. 2018). The pharmacological properties and therapeutic importance of the berberine were studied by Shideman back in 1950; however, after that, several different studies were published from different parts of the globe.

## 20.2 Botanical Sources and Traditional Use

The alkaloid berberine can be produced from many wild plant species growing in high-altitude regions (Neag et al. 2018, Singh et al. 2019). This bioactive constituent was isolated and quantified from various flowering families such as Annonaceae, Berberidaceae, Menispermaceae, Papaveraceae, Ranunculaceae and Rutaceae. The genus *Berberis* L., represented by 595 species (available at http://www.theplantlist.org), is considered as the most extensively distributed natural resources for the alkaloid berberine. This genus is commonly found in Europe, the USA and Asia (Chopra and Vishwakarma 2018). Studies reported that this alkaloid, berberine, is widely synthesized and the most active constituents of barks (stems/roots/ branches), leaflets, young twigs, rhizomes, roots, stems and also flowers in identified medicinal species of plants across the world (Etminan et al. 2005, Abbasi et al. 2010). Study of Andola et al. (2010a, b, c) reported barks and roots as the richest source of this alkaloid (berberine) in comparison with the other parts of plants in different species. Therefore, several studies like of Kosalec et al. (2009) and Kulkarni and Dhir (2010) demonstrated the plant parts such as barks, fruits, leaves and roots as the major ingredients widely used in folklore formulations and medicines, and the most common species employed in traditional medicines belongs to the genus *Argemone*, *Berberis*, *Coscinium* and *Tinospora* (Srivastava et al. 2004, 2006; Neag et al. 2018). The genus *Coscinium* belonging to the family Menispermaceae is represented by only two species, *C. blumeanum* Miers ex Hook.f. and Thomson and *C. fenestratum* (Goetgh.) Colebr. (available at http://www.theplantlist.org), where the genus *Tinospora* constituents 15 species distributed mostly as lianas of the family Menispermaceae (http://www.theplantlist.org). The *Argemone* (Papaveraceae) constitute a total of 40 species (http://www.theplantlist.org), and most of them are utilized in herbal formulations (Abbasi et al. 2009). Two photoplates of eight species of high value in traditional system of medicine yielding the berberine are provided in Fig. 20.2. and Fig. 20.3.

Studies recorded that Rojsanga and Gritsanapan (2005) reported berberine compound from four *Berberis* species (viz. *Berberis aristata*, *B. aquifolium*, *B. heterophylla* and *B. beaniana*). Similarly, Imanshahidi and Hosseinzadeh (2008) reported berberine from four species of *Coscinium* (*Coscinium chinensis*, *Coscinium fenestratum*, *Coscinium japonica*, *Coscinium rhizome*) and the plant species *Hydrastis canadensis*. Besides these, other plant species are *Phellodendron amurense*, *P. chinense* and *Tinospora cordifolia* reported by Khan et al. (2016), whereas the plant species *Xanthorhiza simplicissima* was studied by Bose et al. (1963) as other sources of the alkaloid berberine having wide application in traditional Indian and Chinese system of medicine. Tomè and Colombo (1995) also reported *Chelidonium majus* (Papaveraceae) as the most valued source of berberine in herb community. Chandra and Purohit (1980) recorded the maximum concentration of berberine alkaloid in underground parts, roots, of *Berberis* species, which vary from 1.6 to 4.3 percentages. Rashmi et al. (2009) observed the season-wise variation in berberine contents of *Berberis pseudumbellata* and *Berberis aristata* and conclusively reported the maximum content (2.8%) in summer season harvest and in


**Fig. 20.2** The pictorial representation of the family Berberidaceae yielding the berberine (**a**) *Berberis asiatica*, (**b**) *Berberis lycium*, (**c**) *Berberis himalaica* and (**d**) *Argemone mexicana*

winter harvest (1.8%), respectively, and these differences were because of intraspecific differences and geographical location and importantly depend upon analytical process for extraction and quantification.

A total comprehensive checklist having the name of different botanical sources/species yielding berberine and parts used for extraction or for herbal remedies along with the family of the species in which berberine alkaloid is reported is presented in Table 20.1.

## 20.3 Methods of Extraction

Quaternary protoberberine alkaloid berberine is considered among the majority of broadly spread alkolid, consequently, extractration methods like maceration, decoction, super critical fluid extraction with solvent (e.g. organic, inorganic mixtures, $CO_2$ are employed for extraction (Rojsanga and Gritsanapan 2005; Alothman et al. 2009; Du and Wang 2010; Singh et al. 2010, Guoping et al. 2012, Arawwawala and Wickramaar 2012, Shigwan et al. 2013, Joshi and Kanaki 2013, Mokgadi et al. 2013,

**Fig. 20.3** Pictorial representation of family Rutaceae, Menispermaceae, Ranunculaceae and Papavaraceae yielding the berberine **(a)** *Zanthoxylum armatum*, **(b)** *Tinospora sinensis*, **(c)** *Coptis teeta* and **(d)** *Papaver hybridum*

Liu et al. 2013, Akowuah et al. 2014, Teng and Choi 2013; Xu et al. 2017; Neag et al. 2018). Berberine is recognized as the most light-sensitive alkaloid, and heat is the major challenge during extraction; the rise in temperature leads to deprivation in quality as well as in quantity of berberine. Study by Babu et al. (2012) reported temperature is a critical parameter for extraction and drying treatment just before the extraction. Rojsanga and Gritsanapan (2005) reported the percentage growth of berberine in *C. fenestratum* samples has much better yield in shed dried in comparison with sample dried in oven. Teng and Choi (2013) studied the outcome of diverse inorganic acids such as HCL, $H_3PO_4$, $HNO_3$ acid and $H_2SO_4$ on berberine content in rhizomes of *Coptis chinensis* and 0.34% $H_3PO_4$. The berberine contents can be detected by HPLC method. The highest berberine yield of 13.39% could be obtained by maintaining the optimal extraction conditions such as extraction time of 7.28 h, ethanol ($C_2H_5OH$) concentration of 52.21% and solid solvent ratio of 21.78 ml/g. Reflux and Soxhlet extraction compared to other classical extraction techniques provides more than one time greater berberine yield (Mokgadi et al. 2013). The large solvent volumes are considered as the attribute for other conservative methods. The berberine extracted from *B. aristata* and *B. tinctoria* by Shigwan et al. (2013) and reported hot extraction (HE) is good at 50 °C for 3 h in organic solvent (i.e. $CH_30H$). Several other processes have been developed in recent past including

**Table 20.1** Different sources of berberine from medicinal plants

| Plant species | Family | Life form | Used part | Reference sources |
|---|---|---|---|---|
| *Annickia chlorantha* (Oliv.) Setten and Maas | Annonaceae | Tree | Barks | Mell (1929) |
| *Annickiapilosa* (Exell) Setten and Maas | Annonaceae | Tree | Barks | Buzas and Egnell (1965) |
| *Annickia polycarpa* (DC.) Steen and Maas ex I.M.Turner (=*Unona polycarpa* (DC.), *Xylopia polycarpa* (DC.) Oliv., *Coelocline polycarpa* (DC.) A. DC.) | Annonaceae | Tree | Stem barks | Henry (1949) and Neag et al. (2018) |
| *Argemone albiflora* Hornem. | Papaveraceae | Herb | Aerial parts, roots | Foote (1932) and Israilov and Yunusov (1986) |
| *Argemone mexicana* L. | Papaveraceae | Herb | Epigeal parts, leaves, seeds, roots, capsules, latex | Santos and Adkilen (1932), Haisova and Slavik (1975), Doepke et al. (1976), Pathak et al. (1985), Singh (2014), Kukula-Koch and Mroczek (2015), Santra and Saoji (1971) and Bapna et al. (2015) |
| *Argemone ochroleuca* Sweet | Papaveraceae | Herb | Seeds | Fletcher et al. (1993) |
| *Argemone platyceras* Link & Otto | Papaveraceae | Herb | Leaves, stems | Israilov and Yunusov (1986) |
| *Argemone squarrosa* Greene | Papaveraceae | Herb | Aerial parts | Stermitz (1967) |
| *Argemone subintegrifolia* Ownbey | Papaveraceae | Herb | Aerial parts | Stermitz (1967) |
| *Berberis aetnensis* C. Presl | Berberidaceae | Shrub | Roots, leaves | Henry (1949) and Bonesi et al. (2013) |
| *Berberis amurensis* Rupr. | Berberidaceae | Shrub | Stems, roots | Tomita and Kugo (1956) |
| *Berberis aquifolium* Pursh | Berberidaceae | Shrub | Roots | Parsons (1882) |
| *Berberis aristata* DC. | Berberidaceae | Shrub | Barks, roots, stems, extract, fruits (capsules) | Chakravarti et al. (1950) and Neag et al. (2018) |

(continued)

**Table 20.1** (continued)

| Plant species | Family | Life form | Used part | Reference sources |
|---|---|---|---|---|
| *Berberis asiatica* Roxb. ex DC. | Berberidaceae | Shrub | Roots, stems, barks | Neag et al. (2018) |
| *Berberis barandana* S.Vidal | Berberidaceae | Shrub | Complete plants | Neag et al. (2018) |
| *Berberis beaniana* C. K.Schneid. | Berberidaceae | Shrub | Complete plants | Neag et al. (2018) |
| *Berberis chitria* Buch.-Ham.ex Lindl. | Berberidaceae | Shrub | Complete plants | Neag et al. (2018) |
| *Berberis concinna* Hook.f. | Berberidaceae | Shrub | Barks | Neag et al. (2018) |
| *Berberis congestiflora* Gay | Berberidaceae | Shrub | Stems | Neag et al. (2018) |
| *Berberis coriaria* Royle ex Lindl. | Berberidaceae | Shrub | Barks | Neag et al. (2018) |
| *Berberis croatica* Mart.ex Schult. and Schult.f. | Berberidaceae | Shrub | Roots | Neag et al. (2018) |
| *Berberis darwinii* Hook. | Berberidaceae | Shrub | Roots, leaves, stem barks | Urzúa et al. (1984) |
| *Berberis densiflora* Raf. | Berberidaceae | Shrub | Leaflets | Neag et al. (2018) |
| *Berberis floribunda* Wall. ex G.Don | Berberidaceae | Shrub | Underground parts (roots) | Neag et al. (2018) |
| *Berberis fortune* Lindl. | Berberidaceae | Shrub | Stem woods | Neag et al. (2018) |
| *Berberis guimpelii* K. Koch and C.D.Bouché | Berberidaceae | Shrub | Underground parts (roots) | Neag et al. (2018) |
| *Berberis heteropoda* Schrenk | Berberidaceae | Shrub | Barks | Neag et al. (2018) |
| *Berberis himalaica* Ahrendt | Berberidaceae | Shrub | Barks | Neag et al. (2018) |
| *Berberis horrid* Gay | Berberidaceae | Shrub | Leaves, stems | Neag et al. (2018) |
| *Berberis iliensis* Popov | Berberidaceae | Shrub | Young shoots, roots | Neag et al. (2018) |
| *Berberis integerrima* Bunge | Berberidaceae | Shrub | Roots, leaves | Karimov et al. (1993a, b), Neag et al. (2018) |
| *Berberis jaeschkeana* C.K.Schneid. | Berberidaceae | Shrub | Roots | Neag et al. (2018) |
| *Berberis jamesonii* Lindl | Berberidaceae | Shrub | Roots | Neag et al. (2018) |
| *Berberis kawakamii* Hayata | Berberidaceae | Shrub | Roots | Yang and Lu (1960a) |
| *Berberis koreana* Palib. | Berberidaceae | Shrub | Stem barks, root barks, seeds, roots, leaves | Neag et al. (2018) |

(continued)

**Table 20.1** (continued)

| Plant species | Family | Life form | Used part | Reference sources |
|---|---|---|---|---|
| *Berberisl ambertii* R. Parker | Berberidaceae | Shrub | Roots | Chatterjee and Banerjee (1953) |
| *Berberis laurina* Thunb. | Berberidaceae | Shrub | Roots | Gurguel et al. (1934), Neag et al. (2018) |
| *Berberis leschenaultia* Wall. ex Wight and Arn. | Berberidaceae | Shrub | Barks | Neag et al. (2018) |
| *Berberis libanotica* Ehrenb. ex C.K. Schneid. | Berberidaceae | Shrub | Roots | Bonesi et al. (2013) |
| *Berberis lycium* Royle | Berberidaceae | Shrub | Roots | Andola et al. (2010a, b, c) |
| *Berberis microphylla* G.Forst | Berberidaceae | Shrub | Underground parts (roots) | Neag et al. (2018) |
| *Berberis mingetsensis* Hayata | Berberidaceae | Shrub | Roots | Yang and Lu (1960b) |
| *Berberis morrisonensis* Hayata | Berberidaceae | Shrub | Roots, stems | Yang (1960a, b) |
| *Berberis nepalensis* Spreng. | Berberidaceae | Shrub | Roots | Neag et al. (2018) |
| *Berberis nervosa* Pursh | Berberidaceae | Shrub | Roots | Neag et al. (2018) |
| *Berberis nummularia* Bunge | Berberidaceae | Shrub | Young shoots | Karimov et al. (1993a, b) |
| *Berberis oblonga* (Regel) C.K.Schneid (=*Berberis heteropoda* var. *oblonga* Regel) | Berberidaceae | Shrub | Stems, leaves, roots | Tadzhibaev et al. (1974) |
| *Berberis petiolaris* Wall. ex G.Don | Berberidaceae | Shrub | Roots | Huq and Ikram (1968) |
| *Berberis pseudumbellata* R. Parker | Berberidaceae | Shrub | Roots, stem barks | Andola et al. (2010b), Pant et al. (1986) |
| *Berberis repens* Lindl. | Berberidaceae | Shrub | Roots | Neag et al. (2018) |
| *Berberis sargentiana* C.K.Schneid. | Berberidaceae | Shrub | Stem barks | Liu (1992) |
| *Berberis swaseyi* Buckley | Berberidaceae | Shrub | Stem barks | Neag et al. (2018) |
| *Berberis thunbergii* DC. | Berberidaceae | Shrub | Stems | Neag et al. (2018) |
| *Berberis tinctoria* Lesch. | Berberidaceae | Shrub | Leaves, roots | Srivastava and Rawat (2007) |
| *Berberis trifolia* (Cham. and Schltdl.) Schult. and Schult.f. | Berberidaceae | Shrub | Roots, stems | Neag et al. (2018) |

(continued)

**Table 20.1** (continued)

| Plant species | Family | Life form | Used part | Reference sources |
|---|---|---|---|---|
| *Berberis turcomanica* Kar. ex Ledeb. | Berberidaceae | Shrub | Leaves | Neag et al. (2018) |
| *Berberis umbellata* Wall. ex G.Don | Berberidaceae | Shrub | Roots | Singh et al. (2012a, b) |
| *Berberis vulgaris* L. | Berberidaceae | Shrub | Stems, roots | Neag et al. (2018) |
| *Berberis waziristanica* Hieron. | Berberidaceae | Shrub | Root barks | Atta-ur-Rahma and Ahmad (1992) |
| *Bocconia frutescens* L. | Papaveraceae | Herb | Leaves | Slavik and Slavikova (1975) |
| *Caulophyllum thalictroides* (L.) Michaux (=*Leontice thalictroides* L.) | Berberidaceae | Shrub | Complete plants | Neag et al. (2018) |
| *Chelidonium majus* L. | Papaveraceae | Herb | Roots | Jusiak (1967) |
| *Coptis chinensis* Franch. | Ranunculaceae | Herb | Roots | Neag et al. (2018) |
| *Coptis japonica* (Thunb.) Makino (=*Thalictrum japonicum* Thunb.) | Ranunculaceae | Herb | Rhizomes | Kubota et al. (1980) |
| *Coptis teeta* Wall. | Ranunculaceae | Herb | Rhizomes | Neag et al. (2018) |
| *Corydalis chaerophylla* DC. | Papaveraceae | Herb | Roots | Jha et al. (2009) |
| *Corydalis ophiocarpa* Hook.f. and Thomson | Papaveraceae | Herb | Whole plants | Manske (1939) |
| *Corydalis solida* (L.) Clairv. (=*Fumaria bulbosa* L.) | Papaveraceae | Herb | Aerial parts, rhizomes | Kiryakov et al. (1982) |
| *Corydalis turtschaninovii* Besser. | Papaveraceae | Herb | Tubers | Lee and Kim (1999) |
| *Eschscholzia californica* Cham. | Papaveraceae | Herb | Roots | Gertig (1964) |
| *Evodia meliaefolia* Hance ex Walp. | Rutaceae | Tree | Barks | Perkin and Hummel (1895) |
| *Glaucium corniculatum* (L.) Curtis (=*Chelidonium corniculatum* L.) | Papaveraceae | Herb | Aerial parts | Doncheva et al. (2014), Slavik and Slavikova (1978) |
| *Glaucium grandiflorum* Boiss. and A.Huet | Papaveraceae | Herb | Aerial parts | Neag et al. (2018) |
| *Hunnemannia fumariifolia* Sweet | Papaveraceae | Herb | Roots | Neag et al. (2018) |

(continued)

**Table 20.1** (continued)

| Plant species | Family | Life form | Used part | Reference sources |
|---|---|---|---|---|
| *Hydrastis canadensis* L. | Ranunculaceae | Herb | Whole plants | Baldazzi et al. (1998) |
| *Jeffersonia diphylla* (L.) Pers. (=*Podophyllum diphyllum* L.) | Berberidaceae | Shrub | Whole plants | Neag et al. (2018) |
| *Macleaya cordata* (Willd.) R.Br. (=*Bocconia cordata* Willd.) | Papaveraceae | Herb | Whole plants | Neag et al. (2018) |
| *Macleaya microcarpa* (Maxim.) Fedde (=*Bocconia microcarpa* Maxim.) | Papaveraceae | Herb | Roots | Neag et al. (2018) |
| *Mahonia borealis* Takeda | Berberidaceae | Tree | Stems | Neag et al. (2018) |
| *Mahonia fortunei* (Lindl.) Fedde (=*Berberis fortunei* Lindl.) | Berberidaceae | Tree | Woods | Neag et al. (2018) |
| *Mahonia napaulensis* DC. | Berberidaceae | Shrub | Barks | Neag et al. (2018) |
| *Mahonia simonsii* Takeda | Berberidaceae | Tree | Stems | Neag et al. (2018) |
| *Mahonia bealei* (Fortune) Pynaert (=*Berberis bealei* Fortune) | Berberidaceae | Tree | Woods, roots | Neag et al. (2018) |
| *Nandina domestica* Thunb. | Berberidaceae | Shrub | Barks, roots | Neag et al. (2018) |
| *Papaver dubium* L. | Papaveraceae | Herb | Roots, latex | Slavik (1978) |
| *Papaver hybridum* L. | Papaveraceae | Herb | Aerial part | Neag et al. (2018) |
| *Papaver rhoeas* L. | Papaveraceae | Herb | Roots | Slavík (1978) |
| *Phellodendron amurense* Rupr. | Rutaceae | Tree | Barks, branches, leaves | Neag et al. (2018) |
| *Phellodendron chinense* C.K. Schneid. | Rutaceae | Tree | Barks, branches, leaves | Chen (1981), Chan et al. (2007), Tan et al. (2013) and Chen (1982) |
| *Phellodendron lavallei* Dode | Rutaceae | Tree | Barks | Yavich et al. (1993) |
| *Rollinia mucosa* (Jacq.) Baill. (=*Annona mucosa* Jacq.) | Annonaceae | Tree | Fruits | Chen et al. (1994) |

(continued)

**Table 20.1** (continued)

| Plant species | Family | Life form | Used part | Reference sources |
|---|---|---|---|---|
| *Sanguinaria canadensis* L. | Papaveraceae | Herb | Whole plants | Greathouse (1939) |
| *Tinospora sinensis* (Lour.) Merr. (=*Campylus sinensis* Lour., *Tinospora cordifolia* (Willd.) Miers, *Menispermum cordifolium* Willd.) | Menispermaceae | Liana | Stems | Neag et al. (2018) |
| *Xanthorhiza simplicissima* Marshall | Ranunculaceae | Shrub | Roots, stems, leaves | Okunade et al. (1994) |
| *Zanthoxylum schreberi* (J.F.Gmel.) Reynel ex C. Nelson (=*Curtisia schreberi* J.F.Gmel., Z. *monophyllum* (Lam.) P.Wilson, *Fagara monophylla* Lam.) | Rutaceae | Shrub | Stems, branches | Stermitz and Sharifi (1977) |
| *Zanthoxylum armatum* DC. | Rutaceae | Shrub | Stems | Singh (2019) |
| *Zanthoxylum quinduense* Tul. | Rutaceae | Shrub | Stems, leaves | Ladino and Suárez (2010) |

USE, MAE, UPE, SFE, PLE and few coupled methods. A schematic representation of the extraction process is given in Fig. 20.4. Ultrasonic and microwave-assisted extractions are designated zero waste discharge, easy, competent and economical techniques (Alupului et al. 2009). Teng and Choi (2013) extracted berberine from coptidis rhizome by USE method. Using Response Surface Methodology (RSM), several researchers identified extraction parameters as 59% ethanol concentration at 66.22 °C within 46.57 min. Chang (2013) recorded decrease in extraction time (39.81min) by proper proportion of ionic liquid solutions, as green solvents with USE to extract berberine from *C. chinensis* in order to apply an eco-friendly approach. Xu et al. (2017) studied numerous extraction techniques such as USE, distillation and Soxhlet extraction (SE) to ascertain a highly efficient method three bioactive compounds (viz. phellodendrine, berberine, palmatine) from *Phellodendron* bark and concluded USE and HCL acidified organic solvent (i.e. methanol) as the best. The different processes of development of protocol for extraction of berberine from various plant species are given in Table 20.2.

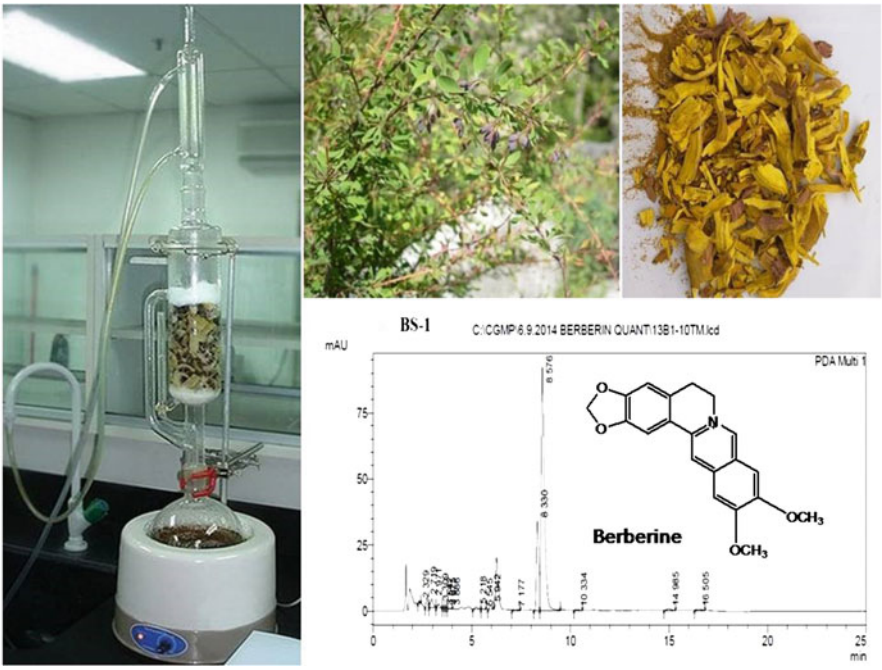


**Fig. 20.4** A schematic representation of the extraction process for berberine alkaloid

## 20.4 Isolation and Quantification Methods

There are several methods available for characterization of berberine from extract of plant materials (Tadzhibaev et al. 1974; Myer and Montg 1995; Marek et al. 2003; Chen et al. 2005; Grycová et al. 2007; Neag et al. 2018). In recent past few decades, several isolation processes have been developed, where the reaction of interconversion of protoberberine and base is main principle. The berberine isolation by classical techniques is based on alcohol and acetic acid, which isolate berberine chloride (Karimov et al. 1993a, b), hydrosulphate or iodide (Tadzhibaev et al. 1974). Different extraction variants proposed by Guo et al. (2017) and Chen et al. (2005) were employed as techniques of microwave radiation or liquid extraction under pressure (Schieffer and Pfeiffer 2001). In chromatographic techniques, acetonitrile and 0.1% formic acid aqueous solution act as the optimal mobile phase. HPLC technique is also taken into consideration for berberine extraction, where it can be seen that the retention time of the investigated compound was 5.67 min. The peak area obtained from the berberine standard was utilized for quantification. The six-point calibration equation of berberine is obtained through plotting LC-MS results (Y) vs. the concentration (X, mg/ml) of calibrators as $Y = 41.996438X-425.714548$ ($R^2 = 0.997$). In case of berberine, the equation in graphs usually shows good linearity in comparison with range (Fig. 20.5).

**Table 20.2** Processes of the development of protocol for extraction of berberine from various plant species

| Extract methods | Plant species | Part used (quantity) | References |
|---|---|---|---|
| ES: $CH_3OH$-$H_2O$ (1:1.v/v); sonication (15 min, RT); CTF (2800 rpm, 15 min); F&E; extr. RS ($CH_3OH$: $H_2O$:: 9:1 v/v) | *Coscinium fenestratum* | Dried stem powder (1 g) | Neag et al. (2018) |
| ES: $CH_3OH$; HE: samp. refl. with ES for 3 h F&E; extr. RS ($CH_3OH$); CE: samp. ext. with ES for 24 h F&E; extr. RS ($CH_3OH$) | *Coscinium fenestratum* | Dried stem powder (10 g) | Neag et al. (2018) |
| ES: abl. $CH_3OH$; CE: samp. ext. at minus (−) 20 °C; HE: WBSE at 50 °C; ES: abl. $C_2H_5OH$; CE samp. ext. at −20 °C; HE: WBSE at 50 °C samp. CTF (10 min at 10 °C after cooling down); samp. filtration | *Coscinium fenestratum* | Dried stems (0.1 g) | Babu et al. (2012) |
| ES: petr. ethr., chlfm., $CH_3OH$ (1L each) sox. extr.: with each ES for 3 days at (30 to 40 °C); ES: ($CH_3OH$) (10 mL) extraction for 1 h F&E; RS in $CH_3OH$ (5 mL) | *Coscinium fenestratum* | Dried stems (1000 g) | Jayaprakasa and Ravi (2014) |
| ES: chlfm.; dried samp. trituration with amm. sol. drying at RT; extraction with ES for 1 h; chlfm. phase extraction with three times $H_2SO_4$ (5%) basification of acid extr. with $Na_2CO_3$ (pH = 9); extraction of basified solution with chlfm. (X3); evap. of chlfm. ph. temp. under 50 °C); resd. solubn. with $CH_3OH$ | *Tinospora cordifolia*, *Tribulus terrestris*, *Emblica officinalis* | Stems (3 g) | Neag et al. (2018) |
| UPE; optimal parameters: ES: $C_2H_5OH$ (69.1%), liquid/solid ratio::31:3, extracting pressure: 243.30 MPa, extr. Tm-: 2 min | *Cortex phellodendri* | Dried trunk bark (2 g) | Neag et al. (2018) |
| SFET: up to 3 h; temp.: 60 °C; pressure: from 200 to 500 bar; flow-rate of $CO_2$: 1 L/min flow-rate of modifier: 0.4 mL/min.; organic solvent modifier systems: $C_2H_5OH$-modif. sup. crt. $CO_2$, $CH_3OH$-modif. sup. crt. $CO_2$, 1,2-propanediol-modif. sup. $CO_2$,5% tween 80 in $CH_3OH$ modif. sup. crt. $CO_2$, 5% tween in $C_2H_5OH$- modif. sup. crt. $CO_2$ sox. extr.; ES: HCL: $CH_3OH$ (1: 100, v/v); time: 8 h | *Coptis chinensis* | Rhizome (1 g) | Liu et al. (2006) |
| UPE; ES: $C_2H_5OH$ (50%), liquid-solid ratio (30:1) extracting; pressure:400 MPa, extr.Tm-: 4 min, extra.Tem.:40 °C; US extr.; ES: | *Cortex pellodendri amurensis* | Barks (1 g) | Neag et al. (2018) |

(continued)

**Table 20.2** (continued)

| Extract methods | Plant species | Part used (quantity) | References |
|---|---|---|---|
| $C_2H_5OH$ (70%); samp. soaking for 24 h in 40 ml ES sonic extraction for 60 min at 30 °C; heat reflux extraction; ES: 70% $C_2H_5OH$; samp. soaking for 24 h in 40 ml ES; samp. ext. for 4 h at boiling state; sox. extr.; ES: 70% $C_2H_5OH$; samp. soaking for 24 hrs in 40 ml ES; samp. extraction: 4 h | | | |
| PHWE; ES: water at 140°C, optimal parameters: pressure: 50 bars and flow rate: 1 mL/min, time: 15 min; reflux extraction ES: $CH_3OH$ (200 mL) sonication: 4 h at 80 °C ultrasonic extraction ES: $CH_3OH$ (50 mL) reflux: 6 h with continuous stirring | *Hydrastis canadensis* | Dried root (2 g) | Neag et al. (2018) |
| CHRE; ES: $CH_3OH$ (100 mL) for 1 h in a water bath filtration; reextraction with ES (50 mL) for 30 min (X2); filtrates combination and concen. to 50 mL; herb extr. ultrasonic extraction ES: $CH_3OH$ (upto 10 mL); sonication; filtration; Ayurvedic form ultrasonic extraction; ES: $CH_3OH$ (upto 25 mL); sonication | *Berberis aristata* | Roots (1.5 g), | Neag et al. (2018) |
| Sox. extr.; ES: $C_2H_5OH$; berberine isolation; $C_2H_5OH$ extract concen. to obtain a syrup mass dissolvation in hot water and filtration acidification (36.5% w/v HCL); cool: ice bath - 30 min, overnight in refrigerator | *Berberis aristata* | Roots | Patel (2013) |
| CE; ES: 80% $CH_3OH$ (1000 mL); stirring at RT extr. concen. | *Mahonia manipurensis* | Stem bark (100 g) | Pfoze et al. (2014) |
| Maceration; ES: 80% $C_2H_5OH$ (500 ml), 160 h; shaken: 80 h (200 rpm), stand: 80 h; reextraction: 48 h, shaken: 24 h, stand: 24 h; combined extra. concen.; evap. to dryness (dry extr.); RS in 80% $C_2H_5OH$ (10 mg dry extr./mL) | *Coscinium fenestratum* | Stem (100 g) | Rojsanga and Gritsanapan (2005) |
| Sox. extr.; ES: $CH_3OH$; evap. to dryness; RS in $CH_3OH$ (known concen.) | *Tinospora cordifolia* | Stems (20 g) | Satija et al. (2015) |
| HE; ES: $CH_3OH$ (2.5 L) (X2); extr.Tm-: 3 h temp.: 50 °C; extr. concen. under vacuum | *Berberis aristata*, *Berberis tinctoria* | Leaves (800 g) | Neag et al. (2018) |
| ASE; ES: several inorganic acids (HCL, $H_3PO_4$, $HNO_3$, $H_2SO_4$) and one; | *Coptis chinensis* | Rhizome (1 g) | Neag et al. (2018) |

(continued)

**Table 20.2** (continued)

| Extract methods | Plant species | Part used (quantity) | References |
|---|---|---|---|
| organic acid ($CH_3COOH$); extr.Tm-:1 to 8 h, acid concen.: 0 to 1% solvent to samp. ratios: 20 to 60 mL/g; maceration at 25 °C; filtration; dilution to 100 mL fin. vol.; sox. extr.; ES: 50% $C_2H_5OH$ (100 mL), 4 h at 70 °C extr. evap. to dryness; RS in ES (up to 100 mL fin. vol.); heating reflux extraction; ES: 50% $C_2H_5OH$; soaked for 1 h; extraction: 4 h at 70 °C (heated water bath); filtration; dilution (up to 100 mL fin. vol.) | | | |

(Abbreviation: *F&E* filtration and evaporation, *CTF* centrifugation, *RT* room temperature, *RS* resolubilization, *HE* hot extraction, *CE* cold extraction, *PHWE* pressurized hot water extraction, *CHRE* crude herb reflux extraction, *ES* extraction solvents, *UPE* ultrahigh pressure extraction, *WBSE* water bath sample extraction, *samp. ext.* sample extraction, *samp. refl.* sample refluxed, *abl.* absolute, *petr. ethr.* petroleum ether, *sox. extr.* Soxhlet extraction, *chlfm.* chloroform, *ph.temp.* phase temperature, *amm. sol.* ammonia solution, *resd. solubn.* residue solubilization, *SFET* supercritical fluid extraction time, *US extr.* ultrasonic extraction, *modif. sup. crt.* $CO_2$-modified supercritical ($CO_2$), *concen.* concentration, *$CH_3OH$* methanol, *$H_2O$* water, *$C_2H_5OH$* ethanol, *extr.* extracts, *ASE* acid assisted extraction, *extr.Tm* extraction time, *evap.* evaporation, *samp.* sample, *Fin. vol.* final volume, *temp.* temperature)

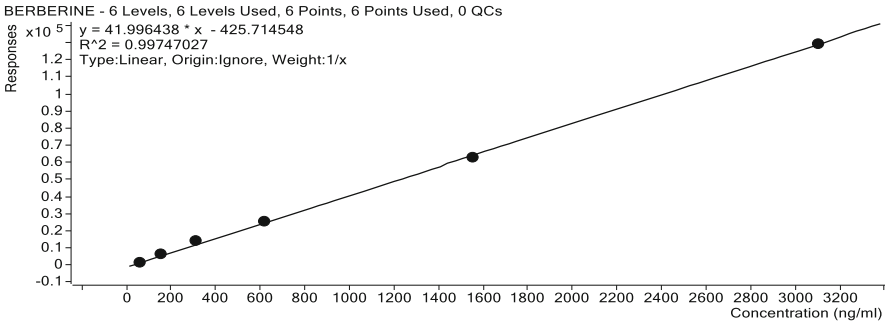


**Fig. 20.5** Calibration linearity range for standardization of berberine

## 20.5 Chemistry Aspects

The molecular formula of berberine is $C_{20}H_{19}NO_5$ (Hussaini and Shoeb 1985). Different alkaloid classes of berberine include jatrorrhizine, columbamine, demethyleneberberine, coptisine, palmatine and canadine (Karimov 1993) (Fig. 20.6). Besides, it also includes oxyberberine, isocorydine, lambertinea and magniflorine (Kirtikar and Basu 1933). These alkaloids are reported for various

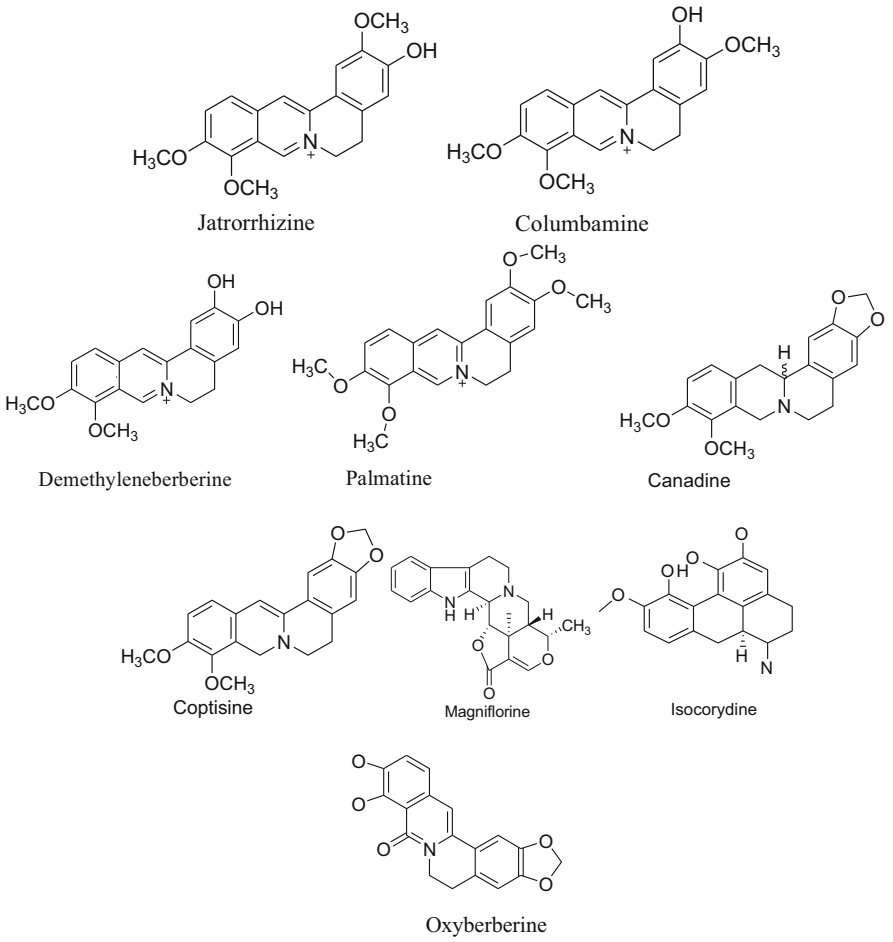


**Fig. 20.6** Structure of different classes of alkaloid of the berberine

biological properties like anticancer (Yamahara 1976, Imanshahidi and Hosseinzadeh 2008), anti-inflammatory (Neag et al. 2018), antioxidant (Kamal et al. 2011), antidiabetic (Imanshahidi and Hosseinzadeh 2008), analgesic and antibacterial (Neag et al. 2018) and hepatoprotective (Imanshahidi and Hosseinzadeh 2008).

The berberine forms yellow needles soluble in water, less soluble in alcohol. Studies (Tiwari and Masood 1979, Chopra and Vishwakarma 2018) reported *Berberis aristata* plant species as the major source of berberine. Besides this alkaloid, this species is reported to contain other bioactive constituents as berbamine, aromoline, karachine, palmatine, oxyacanthine, oxyberberine and taxilamine from roots; also caffeic acid, quercetin, chlorogenic acid, meratin and rutin are reported

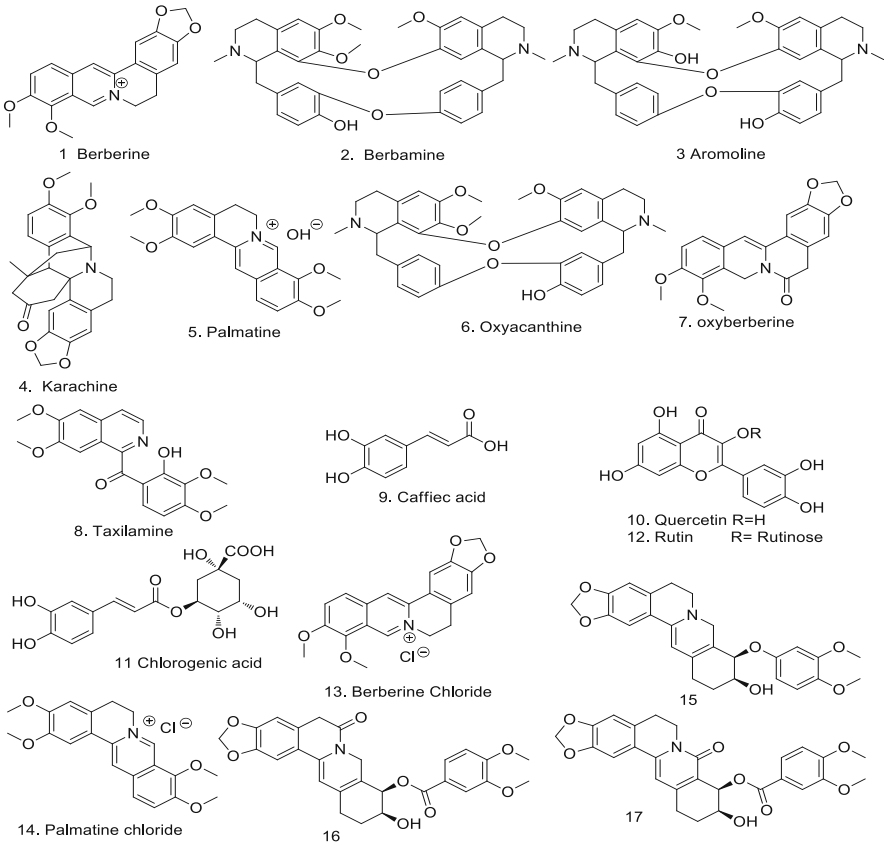


**Fig. 20.7** Different associated chemical constituents with berberine alkaloid: (1.) berberine, (2.) berbamine, (3.) aromoline, (4.) karachine, (5.) palmatine, (6.) oxyacanthine, (7.) oxyberberine, (8.) taxilamine, (9.) e-caffeic acid, (10.) quercetin, (11.) chlorogenic acid, (12.) meratin and rutin, (13.) berberine chloride, (14.) palmatine chloride, (15.) 14β-hydroxy-15β (3′,4′-dimethoxyphenyl ether)-5,6-methylenedioxy-9(10),11(16)-tetradehydroberbinium, (16.) 14β-hydroxy-15β- (3′,4′-dimethoxy-benzoate)-5,6-methylene-dioxy- 9 (10), 11 (16)-tetra-de-hydro-17-one-berbinium and (17.) 14β-hydroxy- 15β- (3′, 4′-dimethoxy-benzoate)- 5, 6-methylene-dioxy- 9(10), 11 (16)- tetradehydro-1-one berbinium (Source: Chopra and Vishwakarma 2018)

from the flowering twigs of genus *Berberis* (Chakravarti et al. 1950; Chandra and Purohit 1980).

Chopra and Vishwakarma (2018) reported the berberine chloride, palmatine chloride, 14β-hydroxy-15β-(3′,4′-di-methoxyphenylether)-5,6-methylene-dioxy-9 (10),11(16)-tetra-dehydro-berbinium,14β-hydroxy-15β-(3′,4′-di-methoxy-benzoate)-5,6-methylene-dioxy-9(10), 11(16)-tetradehydro-17-one-berbinium, 14-β-hydroxy-15β- (3′, 4′-di-methoxy-benzoate)-5,6-methylene-dioxy-9(10),11(16)-tetradehydro-1-one berbinium from stem bark. The chemical structure of these active compounds is given in Fig. 20.7.

## 20.6 Biological Activities

The pharmacological or therapeutic significance of berberine has been stimulated due to intensive research carried out by different studies across the globe. Studies found that berberine and its associated alkaloids, such as berberrubine, columbamine, dimethyleneberberine, etc., show active potential biological functions such as antimicrobial, antidiarrhoeal, immunomodulatory, antihyperglycaemic, antioxidant, hepatoprotective, cardiovascular, antitumor and apoptosis, anticarcinogenic and several miscellaneous functions associated with humans. Few important pharmacological activities associated with berberine are present here in subheads:

### 20.6.1 Antimicrobial Properties

Berberine is recorded to possess antibacterial, antiviral, antiamoebic, antifungal, antihelminthic, leishmanicidal and tuberculostatic properties by various researchers (Zhu and Ahrens 1983; Shin et al. 1993, Takase et al. 1993, Sohni et al. 1996, Musumeci et al. 2003, Freile et al. 2006, Jahnke et al. 2006, Hayashi et al. 2007, Chopra and Vishwakarma 2018). Berberine sulphate (BS) is usually compared with metronidazole and considered as potency with the advantage of being safer and reliable (Chopra and Vishwakarma 2018). This compound may be a very useful drug for curing visceral leishmaniasis (e.g. pentamidine) and inhibits the multiplication of amastigotes in macrophages (in vitro) (Sack and Froehlich 1982, Ghosh et al. 1985). Manometric studies indicated BS and metronidazole possess the action related to inhibition for the endogenous ((Mahajan et al. 1982) and glucose-stimulated respiration of amastigotes (Chopra and Vishwakarma 2018). The alkaloid, berberine, has been recorded to interact with nuclear DNA of *L. donovani* promastigotes (Yi et al. 2007). It has also been shown to be an effective compound against most of the fungi, but the compound shows least effect over *Helminthosporium* species even at the dose of 1500 ppm. Moreover, BS was known for bacteriostatic in case of *Streptococci* (SC), the MICs of berberine blockade SC adherence in the host cells, and considered responsible for immobilization of fibronectin and hexadecane (Sun et al. 1988). The antimicrobial mode of berberine by HPLC/ESI-MS has also been evaluated. An ethanolic extract of traditional formulation containing the plant species of *B. diffusa*, *B. aristata*, *T. cordifolia* and *T. chebula* and species of *Zingiber officinale* has an MIC of 1000 μg/ml, which is found to be effective against *Entamoeba histolytica* (Sohni et al. 1996). BS in concentrations of 10–25 mg/ml repressed the growth of fungi such as species of *Alternaria*, *Aspergillus*, *Candida*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus oryzae*, *Scopulariopsis* and other pathogenic fungi (Singh et al. 2001). Studies have shown that the concentrations of 50 mg/ml BS inhibit *Syncephalastrum*. A study by Nakamoto et al. (1995) recorded that trial denture cleanser prepared with berberine HCL removes 64 to 89% of adhered cells from the surface of acrylic resin and has less effect on other body functions. Berberine can also be used as a stain for locating the malarial parasites found in the blood. It has been seen that the DNA of *Plasmodium vivax* and *P. falciparum* get

fluoresced with berberine. BS was studied with intercellular events after virus penetration into host cells and has been found to have inhibitory function for viral DNA synthesis (Jahnke et al. 2006, Chopra and Vishwakarma 2018).

### 20.6.2 Antidiarrhoeal Activity

Studies by Chopra and Vishwakarma (2018) reported that the berberine was evaluated for antisecretory properties to human colonic (epithelium) cells, and the action mechanism was $K^+$ channel blockades, where it increases the contractility of ileum due to the increase of acetylcholine, which gets released from PGPSN terminal, thereby maximizing the retention of acetylcholine by controlling the cholinesterase functions and also checking α-2-adreno-ceptorsin PGPSN (Takase et al. 1993). It also checks the muscarinic receptors, which concludes the efficacy in intestinal motility reduction, and acts in curing diarrhoea as seen in the case of guinea pig isolated ileum. Previous studies (Zhu and Ahrens 1983; Shin et al. 1993) recorded that berberine when used along with L-phenylephrine showed significant antisecretory activity in pig jejunum Z. It reduces cholera toxin, sodium (Na), chorine (Cl) and $HCO_3$ (Shin et al. 1993, Tsai and Ochillo 1991, Swabb et al. 1981, Zhang and Shen 1989). The berberine antidiarrhoeal properties in Chinese remedies reported by Swabb et al. (1981) suggested actual inhibition level to 70% for *V. cholerae* and *E. coli* in the rabbit by studying ligated intestinal loop model.

### 20.6.3 Immunomodulatory Activity

The immunomodulatory effects of berberine were studied by several researchers (Avolio et al. 2003; Xu et al. 2005; Neag et al. 2018) across the globe. Liu et al. (2016) studied the autoimmune myocarditis model with berberine and reported that this compound alleviated the damage of cardiac functions with restraining antibodies produced by anticardiac myosin, thereby modulating STAT movement; $Th_1$ and $Th_2$ cell differentiation gets blocked, and this is reported to have the essential functions in myocarditis pathogenesis. Some studies (Avolio et al. 2003) recorded autoimmune neuritis via following the model of animals, which is comparable to GBS in human, and reported that berberine helped in humoral as well as cellular immunity by inhibiting the proliferation of lymphocyte (e.g. $CD_4$) and is responsible for decreasing the cytokines of pro-inflammatory such as IL-6, TNFα and other functions. Ma et al. (2010) reported the favourable effect of berberine in the AIEPM, thereby checking functions of MMP9, dipping BBB permeability and decreasing CNS inflammatory cellular permeation.

Luo et al. (2017) studied berberine as immunomodulator, and under this, humoral immunity increased by haemagglutination titre. It is also known that berberine significantly inhibits the DNFB-induced delayed-type hypersensitivity (DTH) in mice. The reduced adhesion between mouse T-lymphocytes and extracellular matrix (ECM) may be one of the mechanisms of suppression of DTH by berberine (Luo

et al. 2017). Previously published study by Minaiyan et al. (2011) reported berberine is responsible for corticosteroid level increasing in rats by induced colitis, which suggested berberine attributed for the increase in endogenous glucocorticoid levels and proves therapeutic effect of berberine as immunomodulatory (Minaiyan et al. 2011). Studies by Schideman (1950) demonstrated that berberine toxicity depends on used animals in experiment (mouse, rat, guinea pig, rabbit). Du et al. (2005) studied berberine effect on DNFB-induced delayed-type hypersensitivity in mice which indicated immunosuppressive effects.

### 20.6.4 Antihyperglycaemic Effect

Several studies (Choi et al. 2006, Cheng et al. 2006, Zhou et al. 2007, Liu et al. 2008a, Chopra and Vishwakarma 2018) indicated antihyperglycaemic effects of berberine on humans. Active berberine exerts a hypoglycaemic effect on glucose uptake in 3 T3-L1 adipocytes (Zhou et al. 2007, Singh and Kakkar 2009, Chopra and Vishwakarma 2018). Study by Choi et al. (2006) reported both the antiadipogenic and 3 T3-L1 adipocyte anti-inflammatory properties. Study by Cheng et al. (2006) reported this alkaloid is responsible for promoting 3 T3-L1 preadipocyte proliferation and also inhibiting terminal adipocyte differentiation, associated with decreasing peroxisome proliferation expression at activated receptor gamma mRNA and protein, and proves obesity treatment having diabetes (Type 2).

### 20.6.5 Antioxidant Properties

The berberine constituent plant is utilized among folklore as herbal medicine (Chopra and Vishwakarma 2018). Several studies of berberine showing antioxidant properties were carried out by various scientist and research scholars (Shirwaikar et al. 2006, Tan et al. 2007a, b, Thirupurasundari et al. 2009, Rahal et al. 2014, Bhattacharyya et al. 2014, Pilch et al. 2014, Ahmed et al. 2015; Neag et al. 2018) across the globe. Tan et al. (2007) studied the berberine antioxidant activity on CCSM cells of cultured rabbit injured by $H_2O_2$. The study reveals that the treatment with 1 mmol/l $H_2O_2$ variably minimized CV, products of NO and SOD activities of CCSM cells from 100 to 48.57 $\pm$ 4.1% (probability$<$0.01), 66.8 $\pm$ 16.3 to 6.7 $\pm$ 2.1 μmol/l (probability$<$0.01) and 49.5 $\pm$ 1.8 to 30.1 $\pm$ 2.6 U/ml (probability $<$0.01), respectively, and maximized LDH release from 497.6 $\pm$ 69.5 to 1100.5 $\pm$ 56.3 μ/l (probability $<$0.01) and malondialdehyde (MDA) content from 3.7 $\pm$ 1.3 to 78.4 $\pm$ 2.9 nmol/mg protein (probability$<$0.01). Various berberine dosage (10 to 1000 μmol/l) treatments inhibit $H_2O_2$ damaging effects with increasing CV (probability$<$0.05 or probability$<$0.01). NO production (probability$<$0.01) and SOD activity (probability$<$0.01) decreased lactate dehydrogenase (LDH) release and MDA content (both probability$<$0.01). Tan et al. (2007) and Liu et al. (2008b) studies concluded that the berberine has antioxidant properties on oxidative stress-induced cultured CCSMC and is responsible for penile dysfunction.

### 20.6.6 Hepatoprotective Properties

Several studies (Wang et al. 1991, Chan 1977, Birdsall and Kelly 1997, Janbaz and Gilani 2000, Ivanovska and Philipov 1996, Upadhyay et al. 2001; Tan et al. 2007; Gulfraz et al. 2008; Domitrovic et al. 2011; Zhao et al. 2012; Imenshahidi and Hosseinzadeh 2008, Neag et al. 2018, Chopra and Vishwakarma 2018) across the globe reported the cardiovascular effects of berberine. The hepatoprotective activities of the alkaloid berberine (bioactive herbal ingredient) were tested on the mice, wherein hepatotoxicity was induced by doxorubicin (Chopra and Vishwakarma 2018). A research indicated that pretreatment berberine reduces efficient hepatic tests as well as histological damage. The study was carried out on carbon tetrachloride ($CCl_4$)-induced hepatotoxicity where this compound decreases oxidative as well as nitrosamine stress, thereby modulating responses of inflammation in the liver of mice having good effects (Zhao et al. 2012). Domitrović et al. (2011) reported that this alkaloid is responsible for superoxide dismutase functioning reduction and peroxidation of lipid and responsible for TNF-α, COX-2 and iNOS level decline.

The berberine effects for hepatic disorders in indigenous drug-containing plants, for example, *T. cordifolia*, *A. paniculata*, *P. kurroa*, *P. niruri* and *B. aristata* (Chopra and Vishwakarma 2018), have also been tested on HPW in the presence of bile salt by TCM (Upadhyay et al. 2001). The studies inferred that these medicinal plants contain CMS properties and treat numerous hepatic disorders (Upadhyay et al. 2001). A study by Tsai and Tsai (2004) recorded that the pretreatment of guinea pig with berberine (4 mg/kg, orally two times a day for 2 days) inhibits $CCl_4$-induced rise in serum levels of ALP (alkaline phosphatase) and AST/ALT (aminotransaminases), proving hepatoprotection. In addition to this, the post-treatment with berberine three oral doses (4 mg/kg interval of 6 h) usually reduces hepatic damage induced by acetaminophen (Tsai and Tsai 2004). Berberine also shows the inhibitory effect with $K^+$ and $Ca^+$ for the selected rat hepatocytes and has hepatoprotective function (Wang et al. 1991, Chan 1977, Janbaz and Gilani 2000).

### 20.6.7 Cardiovascular Functions

The cardiovascular effects of berberine was mentioned in several studies carried out across the globe (Shaffer 1985, Eaker and Sninsky 1989; Huang et al. 1989, Huang 1990a, Wang et al. 1991, Riccioppo 1993, Zhou et al. 1993, Hua and Wang 1994, Li and Wang 1997, Wang and Zheng 1997; Liu et al. 1999, Zhou et al. 2001, Li et al. 2001, Hong et al. 2003, Yang et al. 2004, Abidi et al. 2005, Doggrell 2005, Brusq et al. 2006, Zhao et al. 2007; Park et al. 2014; Zaha et al. 2016; Chang et al. 2016; Chopra aand Vishwakarma 2018, Neag et al. 2018) reported that the mechanism of antihypertensive effect of 6-protoberberine was through the central sympatholytic effect (Liu et al. 1999, Chopra aand Vishwakarma 2018). Berberine derivative 6-protoberberine is an effective antihypertensive agent (Liu et al. 1999). Berberine leads to lowering of low-density lipoprotein cholesterol (Abidi et al. 2005, Doggrell

2005). Studies have revealed that it prevents L-thyroxine-induced cardiac hypertrophy in rats (Chopra and Vishwakarma 2018). It modulates the nervous system (sympathetic) of rats (Hong et al. 2003) and reflects the therapeutic potentials for cardiac hypertrophy and heart failure (Zhou et al. 2001). Berberine is also reported to have antiarrhythmic and inotropic effect (Shaffer 1985). The use of 3–30 μM berberine is responsible for clamping of ventricular myocytes in guinea pig (Xu et al. 2005). It is a blocker of the cardiac ATP-sensitive $K^+$ channels (Hua and Wang 1994). Tetrahydroberberine was reported to protect the myocardium from ischaemic and reperfusion injury (Zhao et al. 2007). Berberine might affect myocardial cell membrane by decreasing impedance and increasing electric coupling (Zhao et al. 2007). Berberine increases the flow of coronary artery and showed protective effect against ischaemia (Eaker and Sninsky 1989). Zeng (1999) and Neag et al. (2018) reported cardiac contractility functioning due to the use of berberine. The berberine alkaloids can endow with cardioprotection in ischemic conditions (Neag et al. 2018) at various levels in human body functions when used, such as modulation of AMPK (AMP-activated kinase) motion (Brusq et al. 2006). Other cardioprotective functioning during ischaemia was studied by Chang et al. (2016); Zaha et al. (2016) studied AMPK, an imperative enzyme, which plays a significant role in cellular metabolism, whereas Mascareno et al. (2001) and Park et al. (2014) studied ischaemia-reperfusion injury.

### 20.6.8 Anticarcinogenic Properties: Antitumor and Apoptosis Functions

The anticarcinogenic (or anticancer) functions of the berberine were studied in details by several researchers (Zhang et al. 1990, Chen et al. 1994, Wu et al. 1999, Lin et al. 1999, Anis et al. 2001, Yount et al. 2004, Kettmann et al. 2004, Inoue et al. 2005; Lin et al. 2006, Peng et al. 2006, Tan et al. 2006, Jantova et al. 2007, Pan et al. 2017, Wojtaszek et al. 2019, Zhang et al. 2020) undertaken in various parts of the world. The berberine potentially represses tumour progression and considered as the most safer, effective and cost-effective agent for cancer patients (Li et al. 2015). The berberine was reported to induce apoptosis in cancer cell line studies (Neag et al. 2018). This alkaloid is known as inhibitor in mevalonate pathways (Issat et al., 2006). Wu et al. (1999) studied berberine and reported that it induces cell apoptosis in HL-60 cell line, through the activation of caspase-3.

Lin et al. (2006) also reported that berberine iodide and berberine from acetone show the maximum cytotoxicity for SCC. Kettmann et al. (2004) reported berberine to have significant cytotoxicity against human uterus HeLa and L1210 cell lines, and the mechanism includes cell cycle arrest and apoptosis. Berberine is preferred compared to nitrosodiethylamine in reducing liver injuries (Kettmann et al. 2004). Inoue et al. (2005) were of the opinion that berberine when tested in vivo to B16 cell lines with a dose ranging from 1 to 10 mg/kg stimulates tumour mass at the lower dose of 1 mg/kg, whereas berberine dose of 5 and 10 mg/kg reduced the overall tumour weight. Chen et al. (1994) found that berberine inhibits the nitrogen-

acetyltransferase function in HCT cells with respect to the dose-dependent manner leading to cell death, which means that at maximum berberine concentration, nitrogen-acetyltransferase and cell death inhibition were higher. Further, the lethal dose ($LD_{50}$) of berberine against 9 L glioma cells was 60 μg/ml, but when used along with low energy laser, $LD_{50}$ markedly decreased to 10μb/ml (Chen et al. 1994). Yount et al. (2004) and Zhao et al. (2017) also reported anticarcinogenic properties of berberine. Zhang et al. (2020) reported berberine maintains the neutrophil N1 phenotype to reverse cancer cell resistance to doxorubicin in a urethane-induced lung cancer model.

### 20.6.9 Miscellaneous Functions of Berberine

Besides the above pharmacological functions, berberine was reported to possess other properties as indicated in several literatures (Peng et al. 1997, Schmeller et al. 1997, Chiou et al. 1998; Xu and Malave 2001, Ko et al. 2000, Tan et al. 2004, Kuo et al. 2004, Kumar and Khanam 2004, Gu et al. 2005, Nechepurenko et al. 2010, Yang et al. 2019, Cui et al. 2007). Berberine is used in treatment of erectile dysfunction because this active molecule inhibits $PDE_5$-$A_2$ mRNA expression (Tan et al. 2004). Study by Xu and Malave (2001) reported this alkaloid is effective in the treatment of CIC (cyclophosphamide-induced cystitis) and helpful in prevention of cyclophosphamide urotoxicity. The berberine inhibitory activity for deoxyribonucleic acid (DNA) cleavage is reported to be better than ascorbic and caffeic acid by a study carried out by Chiou et al. (1998). A study by Peng et al. (1997) proves that this alkaloid, berberine, at dosages of 0.1 and 0.5 g/kg for 1 or 2 weeks significantly improves scopolamine-induced amnesia. It also shows protective effect to the brain damage caused during ischaemic. Lin and Chang (1995) reported berberine induces a relaxant effect in rat isolated mesenteric arteries against the contractile response induced by phenylephrine. Berberine shows the most potent anti-acne activity at MIC of 400 μg/ml (Kumar and Khanam 2004). Alcoholic and aqueous extract ointments of the species also show wound-healing properties (Kuo et al. 2004).

## 20.7 Clinical Trials and Recommendations

Clinical studies of berberine were reported by several studies (Rabbani et al. 1987, Marin-Neto et al. 1988, Huang 1990b, Yuan et al. 1994, Sheng et al. 1997, Zeng et al. 2003, Kong et al. 2004, Wu et al. 2005, Xin et al. 2006, Cicero et al. 2007) from across the globe. A study by Khin-Maung et al. (1985) reported berberine in a double blind clinical trial on 400 peoples having the acute watery diarrhoea, and positive antisecretory and vibriostatic effects were recorded. Rabbani et al. (1987) studied the randomized controlled trial of BS therapy (400 mg, 8 h, single dosage) on 165 patients tested for acute diarrhoea caused by *E. coli* and *V. cholera* and proved

BS (berberine sulphate) as the most safe and effective drug as antisecretory in diarrhoea, without any side effects.

Marin-Neto et al. (1988) have undertaken clinical trial with 12 patients suffering from severe congestive heart failure and reported berberine to be effective in cardiovascular patients. Yuan et al. (1994) studied the berberine effect at a dosages of 1.2 g (probability $<0.001$) in the small intestine of 20 healthy patients and reported antidiarrhoeal functions. The berberine effect on a clinical trial in 100 patients with VT observed with 24–48 h ambulatory monitoring was undertaken by Huang (1990b) and reported 62% cases with 50 percentages or greater VPC suppression and 38% cases with 90 plus percentages greater VPC suppression. The mean observed significantly decreased by using berberine from $452 \pm 421.8$ beats/h to $271 \pm 352.7$ beats/h (probability$<0.001$). No severe side effect recorded which proves that berberine is effective for ventricular tachyarrhythmias. Sheng et al. (1997) studied CLQR malaria by employing pyrimethamine plus berberine, tetracycline or cotrimoxazole in a clinical trial on 215 peoples in 3 groups (82, 84, 69). In the first case, pyrimethamine and berberine were administered; second case, pyrimethamine and tetracycline; and third case, pyrimethamine and cotrimoxazole. The pyrimethamine and berberine group was found to be more effective (74.4%) in clearing stomach parasite than other two cases, which suggested berberine as the best for CLQR malaria. Zeng et al. (2003) performed clinical trials with 156 patients suffering from congestive heart problems and reported that the patients given with berberine 1.2–2.0 g/day have decreased ventricular premature complexes. Kong et al. (2004) recorded this alkaloid as a drug molecule helpful in lowering cholesterol in a clinical trial with 32 hypercholesterolemic patients and concluded that berberine upregulates independently LDLR expression of sterol binding proteins through the mechanism of post-transcription which is usually helpful in stabilizing mRNA. These findings showed the berberine effective as hypolipidemic molecule. Wu et al. (2005) studied the berberine effects in a clinical trial with 52 patients having renal transplant and concluded that 0.2 g berberine thrice in a day for 90 days elevated blood concentration from 88.9 to 98.4%. The eulipidemic effect of berberine as a natural cholesterol-lowering drug molecule was also studied by Cicero et al. (2007) on 40 patients in 2 groups (each group with 20 peoples) and reported that berberine is helpful in reducing total cholesterol content in humans along with policosanol, red yeast extract, folic acid and astaxanthin.

## 20.8 Patents

There are several patents published on the alkaloid berberine, and some important patents on this compound are given in Table 20.3.

**Table 20.3** Patent records on the alkaloid berberine

| Title | Patent no. | Published | Inventor | Date of publication | References |
|---|---|---|---|---|---|
| A process for the extraction of berberine from leaves of *Coscinium fenestratum* with improved yield | IN201611000895 | A61K 8/97 | Shuchishweta, Kendurkar V, Rangaswamy M | 19-01-2018 | Council of Scientific and Industrial Research, India [CSIR-NCL] |
| Administration of berberine metabolites | 20,190,255,028 | 20,190,255,028 | Lowery R, Wilson J, Wells S, LaCore T | 06.05.2019 | United States Patent Application Publication |
| Application of berberine in preparing drug for treating acute soft tissue injury | WO2018161890A1, CN106727542A | WO2018161890A1, CN106727542A | Cai Lixiong, Li Huaiguo, Li Xue, Li Zihong, Liu Dongwen, Liu Lichu, Liu Xiaofang, Yu Junwen, ZhengFanghao | 13.09.2017 | European Patent Specification |
| Bactericidal composition containing berberine and mandipropamid | CN108294023A | CN108294023A | Fan Wangqi, Liu Li, Wu Sha | 20.07.2018 | European Patent Specification |
| Berberine alkaloids as a treatment for chronic, protozoally induced diarrhoea | WO9800018A1 | WO9800018A1 | Mcdevitt, Joseph T | 08.01.1998 | European Patent Specification |
| Berberine compounds and processes for the preparation of berberine compounds | US 2010/0081821 A1 | US 2010/0081821 A1 | Christopher WG, Moser FW, Johnson JE | 01.04.2011 | United States Patent Application Publication |
| Berberine electrostatic composite and preparation method of berberine electrostatic composite | CN102702190A | CN102702190A | Dongjiao Fan, Xing Tang, Yong Ji | 03.10.2012 | European Patent Specification |
| Berberine formulations and uses thereof | WO2015095640A1, US 2015/0174109 A1 | PCT/US2014/071364 | Chen-Mao L, Way-Yu L, Po-Yuan T, Pao-Li W | 25.06.2015 | International Application Published Under the Patent Cooperation Treaty |

(continued)

**Table 20.3** (continued)

| Title | Patent no. | Published | Inventor | Date of publication | References |
|---|---|---|---|---|---|
| Berberine formulations and uses thereof | WO2015095640A1, US 2015/0174109 A1 | PCT/US2014/071364 | Chen-Mao L, way-Yu L, Po-Yuan T, Pao-Li W | 25.06.2015 | International Application Published Under the Patent Cooperation Treaty |
| Berberine hydrochloride drop pill | CN104434843A | CN104434843A | Li Lianghong | 25.03.2015 | European Patent Specification |
| Berberine nanocomposite and preparation method | CN105663040A | CN105663040A | Chen Zhong, Fei Yan, Guo Yihe, Lai Minling, Su Junkai, Tang Qinglin, ZhangMingqing | 15.06.2016 | European Patent Specification |
| Berberine derivative XMU-Ber122 | CN107501259A | CN107501259A | Hu Tianhui, Jiang Xunjin Tian Yuan, Xu Beibei Zhan Yanyan ZhangWenqing Zhang Yandong | 22.12.2017 | European Patent Specification |
| Berberine-eluting stent | CN107158486A | CN107158486A | Chen Shaoliang | 15.09.2107 | European Patent Specification |
| Compositions containing berberine or extracts containing it, for the prevention and treatment of alterations of the lipid and carbohydrate balance | EP2149377A1 | EP2149377A1 | Di Pierro Francesco | 03.02.2010 | European Patent Specification |
| Determination method of embedding rate of berberine hydrochloride in amylase-berberine hydrochloride clathrate compound | CN105181751A | CN105181751A | Bing Fangling, Feng Tao, Gao Linlin, Li Mingming, Liu Yi, Wang Shuai, Xie Kelin, Yi Zhiying | 23.12.2015 | European Patent Specification |

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|---|---|---|---|---|---|
| Mangiferin-berberine salt, manufacturing method and use thereof | EP 2444094 A1 | WO 2010/145192 | Teng HHH | 25.04.2012 | European Patent Application |
| Method for determining berberine | JPS61282100A | JPS61282100A | Hara Yasuhiro, Suzuki Toshiyuki, | 12.12.1986 | European Patent Specification |
| Method of selecting berberine releasing culture cell | JPS61285988A | JPS61285988A | Hara Yasuhiro. Suzuki Toshiyuki, | 16.12.1986 | European Patent Specification |
| Multifunctional compound berberine tablet | CN101313949A | CN101313949A | Yuejin, Wang | 03.12.2018 | European Patent Specification |
| Nanometer berberine tablet | CN106474123A | CN106474123A | Hu Jianhua, Mao Shangwu, Qi Shougang, Wang Jingjing | 08.03.2017 | European Patent Specification |
| Niacin and berberine compositions and methods of use thereof | 20190358211A1 | 20190358211A1 | Mitchell OW | 28.11.2019 | United States Patent Application Publication |
| Pharmaceutical compositions containing berberine for treatment or prevention of weight gain and obesity associated with antipsychotic drugs | US 2011/0281852 A1 | US 2011/0281852 A1 | Davies G, Hu Y | 17.11.2011 | United States Patent Application Publication |
| Pharmaceutical use of berberine | WO2018113080A1 | WO2018113080A1 | Chen S | 28.06.2018 | European Patent Specification |
| The use of berberine as insulin sensitizer | WO2004032924A1 | PCT/CN2002/000715 | Jiang J, Wei J, Wang Z Pan H | 22.04,2204 | Chinese Patent Specification |
| Therapeutic uses of berberine formulations | WO2016210230A1 | WO2016210230A1 | Oscar BC, Chih-Kuang C, I-Yin L, Chen-En T, Po-Yuan T | 29.12.2016 | European Patent Application |

(continued)

**Table 20.3** (continued)

| Title | Patent no. | Published | Inventor | Date of publication | References |
|---|---|---|---|---|---|
| Transdermal formulations for delivery of berberine compounds and their use in the treatment of berberine-responsive diseases and conditions | US20180235870A1 | US20180235870A1 | Gabriele J, Baranowski D, Buchanan B, Zuccolo J, Teris M | 23.08.2018 | United States Patent Application Publication |
| Use of a composition comprising formoterol and beclometasone Dipropionate for the treatment of an exacerbation of asthma | EP 2146704 B1 | WO 2008/128685 | Chiesi P, Rondelli I, Acerbi D, Poli G | 30.10.2008 | European Patent Specification |
| Use of berberine with high solubility in preparation of medicament | AU2003231481A1 | WO03090749A1 | Kaimin W | 06.11.2003 | European Patent Specification |

## 20.9 Conclusion

Since the ancient times, plants containing berberine were utilized by different communities of people for health purposes and to relieve physical sufferings. Herbal botanicals were considered as the most effective and to have safer, lesser or no side effects and easily available. In recent years, due to the renewed interest in herbal products globally, berberine can serve as an important active pharmaceutical compound for future use as drug and their extract as herbal medicine. The presented communication from available scientific reports indicated several traditional medical uses of berberine which were evaluated for modern pharmacological studies, and berberine-rich species have several pharmacological and therapeutic actions, such as immunomodulatory effects; protective action on the cardiovascular system, liver and kidney; endothelial relaxation; and as regulator on glucose metabolism and atherosclerosis. In addition, due to antioxidant and anti-inflammatory effects of berberine, several clinical applications have been formulated from inflammatory conditions to the metabolic syndrome. Berberine-containing plant species accumulating diverse benzylisoquinoline alkaloids may serve as systems to study the intricacies of evolution of enzyme repertoire responsible for the specialization of alkaloid biosynthesis. Such studies will contribute in developing knowledge resource to feed future synthetic biology strategies for metabolic engineering of natural and non-natural novel alkaloids. There is need to carry forward more and more in vivo and in vitro pharmacological studies having primary focus on berberine alkaloid and clinical trials in near future.

**Acknowledgement** We would like to thanks all the authors whose publications have been cited in this review communication. We would also like to thank Director CSIR-IIIM Jammu for the facilities.

**Conflict of Interest** The authors declare no conflict of interest for this publication.

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# Glossary

**Acne** An inflammatory disease occurring in or around the sebaceous glands.

**Aflatoxin** Poisonous carcinogens that are produced by certain molds which grow in soil, decaying vegetation, hay, and grains.

**Allosteric modulator** Drug that binds to a receptor at a site distinct from the active site. Induces a conformational change in the receptor, which alters the affinity of the receptor for the endogenous ligand. Positive allosteric modulators increase the affinity, while negative allosteric modulators decrease the affinity.

**Amino acid** Amino acids are organic compounds containing amine and carboxyl functional groups, along with a side chain specific to each amino acid.

***Am*orphous** Without a clearly defined shape or form.

**Anaemia** Lack of enough blood in the body causing paleness.

**Anesthetic** Inducing loss of feeling or consciousness.

**Analgesic** Relieving pain.

**Analytic studies** Studies with control groups, namely case-control studies, cohort studies, and randomized clinical trials.

**Annual** A type of flower or plant that lives for only 1 year.

**Antagonist** Drug that attenuates the effect of an agonist.

**Antidiarrheal** Preventing or controlling diarrhea.

**Antidote** An agent which neutralizes or opposes the action of a poison.

**Apoptosis** Death of cells which occurs as a normal and controlled part of an organism's growth or development.

**Autophagy** Autophagy (or autophagocytosis) is the natural, regulated mechanism of the cell that disassembles unnecessary or dysfunctional components.

**Bioactivity** Biological activity describes the beneficial or adverse effects of a drug on living matter.

**Biodiversity** Reflects the number, variety, and variability of living organisms.

**Biological resources** Those components of biodiversity of direct, indirect, or potential use to humanity.

**Bovine** Cattle.

**Calibration** The action or process of calibrating something.

**Carbohydrate** Biomolecule consisting of carbon, hydrogen, and oxygen atoms, usually with hydrogen-oxygen atom ratio of 2:1.



B. Singh (ed.), *Botanical Leads for Drug Discovery*,
https://doi.org/10.1007/978-981-15-5917-4

***Chr*omatin** Mass of genetic material composed of DNA and proteins that condense to form chromosomes during eukaryotic cell division.

**Chromatography** Laboratory technique for the separation of mixture.

**Clinical pharmacology** The study of the effects of drugs in humans.

**Coniferous** The conifers are a division of vascular land plants containing gymnosperms, cone-bearing seed plants.

**Crystallization** Chemical solid–liquid separation technique, in which mass transfer of a solute from the liquid solution to a pure solid crystalline phase occurs.

**Cytotoxicity** Quality of being toxic to cells. Examples of toxic agents are an immune cell or some types of venom.

**Decoction** Concentrated liquor resulting from heating or boiling a substance, especially a medicinal preparation made from a plant.

**Distillation** A process of evaporation and re-condensation used for purifying liquids.

**Diuretic** Diuretics, also called water pills, are medications designed to increase the amount of water and salt expelled from the body as urine.

**Dose-response relationship** A relationship in which a change in amount, intensity, or duration of exposure is associated with a change in risk of a specified outcome.

**Drip irrigation** The use of pipes to bring water into contact with the roots of plants.

**Drug product** A finished dosage form, for example, a tablet, capsule, or solution that contains a drug substance.

**Drug substance** An active ingredient that is intended to furnish pharmacological activity or other direct effect in diagnosis, cure, mitigation, treatment, or prevention of diseases or to effect the structure or any function of the human body.

**Drug** An agent that is used therapeutically to treat diseases. It may also be defined as any chemical agent and/or biological product or natural product that affects living processes.

**$EC_{50}$** Molar concentration of an agonist that produces 50% of the maximum possible response for that agonist.

**Enzymes** Proteins that start a chemical reaction.

**$ED_{50}$** In vitro *or* in vivo dose of drug that produces 50% of its maximum response or effect.

**Efficacy** Describes the way that agonists vary in the response they produce when they occupy the same number of receptors. High efficacy agonists produce their maximal response while occupying a relatively low proportion of the total receptor population. Lower efficacy agonists do not activate receptors to the same degree and may not be able to produce the maximal response.

**Endemic** Distribution restricted to a particular area—used to describe a species or organism that is confined to a particular geographical region, for example, an island or river basin.

**Essential oil** Volatile perfumery material derived from a single source of vegetable or animal origin by a process such as hydrodistillation, steam distillation, dry distillation, or expression.

**Ethanol** Form of natural gas that can be produced from corn.

**Experimental studies** Studies in which the investigator controls the therapy that is received by each participant, generally using that control to randomly allocate patients among study groups.

**Extract** A concentrate of dried, less volatile aromatic plant part obtained by solvent extraction with a polar solvent.

**Extraction** The process of isolating essential oil with the help of a volatile solvent.

**Fatty acid** A carboxylic acid consisting of a hydrocarbon chain and a terminal carboxyl group, especially any of those occurring as esters in fats and oils.

**Flavonoid** Class of plant and fungus secondary metabolites.

**Flavor** Refers to that characteristic quality of a material as affects the taste or perception.

**Gastronomist** A connoisseur of good food; a gourmet.

**Glutamic acid** An α-amino acid that is used by almost all living beings in the biosynthesis of proteins. It is non-essential in humans, meaning the body can synthesize it.

**Half-life** Half-life (t½) is an important pharmacokinetic measurement. The metabolic half-life of a drug *in vivo* is the time taken for its concentration in plasma to decline to half its original level. Half-life refers to the duration of action of a drug and depends upon how quickly the drug is eliminated from the plasma. The clearance and distribution of a drug from the plasma are therefore important parameters for the determination of its half-life.

**Heart palpitations** Abnormally rapid and irregular beating of the heart.

**Heterothallism** The term is applied particularly to distinguish heterothallic fungi, which requires two compatible partners to produce sexual spores from homothallic ones.

**Humus** The organic component of soil, formed by the decomposition of leaves and other plant material by soil microorganisms.

**Hydrodistillation** Distillation of a substance carried out in direct contact with boiling water.

**$IC_{50}$** In a functional assay, the molar concentration of an agonist or antagonist which produces 50% of its maximum possible inhibition. In a radioligand binding assay, the molar concentration of competing ligand which reduces the specific binding of a radioligand by 50%.

**In vitro** Taking place in a test tube, culture dish, or elsewhere outside a living organism.

**In vivo** Taking place in a living organism.

**Incidence rate** Measure of the frequency of the disease or outcome. The number of new cases which develop over a defined time period in a defined population at risk, divided by the number of people in that population at risk.

**Infusion** A process of treating a substance with water or organic solvent, with or without heating.

**Insecticide** A type of chemical used to kill insects.

**Liana** Any of various long-stemmed, woody vines that are rooted in the soil at ground level and use trees, as well as other means of vertical support, to climb up to the canopy to get access to well-lit areas of the forest.

**Micro-organism** Tiny living things that can only be seen with a microscope.

**Migraine** A periodic condition with localized headaches, frequently associated with vomiting and sensory disturbances.

**Monitoring** The performance and analysis of routine measurements aimed at detecting changes in the environment or health status of populations.

**Nutraceutical** A food stuff that is held to provide health or medicinal benefits in addition to its basic nutritional value also called functional food.

**Odor** Property of a substance which stimulates and is perceived by the olfactory sense.

**Organic acid** An organic compound with acidic properties.

**Organic farming** Producing foods without the use of laboratory-made fertilizers, growth substances, or pesticides.

**Organic matter** Dead plants, animals, and manure converted by earthworms and bacteria into humus.

**Osteoporotic** A disease where increased bone weakness increases the risk of a broken bone. It is the most common reason for a broken bone among the elderly.

**Perfume** A suitably blended composition of various materials of synthetic and/or natural origin to give a desired odor effect. It is carried in a suitable medium to the extent of not more than 20%.

**pH** A scale of measurement by which the acidity or alkalinity of soil or water is rated. A pH of 6–7.5 is considered "ideal" for most agricultural crops. Each plant (specie-type), however, has its own "ideal" pH range.

**Pharmacology** The study of the effects of drugs. The branch of biology concerned with the study of drug action, where a drug can be broadly defined as any man-made, natural, or endogenous (from within the body) molecule which exerts a biochemical or physiological effect on the cell, tissue, organ, or organism.

**Phytochemistry** The study of phytochemicals, which are chemicals derived from plants.

**Plant** Multicellular predominantly photosynthetic eukaryotes of the kingdom Plantae. Historically, plants were treated as one of two kingdoms including all living things that were not animals, and all algae and fungi were treated as plants.

**Polymorphic** Occurrence of two or more clearly different morphs or forms, also referred to as alternative phenotypes, in the population of a species.

**Polysaccharides** A carbohydrate (e.g., starch, cellulose, or glycogen) whose molecules consist of a number of sugar molecules bonded together.

**Population** The number of living things that live together in the same place. In biology, a population is all the organisms of the same group or species which live in a particular geographical area and have the capability of interbreeding.

**Potency** A measure of the concentrations of a drug at which it is effective.

**Saprophyte** A plant, fungus, or microorganism that lives on dead or decaying organic matter.

**Sclerotia** Compact mass of hardened fungal mycelium containing food reserves.

**Screening** The presumptive identification of unrecognized disease or defect by the application of tests, examinations, or other procedures which can be applied rapidly. Screening is an initial examination only, and positive responders require a second diagnostic examination.

**Side-effect** Any unintended effect of a pharmaceutical product occurring at doses normally used in humans which is related to the pharmacological properties of the drug.

**Signal** Reported information on a possible causal relationship between an adverse event and a drug, the relationship being unknown or incompletely documented previously. Usually more than a single report is required to generate a signal, depending upon the seriousness of the event and the quality of the information.

**Solitary** Existing alone.

**Species** A group of living organisms consisting of similar individuals capable of exchanging genes or interbreeding. The species is the principal natural taxonomic unit, ranking below a genus and denoted by a Latin binomial, for example, *Homo sapiens*.

**Specificity** The ability of a method, system, or tool to correctly classify the proportion of persons who truly do not have a characteristic, as not having it.

**Spectroscopy** The study of the interaction between matter and electromagnetic radiation.

**Steam distillation** Distillation of a substance by bubbling steam through it.

**Stimulant** Making a body organ active.

**Surveillance** Ongoing scrutiny, generally using methods distinguished by their practicability, uniformity, and rapidity, rather than by complete accuracy. Its main purpose is to detect changes in trends or distribution in order to initiate investigative or control measures.

**Tannin** A yellowish or brown bitter tasting organic substance present in some galls, barks, and other plant tissues.

**Taxon (plural taxa)** In biology, a taxon is a group of one or more populations of an organism or organisms seen by taxonomists to form a unit. Although neither is required, a taxon is usually known by a particular name and given a particular ranking, especially if and when it is accepted or becomes established.

**Technology** Instruments, tools, or inventions developed through research to increase efficiency.

**Tincture** A cold alcoholic extract of natural fragrant material of vegetable or animal origin, the solvent being left in the extract as a diluent.

**Topographic** Relating to the arrangement of the physical features of an area.

**Traditional knowledge** The term traditional knowledge generally refers to knowledge systems embedded in the cultural traditions of regional, indigenous, or local community.

**Urbanization** The growth of the city into rural areas.

**Validation** Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determinant specifications and quality attributes.

**Volatile** A material is said to be volatile when it has the property of evaporating at room temperature when exposed to atmosphere.

**Yield** The amount of a crop produced in a given time or from a given place.