

Medicinal and Aromatic Plants of the World

Ákos Máthé
Irfan Ali Khan *Editors*

Medicinal and Aromatic Plants of India Vol. 1

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Medicinal and Aromatic Plants of the World

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Series Editor

Ákos Máthé, Faculty of Agriculture and Food Science,
Széchenyi István University, Mosonmagyaróvár, Hungary

Medicinal and Aromatic Plants (MAPs) have been utilized in various forms since the earliest days of mankind. They have maintained their traditional basic curative role even in our modern societies. Apart from their traditional culinary and food industry uses, MAPs are intensively consumed as food supplements (food additives) and in animal husbandry, where feed additives are used to replace synthetic chemicals and production-increasing hormones. Importantly medicinal plants and their chemical ingredients can serve as starting and/or model materials for pharmaceutical research and medicine production. Current areas of utilization constitute powerful drivers for the exploitation of these natural resources. Today's demands, coupled with the already rather limited availability and potential exhaustion of these natural resources, make it necessary to take stock of them and enrich our knowledge regarding research and development, production, trade and utilization, and especially from the viewpoint of sustainability. The series Medicinal and Aromatic Plants of the World is aimed to look carefully at our present knowledge of this vast interdisciplinary domain, on a global scale. In the era of global climatic change, the series is expected to make an important contribution to the better knowledge and understanding of MAPs.

Budapest, Prof. Dr. Ákos Máthé.

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Editors

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Preface

Medicinal and aromatic plants (MAPs) have been utilized in various forms since the earliest days of mankind. They have maintained their traditional basic curative role even in our modern societies. Apart from their traditional culinary and food industry uses, MAPs are intensively consumed as food supplements (food additives) and in animal husbandry, where feed additives are used to replace synthetic chemicals and production-increasing hormones. Importantly, medicinal plants and their chemical ingredients can serve as starting and/or model materials for pharmaceutical research and medicine production. Current areas of utilization constitute powerful drivers for the exploitation of these natural resources. Today's demands, coupled with the already rather limited availability and potential exhaustion of these natural resources, make it necessary to take stock of them and enrich our knowledge regarding research and development, production, trade, and utilization, especially from the viewpoint of sustainability. The series Medicinal and Aromatic Plants of the World is aimed to look carefully at our present knowledge of this vast interdisciplinary domain, on a global scale. In the era of global climatic change, the series is expected to make an important contribution to the better knowledge and understanding of MAPs.

Mosonmagyaróvár, Hungary
October 2021

Ákos Máthé

Preface

This book, as the eighth volume of the series Medicinal and Aromatic Plants of the World (MAPW), focuses on the medicinal and aromatic plants (MAPs) of India.

The vast country of India, which is frequently called a sub-continent, with its 15 agro-climatic zones, is one of the richest countries in the world regarding its wealth of biodiversity. Out of the documented flora of 17,000–18,000 flowering plant species, more than 7000 are estimated to have medicinal usage in folk and documented systems of medicine like Ayurveda, Unani, Siddha and Homoeopathy (AYUSH System of Medicine).

MAPs are a major resource base for traditional medicine and the herbal industry. In addition, they also provide livelihood and health security to a large proportion of the Indian population. About 242 species have annual consumption levels in excess of 100 metric tons/year.

In India, several attempts have been made to explore and best exploit this huge hidden wealth and to some extent undiscovered/unexplored biodiversity of the rich flora.

In view of the intensive and ever-increasing exploitation (frequently overexploitation) of natural resources of medicinal plants, an increasing number of species are becoming endangered, occasionally facing extinction.

In this scenario, headed by the Ministry of AYUSH, and in collaboration with numerous other relevant scientific institutions, intensive and innovative research and development activities have been launched in India. These are aimed at both exploration and sustainable utilization of natural resources.

The explicit aim of the present volume is to offer insight into the various aspects of these activities that are based on the rich knowledge of past traditions, focusing on the sustainable utilization of the much-needed MAP resources.

The aim of this collective, comprising specialists working in the relevant fields of medicinal and aromatic plants, is to explore/collect/summarize and evaluate/validate the still-available information on these resources.

In the present volume (which is meant to be followed by further volumes), general aspects related to MAP use and research are introduced, while the next volume

will focus on the available knowledge on individual species that are promising success in their conservation/utilization and are native to India.

The editors wish to thank Springer and its editorial staff, in particular Melanie Overbeek, for their support of this daring project entitled *Medicinal and Aromatic Plants of the World*.

Mosonmagyaróvár, Hungary

Ákos Máthé

Hyderabad, India
October 2021

Irfan Ali Khan

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About the Editors



Akos Máthé has a 40 years' background in teaching and research activities in plant ecophysiology and agricultural botany at the University of Horticulture and Food Industry, Budapest, and the Széchenyi István University, Győr, Hungary. Doctor of the Hungarian Academy of Sciences. Two times Fulbright Scholar at University of California Davis and University of Massachusetts, Amherst. Visiting professor also at University of Veterinary Medicine, Vienna (Austria). Courses on the production and ecology of Medicinal Plants, as well as Phytogenic Feed Additives, respectively. Teaching/Research/Consulting and Publication interest and activity include ecophysiology, plant domestication/introduction, production of MAPs, new crops and new uses of plants. International consultant and expertise (e.g.: FAO courses on MAP production – Antalya, Turkey, EU COST, IUCN, CBI committees, etc.). Member of editorial board/reviewer of international scientific journals, Ph.D. Theses, etc. Relevant activities include involvement in both Hungarian and EU funded research projects (e.g.: FEED SEG), education projects (CEEPUS, ERASMUS +, HERB AID, GOOD HERBS, Herbs and Youth, EOHUB, etc.). Founding secretary and Board of Directors member of Hungarian Medicinal Plant Association. President of International Council for Medicinal and Aromatic Plants (ICMAP <http://www.icmap.org>). Chairman of Section for Medicinal and Aromatic Plants, International Society for Horticultural Sciences (2006–2014), Member of Fair Wild Advisory Panel. Professor Máthé has authored some 100 publications in medicinal and

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Irfan Ali Khan obtained his MSc from Aligarh Muslim University and PhD in botany from Osmania University, Hyderabad, specializing in genetics and plant breeding. Professor Khan is the former director of Nawab Shah Alam Khan Centre for Post Graduate Studies and Research (Affiliated to Osmania University), Anwarul Uloom College Campus, Mallepally, Hyderabad. Presently, he is the managing director of Ukaaz Publications, Hyderabad. He has published 163 research papers in reputed national and international journals and is now on the panel of "Experts on Mungbean" for all countries in South-East Asia and the Middle East. Professor Khan has been the editor of *Frontiers in Plant Science*, has edited 74 reference books, and has co-authored 3 textbooks with his wife, Professor Atiya Khanum. He is a Fellow of the Indian Society of Genetics (F.I.S.G.). Besides this, he is the editor-in-chief of *Annals of Phytomedicine* – an international journal. Professor Khan is the senior author of the famous textbook *Fundamentals of Biostatistics* by Khan and Khanum which has been released by world-renowned agricultural scientist Dr. M.S. Swaminathan on February 13, 1994, in Hyderabad. This book has been included as a textbook and also as reference book in more than 400 universities and research institutes in India and abroad. Besides this, he has given a formula of LSD (least significant difference) with suitable examples, which is more or less a substitute for Student's 't' test to compare two treatments.

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Chapter 1

Introduction to Medicinal and Aromatic Plants in India



Ákos Máthé and Irfan Ali Khan

Abstract Since the beginnings of known history of medicinal plants, the production and utilization of medicinal and aromatic plants has seen a tremendous, nearly indescribable progress. In an effort to provide quality healthcare to all, traditional medicine, in particular herbal medicine, has survived as a major healthcare provider, mainly in rural and remote areas.

Indian traditional system of medicine has a vast history that has been acknowledged also by modern research for their effectiveness. Indian traditional medicine or medicinal plants are also considered as vital sources for new drug development. Evidence based incorporation of Indian traditional medicine through clinical practice helps provide quality healthcare to all. In this context, the present chapter provides an insight into various basic aspects of medicinal and aromatic plant verticum spanning the product range - from the sustainable sourcing, conservation, cultivation and trade of raw-materials. Components or actors of the Indian Traditional Medicinal System largely depend on MAPs. The brief survey of the principal actors of Indian MAP sector provides an opportunity to assess the comprehensive and profound activities with which the present Ministry of AYUSH is engaged in promoting sustainable production and utilization of MAPs with the ultimate goal of integrating it into clinical practice to provide safe, efficient and quality healthcare to the people.

Keywords India · Medicinal and Aromatic Plants · Biodiversity hotspots · Flora · Natural conservation · Official and public participants of legal regulation

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Abbreviations

ASU	Ayurveda, Siddha and Unani
CCRAS	Central Council for Research in Ayurvedic Sciences
CCRH	Central Council for Research in Homeopathy
CCRIMH	Central Council for Research on Indian Medicine and Homoeopathy
CCRUM	Central Council for Research in Unani Medicine
CCRYN	Central Council for Research in Yoga and Naturopathy
GMP	Good Manufacturing Practices
ISM & H	Indian System of Medicine and Homeopathy
ISM	Indian System of Medicines
NAM	National Ayush Mission
NCEs	New Chemical Entities
NMPB	National Medicinal Plant Board
RET	Rare, Endangered, Threatened
VCSMPP	Voluntary Certification for Medicinal Plant Produce
WHO	World Health Organization

1.1 Introduction

The known history of medicinal plants in India dates back to the early times before Christ. According to Atal and Kapur (Atal and Kapur 1977) the earliest mention of medicinal plant use can be found in the so called “Rigveda”, one of the oldest repositories of human knowledge, written between 4500 and 1600 B.C.

Mentions on the use of plants for curing diseases can be traced back to the times around 1600 B. C. (Sharma et al. 2021). Some western scholars feel that Ayurveda developed somewhere about 2800–600 B.C. This was in the form of a supplement to Vedas that records definite properties of drugs and their uses, in some details. The eight divisions of the Ayurveda were followed by two books written later by Susruta and Charka, near about 1000 B.C.

According to early records, the primary needs of the sick were met from plants. Since then, the therapeutic value of the plants has become so highly respected that the “holy basil” is still worshipped by Hindus.

Indian traditional medicine and medicinal plants are also considered as vital sources for new drug development. In order to make traditional a mainstream system, several measures have been taken to incorporate traditional medicine in evidence based clinical practice.

In view of above-mentioned, the present chapter aims to offer an introduction to the Indian medicinal and aromatic plant scenery. This is a relatively short insight, that will be farther broadened by the individual chapters that follow.

1.2 Geographic Characteristics of India

India is a country in **South Asia**. With its 3,287,263 km² territory and an estimated population of ca. 1,352,642,280 (2018 census). It is the **seventh-largest country** by land area, **second-most populous** country, and the most populous **democracy** in the world.

India is situated North of the Equator between 66°E to 98°E long. and 8°N to 36°N lat. Border surrounded by Nepal, China and Bhutan in the North; Bangladesh and Myanmar in the east; the Bay of Bengal in the South-East; the Indian Ocean in the South; the Arabian Sea in the West; and Pakistan in the North-West.

India is a quasi “sub-continent”: 2933 kms wide and 3214 kms long. In the North, the ranges of the Himalaya mountains separate the “Indian sub-continent” from the rest of Asia. Southwards, the Indo-Gangetic plains are further crossed over by the Vindhya mountains. The next-lying Deccan Peninsula is bounded by the Arabian sea to the South-West and the Bay of Bengal to the South-East. The southern-most tip of the country projects into the Indian Ocean.

Mountains cover an area of around 100 million ha. Arid and semi-arid zones are spread over 30 million hectares and the coastline is about 8000 km long (MoEF 2009). The three great rivers of Northern India – the Indus, the Ganges and the Brahmaputra, have their sources in the Himalaya.

1.3 Biogeographic Diversity in India

1.3.1 *Biographic Regions of the Indian Subcontinent*

The bio-geographic position of India is so unique that all known types of ecosystems can be found in its territory: these can range from coldest places, like the Nubra Valley, dry cold deserts of Ladakh, temperate and Alpine and subtropical regions of the North-West and trans-Himalayas, rain forests with the world’s highest rainfall in Cherrapunji in Meghalaya, wet evergreen humid tropics of Western Ghats, arid and semi-arid conditions of Peninsular India, dry desert conditions of Rajasthan and Gujarat to the tidal mangroves of the Sunderban which harbors about 47,000 plant species of which 17,000 are angiosperms (S. Sharma and Thokchom 2014) (Fig. 1.1). India is also rich in medicinal plant diversity with all the three levels of biodiversity - such as species-, genetic- and habitat diversity represented (Mukherjee and Wahile 2006).

According to the Indian Medicinal Plants Factsheet (NMPB 2020), out of the ca. 17,000 flowering plant species in India, more than 7000 plants species are known to have been used traditionally, as medicinal plants. Ayurveda, more than 3000 years old system of medicine has widespread acceptance. More than 90% formulations used in the Ayurveda, Siddha and Unani systems of medicine are plant based and about 22% of the MAP-production is sourced through cultivation.

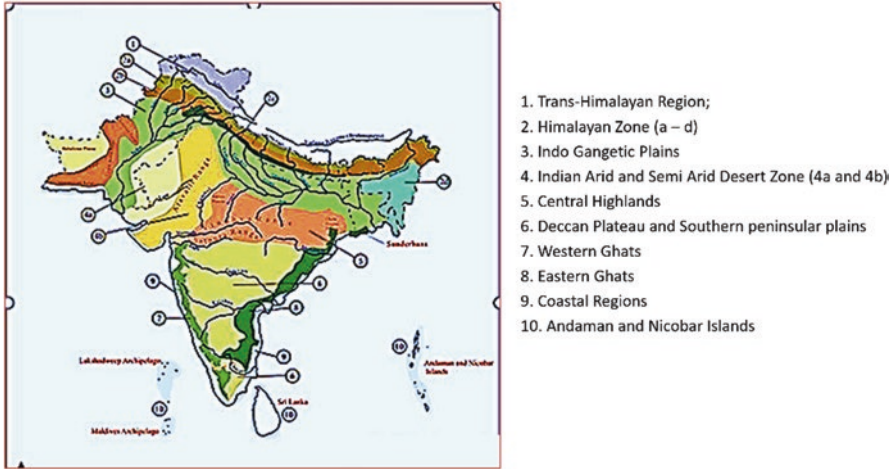


Fig. 1.1 Biogeographic regions of India (After: Manakadan and Khan (2019) Birds of the Indian Subcontinent—In a Nutshell ([researchgate.net](https://www.researchgate.net)))

1.3.2 Biogeographic Diversity of Medicinal Plants, in India

For a better understanding of her vegetation diversity, based on their distinct and unique vegetation, India has been divided into 10 biogeographical regions Singh (2020). The biogeographical regions and their relevant characteristic medicinal plant species are summarized in Table 1.1.

The percentual distribution of bio-geographic zones of India are summarized in Fig. 1.2.

1.4 Biodiversity Hotspots in India

The notion of global biodiversity hotspots can be traced back to the year 2000, when Myers et al. (Myers et al. 2000) identified 25 global biodiversity hotspots in the world, for the first time. Biodiversity hotspots are characterized by exceptional concentrations of **endemic species** that are undergoing exceptional loss of habitat. To qualify as a biodiversity hotspot, a region must have **at least 1500 vascular plants as endemics** and **30% or less of its original natural vegetation**. (In other words, it must be threatened.) In 2009, another 9 hotspots were added to the list of global hotspots).

India accommodates parts of four global biodiversity hotspots, i.e.: the **Himalaya**, the **Western Ghats**, **Indo-Burma** and **Sundaland** (Table 1.2).

- **The Himalaya**, which runs across India's northern tier, is the boundary between two of the Earth's great **biogeographic realms** — the Palearctic, which covers

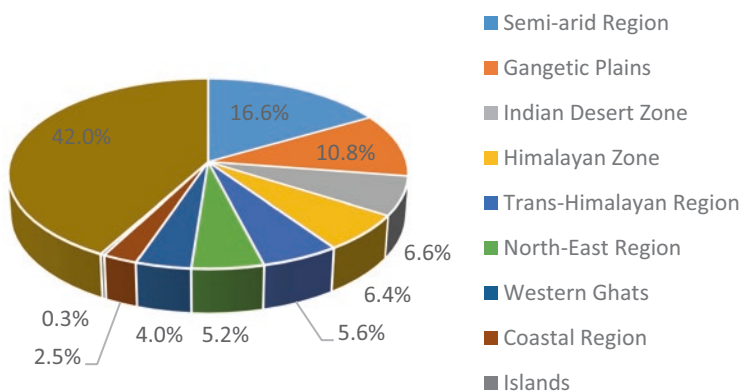
Table 1.1 Biogeographic zones of India with characteristic medicinal genera/species (Singh 2020)

	Biogeographic zone	Characteristic Medicinal Plants
1.	Trans- Himalayan- Ladakh, Lahaulspiti	<i>Ephedra gerardiana</i> , <i>E. intermedia</i> , <i>Fritillaria roylei</i> , <i>Geranium sibiricum</i> , <i>Meconopsis aculeata</i> , <i>Hippophae rhamnoides</i> , <i>Jurinea dolomiaea</i> , <i>Hyoscyamus niger</i> , <i>Juniperus communis</i> , <i>Podophyllum hexandrum</i> , <i>Saussurea gossypiphora</i> , <i>S. lappa</i> , <i>S. obvallata</i> , <i>Rheum webbianum</i> , <i>Bergenia stracheyi</i> , <i>Bunium persicum</i> , <i>Colchicum luteum</i>
2.	Himalayans- Jammu and Kashmir, Ladakh, Himachal Pradesh, Uttarakhand, Sikkim, Arunachal Pradesh, Nagaland, Manipur, Mizoram, Tripura, Meghalaya, Assam and West Bengal.	<i>Rheum</i> , <i>Saussurea</i> , <i>Gentiana</i> , <i>Maconopsis</i> , <i>Cotoneaster</i> , <i>Polygonum</i> , <i>Anemone</i> , <i>Primula</i> , <i>Saxifraga</i> , <i>Allium</i> , <i>Cremanthodium</i> , <i>Corydalis</i> , <i>Juniperus</i> , <i>Ephedra</i> , <i>Hippophae</i> , <i>Aconitium</i> , <i>Podophyllum</i> , <i>Skimmia</i> , <i>Rhodiola</i> , <i>Ainsliaea</i> , <i>Dolomiaea</i> , <i>Dactylorhiza</i>
3.	North-east India– Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim	<i>Oroxylum indicum</i> , <i>Smilax glabra</i> , <i>Paris polyphylla</i> , <i>Berginia cilate</i> , <i>Podophyllum hexandrum</i> , <i>Illicium griffithii</i> , <i>Coptis teeta</i> , <i>Swertia chiryata</i> , <i>Aconitium ferox</i> , <i>Aconitum heterophyllum</i> , <i>Vanda coerulea</i> , <i>Renanthera imschootiana</i> , <i>Rauwolfia serpentine</i> , <i>Aquilaria mallaccensis</i> , <i>Hibiscus Manihot</i> , <i>Abies spectabilis</i> , <i>Acorus calmus</i> , <i>Abutilon indicum</i> , <i>Abroma augusta</i>
4.	Indian Desert – Thar and Kutch.	<i>Glycyrrhiza glabra</i> , <i>Tinospora cordifolia</i> , <i>Plantago ovata</i> , <i>Asparagus racemosus</i> , <i>Cassia angustifolia</i> , <i>Withania somnifera</i> , <i>Achyranthes aspera</i> , <i>Barleria prionitis</i> , <i>Boerhaavia diffusa</i> , <i>Eclipta alba</i> , <i>Euphorbia caducifolia</i> , <i>Evolvulus alsinoides</i> , <i>Pergularia daemia</i> , <i>Sida cordifolia</i> , <i>Solanum surattense</i>
5.	Western Ghats- Kerala, Tamil Nadu, Karnataka, Goa, Maharashtra and Gujarat	<i>Anona squamosa</i> , <i>Buchanania lanzan</i> , <i>Aphanamixis polystachya</i> , <i>Rauwolfia serpentine</i> , <i>Gymnema sylvestre</i> , <i>Gloriosa superba</i> , <i>Phyllanthus neruri</i> , <i>Tridax procumbens</i> , <i>Leucas aspera</i> , <i>Dioscorea bulbifera</i> , <i>Rhicanthus nasuta</i> , <i>Momordica dioica</i> , <i>Mimosa pudica</i> , <i>Hibiscus angulosus</i> , <i>Calotropis gigantea</i> , <i>Parthenium hysterophorus</i> , <i>Saraca asoca</i> , <i>Gloriosa superba</i> , <i>Strycnos nux-vomica</i> , <i>Phyllanthus neruri</i> , <i>Semecarpus anacardium</i>
6.	Semi-Arid regions- Punjab plains, Rajasthan, Haryana, Gujarat, Maharashtra	<i>Bacopa monnieri</i> , <i>Calotropis gigantea</i> , <i>Cannabis sativa</i> , <i>Centella asiatica</i> , <i>Acorus calamus</i> , <i>Cassia fistula</i> , <i>Cardiospermum helicacabum</i> , <i>Bauhinia vahlii</i> , <i>Asparagus racemosus</i> , <i>Asparagus adscendens</i> , <i>Cyperus rotundus</i> , <i>Datura metel</i> , <i>Justicia adhatoda</i> , <i>Eclipta alba</i> , <i>Ricinus communis</i> , <i>Piper betle</i> , <i>Phyllanthus fraternus</i> , <i>Rauwolfia serpentina</i>
7.	Deccan Peninsular- Telangana, Maharashtra, Andhra Pradesh, Karnataka, Kerala and Tamil Nadu	<i>Azadirachta indica</i> , <i>Centella asiatica</i> , <i>Celastrus paniculatus</i> , <i>Chlorophytum tuberosum</i> , <i>Chlorophytum arundinaceum</i> , <i>Curcuma pseudomontana</i> , <i>Cycas circinalis</i> , <i>Drosera burmanii</i> , <i>Gloriosa superba</i> , <i>Oroxylum indicum</i> , <i>Santalum album</i> , <i>Terminalia arjuna</i> , <i>T. chebula</i> , <i>Tinospora cordifolia</i> , <i>Vitex negundo</i> , <i>Withania somnifera</i> , <i>Bixa orellana</i> , <i>Entada pursaetha</i> , <i>Zanthoxylum alatum</i>

(continued)

Table 1.1 (continued)

	Biogeographic zone	Characteristic Medicinal Plants
8.	The Coastal zone- Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Gujarat, Goa, Maharashtra, Odisha, West Bengal, Lakshadweep	<i>Xylocarpus mekongensis</i> , <i>Xylocarpus granatum</i> , <i>Heritiera fomes</i> , <i>Excoecaria agallocha</i> , <i>Avicennia marina</i> , <i>Avicennia alba</i> , <i>Avicennia officinalis</i> , <i>Acanthus bolubilis</i> , <i>Acanthus ilicifolius</i> , <i>Sonneratia caseolaris</i> , <i>Clerodendron inerme</i> , <i>Rhizophora mucronata</i> , <i>Rhizophora apiculata</i> , <i>Salicornia brachiata</i> , <i>Ipomoea pescaprae</i> , <i>Bruguiera gymnorrhiza</i>
9.	Indo- Gangetic planes- Gujarat, Sindh, Punjab, Bihar, Bengal and Assam	<i>Acacia arabica</i> , <i>Holoptela integrifolia</i> , <i>Madhuca indica</i> , <i>Launaea procumbens</i> , <i>Phyllanthus amara</i> , <i>Rosa centifolia</i> , <i>Solanum surattense</i> , <i>Tephrosia purpurea</i> , <i>Tinospora crispa</i> , <i>Tribulus terrestris</i> , <i>Terminalia bellerica</i> , <i>T. chebula</i> , <i>Cassia angustifolia</i> , <i>Cassia occidentalis</i> , <i>Centella asiatica</i> , <i>Crotolaria burhai</i> , <i>Eclipta alba</i>
10.	The Indian Islands- Andaman and Nicobar islands	<i>Semecarpus kurzii</i> , <i>Strobilanthes andamanensis</i> , <i>Uvaria andamanica</i> , <i>Artabotrys nicobarianus</i> , <i>Calamus andamanicus</i> , <i>Aristolachia tagala</i> , <i>Phyllanthus andamanicus</i> , <i>Glochidion calocarpum</i> , <i>Daemonorops manii</i> , <i>Alstonia kurzii</i> , <i>Dichapetalum gelonioides</i> , <i>Dripetes andamanica</i>

**Fig. 1.2** Biogeographic zones of India. (After: Sunit Singh 2020)

most of temperate-to-arctic Eurasia, and Indomalaya. This covers most of the Indian subcontinent and extends into Indochina, Sundaland (Malaysia and western Indonesia) and the Philippines.

The rich plant diversity of the Himalaya numbers over 8000 angiosperms, 44 gymnosperms, 600 pteridophytes, 1737 bryophytes, 1159 lichens etc. (Singh and Hajra 1996) and has been a source of medicinal plant species.

- **Western Ghats** is stretching some 1600 km from the north of Mumbai to the southern tip of India. This biodiversity hotspot contains a large proportion of the country's plant and animal species; many of which are only found here and

Table 1.2 Biodiversity hotspots of India and the number of endemic plant species

Hotspot	Endemic plant species (% of Global total, 300,000)
Tropical Andes*	20,000 (6.7%)
Sundaland*	15,000 (5.0%)
Madagascar*	9704 (3.2%)
Brazil's Atlantic Forest*	8000 (2.7%)
Caribbean*	7000 (2.3%)
Sub-Total (% rounded)	59,704 (19.9%)
Mesoamerica	5000 (1.7%)
Mediterranean Basin	13,000 (4.3%)
Indo-Burma	7000 (2.3%)
Philippines	5832 (1.9%)
Totals	90,536 (30.1%)

* Hotspots with at least 2% of the world's endemic plants and vertebrates? comprising only 0.4% of the Earth's land surface (all nine amount to 1.7% of the Earth's land surface)

† This would total 30.2% but for rounding of numbers in the individual hotspots.

nowhere else in the world. The Western Ghats are one of the world's biodiversity hotspots with over 5000 flowering plants. It is estimated that at least 325 globally threatened species occur here. At 2695 m, Mt. Anamudi in Kerala, India is the highest peak in the Western Ghats. The Western Ghats are being considered as a UNESCO World Heritage Site.

Once covered by dense forests, today, a large part of the Western Ghats-range has been logged or converted to agricultural land area for tea, coffee, rubber and oil palm production, or simply cleared for livestock grazing, reservoirs and roads.

According to Suja (Suja 2005), out of the large variety in the Western Ghats, about 50 species hold a very high value for traditional medicine. The most common species include e.g. *Mimosa pudica*, *Hibiscus angulosus*, *Leucas aspera*, *Phyllanthus neruri*, *Calotropis gigantea*, *Tridax procumbens*, *Parthenium hysterophorus*.

Farther important species are *Anona squamosa*, *Buchanania lanzan*, *Semecarpus anacardium*, *Dioscorea bulbifera* and *Aphanamixis polystachya* (recommended for various forms of tumors), Pepper (fruit) and Cinnamom bark mixed together (recommended for curing Migraine), *Rhincanthus nasuta*, *Momordica dioica*, *Cinnamomum zeylanicum*, *Ophiorhizza mungos* (used to relieve cancer patients).

- **Indo-Burma:** In terms of species diversity and endemism, the Indo-Burma Biodiversity Hotspot is biologically one of the most important regions of the planet. It comprizes all non-marine parts of Cambodia, Lao PDR, Myanmar, Thailand and Vietnam, plus parts of southern China (Tordoff et al. 2012).

There are 309 globally threatened plant species in Indo-Burma (IUCN 2011), comprising two-fifths of the hotspot's globally threatened species. It is estimated that this figure probably represents only a fraction of the plant species of global conservation concern, because of the limited extent of comprehensive global threat

assessments. (Gymnosperms are generally better assessed than angiosperms. Within angiosperms, tree species and particularly commercially valuable timber species are generally better assessed than other groups.)

A total of 40 ethnomedicinal plant species from 35 genera and 25 families have been documented for the first time from Mizoram. *Ardisia polycephala* (VU), *Begonia inflata* (NT), *Blumea lanceolaria* (VU), *Canarium strictum* (NT), *Cautleya gracillis* (EW), *Claoxylon khasianum* (NT), *Curcumorpha longiflora* (VU), *Dalbergia pinnata* (CR/VU), *Dendrobium ariaeflorum* (EN), *Garcinia lancaefolia* (EN), *Gelsemium elegans* (VU), *Helicia excelsa* (NT), *Lepionurus sylvestris*, *Millettia piscidia* (NT), *Musa glauca* (NT), *Pajenela longifolia* (NT), and *Raphidophora hookeri* (NT). *C. gracillis* was extinct in the wild, two were endangered, five were vulnerable, and maximum of nine plant species were of the nearly threatened status (P. K. Rai and Lalramnghinglova 2011).

- **Sundaland:** The Sundaland biodiversity hotspot region covers Indo-Malayan islands (Indonesia and Malaysia). It includes the Nicobar group of Islands–Borneo, Java and Sumatra, Singapore, Philippines.

Sundaland holds about 25,000 species of vascular plants, 15,000 of which are found nowhere else. There are at least 117 endemic plant genera in the hotspot; 59 of these endemic genera are found in Borneo, 17 in Sumatra, and 41 on the Malay Peninsula.

Borneo boasts a spectacular diversity of trees. There are about 3000 species, including more than 265 species of dipterocarps; no less than 155 of these are endemic to the island. The island also has more than 2000 species of orchids.

Notable plants in the hotspot include members of the genus *Rafflesia*, represented by 16 species with very large flowers. One of these, *Rafflesia arnoldii*, has the largest flowers in the world, measuring up to one meter in diameter.

1.5 Flora of India

It is estimated that from the *ca.* 4,65,688 known plant species of the world, 49,441 species, including the bacteria, algae, lichen, virus and fungi, are present in India, with 28% of these species being endemic.

Due to the vast extent of the country and the most varied **topographic/ecological conditions**, as well as the resulting wide range of **habitats**, the flora is extremely diverse. Both these factors and the fact that India – as a country, in the political sense - cannot be defined by only one geographic unit (see: **World Geographical Scheme for Recording Plant Distributions** “Flora of India - Wikipedia” 2021) seem to have made it necessary for botanists to apply a special approach in floristic exploration, i.e.: the Flora of India has been divided into a number of regions.

When surveying the history of Botany of India, it is hard to create a precise picture of the floristic work that had been done in the course of centuries. This dilemma was expressed by Santapau (Santapau 1956), who - whilst holding the post of Chief

Botanist of the Botanical Survey of India - tried to find out the extent of botanical exploration in India. He stated: "It is clear that very few places have been explored methodically in the past; by this I mean that very few areas have been so explored that we may say that we know the complete flora of the area..."

It seems that most of the floristic exploratory work can be traced back to the establishment of Botanical Survey of India (BSI), the apex taxonomic research organization of the country, established in 1890. The organization's mandate was to explore, collect, identify and document the rich plant resources of the erstwhile British India.

In a historical perspective, however, it should be mentioned that the fundamental work "The Flora of British India", by Sir J.D. Hooker and his co-workers included is still instrumental when dealing with plant species of the Republic of India. The following data are meant to illustrate the extent of the exploratory work achieved by Hooker and co-workers: 171 families, 2325 genera and 14,312 species of flowering plants described in the Flora of British India (7 volumes, 1872–1890) covering the areas of present day India, Pakistan, Afghanistan, Nepal, Tibet, Bangladesh, Burma (Myanmar), Ceylon (Sri Lanka) and Malayan Peninsula (Hooker 1875).

1.5.1 Botanical Survey of India (BSI)

The Botanical Survey of India (BSI) was established in 1890 with the main objectives to explore the plant resources of the country and to identify plant species with economic virtues ("Botanical Survey of India" 1891). Following a reorganization by the Government, in 1954, the main objectives of BSI were determined to include: (a) undertaking intensive floristic surveys and collecting accurate and detailed information on the occurrence, distribution, ecology and economic utility of plants in the country, (b) collecting, identifying and distributing materials that may be of use to educational and research institutions and c) acting as the custodian of authentic collections in well planned herbaria and documenting plant resources in the form of local, district, state and national flora.

In 1978, Indian botanists started the publication series, the new National Flora of India. It was published in the form of Fascicles dealing with families, tribes and large genera of flowering plants, as a Series 1 which also includes Flora of India, Series 2 for State Flora, Series 3 for District Flora and Series 4 for Miscellaneous publications on floristic account of special habitat or groups and other aspects dealing with plants ("Flora" n.d.).

To-date, the floristic surveys of many of the Indian states have already been conducted by BSI. Similarly, surveys of Union territories have been completed and the rest are in progress. The floristic survey of 68 protected areas, 26 sacred groves, 01 Ramsar site, 12 fragile ecosystem and 23 Tiger Reserves have also been completed.

About four million plant specimens of different groups are lodged in different herbaria of the BSI. Since the inception of Botanical Survey of India, scientists of BSI have discovered one new family, 43 new genera and more than 1666 new

species and infraspecific taxa including many botanically interested taxa. Population study of about 900 RET taxa of the family Orchidaceae and Sapotaceae have been completed in Eastern Himalayas (BSI n.d.) ([Botanical Survey of India \(bsi.gov.in\)](#)).

Keeping pace with the modern times, requirements and technologies, the present activities of BSI include the development of a digital platform ‘Indian Plant Diversity Information System (IPDIS)’. It has also initiated the web launching of all BSI publications (such as books, records, periodicals, newsletters, reports, archival correspondences, rare books (even not available in any of the Biodiversity library portal) and herbarium specimens.

Farther cutting-edge initiatives are: the development of e-Flora of India and Plant Checklist database, digitization of all BSI publications. The launching of NELUMBO (<http://nelumbo.biocloud.net/>), the online portal of BSI’s official journal, has already been completed.

Till date, BSI has published 10 volumes of Flora of India, 29 volumes of Fascicles, 29 volumes of State Flora for 9 states, 34 volumes of District Flora for 26 District and 140 numbers of Miscellaneous publications.

1.5.1.1 Medicinal Plants in the Botanical Survey of India

Regarding Medicinal and Aromatic Plants, the Environment Information System (ENVIS) hosted by BSI is of great importance. The mandate of ENVIS covers the systematic collection and compilation of data (mainly secondary data) on “Floral Diversity” including the medicinal and aromatic plants. ENVIS collects, collates and disseminates the data through its website (<http://envis.nic.in/>).

1.6 Conservation of MAPs vs. Loss of Natural Diversity

Until relatively recently, medicinal plants were generally lumped into the broad category of Minor Forest Produce (MFP). This terminology was used even by the progressive 1988 Forest Policy Resolution. Remarkably, it was adopted in the very same year, as the Chiang Mai Declaration that internationally recognized the need and called for the sustainable use of natural resources.

1.6.1 Impact of Chiang Mai Declaration

The **Chiang Mai Declaration (1988)** expressed alarm over the consequences in the loss of plant diversity and highlighted “the urgent need for international cooperation and coordination to establish programs for the conservation of medicinal plants to ensure that adequate quantities are available for future generations”. It also called

for a need to coordinate conservation actions based on both *in situ* and *ex situ* strategies (Máthé 2015).

The decades to follow were marked by several declarations and sets of recommendations calling for the Conservation and Sustainable use of biodiversity. In India, also a similar trend could be observed.

In 1999, a Task Force was established to provide policy directives, measures for sustaining the resource base, achieving an equitable marketing system and thriving pharmaceutical industry (Indian System of Medicine and Homeopathy - ISM&H), regulation of domestic and international trade, besides facilitating protection of patent rights and IPR of medicinal plants (Singh 2006). The Task Force emphasized that medicinal plants represent not only a valuable part of India's biodiversity but also a source of great traditional knowledge. Medicinal plants can be viewed as a possible bridge between sustainable economic development, affordable health care and conservation of vital biodiversity. For ensuring sustainable and equitable development of medicinal plants sector, the report recommended the establishment of 200 **Medicinal Plants Conservation Areas** (MPCA), 200 'Vanaspati Vans', in degraded forest areas. The Task Force also recommended the establishment of the **Medicinal Plants Board** for the integrated development of this sector.

Since the publishing of the Chiang Mai Declaration, in 1988, the conservation of medicinal plants has been receiving much more attention in India. This is illustrated by the upsurge in the number of publications detected in the SCOPUS database, under the search term "Conservation + medicinal + plants + India (Fig. 1.3).

Despite of all measures, the majority of supplies continue to be met from wild sources. In an effort to meet the increasing demand for medicinal plants, therefore one of the main aims of NMBP is to focus on both *in-situ* and *ex-situ* conservation of genetic resources (Sharma and Thockhom 2014).

In the course of initiatives, the list of threatened taxa of the Indian flora has been established. It is maintained by the Botanical Survey of India: <https://bsi.gov.in/uploads/documents/research-program/Threatened-plants-of%20India.pdf>. In this database, each plant entry contains information about the purported medicinal values of the species. As an example and just in order to rightly assess the magnitude of the danger plants (including medicinal plants) are facing, it should be mentioned that a total of 560 plant species of India have already been included in the International Union for Conservation of Nature and Natural Resources (IUCN) Red

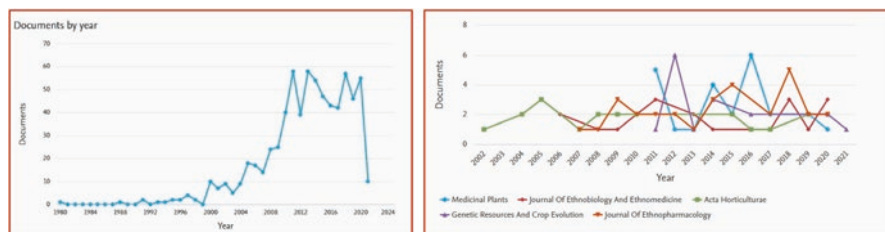


Fig. 1.3 Distribution of records detected in SCOPUS database, under the search term "Conservation of medicinal plants in India"

List of Threatened species: out of these, 247 species have been ranked into the threatened category (Venkatasubramanian et al. 2018).

Farther information on the endangered species can be obtained from the MAPs the database maintained on-line by the ENVIS Centre on Medicinal Plants (<http://envis.frlht.org/implad>). This innovative, search based database stores 7637 botanical names out of which 6198 are medicinal plants.

1.6.2 Germplasm Conservation of MAPs in India

Based on the recognition that traditional medicines are still means of health care for about 65% of the population and there is a need to revitalize local health conditions, the UNDP (United Nations Development Program) and in India the GOI (Government of India) have launched an initiative on ‘Conservation of Medicinal Plants for Health and Livelihood Security’. The project started in 2005 is being implemented in the following eight states: Karnataka, Tamil Nadu, Andhra Pradesh, Kerala, Maharashtra, West Bengal, Rajasthan and Orissa. In Bangalore, the Foundation for Revitalization of Local Health Traditions (FRLHT), is the local partner that has undertaken several efforts through this initiative to strengthen India’s traditional medicine system.

Among the numerous attempts aimed at the conservation of the rich MAPs, the project “Mainstreaming Conservation and Sustainable Use of Medicinal Plant Diversity in Three Indian States” is worth highlighting. This project is jointly funded by the Government of India, National Medicinal Plant Board (NMPB) and the State Governments of Arunachal Pradesh, Chhattisgarh and Uttarakhand. The results that have been reached so far include the Demonstration of replicable models of *in-situ* and *ex-situ* conservation of medicinal plants, the establishment of twenty Medicinal Plant Conservation Development Areas (covering 24,047 hectares, in three project states). The three project states have undertaken plantation of various medicinal plants on 13,130 hectares (United Nations Development Program 2021).

The biogeographic position of India is very unique that has resulted in a wide range of ecosystems and habitat such as forests, grasslands, wetlands, deserts, coastal and marine ecosystems. India is rich in all the three levels of biodiversity viz. species diversity, genetic diversity, and habitat diversity (Mukherjee and Wahile 2006).

India has 426 biomes representing different habitat diversities that gave rise to one of the richest centers for plant genetic resources in the world (Pushpangadan and Narayanan Nair 2001).

In India, similarly to other MAP producing countries, medicinal plant resources are getting depleted at an alarming rate. Today, 90% of the medicinal plants consumed domestically and for export are collected from the wild, and only 70 out of the *ca.* 700 traded species are obtained purely from cultivated sources (Soumya 2012). The continuously increasing demand on herbal products has put the valuable MAP resources under great stress and brought many medicinal plants to the verge

of extinction. MAP natural resources are facing also other threats, like climate change, deforestation, destructive/inexpert harvesting, extensive industrialization, forest fire, etc. It is estimated that in India about 246 plants species are threatened: most of them possess medicinal values (IUCN 2011Tab). These figures have been constantly increasing, so that there is an urgent need to conserve the wild populations of medicinal plant diversity (Ved et al. 2001; Dhar et al. 2000).

1.6.3 The role of Medicinal Plant Conservation Areas (MPCA)

As part of its efforts for biodiversity conservation, the Indian Ministry of Environment and Forestry (MoEF) has set up eight biosphere reserves, 87 national parks and 448 sanctuaries, under Wildlife (Protection) Act, 1972. These cover more than four and half percent geographical area of the country (Singh 2006).

In 2003, Katwal et al. (2003) reported that State Forest Departments (SFDs) of Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Maharashtra, in consultation with the Foundation for Revitalisation of Local Health Traditions (FRLHT) and with the support of DANIDA and UNDP have established 54 forest genebank sites called Medicinal Plant Conservation Areas (MPCA). The network of 54 MPCAs, measuring 200 ha to 500 ha each, has been established gradually since 1993 and represents all forest types with large bio-climatic and soil regime variation. These “gene banks” harbor 45% of recorded populations of flowering and medicinal plants of Peninsular India, including 70% of the red-listed species. The intra-specific diversity of MAP-germplasm conserved in the MPCA network can be used to provide authenticated quality planting material for commercial cultivation to meet rising demands of the herbal industry.

1.6.4 National Medicinal Plant Board Consortia in India

NMPB (NMPB 2021d) has recognized the necessity to maintain connectivity between stakeholders in the both supply and value chain of medicinal plants, while supporting end-to-end conservation and cultivation activities including good post harvesting. In order to establish the desired linkage between the farmers and manufacturers, a ‘Seed to Shelf’ approach is being introduced. In this activity, aspects related to Quality Planting Materials (QPM), Good Agriculture Practices (GAP’s), Good Post Harvest Practices (GPHP’s) are addressed.

In order to meet the ever-increasing demand for medicinal plants, there is a need to focus on both *ex-situ* cultivation and *in-situ* conservation.

In pursuit of above aims, NMPB encourages the foundation of consortia that will engage in the following activities: Production of Quality planting material, Research & Development, Cultivation and Trade of medicinal plants/market linkage.

Eligible partners of consortia are Farmers/ FPOs/ FPCs/QPM centres/ Seed banks/ Nurseries/ SHGs/ NGOs/ Traders/manufactures/Exporters/Pharma/Research institutes / Agriculture Universities.

In the first phase the following consortia have been proposed: Ashwagandha (*Withania somnifera*), Pippali (*Piper longum*), Amla (*Phyllanthus embelica*) Guggulu (*Commiphora wightii*), Satavari (*Asparagus racemosus*).

1.6.5 Conservation Schemes

Conservation, i.e. the study of natural resources and their management for sustainable use and their perpetual existence in their natural habitat is an important component of the schemes represented by NMPB (NMPB 2021a).

Conservation, Development and Sustainable Management of Medicinal Plants forms an important Central Sector Scheme in the activities of the NMPB.

As stated in its Operational Guidelines (NMPB 2015), “natural resources should be protected in such a way that species should be protected from endangerment in their natural habitat. It leads to the understanding and documentation of plants diversity, conserving plant diversity and their sustainable use. Conservation of medicinal plants is an act of careful preservation and protection of natural resources or habitats of medicinal plants especially through planned management”.

As a proposed implementation form, a strategy has been outlined for the XII. Plan period (from 2014 to 2015 onwards) to facilitate conservation and maintenance of wild populations of Medicinal Plants for long term sustainability (NMPB 2015). Important, main elements of the strategy are the following:

- (a) Strengthen the Medicinal Plant Conservation Areas (MPCAs) by systematic survey, geo referencing of existing natural population of medicinal and native aromatic species having medicinal use.
- (b) Enhance conservation through in-situ and ex-situ resource augmentation and artificial re-generation of local populations of medicinal and aromatic plant species.
- (c) Expand area under medicinal and aromatic plants species of medicinal values linked with creation of nurseries to maintain good quality propagation material.
- (d) Promote R & D to address the technology gaps particularly with respect to quality, documentation, identification of substitutes for important medicinal plants including RET (Rare, Endangered Threatened) listed plants and species with high demand in trade and bio-activity guided phyto-chemical studies, etc.
- (e) Improve production, post-harvest technologies, and certification mechanisms for quality standards, Good Agricultural Practices (GAP), Good Field Collection Practices (GFCP) and Good Storage Practices (GSP) value addition and marketing infrastructure.
- (f) Stay abreast of International Developments impacting conservation, availability, trade, quality assurance of medicinal plants.

- (g) Provide livelihoods and economic benefit to forest dwellers, cultivators, local healers and other stakeholders.

1.7 Cultivation of Medicinal and Aromatic Plants in India

The Indian Government has recognized the importance to create alternative means to generate more raw materials by the cultivation of medicinal plants in agriculture fields. The importance of these measures can be underlined by the simple fact that around 500 medicinal plant species are used by the contemporary Ayurvedic industry. Around 80 per cent of these are sourced from wild areas, mostly notified as forest land. Medicinal plants procured from cultivated private fields account for 10% of the total medicinal plants.

In this sense, one of the main goals of NMPB is to encourage both cultivation and sustainable management of medicinal plants across the country and by doing so, to reduce pressure on the collection from wild habitats, mainly in forests. Since 2008, support has been granted in a so called “Mission mode” under Centrally Sponsored Scheme of “National Mission on Medicinal Plants (NMMP)”. Presently, it is continuing under National AYUSH Mission (NAM). The latter is a flagship program launched by the Ministry of AYUSH, Government of India during XII Plan period. The program is being implemented through State Government designated agencies.

Within the framework of the so-called Medicinal Plant Component launched by NBPB, 31 program-implementing agencies spread all over the country offer expert assistance to entrepreneurs wishing to engage in the cultivation of medicinal plants. There is also an extensive list of prioritized plants for cultivation, which distinguishes three levels of subsidies (30%, 50% and 75%) (NMPB 2021c).

1.7.1 *Voluntary Certification Scheme for Medicinal Plant Produce (VCSMPP)*

Reliable quality of the produce is one of the focal points of all medicinal plant related activities in India. To this end, the National AYUSH Mission (NAM) envisions to promote the adoption of Quality standards of AYUSH drugs, thus making available the sustained supply of AYUSH raw-materials. In realizing this vision, one of the major objectives is to support cultivation of medicinal plants by adopting Good Agricultural and Collection Practices (GA(C)P), so as to provide sustained supply of good quality raw materials and support certification mechanism for quality standards, Good Agricultural/Collection/Storage Practices (NAM 2021).

In order to encourage Good Agricultural Practices (GAP) and Good Field Collection Practices (GFCP) in medicinal plants and enhance quality and safety of

these plants, the National Medicinal Plants Board (NMPB), in collaboration with the Quality Council of India (QCI), has launched a **Voluntary Certification Scheme for Medicinal Plant Produce** (VCSMPP) with the following main criteria and services (Birthal et al. 2013):

- Standard For Good Agricultural Practices
- Standard For Good Field Collection Practices
- Certification Process
- Requirements For Certification Bodies
- Approval Procedure For Certification Bodies
- Application Form
- Brochure

The Voluntary certification Scheme is expected to enhance confidence in the quality of India's medicinal plant produce and raw materials. In 2017, the scheme was revised and presently it is in operation. Its main documents are available at: <http://www.nmbp.nic.in>

1.8 Traditional Medicinal Systems, in India

India has one of the richest medicinal plant traditions in the world, where the number of plant-based formulations used in folk medicine by rural communities amounts to some 25,000. The estimated number of practitioners of Indian traditional medicinal systems is around 1.5 million, with more than 500,000 non-allopathic practitioners trained for their respective healthcare systems, in the (>400) medical colleges (Sen et al. 2011). It is also estimated that there are more than 7800 medicinal drug-manufacturing units in India with an annual herb consumption of about 2000 tons (Verma and Singh 2008).

Several **Traditional Healthcare Systems** exist in India. They have developed out of traditional practices, in the course of centuries. To-date **Ayurveda**, **Yoga** and **Naturopathy**, **Unani**, **Siddha** and **Homeopathy** constitute the official traditional systems of medicine. These systems are collectively known as Indian Systems of Medicine (ISM). They are called as AYUSH, the acronym for Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy.

1.8.1 Components of Traditional Medicinal Systems, in India

1.8.1.1 Ayurveda

Ayurveda (the name means the science of life) is one of the ancient health care systems. Its history goes back to 5000 B.C. The Ayurveda was developed through daily life experiences with the mutual relationship between mankind and nature. The

ancient text of Ayurveda reports more than 2000 plant species for their therapeutic potentials.

This system of using natural resources for betterment of health has been developed through day-to-day practice and experiences in the life style of Indian people. It is a holistic system of health care with the concept, that the human body is a matrix composed of **a**) seven basic tissues ('Rasa', 'Rakta', 'Marisa', 'Meda', 'Asthi', 'Majja', 'Shukra'), **b**) the waste products of the body, (e.g. feces, urine and sweat which are derived by the five basic elements ether, air, fire, water and earth), **c**) three basic types of energies or functional principles "vata, pitta and kapha" (Tridosha).

As any imbalances or disturbances in these basic principles of body causes disease, the Ayurvedic disease treatments are employed to regain the balance of basic elements and functional principles of the body. The growth and decay of this body matrix and its constituents revolve around food which gets processed into humors, tissues and wastes. Ingestion, digestion, absorption, assimilation and metabolism of food have interplay in health and disease, which are significantly affected by psychological mechanisms as well as by bio- fire 'agni'. Natural resources which are also believed to be composed of these five elements and three functional principles (Lad 2002) are used for treating the diseases in Ayurveda.

The experiences in the experimentations in day-to-day life are documented in several classical treatises like 'Charak Samihta', 'Sushruta Samhita', etc. Ayurveda is divided into eight major disciplines known as 'Ashtanga Ayurveda', which constitutes eight different disciplines as shown in Table 1.3. In the last 50 years the teaching and training specialties of Ayurveda are more focused towards diagnosis,

Table 1.3 Different streams and specialties in Ayurveda (Mukherjee and Wahile 2006)

Branches in Ayurveda		Specialties for teaching and training of Ayurveda			
Sanskrit	English	Sanskrit	English	Sanskrit	English
Kaya-chikitsa	Internal medicine	Ayurveda-Siddhanta	Fundamental Principals of Ayurveda	Prasuti tantra	Obstetrics and gynecology
Kaumar-bhritya	Pediatrics	Ayurveda-Samhita	Ayurvedic text	Swasth-vritla	Social and preventive medicine
Bhoot-vidya	Psychiatry	Sharira-Rachna	Anatomy	Kayachikitsa	Internal Medicine
Shalakya	Ophthalmology	Sharira-kriya	Physiology	Rog nidan	Pathology
Shalya	Surgery	Dravya guna-vigyan	Materia Medica and Pharmacology	Shalya tantra	Surgery
Agad-tantra	Toxicology	Ras-shastra	Physiochemistry	Shalkya Tantra	Eye & ENT
Rasayana	Geriatrics	Bhaishajya-kalpana	Pharmaceuticals	Mano-roga	Psychiatry
Vajikarana	Eugenics and aphrodisiacs	Kaumar-harita	Pediatrics	Panchkarma	Detoxification of body

treatment and drug development and have developed into sixteen specialties. The treatments are enriched by accepting and adopting the outcomes of experience.

According to Mukherjee (Mukherjee 2001) in an ancient text, entitled “Charak Samhita”, based on their Sanskrit name, 50 groups of plant drugs are distinguished (classified) in Ayurveda (see Table 1.4). In this sense, Ayurveda can be regarded an scientifically organized discipline from the times of its origin. Ayurvedic texts, respected also in neighboring countries, have been translated into Greek (300 B.C.), Tibetan and Chinese (300 A.D.) and several other Asian languages (Mukherjee 2001).

Remarkably, in India, where more than 7500 plant species were used, also other traditional and folklore health care systems have developed besides Ayurveda. The most important of these, like Homeopathy, Siddha and Unani systems will be briefly discussed in the followings.

In India, plants and plant-based formulations are employed for health care and disease treatments with apparent perspectives of safety, efficacy and quality.

1.8.1.2 Homeopathy

Homeopathy, the medical system devised by the German physician Samuel Hahnemann (1755–1843)(Grams 2019), is being practiced since more than 150 years in India. It seems to have blended so well into the roots and traditions of the country that it has been recognized as one of the National Systems of Medicine and plays an important role in providing health care to a large number of people.

1.8.1.3 Siddha

The Siddha system is one of the oldest systems of medicine in India. The term ‘Siddha’ means achievement and the ‘Siddhars’ were saintly figures who achieved results in medicine through the practices. The Siddha Siddha-system of medicine has developed since the ancient human civilization in India. Similarly to Ayurveda,

Table 1.4 Therapeutic classification of plant drugs as per Charak Samhita (Source: (Mukherjee and Wahile 2006)

Sanskrit name	Use
Balya	Promoting strength
Jivaniya	Promoting longevity
Dipaniya	Promoting digestion
Lekhaniya	Promoting anti-obesity Promoting complexion
Verne	Promoting anthelmintic
Krmighna	Galactagogue
Stanyajanana	Emetic
Vamanopaga Kasahara	Antitussive
Svayathihara Javarahara	Anti-inflammatory
Vedanasthapana	Febrifuge, Analgesic, Antiaging
Vayahsthapana	

it is based on the day-to-day experiences of using natural resources for health care (Grams 2019).

The principles and concepts of this system are closely similar to those of Ayurveda, with specialization in iatro-chemistry. This system also considers the human body as a conglomeration of three humors, seven basic tissues and the waste products. The equilibrium of humors is considered as health and its disturbance or imbalance leads to disease or sickness. The system describes 96 principal constituents of a human being which include physical, physio-logical, moral and intellectual components. When there is any change or disturbance in functioning of these principals, body as a system deviates towards the cause of disease (Pillai 1998). The Siddha system is a psycho-somatic system, where attention is given to minerals and metals along with plant constituents (Mukherjee 2001).

Until recent past, the nature was considered as a compendium for templates of new chemical entities (NCEs). The plant species mentioned in the ancient texts of these Ayurveda and other Indian systems of medicines should be explored with the modern scientific approaches for better leads in the health care.

1.8.1.4 Unani

Unani is one of the most well-known traditional medicine systems that draws on the ancient traditional system of medicines of China, Egypt, India, Iraq, Persia and Syria. Once pioneered in Greece, it was developed by Arabs into an elaborate medical science. It is based on the teachings of Buqrat (Hippocrates) and Jalinoos (Galen). Unani Medicine is known as Greco-Arab Medicine. (Ahmad Husain et al. 2010).

More recently, the World Health Organization (WHO) has recognized the Unani System of Medicine (USM) as an alternative system to cater the health care needs of human population (World Health Organization 2010).

WHO in collaboration with the Social-Health Plan of the Lombardy Region (Italy) has launched a series of benchmark documents to ensure that TM/CAM practices meet minimum levels of adequate knowledge, skills and awareness of indications and contraindications (World Health Organization 2010).

Unani medicine encompasses three major forms of practices. **Regimental therapy** (includes: venesection, cupping, the promotion of diaphoresis and diuresis, Turkish baths, massage, cauterization, purging, emesis, exercise and leeching). **Diet therapy** treating ailments with specific diets or by regulating the quantity and quality of food. **Pharmacotherapy** uses naturally occurring medicines, mostly herbal medicines and those of animal and mineral origin. Single medicines or their combination in raw form are preferred over compound formulations.

Unani medicine is popular in South Asian countries and its use is growing in other parts of the world. It has now become part of the mainstream system of medicine in Bangladesh, India, Islamic Republic of Iran, Pakistan, and others.

1.8.1.5 Yoga

Yoga is a discipline that was founded by saints and sages, who presented a rational interpretation of their experiences and formulated a practical and scientifically sound method – not directly related to MAP use - to be available within everyone's reach. With several thousands of years of history, it is regarded as one of the best practices known to calm the inner self. The practice aims to attain self-realization, by improving the inherent power of an individual in a balanced way.

To date, Yoga isn't solely limited to its founders. It has created a worldwide acceptance over the past decades and the techniques and science behind this discipline have been modified to align with the modern lifestyle and sociological needs of individuals. This spiritual discipline is based on a subtle science that aims to bring about harmony between the body and the mind.

1.8.1.6 Sowa Rigpa

Sowa Rigpa (in the Bhoti language = “Knowledge of Healing”) is one of the seven Ayush systems. It is a centuries-old traditional medical system that employs a complex approach to diagnosis, incorporating techniques such as pulse analysis and urinalysis, and utilizes behavior and dietary modification, medicines composed of natural materials (e.g., herbs and minerals) and physical therapies to treat illness.

Four Tantras is the common name for the text of the Secret Tantra Instruction on the Eight Branches, the Immortality Elixir essence. The Four Tantras (Gyushi, rGyu-bzhi) are native Tibetan texts incorporating Indian, Chinese and Greco-Arab medical systems. They are as follows, Root Tantra - Exegetical Tantra - Instructional Tantra - Subsequent Tantra. Although there is clear written instruction in the Four Tantra, the oral transmission of medical knowledge still has remained a strong element in Tibetan Medicine. The Sowa Rigpa System of Medicine is included in the Central Council of Indian Medicine from the year 2012 as per Gazette Notification No. 2345 dated 16.12.2011. (Central Council of Indian Medicine [2011](#)).

1.8.2 *Plants in the Traditional Medicinal Systems*

Indian Materia Medica includes about 2000 drugs of natural origin. Most of these are derived from different traditional systems and folklore practices (Mukherjee and Wahile [2006](#)). Figure 1.4. illustrates the estimated number of plants used by the different medicinal systems in India.

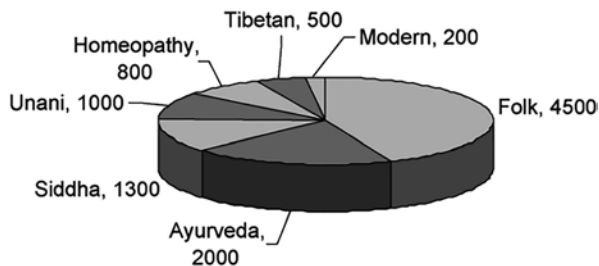


Fig. 1.4 Number of plants used in the Traditional Systems of Medicine, in India (Source: (Mukherjee and Wahile 2006))

1.8.3 *Administrative Bodies for Development of Traditional Medicine*

A good survey of the development of official administrative bodies for the development of traditional medicine was given by Mukherjee et al. (Mukherjee et al. 2012). In this process the following major steps/stages are worth emphasizing:

- Establishment of the Department of Indian Systems of Medicines and Homeopathy (ISM&H), as a separate department in the Ministry of Health and Family Welfare, Govt. of India, in 1995,
- re-naming into Department of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homoeopathy (AYUSH), in 2003,
- registering the Central Council for Research in Ayurveda Science (CCRAS), an autonomous organization, in 1978. CCRAS is working as an AYUSH department and deals with the matters pertaining to quality control and the regulation of Ayurveda, Siddha and Unani drugs.
- AYUSH has been assigned some important Structural components that have the right to govern regulation, development and growth of AYUSH systems in the country and abroad. These components include: a) Five central research councils; b) Two statutory regulatory bodies, namely Central Council of Indian Medicine (CCIM) and Central Council of Homoeopathy (CCH); c) Eleven national institutes for education; d) One drug-manufacturing unit namely as Pharmacopoeial Laboratories, Indian Medicines Pharmaceutical Corporation Limited (IMPCL); e) Eleven national institutes for education.

With the aim of promotion, cultivation and regeneration of medicinal plants and also to develop pharmacopoeial standards, several schemes have been drawn up. As a result, the department of AYUSH is capable of addressing the key issues related to the upgrading of educational standards, the strengthening of existing research institutions and ensuring research programs with a focus on selected diseases (Mukherjee et al. 2012).

At present the Department of AYUSH has become the Ministry of AYUSH. Earlier, the Department of Indian System of Medicine and Homoeopathy (ISM&H) was

responsible for the development of these systems. In 2003, it was renamed as the Department of Ayurveda, Yoga, and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) with focused attention towards education and research in Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homoeopathy.

1.8.4 Traditional Knowledge Digital Library (TKDL)

Traditional Knowledge Digital Library (TKDL), is a pioneering initiative of the Council of Scientific and Industrial Research (CSIR) and the Ministry of AYUSH. It was set up in 2001 with the aim to protect Indian traditional medicinal knowledge and prevent its misappropriation. This digital repository of traditional Indian knowledge contains information about medicinal plants and formulations used in the Indian systems of medicine. It also comprises a Non-patent Database that - by simplifying access to the vast knowledge of remedies or practices - serves the traditional knowledge-based modern research. This large amount of information is structured according to the International Patent Classification (IPC). The TKDL database comprises about 3.6 lakh formulations/ practices that has been transcribed from ISM and Yoga texts. Information from Indian Systems of Medicine (viz., Ayurveda, Unani, Siddha, Sowa Rigpa, as well as Yoga, is available in public domain, whereas traditional knowledge originally available in local languages (e.g.: Sanskrit, Urdu, Arabic, Persian and Tamil) has been converted into digitized format, into five international languages including English, German, Spanish, French and Japanese.

Although, not a component of TKDL, it is worth calling attention to the Catalogue of Indian Synonyms of the Medicinal Plants of India compiled by Moodeen Sheriff (1869), which is the first exhaustive compilation of synonyms of Indian medicinal plants in 12 regional languages, besides Latin and English) (Moodeen Sheriff 2020).

1.8.5 Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT)

The curated database IMPPAT has been constructed via literature mining followed by manual curation of the information gathered from more than 50 specialized books on traditional Indian medicine, more than 7000 abstracts of published research articles and other existing database resources. (<https://cb.imsc.res.in/imp-pat/>) (Sheriff 2018).

To-date, it is considered as the largest database on phytochemicals of Indian medicinal plants. IMPPAT provides an integrated platform to apply cheminformatic approaches to accelerate natural product-based drug discovery. IMPPAT is also expected to enable application of system-level approaches towards future

Fig. 1.5 The logo of e-Charak



elucidation of mechanistic links between phytochemicals of Indian medicinal plants and their therapeutic action.

1.8.6 Government Initiatives in the Development of Traditional Systems of Medicine in India

National Medicinal Plant Board (NMPB) is currently implementing the Central Sector Scheme on „Conservation, Development and Sustainable Management of Medicinal Plants” with a budget of Rs 200.00 crore for the years 2017–2018, 2018–2019 and 2019–2020.

The initiatives of NMPB include „e-charak”: a virtual market-place for MAPs, Collection of mandi price of high Plants and other ICT enabled services (Fig. 1.5).

The online virtual platform www.e-charak.in and a mobile application e-charak were launched to provide an online market portal for the trade of medicinal plants. The on-line portal can be accessed either through internet browsers or the mobile app. In this platform, farmers and collectors could display their produce, so that traders, manufacturers, exporters could look into their requirements. This application and online platform is expected to create vivid transparent, workable trade linkages. Creation of e-charak is a way forward in the Digital India Program.

1.9 Medicinal Plants in the Indian Healthcare System

Plant derived drugs have come into use in the modern medicine through the uses of plant materials in folklore or traditional systems of medicine. They play an important role alongside with modern therapeutics. The list of Indian success stories is rather rich: e.g. serpentine that was isolated from the roots of *Rauwolfia serpentina*, in 1953, marked a revolutionary step in the treatment of hypertension and lowering of blood pressure. Some farther examples, according to Sen et al. (2011) include: reserpine, deserpidine, rescinnamine, vinblastine, vincristine, codeine, morphine, etoposide, guggulsterone, teniposide, nabilone, plaunotol, z-guggulsterone, lectinin, artemisinin and ginkgolides, which have been incorporated into modern medicine.

In 2008, it was estimated that in India, nearly about 70% of modern medicines were derived from natural resources. Furthermore, several other synthetic analogues

were made from prototype compounds isolated from plants (Sharma et al. 2021). Remarkably, about 6% of all described species have been investigated chemically and among them only a small fraction has been investigated pharmacologically (Sen and Chakraborty 2017).

Regarding the vast extent of biodiversity in India, a major task for all the stakeholders - including the policy planners - is the identification and guided development of **new products with large export potential** (as it is frequently the case with medicinal plants).

1.10 Market of Botanicals in India

There are over 3000 medicinal plants in India. This list represents a 1000-odd plants which have been classified as *traded medicinal plants* according to the [ENVIS database](#). Some of the names have been verified against the [Encyclopedia of Life database](#).

In 2017, the Indian Herbal market was Rs 13,470 Crores worth. It is expected to grow with a compound annual growth rate (CAGR) of 19 per cent to Rs 31,660 Crores by the year 2022. This booming sector is an excellent opportunity for the producers of Medicinal and Aromatic plants, since the production and/or collection of MAPs, in India, is characterized, mainly, by the engagement of resource-poor forest dwellers, tribes or small farmers, who cultivate MAPs mostly in wastelands. This practice is characterized also by wide gaps in the adoption of scientific and sustainable practices with no transparency across the supply chain and markets. In general, the trade happens through many layers of middlemen, aggregators and traders, except for a small quantity of MAP produce which is cultivated by the farmers and traded through regulated markets. Government, through its various initiatives, have been trying to collectivize the gatherers, such as formation of Joint Forest Management Committees (JFMCs), primary cooperative societies, state level federations etc., however these initiatives have their own positives and negatives.

India's domestic herbal industry is represented by 8610 licensed herbal units, thousands of cottage level unregulated herbal units and millions of folk healers and household level users of thousands of herbal raw drugs on one hand and a complex trade web on the other that channels the herbal raw drugs from various supply sources to the end users. Thus, to understand the Marketing and trade of the sector, a focus on Demand and Supply of medicinal plants is very important. The canvas portraying demand and supply of medicinal plants in the country is itself very complex.

Currently Marketing of Medicinal Plant produce happens through Mandis and other wholesale markets. There are numerous intermediaries. Trade is rather opaque and information on prices, arrivals and other trends are not easily accessible to farmers/growers. NMPB has been initiating many steps in order to fill this gap.

The commercialization of the production of classical ASU formulations requiring large quantities of wild harvested, cultivated or imported herbal raw drugs has

witnessed the emergence of a thriving raw drug trade. It has become necessary to know the annual consumption levels of the herbal raw drugs and the trends of their use to effectively manage the resource for ensuring sustainable supplies to the herbal industry, folk users and growing global markets. NMPB has been from time-to-time commissioning studies to understand annual trade levels of medicinal plants (NMPB 2021b).

1.11 Actors of Indian Medicinal and Aromatic Plants' Sector

In India, the foundation of the Medicinal Plant Board, in 2000, has marked an important step in the official recognition of MAPs. Previously, MAPs had been ranked among the non-timber forest products (Singh 2006), which together with lack of a central supervising agency for the 'medicinal sector' resulted in an unorganized situation: i.e. the various organizations dealt with different aspects of medicinal plants, without focus and co-ordination, in a *quasi*-disorganized way. Ultimately, this had led to the under-utilization of resources, the overexploitation of wild-harvested species, etc.

This situation was radically changed in 2000, by the foundation of National Medicinal Plant Board.

Prospects and challenges for harnessing the opportunities in medicinal plants sector in India were presented, in an in-depth analysis, by Singh (Singh 2006). This comprehensive study dealt with important aspects like a) the assessment of opportunities and constraints in the medicinal plants sector, particularly under the conditions of changing global scenario, b) issues concerning Intellectual Property Rights (IPRs) protection of medicinal plants and herbal products, c) the policy initiatives at national and international levels d) suggestions for measures and mechanisms to accelerate the development of Medicinal Plants' Sector.

In view of abovementioned, a brief survey of the main actors of Medicinal Plants' Sector is given in the followings:

1.11.1 Ministry of AYUSH

The Ministry of AYUSH was established in 2014, with a vision of reviving the profound knowledge of the Indian ancient systems of medicine and ensuring the optimal development and propagation, with focused attention towards education and research, of the so called AYUSH systems of healthcare. (AYUSH is the acronym of the medical systems that are being practiced in India: Ayurveda, Yoga, and Naturopathy, Unani, Siddha and Homoeopathy). These systems are based on definite medical philosophies and represent a way of healthy living with established concepts on prevention of diseases and promotion of health. The basic approach of all these systems on health, disease and treatment are holistic (https://www.nhp.gov.in/ayush_ms).

1.11.2 Medicinal and Aromatic Plant Association of India (MAPAI)

The Society was founded in 2008 to “promote and cultivate an atmosphere” for the advancement of research on all aspects of Medicinal and Aromatic Plants conservation, production, utilization and trade. MAPAI is meant to promote general interests and interaction among researchers working on Medicinal and Aromatic Plants in different institutions and organizations and to provide a forum for the exchange and dissemination of research information and experiences related to Medicinal and Aromatic Plants. The Open Access Journal of Medicinal and Aromatic Plants (OAJMAP) the only Open Access journal dedicated to the field of medicinal and aromatic plants is accessible at <http://epubs.icar.org.in/ejournal/index.php/JMAP>

1.11.3 National Medicinal Plants Board (NMPB)

In order to promote medicinal plants sector, the Government of India has set up the National Medicinal Plants Board (NMPB), in 2000. Currently the board is located in Ministry of AYUSH, Government of India. The primary mandate of NMPB is to develop an appropriate mechanism for coordination between various ministries/ departments/ organizations in India and implements support policies/programs for overall (conservation, cultivation, trade and export) growth of medicinal plants sector both at the Central /State and International level (Ministry of AYUSH 2015).

1.11.4 Indian Medicinal Plant Database

The medicinal plant species included in the database have been compiled and arranged for electronic search, under the six Indian Systems of Medicine namely: Ayurveda (2559), Siddha (2267), Unani (1049), Homeopathy (460), Sowa-Rigpa (671) and Folk (6403). Within each system of medicine one can browse the data for a specific botanical name or vernacular name. Each of the botanical names has proper author citation and linkage with botanical synonyms where ever applicable (Sheriff 2018).

Validation of specific botanical names has also been incorporated to properly identify the relevant plant entity. Comprehensive lists of botanical names have been arranged alphabetically and appended to the database in respect of each of the six systems of Indian medicine.

1.11.5 Pharmacopoeia Commission for Indian Medicine & Homoeopathy

The Pharmacopoeia Commission for Indian Medicine & Homoeopathy (PCIM&H) is a subordinate office under Ministry of AYUSH, Government of India.

Development of Pharmacopoeias and Formularies as well as acting as Central Drug Testing cum Appellate Laboratory for Indian systems of Medicine & Homoeopathy are the key fields of activity of PCIM&H.

The Commission was originally established as Pharmacopoeia Commission for Indian Medicine (PCIM), in 2010, as an autonomous body under Ministry of AYUSH. Since that time, it has undergone some structural changes. Presently, it is re-registered under the name Pharmacopoeia Commission for Indian Medicine and Homeopathy (PCIM&H 2021).

The main focus of the Pharmacognosy Section is to deal with botanical aspects of drug standardization and development of quality standards for ASU&H single drugs/compound formulations and drug identification protocols, as well as to scientifically validate the quality of single drugs of plant origin and compound formulations.

1.11.6 Ayurvedic Pharmacopoeia of India

The first edition of the Ayurvedic Pharmacopoeia of India was published in 2016 by the Government of India Ministry of AYUSH 2016. It is a book of standards describing the quality of preferred drugs that are manufactured, distributed and sold by licensed drug manufacturers (Fig. 1.6). The Ayurvedic Pharmacopoeia Committee (APC) has already published standards for more than 550 single drugs and 152 classical compound formulations. Vol. IX. contains 45 monographs on the most frequently used plant drugs.

1.11.7 Unani Pharmacopoeia of India

The Unani Traditional Practice is in use in large parts of India. In recent years the demand of “medicine drugs” has been increasing because of people’s faith in the efficacy of the system.

The Ministry of AYISH, with the contribution of Unani Pharmacopoeia Committee, has already published standards for 298 single plant drugs, in six volumes of the Unani Pharmacopoeia of India, Part I. and standards for 100 classical compound formulations, in Part. II. (Formulations) in two volumes (Rai et al. 2020).



Fig. 1.6 Ayurvedic Pharmacopoeia of India

1.11.8 *Homoeopathy Pharmacopoeia of India*

Homoeopathic Pharmacopoeia of India (HPI) is the official book of standards of Homeopathic medicine in terms of Schedule-II of the Drugs and Cosmetics Act, 1940 and Rules, 1945.

The HPI is prepared by the Homeopathic Pharmacopoeia Committee (HPC) constituted by the Government of India.

The Indian manufacturers are legally bound to manufacture Homoeopathic medicines as per standards and methodology given in the Homoeopathic Pharmacopoeia of India. If the standards of some drugs are not included in the Homoeopathic Pharmacopoeia of India, manufacturers are free to undertake manufacturing as per any recognized Pharmacopoeia of the other countries (e.g.: German Homoeopathic Pharmacopoeia (GHP), United States Homoeopathic Pharmacopoeia (HPUS), British Homoeopathic Pharmacopoeia, French Homoeopathic Pharmacopoeia) (Ghosh 2010).

1.11.9 *Siddha Pharmacopoeia of India*

Siddha is one of the oldest medicinal systems originated in Tamil Nadu. The word 'Siddha' is derived from a Tamil word 'Siddhi' ('achievement' or 'perfection'). The Siddha System has established a rich and exceptional catalog of drug knowledge. In this, utmost emphasis is given to the usage of metals and minerals. The Siddha System has published a manual on the treatment for common disorders and illnesses.

Since 2014, the Supreme Court of India and Indian Medical Association have described Siddha medicine as [quackery](#) and there is no governmental recognition of

“siddhars” as legitimate physicians. The Siddha Pharmacopoeia Committee has issued the Siddha Formulary of India, Part I., the first revised edition (Tamil). 150 Siddha classical formulations have been identified for the Siddha Formulary of India, Part III. and translation of Siddha Formulary of India, Part II., in English (CCRS 2018).

1.11.10 Herbal Drug Regulation and Commercialization: An Indian Industry Perspective

According to the World Health Organization of the United Nations (WHO), there are three kinds of herbal medicines: **crude plant materials, processed plant materials and medicinal herbal products**. The earliest recorded evidence of herbal medicine use in India dates back to about 5000 years. The classical Indian texts on herbal medicines include Rigveda, Athurveda, Charak Samhita and Sushruta Samhita (Kumar 2017).

Herbal medicines are used by practitioners of traditional system of medicines due to their well-established and widely acknowledged use. It is the well accepted and accumulated experience of many practitioners and patients over an extended period of time that make herbal medicines popular. Therefore, importantly, the use of herbal medicine is generally and currently regarded as safe (GRAS).

In a recent review by Kumar (2017) herbal medicines are classified into the following categories: a) Indigenous herbal medicines; b) Herbal medicines in systems; c) Modified herbal medicines; d) Imported products with a herbal medicine base.

In India, herbal medicines are regulated under the Drug and Cosmetic Act (D and C) 1940 and Rules 1945, where regulatory provisions for Ayurveda, Unani, Siddha medicine are clearly laid down. The main regulatory authority is the Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH). According to its mandate any manufacture or marketing of herbal drugs have to be done after obtaining manufacturing license, as applicable.

Laws and regulations on herbal medicines are partly the same as those for conventional pharmaceuticals. The D&C Act extends the control over licensing, formulation composition, manufacture, labelling, packing, quality, and export. Schedule “T” of the act lays down the good manufacturing practice (GMP) requirements to be followed for the manufacture of herbal medicines (Kumar 2017). The official pharmacopoeias and formularies are available for the quality standards of the medicines. First schedule of the D & C Act listed authorized texts, which have to be followed for licensing any herbal product under the two categories: Ayurvedic, Siddha or Unani drugs Patent or proprietary medicines (Government of India Ministry of Health And Family Welfare (Department of Health) 2003).

1.11.11 Homeopathic Pharmacopoeia of India (HPI)

Homeopathic Pharmacopoeia of India (HPI) is the official book of standards of Homeopathic medicine. The HPI was published in 1962. A HPI-monograph of each drug contains details on identification, collection, plant part(s) to be used, method of preparation, assessment of purity and limits of impurity. HPI is accessible at [https://www.nhp.gov.in/Homeopathic-Pharmacopoeia-of-India-\(HPI\)_mtl](https://www.nhp.gov.in/Homeopathic-Pharmacopoeia-of-India-(HPI)_mtl)

Indian manufacturers are legally bound to manufacture Homeopathic medicines according to the standards and methodology given in the HPI. Import and export of Homeopathic medicines should also be based on the standards as laid down in the Pharmacopoeia. HPI also helps in the checking/testing of standards of Homeopathic raw-materials and finished products.

1.11.12 National Organic Program (NOP) of India

The National Program for Organic Production (NPOP) provides for Standards for organic production, systems, criteria and procedure for accreditation of Certification Bodies, the National (India Organic) Logo and the regulations governing its use. The standards and procedures have been formulated in harmony with International Standards regulating import and export of organic products.

The National Program for Organic Production is an over-arching architecture and a program of the Government of India which provides an institutional mechanism for implementation of the National Standards for Organic Production (NSOP) and sets out the standards to be followed in the cultivation/ harvest/ production / processing and trading of organic products. Detailed information about the National Standards for Organic Products is available at http://apeda.gov.in/apedawebsite/organic/ORGANIC_CONTENTS/National_Programme_for_Organic_Production.htm%20

1.11.13 Spices Board of India

The Spices Board, under the auspices of the Ministry of Commerce and Industry, Government of India, is the flagship organization for the development and world-wide promotion of Indian spices. The Spices Board was constituted in 1987 under the Spices Board Act 1986. It is an autonomous body responsible for the export promotion of the 52 scheduled spices and development of Cardamom (Small & Large).

The website of Spices Board (<https://www.indianspices.com/about-us/constitution.html>) is a comprehensive storehouse of spice related information, including description, identification, R + D, Quality aspects, marketing *etc.*

1.12 Conclusions

The Indian continent, with its area spanning 9,540,000 sq. miles, makes up about 4.8 percent of the total surface area of the planet. This huge land surface is home to more than 150 identified eco-regions. The bio-geographic position of India is so unique that all known types of ecosystems with their characteristic plant species (including MAPs) can be found in its territory.

India accommodates parts of four global biodiversity hotspots, the Himalaya, Wester Ghats, Indo-Burma and Sundaland. It is estimated that 28% of the *ca.* 4,65,688 known plant species of the world (49,441 species) are endemic.

It seems that utilizing the rich natural resources offered by this diverse and large land surface holds immense opportunities for the stakeholders.

Under the guidance of the Ministry of AYUSH, India is engaged in realizing the vision to revive and farther develop the profound knowledge of the Indian ancient systems of medicine, the so called AYUSH systems of healthcare, with a focus on education and research. In view of the vast (bio)diversity of India, this seems to be a major task for all the stakeholders - including the policy planners. Still, the systematic work of development to utilize – in a sustainable way - the rich medicinal and aromatic plant resources according to the modern age requirements and cutting-edge tools, has already begun. There is good hope that the inherited values of traditional knowledge and resources can be preserved for future generations.

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Chapter 2

History of Research on Medicinal Plants in India



Thadiyan Parambil Ijinu, Varughese George, and Palpu Pushpangadan

Abstract Ancient Indians held a cosmic view of the universe and understood the intrinsic linkage of various elements that constituted the universe. *Homo sapiens* have been utilizing plants for preventive and curative healthcare since time immemorial. They considered biodiversity as an inseparable part of their life and culture. The properties of medicinal plants as described in *Vedic* literatures particularly in *Rig Veda* and *Adharva Veda*, perhaps constitute the first ever written documents available in the history of Indian medicine. Ayurveda, meaning “Science of Life”, is a holistic science of natural healing developed by the ancient *Rishis* of India. Ayurveda accomplishes its goal by treating the disease as well as coordinating the body, mind and soul nexus with the help of a vegetarian diet, herbs, exercise and meditation. The origin of Ayurveda has been placed by Indian scholars to somewhere around 6000 B.C. Through the millennia, Ayurveda developed into a holistic science of healthcare based on sound theoretical, philosophical and practical foundations. Dhanwanthari and Bharadwaj developed the surgical and medical aspects of Ayurveda around ninth century B.C. and their students recorded the details in compendia, which are called *Samhitas*. The *Susrutha Samhita* is the most widely accepted text on surgical aspects of therapy, while the *Charaka Samhita* is the foremost medical text. The steady growth and development of Ayurveda continued till the period of Mughals in India and it suffered further decline during the regime of the Europeans. From the early twentieth century, there has been a steady growth of

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research and development in medicinal plants with the application of modern science and technology. This chapter briefly reviews the historical sequence of events in medicinal plant research in India, from *Vedic* period to the twenty-first century.

Keywords *Vedic* period · *Samhita* period · Ayurveda · Natural product chemistry · Ethnomedicine · Ethnopharmacology

2.1 Introduction

India is rated as one of the 12 mega-diverse countries in the world, with about 7–8% of the total species of plants, animals, fungi and microorganisms. The floristic spectrum of India comprises of approximately 45,500 species, of which over 5285 species are endemic (NBA 2012, 2018). The rich biodiversity of India is matched with an equally rich cultural diversity and a unique wealth of traditional knowledge system, developed, preserved and practiced by millions of ethnic and indigenous people living in the rural and forest areas (Pushpangadan 1998; AICRPE 1998). Local and indigenous people used about 9500 plant species for indigenous medicinal preparations and more than 3900 plant species as food, fiber, fodder, insecticides, pesticides, gums, resins, dyes, perfumes and timber (NBA 2018).

Plants have long been used for medicinal purposes, dating back to the dawn of civilization. The desire to alleviate pain, discomfort and to have better health prompted humans to search for therapeutic or health-promoting agents from nature. Medicinal plants are the primary source of all conventional healthcare systems. Medicinal plants and their preparations are being used as a part of treatment, which eventually evolved into an efficient method of treating ailments and diseases. Plant-based medicines were used as the main therapeutic aid for the whole humankind until eighteenth century. The chemical constituents (metabolites) present in plants are capable of alleviating pain or curing diseases, thus providing better health. Plant based medicines faced a declining trend in the nineteenth century and towards the end of twentieth century, there began a revival of interest in herbal medicine because of the widespread belief that “green medicine” is healthier than the synthetic products (Pushpangadan and Govindarajan 2005).

2.2 Vedic and Samhita Period

The properties of medicinal plants as described in *Vedic* literatures particularly in *Rig Veda* (1500–1000 B.C.) and *Adharva Veda* (1000–900 B.C.) are perhaps the first ever written documents available in the history of medicine. The juice of “Soma” (believed to be a mushroom, *Amanita muscaria*) is mentioned as *Oshadhi*, meaning a heat-producer. While exploring the therapeutic uses of ‘Soma’, the Indo-Aryans

discovered about 100 plants with medicinal properties and the word *Oshadhi* acquired a wider meaning to include all medicines (Jain 1994). In fact, the very conceptualization of Ayurvedic pharmacology begins from the above *Vedic* works. Later, in the *Samhita* period, the great Ayurvedic scholars gave a strong rational basis to the “Science of Life” and postulated various theories such as *Panchabhautik Sidhantha*, *Sapta-Padartha Siddhanta* etc. These theories evolved from the *Sankhya* and *Vaiseshika* School of Thought and were soon accepted as the theoretical foundation of the science of Ayurveda. Description of Ayurvedic pharmacology is available in the ancient Ayurvedic literatures like *Charaka Samhita* (1200–500 B.C.), *Susruta Samhita* (1100–400 B.C.), *Ashtanga Hridaya* (100 A.D.), *Rasa Vaisheshika Sutra* (100 A.D.), *Sarnagadhara Samhita* (1400 A.D.), *Bhavaprakasa Nighantu* (1600 A.D.) etc.

According to Ayurveda, *Vata*, *Pitta* and *Kapha* are the three main physiological regulators of the body and mind, and are termed as *Tridhatu*. *Tridhatu* remains always in a state of equipoise in healthy individuals. In this context, Varier (1987) states that some particular parts of *dhatus* always tend to wax and wane due to difference in factors like food, activities, day and night, age, time and place. Tissues also may wax and wane from diseases affecting particular parts of the body. The parts of the *dhatus* liable to such changes are called *Tridoshas*. It is difficult to comprehend the biological phenomena described in Ayurvedic classics without having a good understanding of the science of material objects and allied aspects enunciated in the ancient philosophical works known as *Shad Darshanas*. It is, therefore, of utmost importance to the students of Ayurveda to acquaint themselves with the fundamental theories of the *Shad Darshanas*. Ayurvedic surgery had unique modalities of treatment for piles, fissure and fistula, which have been followed for centuries. Application of *Kshara Sutra* (medicated thread) has emerged as a preferred alternative in the management of fistula. Some leading hospitals in India have taken up this modality as the only treatment for fistula.

The Siddha system of medicine, with a recorded history from about 2000 B.C., is believed to have originated from *Lord Shiva* and to have been passed on through his wife, *Goddess Parvati* to a number of disciples. According to the scriptures, there were 18 principal Siddhars. Among these, Sage Agasthiya is regarded as the father of Siddha medicine. The use of Siddha medicine is very common in Dravidian culture. The Unani system, which originated in Greece around 400 B.C., came to India through Arab physicians, who accompanied Mughal invaders. At around 1526 A.D., the Vedic and Unani systems interacted and functioned in an integrated manner (Bala 1982, 1985, 1991). The Tibetan healing system, known as Amchi (*Sowa-Rigpa*) was also enriched by Ayurveda, Chinese and Greek medicines. It is practiced widely in Sikkim, Arunachal Pradesh, Darjeeling, Dharamshala, Lahaul and Spiti, and Ladakh regions. The Tibetan Medical and Astrological Institute was established in 1961 by the newly formed Tibetan Government in Exile in Northern India. There are about 2000–2500 plants used for the preparation of medicine by the Indian systems (ISM) including Ayurveda (2000), Siddha (1121), Unani (751), Homeopathy (482) and Amchi (337).

The classical texts of Ayurveda and Siddha provide comprehensive instructions on how to collect and process medicinal plants, including various dos and don'ts. They insisted on collecting certain medicinal plants in certain specific seasons from specific ecosystems and also during certain particular stages of growth and development of the plants. There is increasing evidence that the variety, habitats, and stage of plant development etc. influence the production of secondary metabolites in plants. Almost 35–40% of the medicinal plants found in tropical regions are cross-pollinated and there exists extensive genetic variability, particularly in the secondary metabolites of these species (Pushpangadan 2006). Therefore, in a given population of a medicinal plant species there may be only a few plants which may have the desired therapeutically active constituents. There used to be highly experienced medicinal plant collectors (*bhishagwaras*) in the past who were able to identify the plants with the right therapeutic properties. It is believed that the plant collectors of the *Samhita* period possessed even some kind of intuitive knowledge, so that they were able to pick up the right plants from populations of a species having variations in their therapeutic contents. It is even stated in certain classical texts of Ayurveda that those few plants having the therapeutic property in a large population would speak to those well-experienced medicinal plant collectors with intuitive knowledge that “*I am the one who has the therapeutic ability and therefore collect me*”. We no longer have such intuitive persons who can decipher language of plants. What we have today is the scientific expertise with sophisticated analytical tools. We have to use them appropriately.

During the post-*Vedic* period (after 500 B.C.), medicines had been modified according to rational and scientific principles. Soon afterwards, world trade, particularly with regard to spices, began to intensify, both along sea routes as well as overland routes, resulting in linkages between the Near East, East and Southeast Asia. By the time of the Graeco-Roman era, *Piper nigrum* (Piperaceae; black pepper) and *Zingiber officinale* (Zingiberaceae; ginger) from India, and *Neolitsea cassia* (Lauraceae; cassia), *Cinnamomum verum* (Lauraceae; cinnamon) and *Syzygium aromaticum* (Myrtaceae; clove) from South East Asia, all of which were used for medicinal purposes, were widely known in the Mediterranean. During this post *Vedic* period several important medicinal species were introduced in India including *Cannabis sativa* (Cannabaceae; marijuana, hemp) and *Allium sativum* (Amaryllidaceae; garlic) from Central Asia; *Aloe vera* (Asphodelaceae; aloe), *Cuminum cyminum* (Apiaceae; cumin), *Papaver somniferum* (Papaveraceae; opium poppy) and *Glycyrrhiza glabra* (Fabaceae; liquorice) from the Mediterranean; *Myristica fragrans* (Myristicaceae; nutmeg) from Southeast Asia; *Trigonella foenum-graecum* (Fabaceae; fenugreek), *Crocus sativus* (Iridaceae; saffron), *Carum carvi* (Apiaceae; caraway) and *Medicago sativa* (Fabaceae, alfalfa) from Southwest Asia; *Coriandrum sativum* (Apiaceae; coriander) from the Mediterranean and Southwest Asia; *Ricinus communis* (Euphorbiaceae; castor oil plant), *Solanum nigrum* (Solanaceae; black nightshade) and *Tamarindus indica* (Fabaceae; tamarind) from Africa; *Acorus calamus* (Acoraceae; sweet flag, calamus) from the Eurasian steppes; *Lepidium sativum* (Brassicaceae; cress) from Tibet; and numerous other species (Holley and Cherla 1998).

2.3 Period Up to Nineteenth Century

Until the beginning of the nineteenth century, all medical practices were what we now call “traditional”. The renaissance period in Europe brought great scientific upheavals that began to introduce Cartesian scientific materialism into all human activities, notably into the theory and practice of healthcare. Its approach was to break up complex phenomena into their constituent parts and deal with each one separately. This approach resulted in a search for a single cause for the diseases, and correspondingly, modern pharmacological investigations were aimed at finding a single active principle that could be isolated from the genetic resources, including medicinal plants. The introduction of this kind of abstract medicine in the form of basic chemicals and pharmaceuticals during the eighteenth and nineteenth centuries demonstrated methods for bringing quick relief from suffering and has won instant admiration and popularity. This system known as allopathy or modern medicine, made rapid advances during the nineteenth and twentieth centuries as a result of the advances made in science and technology. New discoveries of sulfa drugs, synthetics, antibiotics, cortisones and other therapeutic agents emerged in quick succession and swept all other systems of medicine off their feet.

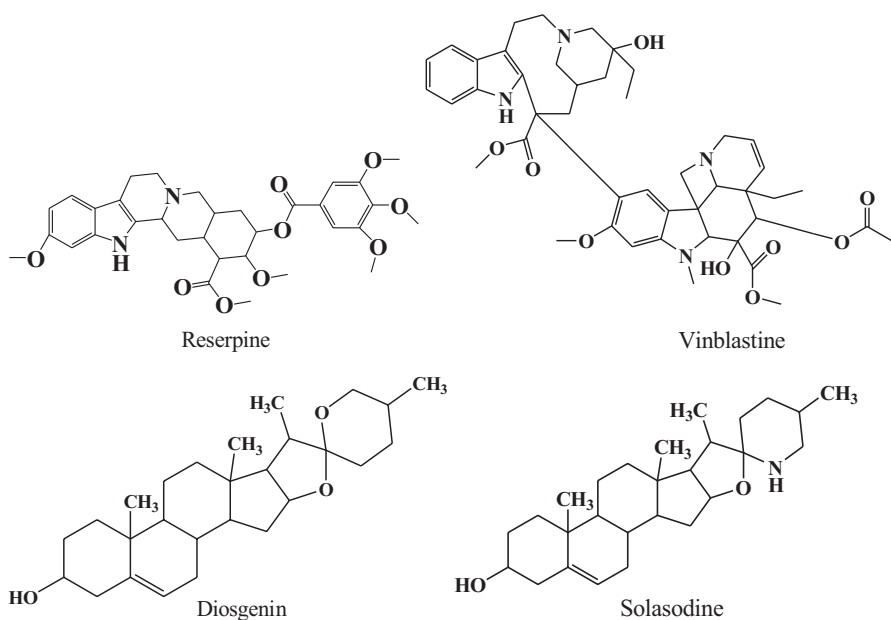
Os coluquios by Garcia da Orta (1560), *Paradisus batavus* by P. Hermann (1698), *Hortus Indicus Malabaricus* by H. van Rheede (1687–1693) and *Thesaurus Zeylanicus* by J. Burmann (1737), *Flora of British India* (1872–1897) by J. D. Hooker are some of pre-independence publications on Indian flora. *Hortus Indicus Malabaricus* is one of the oldest important printed books on Indian medicinal plants (12 volumes, contains descriptions and illustrations of 742 plants belonging to 690 taxa). This botanical treatise on the medicinal properties of flora of Malabar (Kerala) was compiled by Hendrik van Rheede, the Dutch Governor of Malabar, in collaboration with Itty Achudan Vaidyan, Appu Bhat, Vinayaka Pandit and Ranga Bhat. The ethno-medical information presented in *Hortus Indicus Malabaricus* was extracted from palm-leaf manuscripts maintained by Achudan. Later, *Hortus Indicus Malabaricus* has been translated into English and Malayalam languages by Prof. K. S. Manilal (Manilal 2003).

2.4 Nineteenth and Twentieth Century Period

Mitra and Jain (1991) have summarized the English literature produced in the nineteenth century in the form of catalogues, dispensatories, pharmacopoeias and descriptions or illustrations of plants with notes on medicinal uses, which culminated in Sir George Watt’s (1889–1893) *A Dictionary of the Economic Products of India*, six volumes. The timely publication of Hooker’s *Flora of British India* (1872–1897) helped to understand the identity and occurrence of some 15,000 taxa of higher plants in India. The first half of the twentieth century witnessed an increased interest in medicinal plant research. This is illustrated by, for example, the establishment in 1921 of the Calcutta School of Tropical Medicine; the study of

poisonous plants under the auspices of the Indian Council of Agricultural Research (ICAR); and the establishment in 1941 of a Drug Research Laboratory at Jammu. In that year, the ICAR formed a Medicinal Plants Committee to fund agricultural research in State Agriculture and Horticulture Departments and in Directorates of medicinal plants in West Bengal and Tamil Nadu. This led to the All-India Coordinated Research Project on Medicinal and Aromatic Plants (AICRP-MAP 1967), with various centres in different parts of India.

After independence in 1947, interest in herbal medicine was revived and major agencies for medico-botanical research were established or strengthened by the Government of India. To improve understanding of the taxonomy and distribution of the indigenous flora, the Botanical Survey of India (BSI), Calcutta; the Forest Research Institute (FRI), Dehradun; the National Botanic Gardens (NBG), Lucknow and some universities initiated fieldwork in underexplored regions. This resulted in a large number of publications on enumeration of the flora, taxonomic revisions, pharmacognosy, phytochemistry and bioactive molecules of Indian plants. During the turn of the nineteenth century, technological developments in chemistry, particularly in medicinal chemistry, and development of biological and toxicological screening methods led to extraction of active principles and isolation of single biologically active compounds. A report by Dr. Rustom Jal Vakil in *British Heart Journal* on the usefulness of serpina (whole extract of Sarpagandha, *Rauvolfia serpentina*) in the treatment of hypertension aroused the interest of modern medicine in the Ayurvedic Pharmacopoeia (Vakil 1949). The discovery of reserpine from *Rauvolfia* spp., vinblastine from *Catharanthus roseus*, diosgenin from *Dioscorea* spp. and solasodine from *Solanum* spp. further boosted interest in herbal drugs.



Some major publications on medicinal plants were also brought out, viz. *Indian Materia Medica* (Nadkarni 1908), a compilation of drugs used in the indigenous systems of medicine; *Chopra's Indigenous Drugs of India* (Chopra 1933; Chopra et al. 1958); *Indian Medicinal Plants* (Kirtikar and Basu 1935); *Poisonous Plants of India* (Chopra et al. 1949); *Indian Pharmaceutical Codex* (Mukerji 1953); *Glossary of Indian Medicinal Plants* (Chopra et al. 1956, 1969); *Medicinal Plants of India* (Satyavati et al. 1976); *Compendium of Indian Medicinal Plants* Vol. 1 and 2 (Rastogi and Mehrotra 1990, 1991, 1993) and *Indian Herbal Pharmacopoeia* Vol. I and II (Handa 1998, 1999). CSIR published *The Wealth of India - An Encyclopedia of India's Raw Material Resources*, the first volume came out in 1948 (published series of books during 1948–1976). The Indian Council of Medical Research (ICMR) published *Quality Standards of Indian Medicinal Plants* (2003 onwards, 17 volumes) and *Reviews on Indian Medicinal Plants* (2004 onwards, 23 volumes) and *Phytochemical Reference Standards of Selected Indian Medicinal Plants* (2010 onwards, 4 volumes). Khare (2004, 2007) authored books entitled *Encyclopedia of Indian Medicinal Plants* in 2004 and *Indian Medicinal Plants: an Illustrated Dictionary*, 2007.

Several Indian medicinal plants have been investigated in India and abroad and their biological activities were studied. During 1970s, the Central Drug Research Institute (CDRI) started biological activity screening of plants, tested about 3000 species and the results were published in a series of research papers (1968–1990). In 1956, the Indian Pharmacopoeia Commission (IPC) was established under the Ministry of Health and Family Welfare, to set standards for all drugs that are manufactured, sold and consumed in India. The actual process of publishing the first pharmacopoeia started in the year 1944 under the chairmanship of Sir Col. R. N. Chopra. Later, the first edition of Indian pharmacopoeia was published in 1955 under the chairmanship of B. N. Ghosh. The *Ayurvedic Formulary of India* Part I was published in 1978. Later on, the *Ayurvedic Pharmacopoeia of India* was published in 1986 (Part I). The *Siddha Formulary of India* was published in 1984 (Part I, Tamil) and 1992 (Part I, English), and the *Siddha Pharmacopoeia of India* was published in 2008. The *National Formulary of Unani Medicine* was published in 1984 (Part I) and the *Unani Pharmacopoeia of India* was published in 2007 (Part I). The *Homoeopathic Pharmacopoeia of India* was published in 1971 (Part I). The other volumes of each medicinal system were published subsequently in different years.

It was in the beginning of the twentieth century that a few Ayurvedic pharmaceutical companies like Kottakkal Arya Vaidyasala in Kerala for the first time in Ayurvedic medical history began commercial production of Ayurvedic drugs and shelfable products. This transformation from the highly individualized and customized production practice to commercial production of traditional medicine in India confronted the issue of quality and standardization. The traditional experiential wisdom was either eroded or became difficult to operate in such a commercial set up. Therefore, it is necessary to adapt modern science and technology to improve the commercial production of Ayurvedic formulations and also for quality control. This has necessitated extensive and intensive researches in medicinal plant cultivation,

harvesting, post-harvest handling and processing. India has only a minuscule 0.5 percent share in the global herbal medicinal market (AYUSH and value-added products of medicinal plants), worth USD 358.60 million against estimated global trade of USD 70 billion (Economic Times 2016).

2.5 Revival of Traditional Medicine in Twenty-First Century

Ayurveda was one of the most exact sciences developed by ancient Indians. But unfortunately, there came a sudden decline in the magnificent growth of Ayurveda from sixteenth century A.D. onwards. Twentieth century demonstrated the tremendous success of modern medical science, particularly in diagnosis, treatment and surgical measures of a number of diseases that were regarded as incurable in the past. Direct intervention through technological and molecular means has now become possible. Humankind is now harvesting the full benefit of the progress of modern medical science and technology. But we are also equally conscious about the inadequacy of modern medicine in dealing with many metabolic and degenerative disorders and other such ailments associated with old age. Also, modern medicines have very strong side effects, high costs, and are not accessible to majority of human population. The oriental system of traditional medicine has satisfactory management and even cure for many such ailments. Thus, towards the end of twentieth century, there began a revival of interest in traditional medicine. According to a survey conducted by the World Health Organization (WHO), the use of plant remedies is on the increase even in developed countries especially among the younger generation. WHO has estimated that 80% of the world population relies on traditional medicine for primary healthcare.

The technologization and undue objectification of human life and health care systems of the present era have culminated to an extreme nexus between physician and patient by the interpolation of a third entirely mechanical thing, the machine, replacing the creative synthesizing role of the traditional physicians. This has resulted in the dehumanization of the medical system (Pushpangadan 2018). In contrast to this scenario of the modern medicine, the traditional medicine attempts to embody a holistic approach *i.e.*, that of viewing an individual in his totality within society and the ecological environment. It emphasizes the view point that ill health or disease is brought about by an imbalance or disequilibrium of man's physiological, psychological, behavioural, ecological and spiritual environments and not just by an external pathogenic agent, be it a micro-organism or otherwise.

No doubt, the modern medicine has accomplished great strides in developing many new life-saving drugs. The modern health care system has a greater emphasis on the curative and to a lesser degree to the preventive aspects and very little attention paid to health promotion. The promotive and preventive aspects prevalent in oriental medicine, especially in Indian (Ayurveda, Siddha, Unani and Amchi), and Traditional Chinese Medicine (TCM) are finding increasing popularity and acceptance in developed countries. Ayurveda is a treasure house of knowledge for both

preventive and curative healthcare. Longer life expectancy and life-style related problems have brought with them an increased risk of developing chronic or debilitating diseases such as stress, allergies, rheumatic, arthritic and neurological conditions, memory disorders, cancer, diabetes and heart problems. Ayurveda has diversity, flexibility, accessibility and affordability.

The Directorate of Medicinal and Aromatic Plants Research (DMAPR) was established in 1992 by the Government of India for the sustainable production of quality medicinal and aromatic plants through the development of new varieties, good agricultural practices and quality assessment methodologies. The Foundation for Revitalisation of Local Health Traditions (FRLHT) Trust was started in 1993 with an aim to demonstrate contemporary relevance of theory and practice of Indian Systems of Medicine. In the year 2000, the National Medicinal Plant Board (NMPB) was established to coordinate matters concerning medicinal plant programmes, trade, export, conservation and cultivation. The Ministry of AYUSH was formed on November 9, 2014 and under the Ministry they established organizations like Central Council for Research in Ayurvedic Sciences (CCRAS), Central Council for Research in Unani Medicine (CCRUM), Central Council for Research in Siddha (CCRS) and Central Council for Research in Homoeopathy (CCRH). Now-a-days research centres under these organizations are also performing various activities related to medicinal plant research, including quality control and drug development.

2.6 Plant Based Drug Research in India

As per the literature, research in natural product chemistry in India was started by J. L. Simonsen during 1920s. He was a teacher at Presidency College Chennai, during 1910–1920. Then he moved to the Indian Institute of Science, Bengaluru and he left India in 1928. He identified the structure of (+)-3-carene, a major constituent (55–60%) isolated from turpentine oil (*Pinus roxburghii*). His contributions in terpenoids sowed the seeds of natural product research in India. Sir Col. Ram Nath Chopra laid the foundation for research on Indian indigenous drugs and their active constituents. He is the author of *Glossary of Indian Medicinal Plants* brought out by CSIR. He worked in the Calcutta School of Tropical Medicine (1921–1941) and later he moved to the Calcutta Medical College. Siddiqui and Siddiqui (1931) isolated five alkaloids from *Rauvolfia serpentina*, namely ajmaline, ajmalinine, ajmalicine, serpentine and serpentinine. The first systemic pharmacological investigations of *R. serpentina* and its isolated compounds were conducted by Chopra et al. (1933, 1943) and Gupta et al. (1944) along with other collaborators, Sen and Bose (1931). Later, Dr. Rustom Jal Vakil from King Edward Memorial Hospital, Mumbai conducted the clinical trial (1939–1949) of *R. serpentina* in hypertension and published his results in *British Heart Journal* (1949), which aroused the interest of modern medicine in Ayurvedic Pharmacopoeia. But the isolation and detailed pharmacological investigation of reserpine were conducted abroad (Muller et al. 1952; Bein 1953). Reserpine is a unique molecule that blocks both VMAT1 and VMAT2,

thereby exposing biogenic amines to degradation by monoamine oxidase (Bhutani and Gohil 2010; Nagarajan 2014).

Biman Bihari Dey of Presidency College Chennai (1920–1944) isolated thevetin from *Thevetia nerifolia*, heydotine from *Heydotis curicularia* and toddalinine and toddalolactone from *Toddalia aculeata*. Dr. Dey inspired Thiruvengadam Rajendram Seshadri and Krishnaswami Venkataraman to take up natural product research and he sent them to United Kingdom for their doctoral work under Sir Robert Robinson (Nobel Laureate 1947) of the University of Manchester. After completing his doctoral work Dr. Seshadri joined Agricultural College and Research Institute at Coimbatore as a soil analyst (1930). In 1934, he joined Andhra University Waltair and after retirement, he moved to Department of Chemistry, Delhi University (1949). In 1962, the University Grants Commission selected Seshadri's Department as the Centre for Advanced Study in the Chemistry of Natural Products in India and made him its first Director. The Centre has attained great distinction in the chemistry of natural products ever since it came into existence (Nagarajan 2014). His group extensively worked on *Cullen corylifolium* (Fabaceae), *Pongamia pinnata* (Fabaceae), species of *Dalbergia* (Fabaceae), *Pterocarpus* (Fabaceae), *Acacia* (Fabaceae), *Morinda* (Rubiaceae), *Albizia* (Fabaceae), *Hibiscus* (Malvaceae), *Gossypium* (Malvaceae), *Cassia* (Fabaceae), *Citrus* (Rutaceae) and *Pinus* (Pinaceae), and elucidated structure of several flavones, isoflavones and anthocyanins. His work on gossypol, the pigment from cotton seed oil served to clear up structural confusions (Rangaswami 1988). Dr. Venkataraman initially joined at Foreman Christian College, Lahore (1929) and then moved to University Department of Chemical Technology, Bombay (1934–1957). He became the first Indian Director of the National Chemical Laboratory (NCL), Pune in 1957. During his tenure, NCL became a major centre of research on natural products and synthetic organic chemistry. He made significant contributions in isolation and structure elucidation of naturally occurring flavones, isoflavones and naphthoquinones (Nityanand 1995; Nagarajan 2014). They were followed later by Tuticorin Raghavachari Govindachari who got his Ph.D. degree from the Presidency College Chennai under the guidance of Prof. Dey and he joined as a postdoctoral researcher under Prof. Roger Adams at the University of Illinois. Dr. Govindachari returned to India in 1950 and joined the Chemistry Department of Presidency College Chennai as Professor and established School of Natural Products Chemistry. In 1963, he moved to the Ciba Research Centre in Bombay as its head. His group isolated tylophorine, echitamine, tiliacoline, kopsine, ancistrocladine, 9-methoxycamptothecin, shwarone, cedrelone, litso-mentol, wedelolactone etc. Later, he moved to Amruthanjan Research Centre (1977–1986) and Spic Agrochemical Research Centre (1987–2000) devoting much of his attention to the constituents of neem (*Azadirachta indica*) isolating the first ever crystals of azadirachtin A (Govindachari 1984, 2002; Nagarajan 2004, 2014).

At the same time and later, there have been many groups working on medicinal plants including, P. C. Guha (Indian Institute of Science, Bengaluru), P. K. Bose (University of Calcutta and Bose Institute, Calcutta), Asima Chatterjee (University of Calcutta), S. C. Bhattacharyya (Indian Institute of Science; National Chemical Laboratory, Pune; Indian Institute of Technology Bombay; Bose Institute), Sukh

Dev (Indian Institute of Science; National Chemical Laboratory; Indian Institute of Technology Delhi; Delhi University), G. S. R. Subba Rao (Indian Institute of Science), S. Rangaswami (Andhra University and Delhi University), A. C. Jain (Delhi University), G. B. V. Subramanian (Delhi University), S. Neelakantan (Delhi University and Madurai Kamaraj University), R. N. Chakravarti (Bose Institute), C. K. Atal (Indian Institute of Integrative Medicine, Jammu); S. Nityanand (Central Drug Research Institute, Lucknow), K. K. Bhutani (Indian Institute of Integrative Medicine and National Institute of Pharmaceutical Education and Research, Mohali), R. S. Kapil (Indian Institute of Integrative Medicine), D. S. Bhakuni (Central Drug Research Institute), P. Pushpangadan (Indian Institute of Integrative Medicine; Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram and National Botanical Research Institute), V. George (Indian Institute of Integrative Medicine and Jawaharlal Nehru Tropical Botanic Garden and Research Institute), Ram Vishwakarma (Indian Institute of Integrative Medicine), Harkishan Singh (Punjab University), S. Ghosal (Banaras Hindu University), M. M. Dhar (Central Drug Research Institute), R. P. Rastogi (Central Drug Research Institute), B. N. Dhawan (Central Drug Research Institute), S. K. Talapatra (University College of Science, Calcutta), P. Chakraborti (Bose Institute), S. C. Pakrashi (Indian Institute of Chemical Biology, Calcutta), P. Sengupta (Kalyani University), L. Ramachandra Rao (Andhra University), S. Chandrasekhar (Indian Institute of Chemical Technology, Hyderabad), B. R. Pai (Presidency College Chennai), S. N. Chakravarti (Annamalai University), Sankara Subramanian (Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry), R. Uma Shaankar (University of Agricultural Sciences, Bengaluru), C. R. Narayanan (National Chemical Laboratory), N. S. Narasimhan (Savitribai Phule Pune University), B. S. Joshi (Ciba Research Centre), N. Viswanathan (Ciba Research Centre), P. C. Parthasarathy (Ciba Research Centre), M. S. Chadha (Bhabha Atomic Research Centre, Bombay), N. B. Mulchandani (Bhabha Atomic Research Centre), A. Bannerji (Bhabha Atomic Research Centre), V. R. Mamdhapur (Bhabha Atomic Research Centre), S. Chattopadhyay (Bhabha Atomic Research Centre), Pulok K. Mukherjee (Jadavpur University, Kolkata), Mangalam S. Nair (National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram), K. V. Radhakrishnan (National Institute for Interdisciplinary Science and Technology), Ajaikumar B. Kunnumakkara (Indian Institute of Technology Guwahati), Ruby John Anto (Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram), Sharad Srivastava (National Botanical Research Institute), Baby Sabulal (Jawaharlal Nehru Tropical Botanic Garden and Research Institute), K. B. Rameshkumar (Jawaharlal Nehru Tropical Botanic Garden and Research Institute) and many more.

Representative Indian medicinal plants on which significant research leads have been obtained in Indian and foreign laboratories are mentioned in Table 2.1. Among public funded CSIR Institutes, National Chemical Laboratory (NCL, 1950), Pune; Central Drug Research Institute (CDRI, 1951), Lucknow; National Botanical Research Institute (NBRI, 1953), Lucknow; Indian Institute of Chemical Technology (IICT, 1956), Hyderabad; Indian Institute of Integrative Medicine (IIIM, 1957),

Table 2.1 Some important medicinal plants of India investigated in India and abroad

Biological activity	Plant name(s)	Active principle(s)	References
Anti-inflammatory	<i>Withania somnifera</i>	withanolides	Oh and Kwon (2009)
	<i>Boswellia serrata</i>	boswellic acids	Atal et al. (1980, 1981); Takada et al. (2006)
	<i>Berberis aristata</i>	berberine	Kim et al. (2008)
	<i>Curcuma longa</i>	curcumin	Srimal et al. (1971); Chan (1995)
	<i>Commiphora mukul</i>	guggulsterone	Shishodia and Aggarwal (2004)
	<i>Azadirachta indica</i>	nimbidin	Pillai and Santhakumari (1981)
	<i>Embelia ribes</i>	embelin	Gupta et al. (1977)
Cardio-protective	<i>Rauvolfia serpentina</i>	reserpine	Vakil (1949), Muller et al. (1952) and Bein (1953)
	<i>Thevetia neriifolia</i>	thevitin A, thevitin B and peruvoside	Bose et al. (1999)
	<i>Terminalia arjuna</i>	arjunolic acid	Sumitra et al. (2001)
	<i>Coleus forskohlii</i>	forskolin	Tandon et al. (1977) and Dubey et al. (1981)
Anti-diabetic	<i>Momordica charantia</i>	charantin	Krawinkel and Keding (2006)
	<i>Gymnema sylvestre</i>	gymnemic acid IV	Sugihara et al. (2000)
	<i>Andrographis paniculata</i>	andrographolide	Zhang et al. (2009)
Hypolipidaemic	<i>Camellia sinensis</i>	epigallocatechin 3,5-digallate oolonghomobisflavan A oolongtheanin-3'-O-gallate theaflavin-3'-O-gallate	Nakai et al. (2005)
	<i>Alpinia officinarum</i>	3-methylethergalangin 5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-3-heptanone	Shin et al. (2003, 2004)
	<i>Commiphora mukul</i>	guggulipid	Mehta et al. (1968) and Satyavati et al. (1969)

(continued)

Table 2.1 (continued)

Biological activity	Plant name(s)	Active principle(s)	References
Anti-cancer	<i>Arnebia nobilis</i>	arnebin-I	Gupta and Mathur (1972) and Sidhu et al. (1999)
	<i>Roylea calycina</i>	precalyone	Prakash et al. (1979)
	<i>Tithonia tagetiflora</i>	tagitinin F	Pal et al. (1976)
	<i>Dysoxylum binectariferum</i>	rohitukine	Kaur et al. (1992) and Sedlacek (2001)
	<i>Combretum</i> spp.	combretastatin A-4	Pettit et al. (1989)
	<i>Podophyllum emodi</i>	podophyllotoxin	Kusari et al. (2011)
	<i>Tephrosia candida</i>	6-hydroxykaempferol 4'-methyl ether	Sarin et al. (1976)
	<i>Alstonia scholaris</i>	echitamine chloride	Mohana et al. (1985)
	<i>Tylophora indica</i>	tylophorine	Rao et al. (1971)
Antidiarrheal	<i>Parthenium hysterophorus</i>	parthenin	Vaidya et al. (1978) and Narasimhan et al. (1985)
	<i>Holarrhena antidysenterica</i>	conessine	Kavitha et al. (2004)
Anti-asthmatic	<i>Andrographis paniculata</i>	neoandrographolide	Gupta et al. (1990)
	<i>Adhatoda vasica</i>	vasicine	Srinivasarao et al. (2006)
Anti-malarial	<i>Selaginella bryopteris</i>	bilobetin heveaflavone	Kunert et al. (2008)
	<i>Azadirachta indica</i>	nimbolide gedunin	Rochanakij et al. (1985)
	<i>Ancistrocladus heyneanus</i>	ancistrocladidine ancistrotanzanine C ancistroheynine B	Bringmann et al. (2004)
	<i>Cinchona</i> spp.	quinine	Karle and Bhattacharjee (1999)
	<i>Artemisia annua</i>	artemisinin	Klayman et al. (1984), Avery et al. (1992) and Tu (2016)
Anti-leishmanial	<i>Diospyros</i> spp.	diospyrin	Hazra et al. (1987) and Ray et al. (1998)
	<i>Plumbago</i> spp.	plumbagin	Croft et al. (1985)
	<i>Berberis aristata</i>	berberine	Ghosh et al. (1985)
	<i>Piper</i> spp.	piperine	Kapil (1993)
	<i>Swertia chirayita</i>	amarogentin	Ray et al. (1996)
	<i>Picrorhiza kurroa</i>	picroliv (a mixture of iridoid glycosides)	Puri et al. (1992)

(continued)

Table 2.1 (continued)

Biological activity	Plant name(s)	Active principle(s)	References
Antiviral	<i>Thea sinensis</i>	theasinensin D	Hashimoto et al. (1996)
	<i>Terminalia chebula</i>	gallic acid chebulagic acid	Ahn et al. (2002)
	<i>Terminalia bellirica</i>	termilignan thannilignan 7-hydroxy-3',4'-(methylene dioxy)flavan anolignan B	Valsaraj et al. (1997)
Memory enhancers	<i>Bacopa monnieri</i>	bacosides	Rajan et al. (2011) and Shinomol et al. (2011)
	<i>Centella asiatica</i>	asiaticoside madecassoside asiatic acid madecassic acid	Rao et al. (2005) and Shinomol et al. (2011)

Jammu; Central Institute of Medicinal and Aromatic Plants (CIMAP, 1959), Lucknow; North East Institute of Science and Technology (NEIST, 1961), Jorhat and Institute of Himalayan Bioresource Technology (IHBT, 1983), Palampur have taken the lead role in plant-based drug discovery endeavours. Now-a-days, in addition to the above-mentioned laboratories, various labs in the Indian Institute of Technology (IITs), Indian Institutes of Science Education and Research (IISERs), National Institute of Technology (NITs), National Institute of Pharmaceutical Education and Research (NIPERs) also started research on medicinal and aromatic plants.

There are many other initiatives started in India which include “Network Programme on Bioprospecting of Biological Wealth using Biotechnological Tools” during the 9th plan and Biotechnology Industry Research Assistance Council/ Biotechnology Industry Partnership Programme (BIRAC/BIPP) by the Department of Biotechnology, Government of India; New Millenium Indian Technology Leadership Initiative (NMITLI) and Open Source Drug Discovery (OSDD) by the Council of Scientific and Industrial Research, Government of India. The Golden Triangle Partnership (GTP) has been introduced jointly by the Department of Ayush, the Indian Council of Medical Research (ICMR) and the Council of Scientific and Industrial Research (CSIR) for the validation of Ayurvedic drugs and development of new drugs of national and global importance. To protect IPR, CSIR has developed a database called Traditional Knowledge Digital Library (TKDL) launched in 2011 with an aim to prevent the misappropriation of traditional knowledge at international patent offices.

2.7 Ethnomedicinal Research in India

The term “ethnobotany” was coined by Harshberger in 1895 in the Philadelphia Evening Telegraph, but the discipline has existed for ages. There has been a variety of definitions and concepts assigned to the word “ethnobotany”, but now it is universally referred to as the direct relationship between humans and plants. In India, very little organised work had been done before the 1960s. Organised field work and other studies in the subject were started in the Botanical Survey of India (BSI). Also, there has been a reawakened interest in the development of ethnobotanical research in a number of institutions. At BSI, Dr. E. K. Janaki Ammal started ethnobotany research, and she studied food plants of certain tribals of South India. When the senior author of this paper Dr. Pushpangadan joined CSIR-Indian Institute of Integrative Medicine, Jammu under Dr. Janaki Ammal in 1968, she has fondly told him about the importance of doing ethnobotanical studies in South India.

From 1960, Dr. S. K. Jain of BSI started intensive fieldwork among the tribals of Central India. Dr. Jain received his Ph.D. from the University of Pune in 1965, under the supervision of Dr. H. Santapau, then Director of the BSI. Dr. Jain is regarded as the father of Indian ethnobotany. He developed methodology for ethnobotany, especially in the Indian context. The publications from his group in the early sixties triggered the ethnobotanical activity in many other centres, particularly among botanists, anthropologists and medical practitioners in India. He has been Editor-in-Chief of the *Flora of India* series (1978–1984) and the Journal *Ethnobotany*, published by the Society of Ethnobotanists, Lucknow. The establishment of the *Society of Ethnobotanists* by Dr. S. K. Jain in 1982, the *National Society of Ethnopharmacology, India* by Dr. P. Pushpangadan in 1986 and the Journal *Ethnobotany* by Dr. S. K. Jain from 1989 onwards placed the discipline on a firm footing in India. Both the societies and the Journal have international membership and contributions. Dr. Jain laid the foundation for the Institute of Ethnobiology, which started functioning initially in CSIR-National Botanical Research Institute, Lucknow; later shifted to Jiwaji University, Gwalior, now it is under the leadership of Dr. Ashok K. Jain. In 1994, Dr. S. K. Jain organized the 4th Congress of International Society of Ethnobiology (ISE) at CSIR-NBRI. This was one of the most successful congresses and was well attended by over 300 delegates, including 82 foreign ethnobotanists from various parts of the world. Dr. Jain published *Glimpses of Indian Ethnobotany* in 1981. Recently, Jain and Jain (2016) published *Compendium of Indian Folk Medicine and Ethnobotany* (1991–2015), which contains ethnobotanical data of more than 4600 plant species, covering 1000 references. Pushpangadan and Pradeep (2008) published a book entitled *A Glimpse at Tribal India: An Ethnobiological Enquiry*.

Ethnobotanical investigations were carried out by workers like Ammal (1956), Jain (1963), Hajra (1981), Hajra and Baishya (1997), Vartak and Gadgil (1980, 1981), Vartak (1981), Borthakuar (1981, 1996), Borthakur and Gogoi (1994), Jain (1987, 1991, 2002, 2005, 2006, 2010), Jain and Goel (1987, 2005), Jain and Sikarwar (1998), Jain et al. (1994, 1997), Manilal (1978, 1980a, b, c, 1981, 1996,

2005, 2012), Manilal et al. (2003), Joseph and Kharkongor (1981), Pushpangadan and Atal (1984, 1986), Pushpangadan (1986, 1990), Pushpangadan et al. (1988, 1995), Pushpangadan and George (2010), Pushpangadan and Dan (2011), Joshi (1995), Bora and Pandey (1996), Subramoniam et al. (1997, 1998), Mitra (1998a, b), Patil (2000, 2001), Mohanty and Rout (2001), Mohanty (2003, 2010), Bondya et al. (2006), Singh et al. (2011) etc. During the last four decades many ethnobotanical works have been initiated by Institutes such as CSIR-National Botanical Research Institute, ICAR-National Bureau of Plant Genetic Resources, KSCSTE-Jawaharlal Nehru Tropical Garden and Research Institute, Central Council for Research in Ayurvedic Sciences (CCRAS), Central Council for Research in Siddha (CCRS) etc.

2.8 All India Co-ordinated Research Project on Ethnobiology (1982–1998)

The Indian Council of Agricultural Research (ICAR) convened a meeting of its inter-organizational panel for food and agriculture on September 21, 1976 under the chairmanship of Prof. M. S. Swaminathan, the then Director General, ICAR. Having felt the urgent need to undertake an ethnobiological study of the tribals of the country with a view to tap and document the fast-disappearing life-style, knowledge system and wisdom of the tribals, Prof. Swaminathan setup a panel of experts consisting of Dr. T. N. Kushoo and Dr. E. K. Janaki Ammal to submit a report. Consequently, Dr. T. N. Khoshoo along with Dr. E. K. Janaki Ammal drafted the All India Co-ordinated Research Project on Ethnobiology (AICRPE) project proposal, which was approved by the high level committee of Science and Technology, Govt. of India. The project was originally initiated by the Department of Science and Technology (DST) in July 1982 under the Man and Biosphere Programme (MAB) of UNESCO. Later, the MAB programme, along with AICRPE was transferred to the Department of Environment (DOEn, formed on November 1, 1980; presently the Ministry of Environment, Forest and Climate Change, MoEF&CC). In September 1983, DOEn set up a co-ordination unit at CSIR-Indian Institute of Integrative Medicine, Jammu with Dr. P. Pushpangadan as the Chief Co-ordinator of this project for overall supervision, co-ordination and implementation of various programmes included in the AICRPE.

This multi-institutional and multi-disciplinary project was operated in about 27 centres by over 600 scientific personnel located in the different institutions spread over the length and breadth of the country. During the course of its operation (1982–1998, 16 years) AICRPE recorded information on the multi-dimensional perspectives of the life, culture, tradition and knowledge system associated with biotic and abiotic resources of the 550 tribal communities comprising over 83.3 million people belonging to the diverse ethnic group. In India, there are 550 communities of 227 ethnic groups. There are 116 different dialects of 227 subsidiary dialects

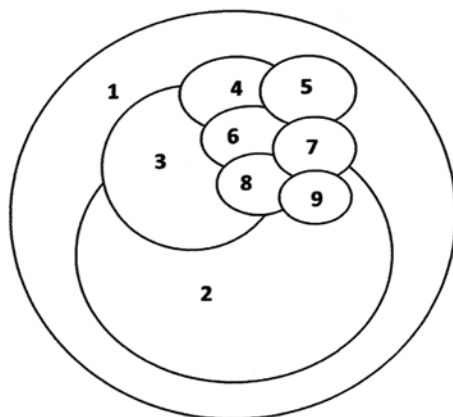


Fig. 2.1 Utilization pattern of wild plants by tribes of India

1. Total (10000); 2. Medicinal (8000); 3. Edible Use (4000); 4. Other Material and Cultural Requirements (750); 5. Fibre and Cordage (600); 6. Fodder (500); 7. Pesticides, Piscicides etc. (325); 8. Gum, Resin and Dye (300) and 9. Incense and Perfumes (100) (AICRPE Report 1998)

spoken by tribals of India. As per recent data, the Indian subcontinent is inhabited by over 104 million tribal people belonging to 704 communities. The knowledge of these communities on the use of wild plants for food, medicine and for meeting many other material requirements are now considered to be potential resource base for appropriate science and technology intervention for developing novel marketable products. Due to a lack of funding, the vast data gathered by the AICRPE team is stored as unattended reports. Over the course of the AICRPE project, traditional informations on approximately 10,000 plants (Fig. 2.1) were collected. It is worth noting that Indian Systems of Medicine (Ayurveda, Siddha, Unani, Amchi, and so on) uses only 2500 plants, while we have a database of 10,000 plants that needs to be scientifically validated. Out of this, 8000 wild plant species used by the tribals for medicinal purposes, about 950 are found to be new claims and worthy of scientific scrutiny. 3900 or more wild plant species are used as subsidiary food/ vegetables by the tribals. Out of the 400 plant species used as fodder, 100 are worth recommending for wider use. About 300 wild species are used by tribals as piscicides or pesticides. At least 175 of them are quite promising to be developed as safe pesticides (Pushpangadan 1998, 2002; Pushpangadan and Pradeep 2008; Pushpangadan et al. 2015, 2017). It is now well recognized that the traditional wisdom and knowledge on utilization of the biological resources is of immense value to biodiversity planners and scientists in developing strategies for conservation, utilization and generation of wealth from the bioresources. Bioscientists consider that ethnobiological/ ethnobotanical knowledge system as a first effective means for identifying as well as locating alternative food sources and leads for drugs and pharmaceuticals, natural dyes, colours, gums, resins, etc.

2.9 Genesis of the Subject Ethnopharmacology in India

The concept “ethnopharmacology” was first used at an international symposium held in San Francisco in 1967, during the discussion on the theme “Traditional Psychoactive Drugs” (Efron et al. 1967). However, Rivier and Bruhn (1979) attempted to describe “ethnopharmacology” as “a multi-disciplinary area of research concerned with observation, description and experimental investigation of indigenous drugs and their biological activities”. Bruhn and Holmstedt (1981) later redefined it as “the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man”. However, none of these said definitions captures the true spirit of this interdisciplinary subject. Ethno- (Gr., culture or people) pharmacology (Gr., drug) is concerned with the intersection of medical ethnography and the biology of therapeutic action, i.e., a transdisciplinary exploration that spans the biological and social sciences. This indicates that ethnopharmacologists are professionally cross-trained - for example, in pharmacology and anthropology - or that ethnopharmacological research is the result of partnerships between people who acquired formal training in two or more traditional disciplines.

Ethnopharmacology research in India was initiated at CSIR-Indian Institute of Integrative Medicine (IIIM), Jammu in 1985 by the then Director, Dr. C. K. Atal, along with his student, Dr. P. Pushpangadan (Chief Coordinator, AICRPE), the senior author of this communication. Dr. Atal, however, left RRL in mid 1980s. But Dr. Pushpangadan and his students, colleagues and a few other enthusiasts, notably Dr. A. K. Sharma, Dr. S. Rajasekharan, Dr. V. George, Dr. P. G. Latha, Dr. K. Narayanan Nair, Dr. B. G. Nagavi, Shri. P. R. Krishnakumar etc., continued their effort to develop ethnopharmacology research. They observed that subjecting traditional herbal remedies, including those of the classical systems like Ayurveda, Siddha and Unani to the parameters of modern medicine is illogical. Both these systems are conceptually quite different. In contrast to the reductionist approach of modern medicine, the concept of disease, its etiology, manifestation and approach to treatment etc. in classical systems are all viewed on a holistic basis. An integrated approach combining the classical systems of medicine with the modern science and technology may lead to develop the desired results. The concept and methods of ethnopharmacology research thus developed by the authors contain experts from diverse disciplines like Ayurveda, Siddha, scholars of Sanskrit and Tamil languages (who can correctly interpret the classical texts of Ayurveda and also its theoretical basis like *Sankhya* and *Vaiseshika* philosophy), ethnobotany/ ethnomedicine, chemistry, pharmacognosy, pharmacology, biochemistry, molecular biology, pharmacy, etc. The main goal of this approach was to establish suitable techniques for evaluating traditional remedies using Ayurvedic pharmacy and pharmacology principles such as *rasa*, *guna*, *veerya*, *vipaka*, and *prabhava*, in other words *Samagrah Guna* of the *Draya Guna* concept of Ayurveda (Pushpangadan et al. 2014; Pushpangadan et al. 2016a, b).

The senior author was successful in convincing Prof. M. G. K. Menon, in 1985, to be the Chief Patron of the newly formed National Society of Ethnopharmacology

(NSE), India. This society was formally registered in 1986 with the senior author as its founder president. The first ethnopharmacology laboratory started functioning at IIM under the All India Coordinated Research Project on Ethnobiology (AICRPE) funded by the Ministry of Environment, Forest and Climate Change, Govt. of India. However, the first full-fledged ethnopharmacology laboratory was started in 1992 at Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) where the senior author joined in 1990, as its Director. At JNTBGRI, with a proper understanding of the concepts and theories of the classical systems of medicine and a logical linking and integration with the advanced technologies of modern science resulted in developing several standardised marketable herbal products such as Jeevani, Sisairosp etc. (Pushpangadan et al. 1988; Sharma et al. 1989; Mashelkar 2001; George et al. 2003).

The first National Conference on Ethnopharmacology was held in Thiruvananthapuram, Kerala, from 24th to 26th May, 1993, by the NSE, India in collaboration with JNTBGRI and with funding from DANIDA. In 1995, an edited book *Glimpses of Indian Ethnopharmacology* was published based on the selected papers presented at the conference. In February 1999, the senior author moved from JNTBGRI to CSIR-NBRI as its Director. At NBRI, the senior author has established a state-of-the-art ethnopharmacology laboratory and herbal product development division where the latest analytical techniques such as HPLC, HPTLC, high-throughput analysis, activity guided isolation techniques and similar other innovative new techniques in validating, formulating and standardizing the herbal products etc. were introduced (Pushpangadan 1998, 2002; Pushpangadan and Pradeep 2008; Pushpangadan et al. 2015, 2017).

2.10 Conclusion

Medicinal plants and natural products have played an important role in taking care of the healthcare needs of the people of India for several millennia. The traditional systems of medicine have been enriched by successive generations through experiments, observations, inferences and judicial applications. With the advancement of modern science and technology and with the massive support of the Government of India, intensive efforts have been initiated in Indian research and development institutions, as well as universities to scientifically validate the traditional medicines used in ISM. Currently, India's share in international drug exports is much lower, as compared to its competitors (e.g. China), which is due to the lack of scientific data to support the efficacy claims of good quality Indian crude drugs. This lacuna is now being seriously addressed by the Government of India and the scientific community, so that they may soon come up with scientific dossiers on the classical Indian herbal medicines. With concerted efforts, India will be able to claim its rightful share in the international herbal drug trade, in the near future.

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Chapter 3

Biodiversity of Medicinal and Aromatic Plants with Special Reference to Endemic Plants of India



Atiya Khanum

Abstract The exponentially increasing human population and their multifarious activities result in serious threats to the species of various habitats. Hence, there is a need to preserve the ‘gene pool’ of wild relatives of modern crop plants. There are several wild species which have potential to be used as industrial raw material. Around 119 pure chemical substances extracted from 90 plant species are used in modern medicine, of which 40 are derived only from wild. Identification and exploration of such wild plant species could enhance the economy of a country. The genetic code of the species which enable it to survive and evolve is instrumental for the genetic engineers to develop “miracle drugs” and ‘wonder foods’. The Indian system of medicine uses more than 1100 medicinal plants and most of them are sourced from the wild. There is a tremendous demand for raw materials from the drug industry. Inventorization, identification, exploration, assessment of the genetic diversity and enhancement of the production of raw material is the need of the hour. In the present chapter, the diversity of medicinal and aromatic plants is described in accordance with the phytogeographical regions of India. Each phytogeographical region is characterised by its characteristic floristic composition. India is one of the 12 centres of diversity and origin of several cultivated plants in the world. About 49,441 plant species representing 11.4% of the world flora can be found in India. Two of the phytogeographical regions of India, viz., Eastern Himalayas and the Western Ghats are included in the hot-spots of the World and are a rich repository of medicinal and aromatic plants.

India possesses the largest number of endemic species in the world, next to Australia. This is due to its unique topography, extreme climatic conditions and intense geographical isolation describing the major causes of endemism along with a wide list of endemic species from different centres of endemism. The author has

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tried to highlight the variability and confinement of medicinal and aromatic plants. It is expected that this chapter will be a good asset for future taxonomist, ecologist, phytochemist, genetic engineers to evaluate the rich floristic diversity.

Keywords Biodiversity · Medicinal and aromatic plants · Endemism · Miracle drugs · Wonder foods · Genetic diversity · Floristic diversity

3.1 Introduction

Biodiversity refers to the variability among the living organisms; plants, animals and micro-organisms inhabiting the earth in terrestrial and aquatic ecosystems. The term is defined in the Convention on Biological Diversity as “The variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems, and the ecological complexes of which they are part; this includes diversity within species, between species and ecosystems” (Chowdhery and Murti 2000).

Since life began on earth (3.5 billion years ago), as many as 500 million species of plants, animals and microbes were inhabiting on it. But many of these disappeared in the normal evolutionary process; some of them survive as living fossils, e.g., *Ginkgo* and also many more are evolved as new species. At present, it has been estimated that more than 50 million species of plants, animals and microbes are existing on earth. But unfortunately, only about 1.5 million species have been identified and described so far (Sinha 1997). There is a need for inventorisation and monitoring of plant biodiversity. Plant collection, inventorisation and its monitoring provide necessary information for its effective management and conservation. It is of utmost importance for the survival of some unique species for which there is no alternative. For one cannot think off what would have happened, if the life saving antibiotic Penicillin or if *Ancistrocladus korupensis* had been eliminated from the nature, before the discovery of Michellamine B to combat HIV AID virus.

3.2 Significance of Biodiversity

The exponentially increasing human population and their multifarious activities result in decreasing earth's carrying capacity to support life, which in turn causes serious threat to the species of all habitats. Hence, the earth's biological resources are under severe pressure and fast dwindling. In this scenario, conservation and sustainable development of species is of utmost importance.

There are several wild species which have the potential to be used as industrial raw materials. Around 119 pure chemical substances extracted from 90 species of

plants are used in modern medicine in Asia, Africa and Latin America: more than 40 of these are available only from the wild. Identification and exploration of such wild plants from various habitats like deserts, swamps, *etc.*, enhances the economy of a country (Sinha 1997) (Table 3.1).

India (8–30°N and 60–97.5°E) with a total area of 329 million hectares is very rich in biological diversity. This richness is due to vast variety of climatic and altitudinal conditions coupled with different ecological habitats, varying from humid tropical Western Ghats, hot deserts of Rajasthan, Cold deserts of Ladakh and icy mountains of Himalayas. India is one of the 12 Centres of diversity and origin of several cultivated plants of the world. It also has two of the 18 “hot spots” of the world – the Eastern Himalayas and the Western Ghats (Myers 1988).

About 49,441 species of plants representing 11.4% of the world flora found in India. A very small proportion of medicinal plants of India are from lower group of plants: such as algae, fungi, lichens, *etc.*, while the higher group constitutes the major portion (Singh and Mudgal 1997). Marine algae are well known due to their high proteins, vitamins, and carbohydrate contents. Over 45% species of marine algae are the important source of Agar-Agar, *e.g.*, *Gelidium*, *Gracilaria*, *Dictyota*, *Padina*, *etc.*, *Chlorella* and *Spirulina* are used their food for their medicinal value. A number of sterols- Fucosterol, Chondrillasterol, and Sargasterol are obtained from different marine algae.

India is rich in diverse germplasm which includes 51 species of cereals and millets, 104 species of fruits, 27 species of spices and condiments, 55 species of vegetable and pulses, 24 species of fibre crops, 12 species of oil seeds, and almost 3000 species of medicinal plants.

Figure 3.1 gives an overview of the Plant Diversity Centres of India.

3.3 Medicinal and Aromatic Plant Diversity in Relation to Phytogeographical Regions of India

India exhibits a wide range of altitudes, topographic and climatic conditions, hence medicinal plants are distributed into seven phytogeographical regions (Chowdhery and Murti 2000).

3.3.1 North Western Himalayas

It is a rich repository of medicinal and aromatic plants occupying the three states, Jammu and Kashmir, Himachal Pradesh and Uttarakhand of India. In the foot hills of North Western Himalayas (1000–5000 ft) in the region of Siwaliks and adjacent areas Subtropical dry evergreen forest having *Olea ferruginea*, *Shorea robusta* and *Punica*, *Acacia*, *Pistacia* species; in riverine region *Dalbergia sissoo* while in more

Table 3.1 Significance of wild species exhibited by their raw materials

S.No.	Species	Raw material	Use
A. Medicines			
1.	<i>Ancistrocladus korupensis</i>	Michellamine B	Combating the HIV AIDS virus
2.	<i>Arnebia hispidissima</i>	5 iso hexenyl naphthazarium	Anti cancer activity
3.	<i>Artemisia annua</i>	Artemesin	Effective in malaria.
4.	<i>Artemisia scoparia</i> (inflorescence)	Scoparone	Hypotensive tranquillising agent
5.	<i>Atropa belladonna</i>	Atropine	Dilation of eye
6.	<i>Bacopa monnieri</i>	Bacosides	Mental, toxic, improve memory power
7.	<i>Balanites aegyptiaca</i> (roots)	Diosgenin	Steroid hormones oral contraceptive
8.	<i>Catharanthus roseus</i>	Vinblastine	Antileukemic(80% survival of leukemic children)
9.	<i>Chondrodendron tomentosum</i>	Tubocurarine	Muscle relaxant in surgeries
10.	<i>Commiphora wightii</i> (resin from incision in the bark)	Bedellium	Antiseptic on old wounds, as urine stimulant
11.	<i>Digitalis purpurea</i> (foxglove)	Digitoxin	Cardiac stimulant and tonic
12.	<i>Ephedra foliata</i>	Ephedrine	Bronchial disorders
13.	<i>Ephedra gerardiana</i>	Ephedrine	Bronchio – dilator in asthma, prevent heart block
14.	<i>Erythroxylum coca</i> (leaves)	Cocaine	Use as local anaesthetic
15.	<i>Gloriosa superba</i>	Gloriosin, colchicine	Anti- inflammatory, antiarthritis, muscle relaxant
16.	<i>Gymnema sylvestre</i>	Gymnemic acid	Antidiabetic
17.	<i>Homolanthus nutans</i>	Prostatin	Combating the HIV, AIDS virus
18.	<i>Panax ginseng</i>	Ginseng	Tumors, corneal opacity, resistance
19.	<i>Papaver somniferum</i>	Morphine	Sedative and pain killer
20.	<i>Plantago ovata</i>	Mucin (Isabgol) D-glucosamine, D-galactosamine	Laxative
21.	<i>Psoralea corylifolia</i>	Psoralen	Highly effective in treatment of leukoderma
22.	<i>Rauwolfia serpentine</i>	Reserpine	Reduce B.P.
23.	<i>Salix</i> spp.	Aspirin	Dissolving blood
24.	<i>Sapium sebiferum</i>	Tannin	Anti AIDS
25.	<i>Tabebuia avellandedae</i>	Hapaehol (naphthoquinones)	Carcino sarcoma
26.	<i>Taxus baccata</i>	Taxol	In treatment of breast cancer
27.	<i>Tinospora cordifolia</i>	Insulin	For cure of diabetics, (increase secretion by pancreas)
28.	<i>Vitex negundo</i>	Chrysofenol D	Antihistamine, muscle relaxant

(continued)

Table 3.1 (continued)

S.No.	Species	Raw material	Use
29.	<i>Withania somnifera</i>	Withanolide DWithaferin A	Antitumor against sarcoma, anabolic
B. Perfumeries			
1.	<i>Commiphora weightii</i>	Gum resin exudates	Incense and perfume (Agarbatti)
2.	<i>Cymbopogon flexuosus</i>	Citral	Confectionary, culinary, flavouring, perfumery
3.	<i>C. martinii</i> var. <i>motia</i>	Geraniol	Perfumery
4.	<i>C. winterianus</i>	Citronellol, geraniol	Confectionary, flavouring
5.	<i>Lavandula</i> spp.	Lavender oil	Cosmetics, perfumery, disinfectant
6.	<i>Mentha arvensis</i>	Menthol	Cosmetics, culinary, perfumery
7.	<i>Pandanus fascicularis</i>	Kewda oil	Flavouring for food and beverages, perfumery
8.	<i>Santalum album</i>	Sandal oil	Carving, incense, perfumery
9.	<i>Vetiveria zizanioides</i>	Vetiver oil	Perfumery, cosmetics, drugs, screens and blinds

Source: Sinha (1997)

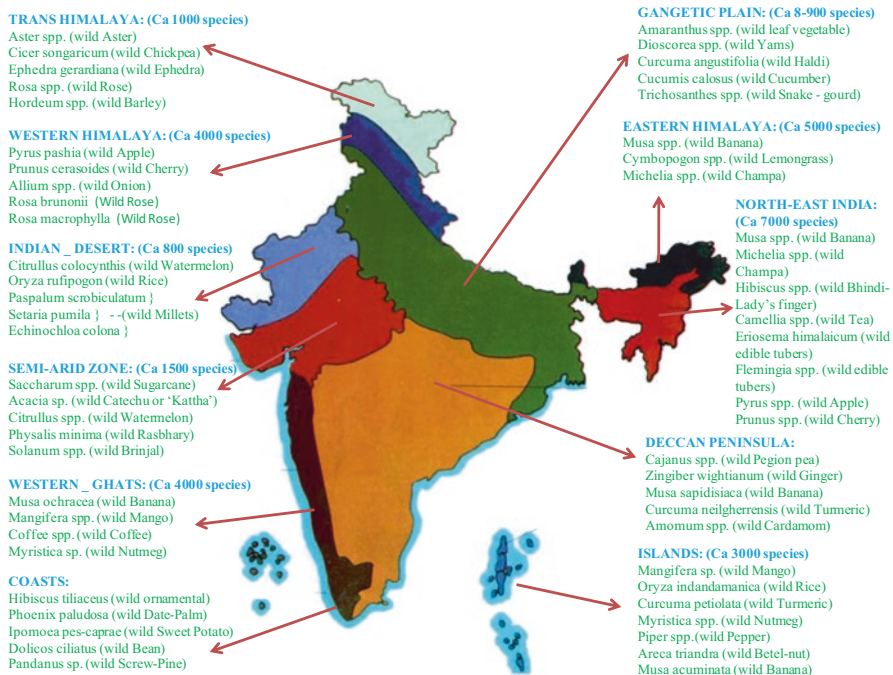


Fig. 3.1 Plant Diversity Centres of India. (Source: Chowdhery and Murti 2000)

moist places *Cedrela toona*, *Ficus glomerata* and *Eugenia jambolina* along with *Acacia catachu*, *Butea monosperma* occur. In western dry areas *Zizyphus*, *Carissa*, *Acacia*, *Pinus roxburghiana* appears.

In the temperate montane zone (5000–11,500 ft above sea level), *Cedrus deodara*, *Quercus incana*, *Aesculus indica*, *Abies pindrow*, *Picea morinda*, *Quercus semicarpifolia*, *Q. dilata*, *Taxus baccata* are most common. *Pinus gerardiana* is found in the inner valley on dry montane. In Kashmir *Betula*, *Salix*, *Poplar*, *Birch* are abundant. Other common plants grown in Kashmir are: *Crocus sativus*, *Apple*, *Peaches*, *Walnut*, *Almonds*. On the lower level of alpine zone, many herbs such as *Primula*, *Potentilla*, *Polygonum*, *Geranium*, *Saxifraga*, *Artemisia* sp. occur.

Most important medicinal and aromatic plants of North Western Himalayas are: *Acorus calamus*, *Aegle marmelos*, *Abelmoschus moschatus*, *Artemisia maritima*, *Aconitum heterophyllum*, *Bergenia ciliata*, *Boerhaavia*, *Bombax*, *Boswellia*, *Butea*, *Buchanania chinensis*, *Betula utilis*, *Chonemorpha fragrans*, *Cleome viscosa*, *Calotropis*, *Carissa Spinarium*, *Datura* spp., *Ephedra gerardiana*, *Ferula jaeschkeana*, *Ficus* spp., *Geranium*, *Gloriosa superba*, *Gymnema sylvestre*, *Juniperus polycarpes*, *Nelumbo nucifera*, *Oxoxylum indicum*, *Plantago major*, *Piper longum*, *Pistacia chinensis*, *Polygonatum*, *Potentilla*, *Podophyllum*, *Prunus armeniaea*, *Punica granatum*, *Rhododendron* spp., *Swertia chirayita*, *Viola*, *Vitex negando*, *Xanthoxylum armatum*.

Different altitudinal zones are occupied by different medicinal and aromatic plants. Samant et al. (2007) listed the representative species of medicinal plants of Himachal Pradesh occupying the different altitudinal zones (Table 3.2).

3.3.2 Eastern Himalayas

It comprises of Sikkim, North of West Bengal (Darjeeling), Arunachal Pradesh. Eastern Himalayan region of India accomodates a large number of medicinal plants of economic value. Due to warmer and wet climate, diversity is higher in the Eastern as compared to Western Himalayas. Eastern Himalayan region is one of the biodiversity hotspots of the globe. This region is rich in Orchids.

Most valued medicinal and aromatic plants of Eastern Himalayas are *Abies spectabilis*, *Abrus precatorius*, *Aconus calamus*, *Aegle marmelos*, *Adhatoda vasica*, *Allium wallichii*, *Aloe barbadensis*, *Amomum subulatum*, *Artemisia vulgaris*, *Azadirachta indica*, *Bacopa monneri*, *Bauhinia* ssp., *Begonia picta*, *Calendula officinalis*, *Catharanthus roseus*, *Carica papaya*, *Cassia fistula*, *Centella asiatica*, *Chenopodium album*, *Cinchona officinalis*, *Citrus* ssp., *Chenopodium album*, *Coptis teeta* (Endemic, found only in Arunachal Pradesh), *Curcuma* ssp., *Digitalis purpuraria*, *Dillenia indica*, *Dioscoria* ssp., *Embllica officinalis*, *Ephedra gerardiana*, *Eucalyptus globulus*, *Eupatorium* ssp., *Ficus* ssp., *Foeniculum vulgare*, *Glycyrrhiza glabra*, *Juglans regia*, *Juniperus macropoda*, *Melia azedarach*, *Mucuna pruriens*, *Musa sapientum*, *Oroxylum indicum*, *Panax pseudoginseng*, *Piper longum*, *Rheum australe*, *Rhododendron*, *Sapindus mukrossi*, *Solanum nigrum*, *Swertia chirayita*,

Table 3.2 Medicinal plants of Himachal Pradesh occupying the different altitudinal zones

Tropical and sub-tropical 1800 m	<i>Achyranthes aspera</i> , <i>Mangifera indica</i> , <i>Apium graveolens</i> , <i>Rauwolfia serpentina</i> , <i>Acorus calamus</i> , <i>Asparagus racemosus</i> , <i>Azardirachta indica</i> , <i>Artemisia absinthium</i> , <i>A. japonica</i> , <i>Tagetes minuta</i> , <i>Berberis asiatica</i> , <i>Rorippa indica</i> , <i>Bauhinia variegata</i> , <i>B.vahlii</i> , <i>Caesalpinia ecapitata</i> , <i>Terminalia alata</i> , <i>T. arjuna</i> , <i>T.chibula</i> , <i>Dioscorea bulbifera</i> , <i>Embllica officinalis</i> , <i>Swertia angustifolia</i> , <i>Curculigo orchioides</i> , <i>Ajuga parviflora</i> , <i>Mentha arvensis</i> , <i>M. piperata</i> , <i>Ocimum sanctum</i> , <i>Salvia plebia</i> , <i>S. lanata</i> , <i>Cinnamomum lamala</i> , <i>Aloe barbadensis</i> , <i>Tinospora cordifolia</i> , <i>Syzygium cuminii</i> , <i>Zizyphus mauritiana</i> , <i>Rubia cordifolia</i> , <i>Zanthoxylum arnatum</i> , <i>Sapindus mukorossii</i> , <i>Bergenia ligulata</i> , <i>Bacopa monnieri</i> , <i>Withania somnifera</i> , <i>Atropa belladonna</i> , <i>Urtica parviflora</i> , <i>Hedychium spicatum</i> .
Temperate 1801–2800 m	<i>Ferula jaeschkeana</i> , <i>Heracleum candicans</i> , <i>Berberis aristata</i> , <i>Betula alnoides</i> , <i>Corylus jacquemontii</i> , <i>Skimmia laureola</i> , <i>Geranium nepalense</i> , <i>Rhododendron arboreum</i> , <i>Malva verticillata</i> , <i>Oxalis corniculata</i> , <i>Phytolacca acinosa</i> , <i>Polygala sibirica</i> and <i>Taxus baccata</i> subsp <i>wallichiana</i> .
Sub-alpine 2801–3800 m	<i>Allium humile</i> , <i>Malaxis muscifera</i> , <i>Carum carvii</i> , <i>Geranium wallichianum</i> , <i>Angelica glauca</i> , <i>Heracleum lanatum</i> , <i>Arisaema flavum</i> , <i>Saussurea auriculata</i> , <i>S. costus</i> , <i>T. tomentosum</i> , <i>Impatiens glandulifera</i> , <i>Arnebia benthamii</i> , <i>Eritrichium canum</i> , <i>Rhododendron campanulatum</i> , <i>Ribes orientale</i> , <i>Polygonatum multiflorum</i> , <i>P. verticillatum</i> , <i>Plantago depressa</i> , <i>Aconitum ferox</i> , <i>Pedicularis pectinata</i> , <i>Polygonatum verticillatum</i> , <i>Dactylorhiza hatagirea</i> , <i>Picrorhiza kurroa</i> .
Alpine 3801 m	<i>Cortia depressa</i> , <i>Selinum tenuifolium</i> , <i>Heracleum wallichii</i> , <i>Inula royleana</i> , <i>Saussurea obvallata</i> , <i>Arnebia euchroma</i> , <i>Iris kumaonensis</i> , <i>Frilillaria roylei</i> , <i>Polygonum affinee</i> , <i>Rhododendron anthopogon</i> , <i>Rheum australe</i> , <i>Picrorhiza kurroa</i> , <i>Aconitum heterophyllum</i> , <i>A. rotundifolium</i> , <i>Nardostachys grandiflora</i>

Tamarindus indica, *Taxus baccata*, *Taxus wallichiana*, *Valeriana hardwickii*, *Withania somnifera*.

3.3.3 Indus Plains (Western Arid Region)

It covers the parts of Southeastern Punjab, Western Rajasthan, Kutch, parts of Gujarat, and Delhi. Annual rainfall is less than 70 cm and the climate is hot and dry summers, along with cold winters. This region is consisting of sandy plains and dunes, gravelly plains, rocky habitat and saline depressions. Xerophytic plants such as *Acacia nilotica*, *Prosopis* ssp., *Saccharum munja*, *Capparis aphylla*, *Zizyphus nummularia* are common. Sandy plains also have species of *Calligonum*, *Lycium*, *Aerva*, *Leptadenia*. The rocky areas have species of *Calotropis*, *Grewia*, *Panicum*, *Eleusine*, *Tribulus*, *Cassia*, *Commiphora*, *Indigofera*, *Euphorbias*. In moist areas, species of *Anogeissus*, *Prosopis*, *Acacia* and *Zizyphus* are found.

However, the Indus plain also shows the presence of several medicinal plant species, such as: *Abutilon indicum*, *A. hirsutum*, *Acacia nilotica*, *A.leucophloea*, *A.senegal*, *Acalypha indica*, *Achyranthus aspera*, *Adhatoda zeylanica*, *Aegle marmelos*, *Aerva lanata*, *Ageratum conizoides*, *Ailanthus excelsa*, *Aloe vera*, *Amaranthus* ssp., *Andrographis paniculata*, *Anogeisus pendula*, *Argemone*

mexicana, *Azadirachta indica*, *Bacopa monieri*, *Balanites aegytiaca*, *Barleria prionites*, *Blepharis ssp.*, *Boerhaavia diffusa*, *Boswellia serrata*, *Buchanania jinsang*, *Butea monosperma*, *Cassia auriculata*, *C. tora*, *Centella asiatica*, *Cleome ssp.*, *Commiphora weightii*, *Convolvulus arvensis*, *Cynodon dactylon*, *Datura metel*, *Echinops echinatus*, *Eclipta prostrata*, *Evolvulus alsinoides*, *Melia azedarch*, *Miriabilis jalapa*, *Moringa oleifera*, *Ocimum sanctum*, *Oroxylum indicum*, *Pergularia ssp.*, *Plantago ovata*, *Phyllanthus niruri*, *Portulaca oleracea*, *Pterocarpus marsupium*, *Ricinus communis*, *Santalum album*, *Sesamum indicum*, *Sida ssp.*, *Sonchus ssp.*, *Tecomella undulata*, *Tephrosia purpuria*, *Tinospora cordifolia*, *Trichodesma zeylanica*, *Vernonia cinerea*, *Vitex negundo*, *Withania somnifera*, *Wrightia tinctoria*, *Ziziphus mauritiana*, *Z. nummularia*, *Zygophyllum simplex*.

3.3.4 Gangetic Plains

It includes parts of north Punjab, most of Haryana, and stretches from eastern Rajasthan through western parts of Uttar Pradesh to Bihar and Bengal and considered to be most fertile region. The vegetation is chiefly of “Tropical moist deciduous forests” and “Dry deciduous forests”.

The Gangetic plain region offers a great variability in medicinal and aromatic plants. For example, *Acacia catechu*, *A. concinina*, *A. arabica*, *Achrolichum aureum*, *Acorus calamus*, *Adhatoda vasica*, *A. zeylanica*, *Aegle marmelos*, *Albizia lebbek*, *Aloe barbadensis*, *Andrographis paniculata*, *Anogeissus latifolia*, *Asparagus curtilus*, *A. sarmentosus*, *Azadirachta indica*, *Berberis asiatica*, *Butea monosperma*, *B. frondosa*, *Cassia fistula*, *C. tora*, *Catharanthus roseus*, *Chrysopogon aciculatus*, *Cinnamomum tamala*, *Clerodendron vesica*, *Curcuma longa*, *Cymopogon martinii*, *Dalbergia sissoo*, *Datura stramonium*, *Desmodium gangeticum*, *Desmostachya bipinnata*, *Embllica officinalis*, *Eragrostis viscosa*, *Ficus bengalensis*, *F. religiosa*, *Glycirriza glabra*, *Gmelina arborea*, *Grewia optiva*, *Haldina cordifolia*, *Helicteris isora*, *Hiptage bengalensis*, *Imperata cylindrica*, *Lagerstroemia parviflora*, *Mangifera indica*, *Mentha piperata*, *Mesua ferea*, *Mucuna pruriens*, *Muraya exotica*, *M. koenigii*, *Nelumbo nucifera*, *Oldenlandia diffusa*, *Panicum notatum*, *Papaver somniferum*, *Peganum harmala*, *Phoenix sylvestre*, *Phragmites karka*, *Phyllanthus emblica*, *Pluchea lanceolata*, *Plumbago zeylanica*, *Portulaca oleracea*, *Psoralea corylifolia*, *Rauwolfia serpentina*, *Ricinus communis*, *R. cordifolia*, *Rubia cordifolia*, *Saccharum spontaneum*, *Santalum album*, *Saraca indica*, *Sesbania cannabina*, *Semecarpus anacardium*, *Terminalia arjuna*, *Terminalia bellirica*, *T. tomentosa*, *Tinospora cordifolia*, *Vetiveria zizanioides*, *Withania somnifera*, *Zingiber officinalis*.

3.3.5 *North-Eastern India*

It includes the plain region of Arunachal Pradesh and the six eastern states Assam, Meghalaya, Tripura, Mizoram, Manipur and Nagaland. Most of this region is covered by Himalayas and receives heavy rainfall (1000–3000 mm) per annum; hence exhibit high wetness, resulting in dense Tropical evergreen forests in Eastern and Southern regions.

Medicinal and aromatic plants to flourish in this region are:

Abroma augusta, *Abies spectabilis*, *Acacia concina*, *A. pennata*, *A. polycantha*, *Acalypha indica*, *Acanthus leucostachys*, *Achyranthus aspera*, *Ageratum conyzoides*, *Aloe barbadens*, *Alnus nepalensis*, *Alstonia nilagirica*, *Amaranthus spinosus*, *Amorphophallus companulatus*, *Ananas comosus*, *Andrographis paniculata*, *Artemesia nilagirica*, *A. indica*, *Barringtonia acutangula*, *Bauhinia variegata*, *Betula alnoides*, *Boehmeria macrophylla*, *Bulbophyllum guttulatatum*, *Callicarpa arborea*, *Camellia caduca*, *Caryota urens*, *Cassia alata*, *Centella asiatica*, *Chromotaena odoratum*, *Citrus medica*, *C. assamensis*, *C. latipes*, *Clematis b Buchananiana*, *Clerodendron serratum*, *Colocasia esculenta*, *Coptis teeta* (Endemic to Arunachal Pradesh), *Costos speciosus*, *Cryptolepis b Buchananiana*, *Curcuma longa*, *Cynodon dactylon*, *Dillenia indica*, *Dioscoria floribunda*, *D. bulbifera*, *Eleucine coracana*, *Eryngium foetidum*, *Erythrina stricta*, *Euphorbia nerifolia*, *Gmelina arborea*, *Gnaphalium affine*, *Gymnadaenia orchidis*, *Hemidesmus indicus*, *Ilex khasiana*, *Illicium griffithii*, *Impatiens chinensis*, *Jatropha curcas*, *Kaempferia galanga*, *Leucas lavandulaefolia*, *Litsea cubeba*, *Mentha arvensis*, *Mesua ferrea*, *Michelia champaca*, *Mikania scandens*, *Mirabilis jalapa*, *Moringa oleifera*, *Mucuna pruriens*, *Murraya paniculata*, *Musa sapientum*, *M. acuminata*, *M. paradisiaca*, *Mussaenda roxburghii*, *Nepenthes khasiana* (Endemic to Meghalaya), *Ocimum sanctum*, *Oroxylum indicum*, *Osbeckia capitata* (Endemic), *Oxalis corniculata*, *Oxyspora paniculata*, *Panax pseudo-ginseng*, *P. skikimensis*, *Picorhiza kurroa*, *Pinus kesiya*, *Piper betel*, *P. griffithii*, *Plantago erosa*, *Podophyllum hexandrum*, *Prunus nepalensis*, *Rauwolfia serpentina*, *Ricinus communis*, *Rubia manjith*, *Saraca indica*, *Schima khasiana*, *Scoparia dulcis*, *Shorea robusta*, *Solanum khasianum*, *S. nigrum*, *S. indicum*, *Spilanthus acmella*, *Swertia chirayata*, *Taxus baccata*, *T. wallichiana*, *Terminalia myriocarpa*, *Tinospora cordifolia*, *Urena lobata*, *Urtica urens*, *Vanda coerulea*, *Verbena officinalis*, *Vitex peduncularis*, *Wadellia calendulacea*, *Withania somnifera*, *Zingiber officinale*.

3.3.6 *Plateau Region: Central India and Deccan*

The plateau region of India is almost triangular in shape and extends from northern plain to Kanyakumari in south. The northwest of this plateau lies in Aravalli hills. The Western Ghats and Eastern Ghat lies to west and east of the peninsular,

respectively. The peninsular plateau is differentiated into the **Central Highlands** and **Deccan Plateau**.

The **Central India** comprises the states of Madhya Pradesh, Chattisgarh, Maharashtra, Odisha, and Jharkhand. The forest vegetation is chiefly constituted by *Tectona grandis*, *Diospyros melanoxylon*, *Butea monosperma*, *Terminalia* spp., and *Dalbergia latifolia*. The thorny vegetation consists of *Carissa spinarum*, *Zizyphus rotundifolia*, *Acacia leucophloea*, *A. catechu*, etc.

The Satpura Plateau of Central India is home of variety of plants and herbs and inhabit a lot of rare species. The WHO (1996) has advised the various Nations to take up major initiative on ethnobiological studies on plants being used by tribals for medicinal use. An inventory of various medicinal plant species used by Gond, Bharia and Koru tribes inhabiting the forests of Madhya Pradesh, and also use of medicinal plants by traditional herbal healers in Central India reveals the presence of following species:

Abrus precatorius, *Acacia catechu*, *A. nilotica*, *Acalypha indica*, *Acanthospermum hispidum*, *Acanthus aspera*, *Achyranthus aspera*, *Acorus calamus*, *Adhatoda vasica*, *Aegle marmelos*, *Ailanthus excelsa*, *Aloe vera*, *Andrographis paniculata*, *Anisomeles indica*, *Anogeisus latifolia*, *Argemone mexicana*, *Argyrea speciosa*, *Asparagus racemosus*, *Bauhinia variegata*, *B. purpurea*, *Berberis aristata*, *Boerhaavia diffusa*, *Boswellia serrata*, *Bryonia laciniosa*, *Calotropis gigantea*, *C. procera*, *Cardiospermum helicacabum*, *Cassia fistula*, *C. tora*, *Catharanthus roseus*, *Celastrus paniculata*, *Centella asiatica*, *Chlorophytum arundinaceum*, *Cissampelos pariera*, *Cissus quadrangularis*, *Citrullus colocynthis*, *Clitoria turnacea*, *Costus speciosus*, *Cryptolepis buchananni*, *Curcuma amada*, *C. augustifolia*, *Cuscuta reflexa*, *Cyperus scariosus*, *Datura metel*, *Dioscorea hispidum*, *D. pentaphylla*, *Diophyros melanoxylon*, *Eclipta alba*, *Elephantopus scaber*, *Embellia ribes*, *Evolvulus alsinoides*, *Gloriosa superba*, *Gymnema sylvestre*, *Hedychium spicatum*, *Helicteris isora*, *Hemidesmus indicus*, *Hollarrhena pubescens*, *Hygrophila auriculata*, *Hyptis suaveolens*, *Justicia adhatoda*, *Jatropha curcas*, *Mallotus philippensis*, *Melia azedarach*, *Mitragyria parviflora*, *Mimordica charantia*, *Moringa oleifera*, *Nyctanthus arbortritis*, *Ocimum sanctum*, *Pergularia daemia*, *Phyllanthus amarus*, *P. emblica*, *Plumbago zeylanica*, *Pongamia pinnata*, *Psoralea corylifolia*, *Pterocarpus marsupium*, *Rauwolfia serpentina*, *Rheum australe*, *Ruta graveolens*, *Semecarpus anacardium*, *Shutaria hirsuta*, *Sida acuta*, *S. cordifolia*, *S. rhombifolia*, *Smilax* spp., *Solanum nigrum*, *Sphaeranthus indicus*, *Spilanthus acmeda*, *Syzygium cumini*, *Tectona grandis*, *Terminalia arjuna*, *T. bellirica*, *T. Chebula*, *Thumbergia fragrance*, *Tinospora cardifolia*, *Tribulus terrestris*, *Trichodesma indicum*, *Tridax procumbens*, *Tylophora indica*, *Urginea indica*, *Ventilago caliculata*, *Vitex negundo*, *Vitis tomentosa*, *Withania somnifera*, *Zingiber officinale*.

The **Deccan plateau** extends from the base of the Satpura to Kanyakumari. It comprises the states of Maharashtra, parts of Chattisgarh and Odisha, Telangana, Andhra Pradesh, Karnataka, and Tamilnadu. Larger part is covered with Tropical thorn forests.

Though, most of this region comes under Tropical dry deciduous forest, still it provides the favourable habitat for the growth of majority of medicinal plants including wild herbs such as: *Acacia leucophloea*, *A.arabica*, *Acalypha indica*, *Achyranthus aspera*, *Adhatoda zeylanica*, *Aegle marmelos*, *Albizzia* spp., *Aloe succotina*, *A. vera*, *Alternanthera sessilis*, *Andrographis paniculata*, *Annona squamosa*, *Anthocephalus chinensis*, *Aristolochia indica*, *Artemisea parviflora*, *Artocarpus heterophyllus*, *Atropa belladonna*, *Bacopa monneiri*, *Basella alba*, *Bauhinia purpuria*, *B. Variegata*, *Boerhaavia diffusa*, *Boswellia*, *Brasica juncea*, *Calotropis procera*, *C. gigantia*, *Cardiospermum halicacabum*, *Carica papaya*, *Carissa carundas*, *Cassia alata*, *C. fistula*, *C. Senna*, *Catharanthus roseus*, *Ceiba pendandra*, *Celastrus paniculatus*, *Centella asiatica*, *Chenopodium ambrosioides*, *Chloroxylon swertania*, *Citrus lemon*, *Cleistanthus collinus*, *Clerodendron inerme*, *Clitoria ternatea*, *Coccinia grandis*, *Coleus forskohlii*, *Cocos nucifera*, *Curculigo orchoides*, *Dalbergia paniculata*, *Decalepis hameltonii*, *Desmodium gangeticum*, *Diospyros melanoxylon*, *Dodonaea viscosa*, *Erythrina variegata*, *E. stricta*, *Eucalyptus globulus*, *Garcinia gummigatta*, *Gardenia gummifera*, *Gliricidia maculate*, *Gloriosa superba*, *Glossocardia basvallia*, *Gmelina arborea*, *Hibiscus rosasinensis*, *Hemidesmus indicus*, *Jatropha curcas*, *Justicia adhatoda*, *Lagerstomia parviflora*, *Lansea coromandelica*, *Lantana camara*, *Lawsonia inermis*, *Litsea glutinosa*, *Madhuca longifolia*, *Mangifera indica*, *Mimosops elengi*, *Morinda tinctoria*, *Moringa oleifera*, *Murraya koenigii*, *Nerium oleander*, *Nyctanthes arbortristis*, *Ocimum* spp., *Oxalis corniculata*, *Oxyxylum indicum*, *Phyllanthus acidus*, *P. emblica*, *Physalis minima*, *Piper longum*, *Pongamia pinnata*, *Pterocarpus santalinus*, *Putranjiva roxburghii*, *Rheum australe*, *Ruta chalepensis*, *Saraca asoca*, *Sapindus emarginatus*, *Sesbania grandiflora*, *Smilax perfoliata*, *Solanum* spp., *Strychnos nuxvomica*, *Swertia chirayata*, *Tabernamontana divaricata*, *Tagetes erecta*, *Tamarindus indica*, *Tectona grandis*, *Terminalia alata*, *T.bellireca*, *Thespesia populnea*, *Tinospora cordifolia*, *Tridax procumbens*, *Tylophora indica*, *Viteveria*, *Wrightia arborea*, *Xylia xylocarpa*, *Ziziphus jujuba*.

3.3.7 Western Ghats

Western Ghats are one of the biodiversity hot spots of the world. It receives an annual rainfall of 2000–8000 mm, hence exhibit luxuriant forest with approximately 5800 species of angiosperms of which nearly 2100 are endemic. It comprises the western coast of India extending from south of Tapti River, runs approximately 1600 km through the states of Maharashtra, Goa, Karnataka, Kerala and Tamil Nadu (upto Kanyakumari). The Western Ghats descend to the dry Deccan Plateau in the east. Most outstanding features of Western Ghats are the formation of Tropical rainforest in the southern Western Ghats, *i.e.*, “**Silent Valley**” (rainforest in Kerala) which is considered as the seat of origin and evolution of biological species on Earth, and the “**Sholas**” which are temperate evergreen forests and grasslands above an altitude of 1800 m in Palni, Nilgiris and Anamalai hills.

Western Ghats represents a store house of several economically important plants. It is difficult to enlist the medicinal plants of different biomes of Western Ghats individually; hence a few important species are listed in alphabetical order as under:

Acorus calamus, *Acrocarpus fraxinifolius*, *Adina hondala*, *Adhatoda beddomei*, *Aegle marmelos*, *Aglaiia elaeognoidea*, *Alangium salvifolium*, *Alpinia galanga*, *Alstonia scholaris*, *Amorphophallus commutatus*, *Anamirta cocculus*, *Anisochilus carnosus*, *Aphanomyxis polystachya*, *Aristolochia indica*, *A.bracteata*, *A.heterophyllus*, *A.hirsutus* (Endemic), *Asclepias curassavica*, *Asparagus racemosus*, *Atlantia wightii*, *Bacopa monnieri*, *Baliospermum montanum*, *Biophytum* ssp., *Bischofia javanica*, *Boerhaavia diffusa*, *Boswellia cordifolia*, *Bridelia squamosa*, *Buchanania lanzan*, *B.lanceolata*, *Butea monosperma*, *Caesalpinia bonduie*, *Calophyllum elatum*, *Canarium strictum*, *Canavalia stockssi*, *Capparis mooni*, *Cassia fistula*, *Cayratia pedata*, *Celastrus paniculata*, *Centella asiatica*, *Ceropegia* ssp., *Chenopodium ambrosoides*, *Chlorophytum borivilianum*, *Cinnamomum travancoricum*, *C.verum*, *C.wightii*, *C.zeylanicum*, *Commiphora mukul*, *C. slocksiana*, *Coscinium fenestratum*, *Crateva magna*, *Crinum brachynema*, *Cullenia exarittata*, *Curculigo orchioides*, *Curcuma amarissima*, *C.inodora*, *C.zedoaria*, *Cyperus rotundus*, *Daemia extensa*, *Desmodeum gangeticum*, *Dioscoria wightii*, *Diospyros paniculata*, *Dolichos bracteatus*, *Drosera peltata*, *Dysoxylum malabaricum*, *Emblica officinalis*, *Eratamia heyneana*, *Euodia unuankenda*, *Eurya nitida*, *Flemingera peltata*, *Garcinia cambogia*, *G.gummi-gutta*, *G.inida*, *Gardinia obtusa*, *Gaulthera fragrantissima*, *Gluta travancorica*, *Gloriosa superba*, *Gmelina arborea*, *Gymnema sylvestre*, *Gymnosporia montana*, *Haldina Cordifolia*, *Helicteris isora*, *Hemidesmus indicus*, *Heracleum candolleianum*, *Hibiscus angulosus*, *Holarrhena superba*, *Holigarna arnottiana*, *Holoptelia integrifolia*, *Holostemma ada kodien*, *Hopea parviflora*, *Hydrocarpus alpina*, *Hydrocotyle asiatica*, *Hymenodictyon orixense*, *Ichnocarpus frutescens*, *Ilex denticulata*, *Impatiens nilagirica*, *Ipomoea pestigridis*, *Kaempferia rotunda*, *Knema attenuata*, *Leucas aspera*, *Limonia acidissima*, *Lobelia inflata*, *L.nicotianaefolia*, *Mahonia leschenaultii*, *Mallotus phillipensis*, *Memeylon malabaricum*, *Mesua nagassarium*, *Michelia nilagirica*, *Microtropis ramiflora*, *Mimosa pudica*, *Mucusa monosperma*, *M.pruriens*, *Mukia scabra*, *Murraya paniculata*, *Myristica malabarica*, *Narenga alata*, *Neolamarkia cadamba*, *Nervilea aragoana*, *Nilgiranthes ciliatus*, *Nothapodytes foetida*, *Ocimum sanctum*, *Ophiorhiza mungos*, *Oroxylum indicum*, *Oryza jey-porensis*, *O.nivara*, *Osbeckia cupularis*, *Passiflora edulis*, *Phyllanthus amarus*, *Piper attenuatum*, *Piper barberi*, *P.bracteatum*, *P.nigrum*, *Plantago erusa*, *Plecosperrum spinosum*, *Plumbago indica*, *P. zeylanica*, *Poeciloneuron indicum*, *Pongamia pinnata*, *Pseudarthria viscida*, *Psoralea* ssp., *Pterocarpus marsupium*, *P.santalinus*, *Pterolobium hexapetalum*, *Rauwolfia barberi*, *R. serpentina*, *Rhincanthus nasuta*, *Rhododendron arboreum*, *Rhus mysorensis*, *Rotula aquatica*, *Ruta cordifolia*, *Ruta graveolens*, *Sageraea dalzelli*, *Salacia fruticosa*, *Saraca asoca*, *Sarcostemma acidum*, *Schlechera oleosa*, *Scutia circumscissa*, *Semecarpus anacardium*, *Sesamum indicum*, *S.prostratum*, *Shorea tumbuggaia*,

Sida cordifolia, *Spondias pinnata*, *Stepharia japonica*, *Sterospermum colais*, *Strobilanthes ciliatus*, *Strychnos nux-vomica*, *Symplocos cochinchinensis*, *Syzygium cumini*, *S.travancoricum*, *S.mungudam*, *Tabernamontana heyneana*, *Tephrosia collina*, *Terminalia arjuna*, *T. pallida*, *Tinospora cordifolia*, *T. malabarica*, *Toddalia asiatica*, *Trichopus zeylanicus*, *Trichosanthes lobata*, *Tylophora indica*, *Urginea indica*. *Uleria salicifolia*, *Vigna mungo* var. *sylvestris*, *Vigna radiata* var. *setulosa*, *Vernonia travancorica*, *Vateria indica*, *Withania coagulans*, *W. somnifera*, *Wrightia tinctoria*, *Zanthoxylum rhetusa*.

3.4 Plant Endemism in India: Important Centres

Endemism denotes confinement of taxa to a restricted area which may range from a small habitat to a biogeographical region, usually isolated by geographical or temporal barriers. Such species are called endemic in contrast to wides or cosmopolitan species. Endemism indicates the importance and uniqueness of flora of a region.

Due to high degree of endemism, rich diversity and presence of threatened species (as a result of overexploitation of plants for pharmaceutical and various other purposes), Eastern Himalayas and Western Ghats are included in the list of 18 hot spots of the world. Different phytogeographical zones of India have over 40 sites that exhibit high endemism. The flora of North India can not migrate to the neighbouring countries due to the high ranges of Himalayan Mountains. Moreover, the warm alluvial Indo-Gangetic region and the Brahmaputra basin on the south, arid and semiarid region on southwest acts as a barrier for migration of species from north. Similarly, the peninsular region of India which consists of Western and Eastern Ghats and the Deccan plateau have high degree of endemism. This region is surrounded by physical barriers on three sides as Arabian Sea in the West, Bay of Bengal in the East, and Indian Ocean in the south (Fig. 3.2). Moreover, Vindhya and Satpura range does not allow the peninsular flora to migrate to the central and north India. The Peninsular region is considered next to the oceanic islands in possessing high degree of endemism.

Even the Western Ghats including the Silent Vally is protected by vast Arabian Sea on the Western side, semi arid Deccan Plateau on the eastern side, Vindhya and Satpura ranges on north and Indian Ocean on the south. These entire physical conditions act as a barrier for the migration of flora, hence support a large number of endemic plants that are considered next only to Himalayas in holding the endemic species. Though, the Silent Valley of Western Ghats covers only 5% of Indian land but endowed with 4000 species of plants, out of which about 1800 are said to be endemic (Sinha 1997).

Chatterjee (1939, 1962) is the pioneer in the study of endemism in Indian flora, estimated 11,124 dicot species of flowering plants in Indian Region, which included Pakistan, Nepal, Bhutan, Myanmar, present day Bangladesh and Srilanka; out of these 6850 species representing 61% were endemic. He also estimated that out of total 134 genera endemic to Indian Region, 34 genera to be endemic to Peninsular



Fig. 3.2 Map of India physical – to highlight barriers of endemism. (<https://www.mapsofindia.com/maps/india/physical-map.html>)

India pertaining to the political boundaries of present-day India. Balakrishnan (1996) mentioned more than 6100 species out of about 17,500 species of flowering plants which are endemic to India, about 800 species in the North-West and western Himalayas, about 2500 species in eastern Himalayas and north east India, about 2600 species in Peninsular India and more than 200 species in Andaman and Nicobar Islands.

3.4.1 *Endemism in Western and North Western Himalaya*

This region comprises of the states of Jammu and Kashmir, Himachal Pradesh and North – West Uttar Pradesh. The geographical position, physiography and geology of this region together contributed to very high endemism.

Some important endemic species from this region are: *Agropyron dentatum*, *Anemone narcissifolia*, *A. tetrapetala*, *Aquilegia nivalis*, *Arabidopsis russelliana*, *Arabis nova*, *A. tenuirostris*, *Astragalus grahamianus*, *A. agacanthoides*, *A. pin-dreensis*, *A. kashmirensis*, *Berberis glaucocarpa*, *Cotoneaster pangiensis*, *C. wattii*, *C. stracheyi*, *C. prostratus*, *C. osmastonii*, *C. gilgitensis*, *Carex borii*, *Ceratosepala falcata*, *Christolea stewartii*, *Crepis naniforma*, *Delphinium koelzii*, *D. roylei*, *Dianthus orientalis*, *Dicranostigma lactucoides*, *Draba cachemirica*, *D. lasiophylla*, *Eriocycla thomsonii*, *Erophila verna*, *Euclidium syriacum*, *Euphrasia jaeschkei*, *E. pauciflora*, *E. platyphylla*, *Ferula jaeschkeana*, *Galium serpylloides*, *Halerpestes salsuginosa*, *Hedysarum astragaloides*, *Heracleum thomsonii*, *Lancea tibetica*, *Paraquilegia uniflora*, *Pedicularis albida*, *P. purpurea*, *Potentilla curviseta*, *Ranunculus arvensis*, *Seseli trilobum*, *Silene cancellata*, *S. eduardi*, *Thalictrum reniforme*, *T. rostellatum*, *T. saniculaeforme*, *Veronica hirta*, *Viola jangiensis*, *Waldheimia stoliczkai*, *Minuartia ebracteolata*, *Astragalus maxwellii*, *Eremurus himalaicus*, *Festuca simlensis*, *Puccinellia himalaica*, *Primula obtusifolia*, *Alchemilla chthamalea*, *Spiraea rhamniphylla*, *Saxifraga duthiei*, *Euphrasia densiflora*, *E. flabellata*, *Scrophularia dentata*, *Indigofera cedrorum*, *Poa jaunsarensis*, etc.

Endemic species restricted to **Jammu and Kashmir** worth mentioning are: *Anaphalis kashmiriana*, *Bidens tetraspinosa*, *Chondrilla setulosa*, *Lactuca benthamii*, *L. kashmiriana*, *Olgaea thomsonii*, *Lavatera kashmiriana*, *Saussurea clarkii*, *Tragopogon kashmirianus*, *Impatiens meeboldii*, *I. pahalgamensis*, *Berberis huegeliana*, *B. kashmiriana*, *B. pseudoumbellata ssp. gilgitica*, *B. stewartiana*, *Actinocarya tibetica*, *Cynoglossum flexuosum*, *Eritrichium spathulatum*, *E. spathulatum var. thomsonii*, *Hackelia meeboldii*, *H. stewartii*, *Heliotropium dasycarpum var. gymnostomum*, *Pseudomertensia drummondii*, *Arabis tenuirostris*, *Draba aubrietoides*, *D. ludlowiana*, *Erophila tenerrima*, *Lignariella duthiei*, *Callitriche fehmedianii*, *Gentiana harwanensis*, *G. marginata var. hugelii*, *Astragalus gilgitensis*, *Hedysarum cachemirianum*, *Oxytropis shivai*, *Allium gilgiticum*, *Neottia kashmiriana*, *Bromus barobalianus*, *Calamagrostis decora*, *C. stoliczkai*, *Digitaria stewartiana*, *Festuca levingii*, *Poa ladakhensis*, *P. suruana*, *Puccinellia thomsonii*, *Rostraria clarkeana*, *Schizachyrium impressum*, *Primula clarkei*, *Androsace studiosorum*, *Aconitum moschatum*, *Consolida schlagintweitiana*, *Ranunculus stewartii*, *R. palmatifidus*, *R. glacialiformis*, *Rhamnella gilgitica*, *Sageretia kashmirensis*, *S. kishwarensis*, *Alchemilla aksharmae*, *A. brummittii*, *A. cecillii*, *A. duthieana*, *A. gilgitensis*, *A. kishengangensis*, *A. mantonii*, *A. nicolsonii*, *A. niltarensis*, *A. plocekii*, *A. rothmaleri*, *A. samantraii*, *A. sarojinii*, *A. sojakii*, *A. waltersii*, *Cotoneaster lambertii*, *Prunus bokhariensis*, *Galium harwanensis*, *G. mahadivensis*, *Saxifraga asarifolia*, *S. flagellaris ssp. mucronulata*, *Euphrasia alba*, *E. incisa*, *E. kashmiriana*, *E. secundiflora*, *Pedicularis breviro*, *P. canescens*,

Scrophularia nudata, *Veronica cachemirica*, *V. koelzii*, *V. nana*, *V. uncinata*, *Viola fedtschenkoana* ssp. *muzaffarabadensis*, *V. jordani* var. *falconeri*, *V. rupestris* var. *himalayensis*, etc.

Some endemic species restricted to **Himachal Pradesh** are: *Aconitum falconeri* var. *latilobatum*, *Delphinium koelzii*, *Viola indica* var. *barbata*, *V. jangiensis*, *Silene kunawurensis*, *Cotoneaster glacialis*, *Jasminum parkeri*, *Tanacetum himachalensis*, *Deyeuxia simlensis*, *Epilobium semiamplexicaule*, *E. spitianum*, *Lagotis kunawurensis*, *Juncus rohtangensis*, *Meconopsis bikramii*, *Microsisymbrium axillare* ssp. *brevipedicellatum*, *Poa lahulensis*, *Pseudomertensia lahulensis*, *Ranunculus bikramii*, etc.

Some endemic species are restricted to **U.P. Himalayas** only. These are: *Arenaria ferruginea*, *Astragalus agacanthoides*, *Berberis lambertii*, *B. affinis*, *B. osmastonii*, *Chimonobambusa jaunsarensis*, *Cicerbita filicina*, *Clarkella nana*, *Cotoneaster garhwalensis*, *Gentiana tetrasepala*, *G. saginoides*, *Ilea nutans*, *Ivanjohnstonia jaunsarensis*, *Mahonia jaunsarensis*, *Meeboldia selinoides*, *Poa rhadiana*, *P. pseudamoena*, *Pueraria stracheyi*, *Trachycarpus takil*, *Pseudodanthonia himalaica*, etc. (Chowdhery and Murti 2000).

3.4.2 Endemism in North-Eastern Himalayas and North Eastern India

Eastern Himalayas and north-east India is another major centre of endemism. It includes Sikkim, Arunachal Pradesh, Assam, Meghalaya, Nagaland, Manipur, Mizoram and Tripura. Balakrishnan (1996) reported about 2500 endemic species from this region.

Some important **Eastern Himalayan** endemic species are: *Abies densa*, *Agapetes incurvata*, *A. sikkimensis*, *Dipsacus atratus*, *Eriobotrya hookeriana*, *Geum macrosepalum*, *Larix griffithiana*, *Lindera heterophylla*, *Liparis perpusilla*, *Lloydia flavonutans*, *Maddenia himalaica*, *Meconopsis grandis*, *M. superba*, *M. villosa*, *Myricaria albiflora*, *Primula whitei*, *Rhododendron baileyi*, *R. camelliaeflorum*, *R. ciliatum*, *R. glaucophyllum*, *R. grande*, *R. lanatum*, *R. lindleyi*, *R. wallichii*, *R. wightii*, *Rubus fragarioides*, *Saussurea conica*, *Acanthus leucostachys*, *Aconitum assamicum*, *Anoectochilus sikkimensis*, *Aeschynanthus parasiticus*, *Baliospermum micranthum*, *Berberis dasyclada*, *Calamus leptospadix*, *Calanthe densiflora*, *Capparis acutifolia*, *Cotoneaster assamensis*, etc.

Taxa restricted to **Eastern Himalaya, Assam** and **Tripura** include: *Cryptocoryn amygdalina*, *Phoebe attenuata*, *Rubus hamiltonii*, *Dalbergia lanceolaria*, *D. rimosa*, *Lonicera glabrata*, *Elatostema papillosum*, *Sloania dasycarpa*, *Actephila excelsa*, *Leea bracteata*, *L. trifoliata*, *Ardisia neriifolia*, *Jasminum caudatum*, *J. subtriplinum*, *Ophiorrhiza lucida*, *Wendlandia wallichii*, *Clerodendrum bracteatum*, *Stephania glandulifera*, *Dichrocephala hamiltonii*, *Lycianthus macrodon*, *Eranthemum palatiferum*, *Strobilanthes capitatus*, *Aeschynanthus grandiflorus*,

Melissa axillaria, *Globba clarkei*, *G. multiflora*, *Rhaphidophora decursiva*, *Calamus leptospadix*, *Oberonia pachyrachis*, *Scleria terrestris*, etc. (Chowdhery and Murti 2000).

Taxa restricted to **Assam** and **Tripura** include *Dalbergia thomsonii*, *Desmodium griffithianum*, *Fissistigma verrucosum* (extending to Meghalaya and Mizoram also), *Ixora subsessilis*, *Lasianthus tubiflorus*, *Litsea meissneri*, *Ophiorrhiza subcapitata*, *Nycticalos thomsonae*, *Phlogacanthus guttatus*, *P. tubiflorus*, *Pogostemon hispidus*, *Rhaphidophora lancifolia*, *Stuednera assamica*, *Tetrastigma obovata*, *Xantolis assamica*, etc. *Cycas pectinata* is distributed in Tripura, Manipur and Assam. *Gnetum montanum* and *G. oblongum* occur in Tripura and other parts of eastern India.

Some important endemic taxa of **Assam** region are: *Acacia diadenia*, *A. pennata* ssp. *herrii*, *Agapetes kanjilali*, *A. variegata* var. *bhareliana*, *Bambusa cacharensis*, *B. mastersii*, *Camellia sinensis* var. *assamica*, *Calamus kingianus*, *C. nabariensis*, *Chonemorpha assamensis*, *Chrysoglossum assamicum*, *Cinnamomum cacharensis*, *Citrus assamensis*, *Dendrobium assamicum*, *Dioscorea pentaphylla* var. *communis*, *Diospyros cacharensis*, *Dipterocarpus mannii*, *Drypetes assamica*, *Eulophia santapau*, *Euonymus assamicus*, *Fimbristylis circumciliata*, *Fissistigma santapau*, *Flacourtia helferi*, *Gigantochloa macrostachya*, *Glochidion assamicum*, *Glycosmis singuliflora*, *Heritiera dubia*, *Hymenachne assamica*, *Hypericum assamicum*, *Ixora goalparensis*, *Justicia craibii*, *Litsea assamica*, *Maba cacharensis*, *Maesa kurzii*, *M. maxima*, *Magnolia baillonii*, *M. caveana*, *M. gustavi*, *Mesua assamica*, *Michelia mannii*, *M. montana*, *Myristica clarkeana*, *Pandanus assamensis*, *Parakaemferia synantha*, *Pavetta assamica*, *Persea dubia*, *Phoebe cooperiana*, *P. goalparensis*, *Phyllostachys assamica*, *Piper clarkei*, *P. gullatlyi*, *P. gammei*, *P. jankinsii*, *P. listeri*, *Poa wardiana*, *Polygonum sarbhanganicum*, *Rotboellia goalparensis*, *Salacia jenkinsii*, *Syzygium assamicum*, *Thamnocalamus prainii*, *Trachelospermum assamense*, *Amblyanthus multiflorus*, *Begonia tessaricarpa*, *Carex fuscifructa*, *Galeola altissima*, *Saurauia griffithii*, *Mycetia mukerjiana*, etc.

Some endemic species, confined to **Arunachal Pradesh** are: *Acer oblongum* var. *microcarpum*, *Aconogonum pangianum*, *Agapetes aborensis*, *A. subansirica*, *Albizia arunachalensis*, *Aspidopterys glabriuscula* var. *lohitensis*, *Begonia aborensis*, *Blechnidium melanopus*, *Boehmeria tirapensis*, *Camellia siangensis*, *Cheirostylis munnacampensis*, *C. sessanica*, *Chirita macrophylla*, *C. mishmiensis*, *Cleisostoma tricallosum*, *Coelogyne arunachalensis*, *Coptis teeta*, *Epipogium indicum*, *E. sessanum*, *Eria jengingensis*, *E. lohitensis*, *E. sharmae*, *Eurya arunachalensis*, *Gastrodia arunachalensis*, *Globba multiflora*, *Gomphostemma aborensis*, *Impatiens mishmiensis*, *Leycesteria dibangvalliensis*, *Lithocarpus kamengensis*, *Litsea mishmiensis*, *Lobelia mishmica*, *Maesa arunachalensis*, *Mapania arunachalensis*, *Paphiopedilum wardii*, *Petasites kamengicus*, *Pholidota wattii*, *Pileostegia subansiriana*, *Primula mishmiensis*, *Psychotria aborensis*, *Rhododendron falconeri* spp. *eximium*, *R. santapau*, *R. subansiriensis*, *R. twangensis*, *Sapria himalayana*, *Senecio mishmi*, *Strobilanthes aborensis*, *Syzygium mishmiense*, etc.

There are some species which are strictly confined to **Sikkim** such as *Acronema pseudotenera*, *Anaphalis cavei*, *A. hookeri*, *A. subumbellata*, *Anemone demissa* var.

monantha, *Angelica nubigena*, *Arenaria thangoensis*, *Astragalus zemuensis*, *Berberis umbellata* var. *branii*, *Blumea sikkimensis*, *Cacalia chola*, *Calamus inermis*, *Caragana spinifera*, *Carex kingiana*, *Codonopsis affinis*, *Coelogyne treutleri*, *Cremanthodium palmatum* ssp. *benthamii*, *Crepis atropappa*, *Inula macrosperma*, *Juncus sikkimensis*, *Jurinea cooperi*, *Lactuca cooperi*, *Ligularia kingiana*, *L. pachycarpa*, *L. yakla*, *Mahonia sikkimensis*, *Podophyllum sikkimensis*, *Agrostis neodebilis*, *Catabrosa aquatica*, *Cyathopus sikkimensis*, *Trisetum sikkimensis*, *Rhynchospora sikkimensis*, *Ranunculus sikkimensis*, *Rhododendron sikkimensis*, *Uvaria lurida* var. *sikkimensis*, etc.

Meghalaya is also quite rich in endemic species which include several species restricted exclusively to Meghalaya. The wide separation of the Garo, Khasi and Jaintea hills by the Brahmaputra-river basin and Surma valley and ridges might have contributed to high degree of endemism in Meghalaya (Balakrishnan 1981–83). Some important endemic taxa are: *Aphyllorchis vaginata*, *Adinandra griffithii*, *Alsodeia racemosa*, *Aechmanthera leiosperma*, *Anacolosa ilicioides*, *Baliospermum micranthus*, *Calliandra griffithii*, *Callicarpa psilocalyx*, *Camellia caduca*, *Alseodaphne khasyana*, *Ardisia quinquangularis*, *Aspidoptery oxyphylla*, *Begonia brevicaulis*, *Capparis cinerea*, *Carex repanda*, *Clematis apiculata*, *Cleyera japonica*, *Coelogyne viscosa*, *C. purpurea*, *Cotoneaster khasiensis*, *Crotalaria noveoloides*, *Cyclea debiliflora*, *C. meeboldii*, *Digitaria jubata*, *Dipsacus asper*, *Distylium indicum*, *Dysoxylum khasianum*, *Engelhardtia wallichiana*, *Eria crassicaulis*, *Festuca rubra* ssp. *clarkei*, *Gymnocladus assamicus*, *Goniothalamus simensii*, *Hedychium calcaratum*, *H. rubrum*, *Holboellia khasiana*, *Ixonanthes khasiana*, *Neanotis oxyphylla*, *Ceropegia angustifolia*, *Citrus latipes*, *Nepenthes khasiana*, *Ophiorrhiza subcapitata*, *Pyrenaria khasiana*, *Silene khasiana*, *Sterculia khasiana*, *Corybas purpureus*, *Cynanchum wallichii*, *Dactylicapnos torulosa*, *Daphne shillong*, *Eria ferruginea*, *E. pusilla*, *Eriobotrya angustissima*, *Gastrodia exilis*, *Glochidion thomsonii*, *Goldfussia glabrata*, *Goodyera recurva*, *G. robusta*, *Gymnostachyum venustum*, *Habenaria concinna*, *H. furfuracea*, *H. khasiana*, *Hedychium dekianum*, *Ilex enibelioides*, *I. venulosa*, *Impatiens acuminata*, *I. khasiana*, *I. laevigata*, *I. porrecta*, *Ischaemum hirtum*, *I. hubbardii*, *Lindera latifolia*, *Liparis acuminata*, *Micropera mannii*, *Paramignya micrantha*, *Pantlingia serrata*, *Phlogacanthus wallichii*, *Photinia cuspidata*, *P. polycarpa*, *Pogonatherum rufobarbatum*, *Pogostemon strigosus*, *Pteracanthus griffithianus*, *P. nobilis*, *Rhynchospora griffithii*, *Rubus khasianus*, *Senecio jowaiensis*, *Taeniophyllum khasianum*, *Tetrastigma obovatum*, *Trachyspermum khasianum*, *Trias pusilla*, *Trivalvaria kanjilalii*, etc.

Some endemic species restricted to **Nagaland** are: *Begonia wattii*, *Capillipedium nagense*, *C. pteropechys*, *Chaerophyllum orientalis*, *Clematis meyeniana*, *C. meyeniana* var. *insularis*, *Cocculus prainianus*, *Coelogyne hitendrae*, *Corydalis borii*, *Cotoneaster nagensis*, *Crotalaria meeboldii*, *Cyclea wattii*, *Deyeuxia borii*, *D. nagarum*, *Hedychium marginatum*, *Pholidota imbricata*, *Pimpinella evgoluta*, *P. flaccida*, *Silene vagans*, etc.

There are taxa exclusively confined to **Manipur**. Some such important taxa include: *Actinodaphne obovata* var. *wattii*, *Arisaema wattii*, *Aster ageratoides*,

Begonia obversa, *Baliospermum suffruticosum*, *Beaumontia longituba*, *Berberis manipurana*, *B. sublevis*, *Carex manipurensis*, *Clematis wattii*, *Craibiodendron stellatum*, *Dalbergia wattii*, *Dischidia micholitzii*, *Elatostema ciliatum*, *Elaeagnus loureirii*, *Euphorbia serrulata*, *Gleadovia banerjiana*, *Ilex wattii*, *Illicium manipurense*, *Impatiens gibbisejala*, *I. longirama*, *I. rubro-lineata*, *I. spissiflora*, *I. teneriflora*, *Iris bakeri*, *I. wattii*, *Kalanchoe rosea*, *Mahonia feddei*, *M. magnifica*, *M. manipurensis*, *Pilea minuta*, *Piper aurorubrum*, *P. kapruannum*, *P. lainatakannum*, *P. makruense*, *P. meeboldii*, *P. muneyporensis*, *Polygonum stellato-tomentosum*, *Potentilla manipurensis*, *Prunus wattii*, *Pyrus wattii*, *Spodiopogon lacei*, *Strychnos nuxblanda*, *Vaccinium lamellatum*, *Vernonia clivorum*, *Zanthoxylum pseudoxyphyllum*, etc.

Endemic species to **Mizoram** include: *Arundinaria phar*, *Begonia lushaiensis*, *Bulbophyllum parryae*, *Chasalia lushaiensis*, *Derris lushaiensis*, *Dichrocephala minutifolia*, *Didymocarpus adenocarpa*, *D. rodgeri*, *D. parryorum*, *D. wengeri*, *Eria lacei*, *Glycosmis cyanocarpa* var. *linearifolia*, *Jasminum wengeri*, *Mahonia borealis* var. *parryii*, *Mantisia wengeri*, *Mussaenda parryorum*, *M. pentasaemia*, *Orthosiphon glandulosus*, *Petrocosmea parryorum*, *Rhododendron veitchianum*, *Senecio lushaiensis*, *Sonerila villosa*, *Stereogyne lushaiensis*, *Strobilanthes parryorum*, *Trisepalum lineicarpa*, *Veronica parryae*, etc.

3.4.3 Endemism in Peninsular India

The Peninsular region of India is a triangular plateau, bordered by the Vindhya and Satpura ranges in the north and surrounded by water on three sides – eastern by Bay of Bengal, western by Arabian Sea and south by Indian Ocean. It is highest in the south and west and sloping eastward. It represents ancient table-land, bordered on the west and east by Western Ghats and Eastern Ghats, respectively.

The Western Ghats are only next to Himalayas in having high number of endemic plants. Nayar (1982) described the presence of 2100 endemic species in Peninsular India. Ahmed Ullah and Nayar (1986) have made extensive studies on the endemic flora of Peninsular India. Presence of about 60 endemic genera including 49 monotypic genera makes this region unique and significant (Rao 2013). On the basis of the distribution of endemic species, the main centres of endemism in Peninsular India are: (i) North Western Ghats, (ii) Central Western Ghats, (iii) South Western Ghats, (iv) Eastern Ghats.

- (i) **North Western Ghats:** It includes the area from Tapti River to Goa. Some of the important rare endemic species in this area are: *Abutilon ranadei*, *Achyranthes coynei*, *Barleria sepalosa*, *Cissus arenosus*, *Dichanthium compressum*, *D. woodrowii*, *Dysophylla stocksii*, *Glyphochloa santapau*, *Habenaria caranjensis*, *Salacia brunoniana*, *Synnema anomalum*, *Viscum mysorensis*, *Arisaema caudatum*, *Canscora concanensis*, *Ceropegia fantastica*, *C. huberi*, *C. lawii*, *C. odorata*, *Dichanthium maccannii*, *Dysophylla salicifo-*

lia, *Eriocaulon humile*, *Heracleum concanense*, *H. pinda*, *Dipcadi minor*, *D. ursulae*, *Gymnema cuspidatum*, *G. khandalense*, *Hypoestis lanata*, *Lepidagathis lutea*, *Maytenus puberula*, *Oianthus deccanensis*, *O. urceolatus* and *Scurrula stocksii*.

Some of the monotypic genera strictly confined to this area are: *Bhidea*, *Bonnayodes*, *Frerea*, *Pinda*, *Helicanthes*, *Pogonachne*, *Erinocarpus*, *Pseudodichanthium*, and *Seshagiria*.

- (ii) **Central Western Ghats:** It includes the area from river Kalinadi to Coorg. Some of the important endemic species of this region are: *Acanthopale jogensis*, *Aglaia littoralis*, *Bulbophyllum mysorensis*, *Calamus nagbettai*, *Croton lawianus*, *Eulophia emiliana*, *Glyphochloa mysorensis*, *Hoya retusa*, *Hugonia belli*, *Iphigenia mysorensis*, *I. veldkampii*, *Ischaemum dalzellii*, *Loxoma maculata*, *Luisia macrantha*, *Memecylon terminale*, *Marsdenia raziana*, *Nervilia hispida*, *Oberonia brachyphylla*, *O. josephii*, *Ochlandra talbotii*, *Oldenlandia prainiana*, *O. sedgewickii*, *Paracautleya bhattii*, *Phalaenopsis mysorensis*, *Phlebophyllum canaricum*, *Phyllanthus talbotii*, *Psychotria canarensis*, *Schizachyrium sudhanshui*, *Tarenna agumbensis*, *Theriophonum uniseriatum*, *Vernonia dalzelliana*, *V. ornata*, etc. Some of the very rare endemic species of this region are: *Caralluma truncato-coronata*, *Cynoglossum ritchiei*, *Hubbardia heptaneuron*, *Leea talbotii*, *Leucas angustissima*, *Neanotis ritchiei* and *Viscum mysorensis*.
- (iii) **South Western Ghats:** This region is a conglomeration of several hill ranges particularly Travancore, hills of Kerala and Nilgiri, Anamalais, Palni, Tirunelveli hills of Tamil Nadu. There are about 1286 endemic genera in southern Western Ghats alone (Nayar 1996).

Some of the important endemic genera of this region include: *Anaphyllum*, *Ascopholis*, *Baeolepis*, *Blepharistemma*, *Carvia*, *Danthonidium*, *Diplocentrum*, *Erinocarpus*, *Haplothismia*, *Hubbardia*, *Hyalisma*, *Indobanalia*, *Indotristicha*, *Jardonia*, *Meteromyrtus*, *Nanothamnus*, *Nilgirianthus*, *Taeniandra*, *Uleria*, *Willisia*, *Janakia*, *Kanjaram*, *Limnopoa*, *Otonephium* and *Silentvalleya*.

Some of the important endemic species in the **Travancore region** are: *Aglaia maiae*, *Asteriastigma macrocarpa*, *Begonia aliciae*, *Bombax scopulorum*, *Buchanania barberi*, *B. lanceolata*, *Clematis bourdillonii*, *Colubrina travancorica*, *Cosciniium fenestratum*, *Cyclea fissicalyx*, *Cynometra beddomei*, *Dysoxylon beddomei*, *D. ficiforme*, *Garcinia imbertii*, *Impatiens aliciae*, *I. anaimudica*, *I. cochinnica*, *I. coelotropis*, *I. johnii*, *I. leptura*, *I. macrocarpa*, *I. murmarensis*, *I. pallidiflora*, *I. Pandata*, *I. Platyadena*, *I. rivulicola*, *Loeseneriella bourdillonii*, *Paphiopedilum druryi*, *Phaeanthus malabaricus*, *Polyalthia rufescens*, *Pterospermum reticulatum*, *Sageraea grandiflora*, *Schefflera bourdillonii*, *Smithia venkoborowii*, *Taeniophyllum scaberulum*, *Vanilla wightiana*, etc.

Some rare endemic species in the **Nilgiri region** are: *Agrostis schmidii*, *Andropogon longipes*, *Andrographis lawsonii*, *Arisaema tuberculatum*, *A. tylophorum*, *Bulbophyllum fuscopurpureum*, *Carex christi*, *C. pseudoaperrata*, *Cinnamomum perrottetii*, *Clematis theobromina*, *Coelogyne angustifolia*, *Eria albiflora*, *Eriocaulon gamblei*, *Garnotia schmidii*, *Habenaria denticu-*

lata, *H. fimbriata*, *Impatiens neo-barnesii*, *I. nilagirica*, *Lasianthus ciliatus*, *Liparis biloba*, *L. duthiei*, *Mackenzica homotropa*, *Memecylon sisparensense*, *Microtropis densiflora*, *Ophiorrhiza pykorensis*, *Ochlandra beddomei*, *O. setigera*, *Rhododendron arboreum ssp. nilagiricum*, *Pavetta hoheneckeri*, *Senecio kundaicus*, *Sonerila elegans*, *Thunbergia bicolor* and *Youngia nilagiriensis*.

Some rare endemic species of **Anamalais** are: *Acrocephalus wightii*, *Antistrophe serratifolia*, *Desmos viridiflorus*, *Didymocarpus fischeri*, *Didyplosandra andersonii*, *Hedyotis anamalayana*, *Helichrysum perlanigerum*, *Impatiens wightiana*, *Liparis platyphylla*, *Peucedanum anamallayense*, *Premna paucinervis*, *Pseudoglochidion anamalayanum*, *Salacia beddomei*, *Symplocos anamalayana*, *Trichosanthes anamallayana*, and *Vernonia recurva*.

Some endemic species restricted to **Palni hills** are: *Anaphalis beddomei*, *Crotalaria conferta*, *C. fysoni*, *Isachne angladei*, *Ixora saultierei*, *Liparis beddomei*, *Ophiorrhiza roxburghiana*, *Pimpinella pulneyensis*, *Sonerilla pulneyensis*, *Vernonia fysoni*, *V. pulneyensis*, etc.

Endemic species of **Tirunelveli hills**, which form the Southern most tip of Western Ghats include: *Aerva wightii*, *Ehretia wightiana*, *Exacum courtalense*, *Fimbristylis contorta*, *Hedyotis barberi*, *Hetaeria ovalifolia*, *Hopea erosa*, *Nothopegia aureofulva*, *Piper barberi*, *Plectranthus beddomei*, *Psychotria globicephala*, *Symplocos barberi*, etc.

- (iv) **Eastern Ghats:** It can be divided into northern Eastern Ghats which include regions such as Ganjam-Koraput and Visakapatnam hill and southern Eastern Ghats, which include regions such as Nallamalais, Cuddpah, Tirupathi and Shevaroy hills.

The hills of Ganjam – Koraput ranges have endemic species like: *Acacia donaldii*, *Aglaiia haslettiana*, *Aspidopteris hutchinsonii*, *Dimeria orissensis*, *Mucuna minima*, *Oryza jeyporensis*, *Themeda mooneyi*, *T. saxicola*, *Tragia gagei*, *Uvaria eucineta*, etc. The hills of Visakhapatnam harbour endemic species like *Nilgirianthus circarensis*, *Toxocarpus roxburghii*, *Argyreia arakuenensis*, *Kalanchoe cherukondensis*, *Phyllanthus narayanswamii*, *Memecylon madgolense*, etc. Other important endemic species include: *Maytenus bailadiliana*, *Atylosia cajanifolia*, *Wendlandia gamblei*, *Phlebophyllum jeyporensis*, *Leucas mukerjiana*, *Bupleurum andhricum*, etc.

The southern Eastern Ghats have many interesting endemic plants. The important ones are: *Pterocarpus santalinus*, *Boswellia ovalifoliolata*, *Pimpinella tirupatiensis*, *Shorea tumbuggaia*, *Argyreia choisyana*, *Boswellia ovalifolia*, *Crotalaria sandoorensis*, *Chamaesyce senguptae*, *Andrographis nallamalayana*, *Eriolaena lushingtonii*, *Crotalaria madurensis var. kurnoolica*, *Dicliptera beddomei*, *Premna hamiltonii*, *Dioscorea kalkapershadii*, *Neuracanthus neesianus*, *Cordia domestica*, *C. evolutior*, *Cycas beddomei*, etc. (Chowdhery and Murti 2000).

Andaman and Nicobar Islands exhibits a high percentage, i.e., more than 11% of endemic species. This is due to their isolation from other land areas, extreme competition for space and light result in their confinement to very limited areas. Rao

(1986) reported 187 species as endemic out of 1454. Balakrishnan et al. (1989) reported 144 species, endemic to Andaman group of Islands and 74 species endemic to Nicobar Islands.

Some of the endemic, rare and endangered species found in Andaman and Nicobar Island are: *Antidesma andamanicum*, *Ardisia andamanica* var. *effusa*, *Adenia heterophylla* ssp. *andamanica*, *Aerides emericii*, *Anoectochilus nicobaricus*, *Artabotrys nicobarianus*, *Aglaonema nicobaricum*, *Amorphophallus carnosus*, *A. longistylus*, *A. oncophyllus*, *Bentinckia nicobarica*, *Bombax insigne* var. *polystemon*, *Bridelia kurzii*, *Boesenbergia albo-lutea*, *Crinum pusillum*, *Calamus dilaceratus*, *C. nicobaricus*, *Corypha macropoda*, *Connarus nicobaricus*, *Cleistocalyx nicobaricus*, *Cyperus kurzii*, *Cyrtandra burtii*, *C. occidentalis*, *Dendrobium tenuicaule*, *Diplospora andamanica*, *Ellipanthus calophyllus*, *Excoecaria rectinervis*, *Embelica microcalyx*, *Eulophia nicobarica*, *Ficus andamanica*, *Garcinia cadelliana*, *G. calycina*, *G. kingii*, *Glochidion andamanicum*, *Ginolla andamanica*, *Globba pauciflora*, *Hippocratea andamanica*, *H. nicobarica*, *Hypolytrum balakrishnanii*, *Habenaria andamanica*, *Hedyotis andamanica*, *H. congesta* var. *nicobarica*, *Henslowia erythrocarpa*, *Ixora andamanica*, *I. hymenophylla*, *I. longibractea*, *Jasminum andamanicum*, *J. unifoliolatum*, *Kaempferia siphonantha*, *Korthalsia rogersii*, *Litsea leiantha*, *Mangifera andamanica*, *Mimusops andamanensis*, *Miliusa tectona*, *Mitrephora andamanica*, *Mesua manii*, *Malleola andamanica*, *Neolitsea andamanica*, *N. nicobarica*, *Orophaea salicifolia*, *O. torulosa*, *Ophiorrhiza nicobarica*, *Phalaenopsis speciosa*, *Prismatomeris andamanica*, *Psychotria andamanica*, *P. tylophora*, *Pubistylis andamanensis*, *Sageraea listeri* var. *andamanica*, *Stephania andamanica*, *Strychnos narcondamensis*, *Syzygium andamanicum*, *S. kurzii* var. *andamanica*, *Scutellaria andamanica*, *Taeniophyllum andamanicum*, *Uvaria nicobarica*, *Urophyllum andamanicum*, *Wendlandia andamanica*, *Zeuxine andamanica*, and *Z. rolfiana*, etc. (Chowdhery and Murti 2000).

3.5 Conclusions

India with its wide range of climatic, topographic and ecological habitats is endowed with a rich diversity of medicinal and aromatic plants. Unfortunately, this has not been satisfactorily documented and utilized. Different phytogeographical regions provide different habitats for the variety of MAPs, which in turn provide the raw materials for pharmaceutical and flavoring industries. In this chapter, along with the major vegetation of each phytogeographical region, the list of wild species of MAP's is provided which can certainly help the future scientists to excavate the rich resources and produce the health care products.

India has over 40 sites of high endemism. The author tried to enlist a number of endemic plants at different sites with specific reference to Western Himalayas, Eastern Himalayas, Eastern India, Peninsular India including Eastern and the Western Ghats. Knowing the facts about the confinement of these endemic species

in particular regions of India, our future taxonomists, ecologists, phytochemists, and genetic engineers can definitely concentrate on these species to improve the production of raw materials. Moreover, the conservation of these highly valuable endemic medicinal and aromatic plants is a challenging task to be taken by future biological researchers/scientists.

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Chapter 4

Chemical Diversity and Ethnobotanical Survey of Indian Medicinal and Aromatic Plants Species



Satyanshu Kumar, Ashish Kar, Jinal Patel, Sharad Kumar Tripathi, Raghuraj Singh, and Padamnabhi Shanker Nagar

Abstract Plants are the source of important drugs. Their secondary metabolite content is subject to environmental influences. Scientific evaluation of the chemical diversity of plants may be useful in exploring their medicinal as well as other uses. Still there are numerous medicinal plants for which no results either of ethnopharmacological uses or phytochemical studies could be found in the literature. Ethnobotany deals with the relationship between humans and plants. It has played an important role in the development of new drugs for centuries. Ethnobotany is attracting professionals from diverse academic backgrounds and interest. Ethnobotany may play an important role in securing sustainable supplies, and it can also be of use in the search for new medicinal receipts which could be used to treat diseases for which no standard therapy has been reported. Harnessing chemical diversity based on phytochemical research of species containing potentially active principles would be more relevant in context of ethnobotanical research.

Keywords Biodiversity · Secondary metabolites · Ethnomedicine · Traditional medicine

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4.1 Introduction

Secondary metabolites from plants are rich sources of bioactive compounds bringing forth many health beneficial effects in man and animals. Worldwide, medicinal plants have played a key role in providing health care. Since antiquity, medicinal plants have been employed in the treatment of a wide range of diseases and health conditions. Plants still continue to make important contributions both in providing lead molecules for further pharmaceutical research and as alternative sources of efficacious medication even in spite of the spectacular advances in the discovery of novel drugs that have occurred over the last few decades (Cragg et al. 1997; Shu 1998). Since time immemorial, plant products have been part of phytomedicines. Phytomedicines are composed of plant organs supplied either in natura (generally leaves, root or bark) or in processed form (typically liquid or powdered extracts). Commercial extracts normally contain the active principles of the plant material in crude or processed state, together with excipients, i.e. solvents, diluents or preservatives. The source and quality of the raw materials play a pivotal role in guaranteeing the quality and stability of the herbal preparations (Calixto 2000).

Scarcity coupled with strong demand on drugs has led to the cultivation of medicinal plants (Chopra et al. 1958). In an attempt to discover new drugs, multinational pharmaceutical companies typically spend an annual amount of US\$ 110 billion (Nair et al. 2014). A vast majority of medicinal plants have been recklessly exploited. Therefore it is an imperative to rationalize the use of important medicinal plants (Sultan et al. 2008).

Bioactive phytochemicals are naturally occurring compounds present in or derived from a plant (Hardy 2000). Bioactive compounds of plant origin are those secondary metabolites that possess desired health/ wellness benefits for man and/or animals. These metabolites are both chemically and taxonomically extremely diverse compounds with frequently obscure functions (Yadav and Agarwala 2011).

Phytochemicals may function as antioxidant (protect cells against oxidative damage), antiproliferative (interfere with replication of undesirable cancerous cell), carcinogen detoxifier, hypocholesterolemic, stimulant of enzymes and hormones, antibacterial and antiviral, anti-inflammatory, ligand to cell wall (some phytochemicals bind physically to human cell thereby preventing the adhesion of pathogens) and potential inhibitor of different actions affecting the initiation and progression of several pathogenic processes (Kaur and Das 2011).

Secondary metabolites are frequently called the vast “Chemical library” of biological systems. Most of the drugs, herbs, ethnomedicines, essential oils, perfumes and cosmetics derive from them. Cultivation is an important practice to conserve endangered medicinal plants growing in the wild and it works as a practical method to make available natural raw materials without affecting their actual habitat (IUCN 1993). A prerequisite for breeding is the study of genetic diversity of available plant germplasm (Bernáth 2002). Germplasm characterization is necessary to enhance

germplasm management and utilization. Genetic diversity is influenced by habitat types and the altitudinal range (Jugran et al. 2013, 2015). Diversity classification in germplasm collections is important for both plant breeding and germplasm collection. High diversity is an indicator of better adaptability of a population as a result of more fitness under rapidly changing environment. Wild plant species which can adapt easily in any conditions are always suitable for domestication or cultivation (Dhiman et al. 2020). The most important goals of any medicinal plant breeding program are to improve the morphological characteristics and increase the accumulation of biologically active substances. The quantitative and qualitative status of active constituents along with genetic diversity for a medicinal plant is the basis to devise conservation strategies and select right samples for maximum yields. Conservation strategies for populations should take into account both genetic diversity and chemical variation levels, especially in the case of populations having high differentiation to bioclimatic factors and the geographic location of populations (Nair et al. 2014).

Chemical constituents of MAP are the basis of their exploitation (Heywood 2002). Chemical markers are the group of chemical constituents derived from herbal/medicinal products. Chemical markers play an important role also in the quality control of herbal products and medicines. Chemical diversity plays an important role also in plant adaptation (Dhiman et al. 2020). Species, strains and geographical origin can be distinguished using chemical fingerprinting. It is imperative to identify elite plants/population based on their chemical attributes to ensure the quality of plant material (Jugran et al. 2015).

Association between the molecular markers and the phytochemical markers has been found to provide the best method of assessment of plant genetic diversity. This approach is used to screen and improve the gene pool of elite genotypes (Ray et al. 2019; Qaderi et al. 2019; Nair et al. 2014; Hennenke et al. 2016).

4.2 Chemical Diversity in Selected Threatened Indian Medicinal Plants

A species that has been described as species with small population is not presently endangered but is at risk. A species which is in danger of extinction throughout all or a significant portion of its range has been categorized as an endangered species. Threatened is a species that is likely to become endangered in the foreseeable future (IUCN 1978; Bryde 1979; Nayar and Sastry 1990). The chemical diversity in nine high value endangered medicinal plants of India is briefly described in terms of their major phytochemical principles. These species are not found in cultivation. They are collected from nature; therefore, their ecological study needs to be brought to the forefront, in addition to their *in-situ* conservation.

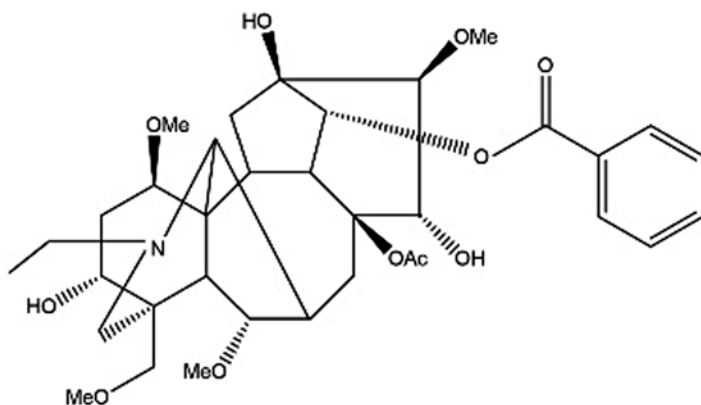


Fig. 4.1 Chemical structure of aconitine

4.2.1 *Aconitum heterophyllum*

Aconitum heterophyllum (Family: Ranunculaceae) is widely distributed across North Asia and North America. Worldwide about 300 species of *Aconitum* are found and 27 species, in India (Paramanick et al. 2017; Sharma et al. 1993). *A. heterophyllum* possesses potential immunomodulatory activity (Murayama and Hikino 1984; Weiner 1990). Content of aconitine (Fig. 4.1), an alkaloid, varies from species to species and also with place of origin (Prasad 2000; Hikino et al. 1983; Iwasa and Naruto 1996, Song et al. 1984). Variation of aconitine content in *A. chasmanthum* and *A. heterophyllum* from Kashmir Himalayas was reported by Jabeen et al. (2011). Aconitine content varied from 0.0310% to 0.0320%, 0.0014% to 0.0018% in *A. chasmanthum* and *A. heterophyllum*, respectively.

4.2.2 *Ephedera foliata*

Ephedera Linnaeus is a genus of about 40 species. Eight *Ephedera* species from India and adjoining regions were listed by Sahni (1990). Three additional species namely *E. kardangensis*, *E. khurickensis* and *E. sumlingensis* were also reported recently (Sharma and Singh 2015). *E. foliata* is a typical component of arid and semi arid regions of North-Western parts of India (Bhandari 1978). It is harvested on commercial basis in Gujarat (Gavali and Sharma 2004). However, over exploitation, extensive habitat destruction, very slow growth rate, poor regeneration, grazing and other anthropogenic pressure have caused tremendous reduction in its natural populations. It has now become a rare or endangered species from a vulnerable category (Kharin 2002; Joshi et al. 2013) and it is considered as a threatened

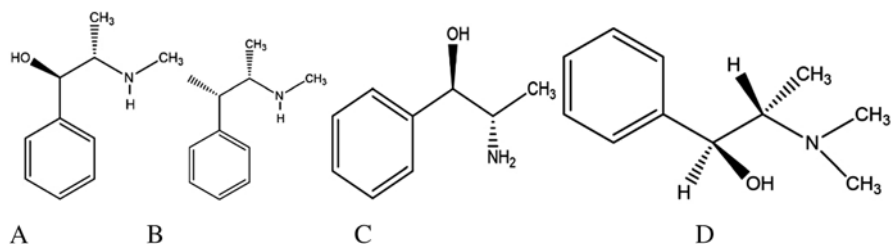


Fig. 4.2 Chemical structure of (a) (–)-ephedrine (b) (+)-pseudoephedrine (c) (–)-norephedrine (d) (+)-methylephedrine

species in India (Meena et al. 2019). Whole plant of *E. foliata* is used in fever, blood purification, asthma, dropsy, snake bite and as cardio tonic (Silori et al. 2005; Quattrocchi 2012). The major active principle in *Ephedra* is (–)-ephedrine and (+)-pseudoephedrine. Other minor alkaloids include (–)-norephedrine, (+)-methylephedrine (Fig. 4.2). Depending upon the species, the total alkaloid content in *Ephedra* can exceed 2% (Bruneton 1995; Chaudhary et al. 2020). The alkaloid content in Indian *Ephedra* ranged from 0.28–2.79% (Chauhan 1999; Polunin et al. 1987). Chaudhary et al. (2020) reported variation of metabolite content in *Ephedra* within same phytogeographical region of Kashmir Himalayas. The climatic conditions, physical and chemical property of soil (pH, soil moisture, macro-micro-nutrients, etc.) and other edaphic factors were attributed for the variation of metabolites content in *Ephedra* from same phytogeographical region.

4.2.3 *Malaxis acuminata*

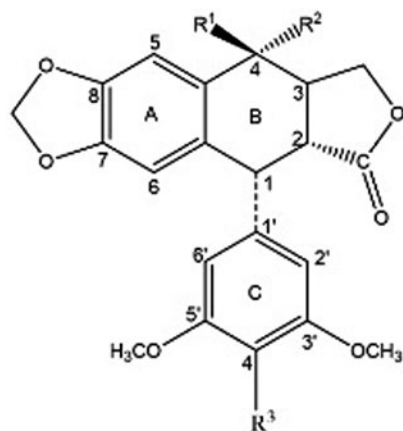
Malaxis acuminata is commonly known as “Jeevaka”. It is a small, perennial, pseudobulbous terrestrial orchid with pale yellowish-green to pinkish flowers in terminal racemes (Bose et al. 2017). The dried pseudobulbs are important ingredient of very reputed Ayurvedic drug “Ashtavarga” and also used in the preparation of polyherbal tonic “Chyavanprash” (Cheruvathur et al. 2010; Govindarajan et al. 2007). Due to the rapid loss of forest cover, jhum cultivation, etc., *M. acuminata* has become threatened in the nature. It has been listed in CITES (Conservation of International Trade of Endangered Species of Wild Fauna and Flora) Appendix II (Jalal 2012; Lohani et al. 2013). Secondary metabolite profiling in various parts of *M. acuminata* was reported by Bose et al. (2017). Presence of fatty acids, α -hydroxy acids, phenolic acids, sterols, amino acids, sugars and glycosides were reported using GC-MS analysis of methanolic extracts of leaves and stems of wild as well as *in vitro* plantlets of *M. acuminata*.

4.2.4 *Pterocarpus marsupium*

Pterocarpus marsupium is distributed in Central, Western and Southern regions of India (Devgun et al. 2009). It is listed as vulnerable plant in the INCN red data list (IUCN 2017). The Ayurvedic System of Medicine strongly recommends water stored in a tumbler made from hardwood of *P. marsupium* for effective diabetes control (Chopra et al. 1958; Jain 1968; Satyavathi et al. 1987). Mohankumar et al. (2012) reported that a high molecular constituent obtained from the fractionation of the aqueous extract of *P. marsupium* hardwood had potent insulinotropic and insulin like properties. The heartwood of *P. marsupium* is important source of pterostilbene. Other secondary metabolites such as epicatechin, pterocarpol, pterosupin, pterocarposide and marsuposides have also been reported from *P. marsupium* (Teixeira da Sliva et al. 2018).

4.2.5 *Podophyllum hexandrum*

The rhizomes of *Podophyllum hexandrum* are well known in medicine, as a source of podophyllin resin. Podophyllotoxin (Fig. 4.3) is the major lignan present in the resin and it is the starting material of etoposide. Vepesid, commercial name of etoposide, is an FDA approved anticancer drug used to treat testicular as well as lung



Compound	R ¹	R ²	R ³
1 DPT	H	H	OCH ₃
2 PPT	H	OH	OCH ₃
3 4'-DMEP	OH	H	OH

Fig. 4.3 Chemical structure of podophyllotoxin

cancer by inhibiting replication of cancer cells (Becker 2000; Henderson 2000; Jackson and Dewick 1984). Podophyllotoxin preparations are also commercially available to treat genital warts (Beutner 1996). The Indian *P. hexandrum* is superior to its American species *P. peltatum* in terms of its higher podophyllotoxin content (higher than 5%) in dried roots in comparison to only 0.25% of *P. peltatum* (Panda et al. 1992; Mishra et al. 2005). Sultan et al. (2008) reported high diversity in the concentration of marker compounds (podophyllotoxin β -D-glycoside and podophyllotoxin) in 36 individuals from 12 accession of *P. hexandrum*.

4.2.6 *Rauvolfia serpentina*

The genus *Rauvolfia* comprises of 80 species and is represented by five species namely *R. hookeri*, *R. micrantha*, *R. serpentina*, *R. verticillata* and *R. tetraphylla* (Bindu et al. 2014). *R. serpentina* has been designated as critically endangered in India and is included in Appendix II of CITES, which restricts its export (Singh et al. 2010). Population of *R. serpentina* declined more than 50% during the period of 1985–1995 due to loss of habitat and overexploitation (Bindu et al. 2014). Government of India has banned export of this species from the wild in order to prevent over exploitation of this species (Sukumaran and Raj 2008). Roots are being indiscriminately collected from the wild to meet the growing demands of the pharmaceutical industry and this has rendered listing the species “endangered”.

Indole alkaloids such as reserpine, reserpiline, rescinnamine, ajmaline, ajmalicine, rauwolfinine, serpentine, serpentinine and yohimbine, etc. have been reported from *Rauvolfia* species (Sahu 1983; Gao et al. 2012). Reserpine is the most prominent among these alkaloids (Nair et al. 2014). Reserpine (Fig. 4.4) has been known, documented and used to treat snakebites and insanity, however, the main use of the drug is as a sedative and hypnotic and for reducing blood pressure. The drug is now largely used in insanity and high blood pressure. It is more suitable for cases of mild anxiety or patients of chronic mental illness (Bleuler and Stoll 1955).

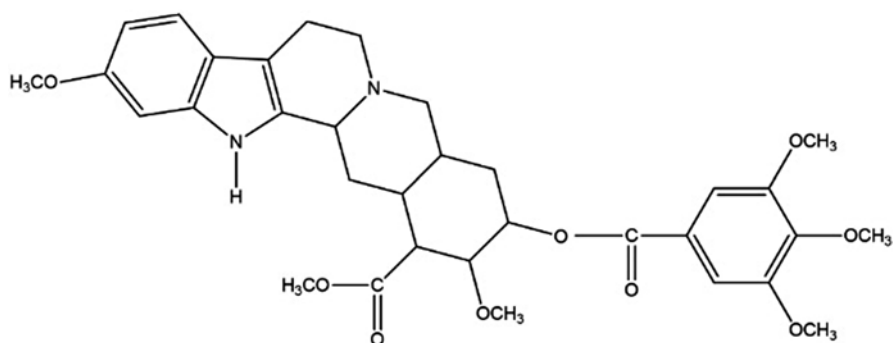


Fig. 4.4 Chemical structure of reserpine

Bindu et al. (2014) reported that reserpine content was highest for *R. tetraphylla* (450.7 µg/g dry wt.) among five *Rauvolfia* species from India. Reserpine content was comparatively low in the *R. serpentina* (254.8 µg/g dry wt.). Reserpine content in 10 population of *R. serpentina* ranged from 0.192 g/100 g to 1.312 g/100 g. *R. micrantha*, which is an endemic *Rauvolfia* species in India, had significantly higher reserpine (422.1 µg/g dry wt.) content (Bindu et al. 2014). Chemical synthesis of reserpine is economically still not feasible (Farooqi and Sreeramu 2001). Significantly higher reserpine content in *R. micrantha* makes this species a suitable candidate as a source of reserpine, replacing *R. serpentina* and *R. tetraphylla*.

4.2.7 *Rheum emodii*

The genus *Rheum* belonging to family *Polygonaceae* encompasses about 60 species. It is mainly distributed in the temperate and subtropical Asia (Anjen et al. 2003). Seven species from this genus have been reported from India (Hooker 1885). *Rheum emodii* is found at an elevation of 2000–3800 m in the temperate Himalayas from Kashmir to Sikkim (Zargar et al. 2011). Phytochemicals from *Rheum emodi* have been reported to possess antioxidant, antidiabetic, antimicrobial, antifungal, cytotoxic, hepatoprotective and nephroprotective activities. *R. emodii* has been used an ingredient in many herbal formulations used for treatment of various diseases, in particular for the regulation of blood fat, hepatitis and cancer (Zargar et al. 2011). Rhizome is the source of major phytochemicals from *R. emodii*. Free anthraquinones and their glycosides are the major phytochemicals from *R. emodii* (Fig. 4.5). Anthraquinone with carboxyl group (Rhein) and without carboxyl group including chrysophanol, aloe-emodin, emodin, physcion, chrysophanein and emodin glycoside and alkyl derivatives of anthraquinone namely 6- methyl rhein and 6-methyl aloe-emodin have also been reported from *R. emodii* (Malik et al. 2010; Singh et al. 2005). Anthrone C-glycoside derivatives, such as oxanthrone, ether (revandchinone-2) and revandchinone-3 have also been reported from *R. emodii* (Babu et al. 2003; Singh et al. 2005).

4.2.8 *Swertia chirata*

About 40 *Swertia* species are present in India. These species are randomly dispersed in the Western and Eastern Himalayan regions and Western Ghats. *Swertia* herb is used as a principal component in several commercial polyherbal formulations. *S. chirata* is known as the most well-known and elite species of *Swertia* (Kaur et al. 2019b). Amarogentin, swertiamarin (Fig. 4.6) and mangiferin are responsible for the therapeutic potential of *Swertia* (Kumar and Van Staden 2015). Kaur et al. (2019b) reported phytochemical diversity among 48 accessions of five *Swertia*

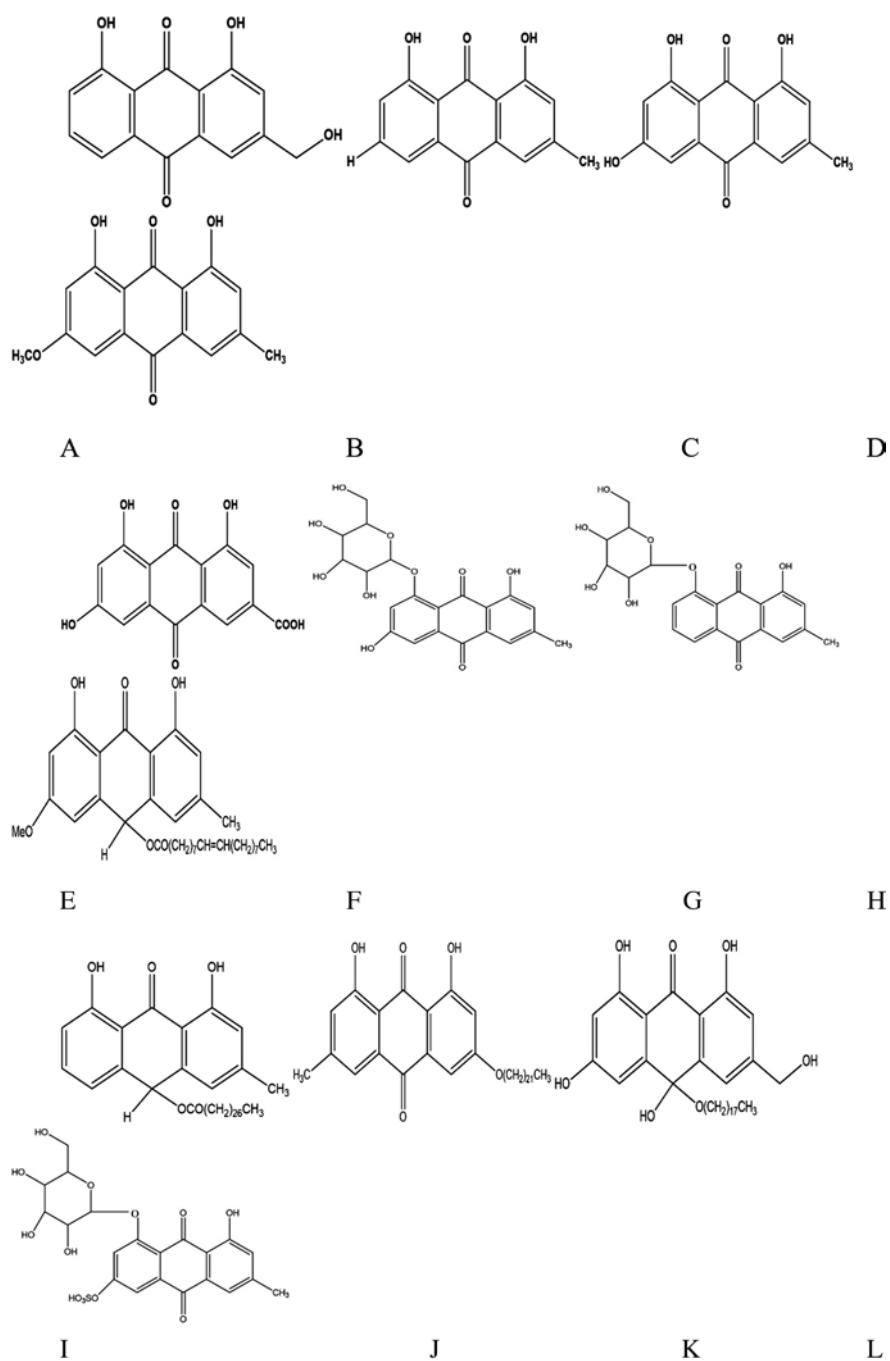


Fig. 4.5 Chemical structures of (a) aloemodin (b) crysophanol (c) emodin (d) physicon (e) rhein (f) emodin glucoside (g) crysophanol glucoside (h) revandchinone-I, (i) revandchinone-II, (j) revandchinone-III (k) revandchinone-IV and (l) sulfemodin -8-O-glucoside

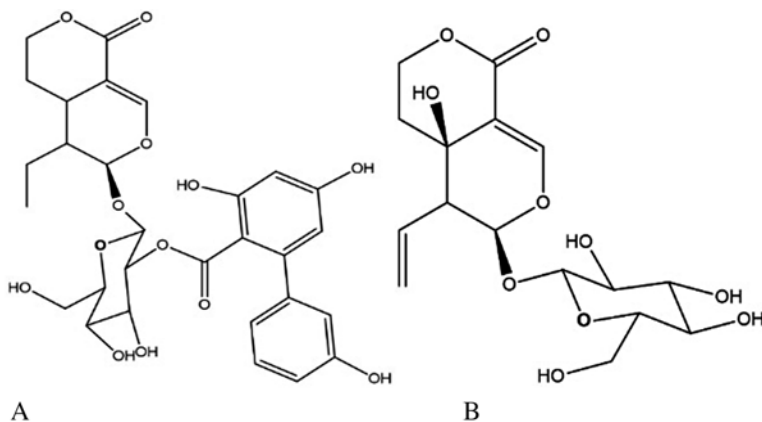


Fig. 4.6 Chemical structure of (a) amarogentin and (b) swertiamarin

species collected from Western Himalaya, India (*S. chirata*, *S. cordata*, *S. nervosa*, *S. paniculata* and *S. angustifolia*). Maximum yield of amarogentin ($0.75 \pm 0.20\%$) and swertiamarin ($6.68 \pm 0.10\%$) was found in *S. chirata* accessions and it was followed by *S. paniculata* accessions that had 0.66 ± 0.10 amarogentin and $5.76 \pm 0.03\%$ swertiamarin content. Also, substantial amount of secoiridoid glycosides were recorded in accessions of *S. angustifolia*. Similar results were reported in another study that represented high level of phytochemical diversity in the *Swertia* species/population on the basis of four triterpenoids namely oleanolic acid, ursolic acid, betulinic acid and lupeol (Kaur et al. 2019a).

4.2.9 *Valeriana jatamansi*

Valeriana jatamansi belonging to family *Valerinaceae*, is commonly known as the Indian Valerian of Tagar. *V. jatamansi* is used in both traditional and modern systems of medicine. Among the top selling herbal supplements, the drug valerian ranks at eighth place (Blumenthal 2001). *V. jatamansi* has a long history of uses as a medicine in the Rigveda, Charak Samhita and modern medicine (Jugran et al. 2019). It is used as an ingredient in the preparation of 39 Ayurvedic formulations (Prakash and Mehrotra 1991; Rawat and Vashishta 2011). Valepotriates and valerenic acid (Fig. 4.7) derived from the roots/rhizomes of *V. jatamansi* and other related species of the genus are considered to constitute the chemical fingerprint of these species. These two phytochemicals are used for assuring the quality of the plants (Singh et al. 2006, 2010; Jugran et al. 2015). Valerenic acid has been reported to possess sedative and antispasmodic properties (Houghton 1999). Content of valerenic acid in *V. jatamansi* has been explored (Singh et al. 2006, 2010; Jugran et al. 2015). Jugran et al. (2015) reported significant variation in valerenic acid content (%) in aerial and root portions of 25 population of *V. jatamansi*. It was in the

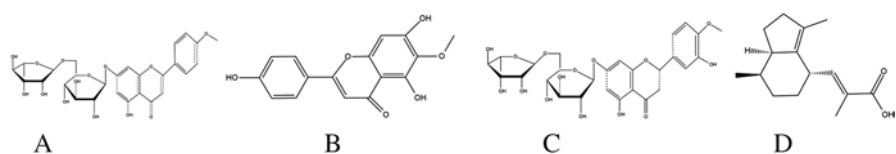


Fig. 4.7 Chemical structure of valepotriates (a) linarin (b) 6-methylapigenin (hispidulin) (c) hesperidin and valeric acid (d)

Table 4.1 Chemical diversity of nine selected Indian medicinal plants

Medicinal plant	Selected phytochemical markers	Reference
<i>Aconitum heterophyllum</i>	Aconitine	Jabeen et al. (2011)
<i>Ephedera foliata</i>	Alkaloid (ephedrine, (-)-ephedrine and (+)-pseudoephedrine, (-)-norephedrine, (+)-methylephedrine)	Bruneton (1995), Chaudhary et al. (2020)
<i>Malaxis acuminata</i>	Fatty acids, α -hydroxy acids, phenolic acids, sterols, amino acids, sugars and glycosides	Bose et al. (2017)
<i>Pterocarpus marsupium</i>	Pterostilbene, epicatechin, pterocarpol, pterosupin, pterocarposide and marsuposides	Teixeira da Sliva et al. (2018)
<i>Podophyllum hexandrum</i>	Podophyllotoxin	Sultan et al. (2008)
<i>Rauwolfia serpentina</i>	Reserpine, reserpiline, rescinnamine, ajmaline, ajmalacine, rauwolfinine, serpentine, serpentinine and yohimbine	Sahu (1983), Gao et al. (2012), Nair et al. (2014)
<i>Rheum emodi</i>	Anthraquinone, alkyl derivatives of anthraquinone, anthrone C-glucosides	Malik et al. (2010), Singh et al. (2005), Babu et al. (2003)
<i>Swertia species</i> (<i>S. chirata</i> , <i>S. cordata</i> , <i>S. nervosa</i> , <i>S. paniculata</i> and <i>S. angustifolia</i>)	Amarogentin, swertiamarin Oleanolic acid, ursolic acid, betulinic acid and lupeol	Kaur et al. (2019b) Kaur et al. (2019a)
<i>Valeriana jatamansi</i>	Valeric acid	Jugran et al. (2015)

range of 0.13 ± 0.01 to 0.57 ± 0.04 and 0.20 ± 0.03 to 1.70 ± 0.02 for aerial parts and roots, respectively.

The major phytochemicals present in the selected nine medicinal plants have been described in Table 4.1. Selected major phytochemical marker compounds may find use as reference for the purpose of certification of authentic materials, processing, quality control and value addition for post cultivation management. This information could be utilized to explore the cultivation prospects of these species in terms of technical and economical feasibility as well as marketability.

4.3 Ethnobotanical Research of MAP in India

4.3.1 Role of Ethnic Knowledge in Drug Discovery

Ethnobotanical knowledge is still transmitted from generation to generation chiefly by word of mouth. The botanical collections of early explorers and the later ethnobotany have played an important role in the development of new drugs for many centuries. In the middle of the last century interest in this approach had declined dramatically, but has risen again during last decade, when also new focuses have developed. Paul and Balick (1994) pointed out some important drugs discovered in different parts of the world, based on the ethnomedicinal knowledge (Table 4.2). Historically, much corporate drug discovery has depended on indigenous knowledge delivered to modern science through ethnobotany. Over 50% of modern prescription medicines have originally been discovered from plants and the reason behind that is that the plants were used in indigenous medicine and some common drugs were first used only on a local scale. In Europe, for example, aspirin was first isolated from *Filipendula ulmaria* because it had long been used in folk medicine. Another European folk cure that has become a drug was derived from *Digitalis purpurea*. The leaves of this plant were first used to treat congestive heart failure. Its active ingredients, digitoxin and digoxin have remained an important treatment for heart ailments. Balick and Cox (1996) showed that at least 89 plant-derived medicines used in the industrial world had originally been discovered by studying indigenous medicine. Among them, the best known is quinine, used in South America to treat fever. This has been the single most effective cure for malaria. Quinine comes from the bark of *Cinchona* trees that grow in the Andean region. More recently, the drugs vincristine and vinblastine were discovered in the rosy periwinkle (*Catharanthus roseus*) from Madagascar. When the Eli Lilly Company studied this plant, they found that the periwinkle had anti-cancer properties. Vincristine has given children with leukemia a likelihood of remission and vinblastine has cured many people with Hodgkin's disease. Native American peoples used the mayapple (*Podophyllum peltatum*) to treat warts. Two important drugs have been derived from

Table 4.2 Important drugs discovered on the basis of ethnomedicinal knowledge

Drug	Plant name	Medicinal use
Aspirin	<i>Filipendula ulmaria</i>	Reduces pain and inflammation
Codeine	<i>Papaver somniferum</i>	Eases pain and suppresses coughing
Ipecac	<i>Psychotria ipecacuanha</i>	Induces vomiting
Pilocarpine	<i>Pilocarpus jaborandi</i>	Reduces pressure in the eye
Pseudoephedrine	<i>Ephedra sinica</i>	Reduces nasal congestion
Quinine	<i>Cinchona pubescens</i>	Against malarial fever
Reserpine	<i>Rauwolfia serpentina</i>	Lowers blood pressure
Scopolamine	<i>Datura stramonium</i>	Eases motion sickness
Theophylline	<i>Camellia sinensis</i>	Open bronchial passages
Vinblastine	<i>Catharanthus roseus</i>	Combats Hodgkin's disease

it and are *teniposide* to treat bladder cancer and podophyllotoxin from which a powerful anti-tumor agent has been synthesized (Balick and Cox 1996).

To date, in India, based on ethnomedicinal knowledge, several drugs have been developed (Table 4.3). These are marketed by the pharmaceutical companies and research is going on in the development of drugs for some ailments including cardiovascular drugs from *Terminalia arjuna*; antidiabetic drugs from *Momordica charantia*, *Gymnema sylvestre* and *Andrographis paniculata*; antiprotozoal drugs from *Selaginella bryopteris*; anti malaria drugs from *Azadirachta indica*, *Ancistrocladus heyneanus*; for antileishmanial drugs *Diospyros spp.*, and *Plumbago spp.* (Bhutani and Gohil 2010).

4.3.2 Future Scope of Ethbotanical Research in India

Medicinal plant species still unknown from a phytochemical point of view, have been used to cure of ailments of different types. Ethnic population makes resort to traditional medicine because of difficult access to Western medicine as well as their high cost. These people use a wide range of plants therapeutically to maintain their health. There is great promise for new drug discovery based on traditional plant uses. Globally, 119 plant derived drugs from 90 plants are in use (Farnsworth and Morris 1985). Significantly, 77% of these were obtained as a result of examining the plants based on ethnomedical uses (Cordell 2000).

The value of ethnomedicine was recognized about six decades back in India with the pioneering work of Jain (1994). The Tropical Botanic Garden and Research Institute (TBGRI) conducted an ethnobotanical field study in the forests of south-west India in 1987. These forests are home to the Kani tribe, nomadic traditional

Table 4.3 Important drugs developed in India based on ethnomedicinal knowledge

Drug	Plant name	Medicinal use
Vasicine	<i>Adathoda zeylanica</i>	Bronchial disorder
Flavonoids	<i>Euphorbia prostrata</i>	Piles
Sennosides	<i>Cassia spp.</i>	Constipation
Baccosides	<i>Bacopa monnieri</i>	Memoray enhancer
Tylophorine	<i>Tylophora indica</i>	Bronchial disorder
Conessine	<i>Holarrhena antidysenterica</i>	Antiamoebic
Shatavarin	<i>Asparagus racemosus</i>	Tonic
Monoterpenes	<i>Ocimum sanctum</i>	Respiratory disease
Flavonoids	<i>Bauhinia variegata</i>	Diarrhoea, piles
Monoterpenes	<i>Cyperus rotundus</i>	Antibacterial
Boerhavinones	<i>Boerhavia diffusa</i>	Hepatoprotective
Anthocyanins	<i>Syzygium cumini</i>	Anti diabetic
Flavonoids	<i>Vitex negundo</i>	Anti inflammatory

collectors of non-timber forest products. The Kanis use a wild plant species for energy that they called arogyapacha, identified as *Trichopus zeylanicus* by the TBGRI. It provided a lead in the development of the drug “Jeevani” (giver of life) after the TBGRI transferred the manufacturing license to an Ayurvedic drug company in India. The TBGRI agreed to share 50% of the license fee and the 2% royalty on profits with the Kani (Anuradha 1998). Ethnobotany remains a fascinating and promising area of study for northeast India. The information about folk medicine of North-East India are still not gathered in systemic way or not documented in old literature, these are generally passed over generation to generation vocally. Multidisciplinary research and development work using the traditional folk medicinal plants based upon their traditional knowledge can provide deep motivation for identification of new pharmacophores. Newer approaches utilizing collaborative, multidisciplinary research on ethnomedicinal knowledge will help in near future in improving healthcare worldwide particularly from northeastern region of India. Some of the preliminary laboratory works carried out based on ethnobotanical knowledge in northeast India has been described here. Kar et al. 2005 carried out investigation based on traditional knowledge of Karbi and Hmar tribe of Assam on antimicrobial properties of extracts of *Curunga amara* Juss. against human pathogenic microorganisms. Tayung and Kar (2005) carried out investigation based on traditional knowledge of Monpa tribe of Arunachal Pradesh on antimicrobial activity of *Thalictrum javanicum* (Blume) root extracts against some human pathogens. Kalita et al. (2012) carried out antimicrobial study on *Paederia foetida* and *Hibiscus esculentus* which are generally used against stomach troubles, diarrhoea, hypertension, skin diseases, in urinary troubles and dental problem by the tribes of Assam. Similarly, *Hibiscus esculentus* extract was tested against the growth of *Staphylococcus aureus*. It possessed the potentiality against the growth of *E. coli*. Vijayakumar et al. (2012) carried out investigation on the *Illicium griffithii* fruit and seed from Arunachal Pradesh based on the ethnic knowledge. Phytochemical qualitative analysis revealed the presence of phenols, tannins, flavonoids, triterpenoids, steroids, alkaloids in the seeds. Ethyl acetate extract of fruits showed significant activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Yersinia enterocolitica*, *Vibrio parahaemolyticus*, *Salmonella paratyphi*, *Xanthomonas oryzae* and *Pseudomonas aeruginosa*; methanol extract showed activity against *S. aureus*, *Bacillus subtilis* and *Xanthomonas oryzae*. Haripyaree et al. (2013) carried out microbial investigation *Mimosa pudica* L, *Vitex trifolia* Linn, *Leucas aspera* Spreng, *Centella asiatica* (L) Urban and *Plantago major* Linn against antimicrobial screening of six organisms viz., *Ceratocystis paradoxa*, *Aspergillus niger*, *Penicillium citrinum*, *Macrophomina phaseoli*, *Trichoderma viride* and *Rhizopus nigricans* in Manipur and reported that *M. pudica* showed highest antifungal activities against more than one micro-organism. Methanol and hexane extract of *M. pudica* and *V. trifolia* showed moderate and strong activities against *C. paradoxa*. *C. asiatica* extract showed activity against *M. phaseoli*; *Leucas aspera* exhibited antifungal activity against pineapple fruit rotting fungus *C. paradoxa*. Kalita et al. (2012) carried out an investigation on local medicinal plants of Assam. Some of the research institutes of northeast India are doing research on drug formulation based on ethnic knowledge and few of them

are *Cajanus cajan* against jaundice, *Gomphostemma spp.* against malarial fever, *Terminalia spp.* against fungal diseases, *Oroxylum indicum* against cancer, *Dillenia indica* against diabetes. In addition to that some of research institute outside of northeast India collect plant sample from northeastern states and doing research in their laboratory. The above-mentioned species are some of the numerous examples only and there seems to be a possibility to explore more number of medicinal plants from this part of India for the development of useful drugs.

Existing rich ethnic heritage of India could be further explored through more dedicated ethnobotanical studies. It has also been gratifying that integrated forms of modern and traditional medicine have remained a part of reality. Information gathered from Ethnobotanical study would also be useful for conservation of traditional knowledge which is essentially required to save the cultural heritage of the natives. In this context confirmation of the therapeutical uses of the plants with scientific criteria and fostering phytochemical research on species containing potentially active principles would be more relevant for harnessing the value of ethnobotanical research carried out.

Silambarasan and Ayyanar (2015) carried out ethnobotanical studies in Eastern Ghats and recorded a total of 118 plants. Gairola et al. (2014) recorded a total of 948 plant taxa (923 angiosperms, 12 gymnosperms and 13 pteridophytes) belonging to 129 families, 509 genera, 937 species and 11 varieties from Jammu and Kashmir and Ladhakh for which no traditional medicinal use by indigenous communities of have been reported. Dey and De (2012) reported 56 plant species used against different types of gastrointestinal disorders like indigestion, stomach pain, vomiting tendency, constipation, piles, diarrhea, dysentery, cholera, loss of appetite, liver complaints, intestinal worms etc. Tetali et al. (2009) recorded 182 plants from Pune district of Maharashtra used by tribes and natives for different ailments. From these plants, 28 flowering plants were documented for diarrhoea. Amongst the 28 plants, antidiarrhoeal activity of five plants viz., *Caesalpinia sepiaria*, *Dioscorea pentaphylla*, *Launaea pinnatifida*, *Syzygium rubicundum* and *Ziziphus jujuba* has not been reported previously. Two species viz., *Ziziphus xylopyra* and *Syzygium rubicundum* are endemic to India. Bisht and Adhikari (2018) reported 70 medicinal plants from Uttarakhand which have been used against 31 ailments. Kaur et al. (2011) recorded 15 medicinal plant species used to treat leprosy, arthritis, nasal bleeding, ulcer etc. from Himachal Pradesh. Kaur et al. (2020) reported 51 plant species used to treat gastrointestinal disorder. Parul et al. (2017) recorded 18 medicinal plants from Harayana which have been used against digestive disorder.

4.4 Conclusions

Increasing demand on MAPs frequently comes along with the illegal overharvesting and unscrupulous collection practice of endangered plant species from the wild. Future strategies, regarding the conservation of the endangered plant species, are a major concern. Adaptation of some advanced plant biotechnological techniques,

namely micro-propagation, hairy root technologies and synthetic seed production may be useful in securing surplus supplies of such plant species to meet its future demand. Isolation of most effective compounds and development of analytical tools of various *in vitro* and *in vivo* studies may result numerous opportunities to further unravel the potential bioactivities of the species. Additionally, *in silico* molecular docking techniques may play an important role in the identification/design of the most effective molecules. These effective molecules may be synthesized from their analogues available in higher quantity to reduce the pressure on their natural habitats. Also, research on chemical diversity to identify the active constituents will open up opportunities to discover new chemotypes, as promising sources of drugs. There is a great scope for ethnobotanical studies as it points out to the species which most urgently should be studied scientifically. However, this approach in search of new pharmaceuticals is woefully underutilized.

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Chapter 5

Traditional Uses of Medicinal and Aromatic Plants Among the Tribes of India



Afroz Alam, Medha Jha, and Shah Faisal

Abstract Tribes of India are the centers of information on plant wealth and their different uses for the livelihood. They tend to use plants in various ways during their life. Herbal drug formulations used by these tribes are important also to the society. India is a rich diversity center of medicinal plants. Around 45,000 plant species nearly 15,000 plants are used for their medicinal values. Many of these plants are aromatic, but there has been nearly no such report available till date. Therefore, an attempt is made to compile a list of aromatic plants that are still being used by the Indian tribes for medicinal purposes. After a thorough study, overall 134 valid plant species are listed.

Keywords Aromatic plants · India · Medicinal plants · Traditional · Tribes

5.1 Introduction

Nature has an amazing blessing on India where almost all the climatic zones are found with huge diversity in terms of flora and fauna. The biodiversity hot spots are evidential proofs of this amazing country. As the existence of human beings is dependent upon the surroundings of any place hence the settlements of human beings have evolved in accordance with the various geographical zones. That is why, in India there is a huge difference among various communities that belong to different states in terms of their languages, costumes, living standards, rituals, food habits, etc.

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Though, over the years, the increasing urbanization throughout the country somewhat successful in lessening these differences among various communities yet the tribes of India are still maintaining their unique identity by conserving their ancient mode of living in forests. These tribes are the treasures of India and many efforts are going on to protect them in their natural habitats.

Like its floral and faunal diversity, India is very rich in tribal communities and at present most of states of the country have appreciable tribal populations. The majority of the tribal persons is far away from the much needed education and healthcare systems. They are still dependent on the natural resources to fulfil their daily needs except the formal education. However, they are very well educated by their surroundings and as a result their knowledge about the biodiversity is deeper than that of the so called modern and well-educated society (Pei 2001).

Especially in terms of floral diversity and its sustainable use these tribes are knowledgeable and in the era of herbal medication they are reservoirs of treasured information. Whenever, any researcher collects data about the plant uses by various tribes, the term ethnobotany appears. In the past, various ethnobotanists attempted to collect information from these tribes of the country and published very good articles. Those articles tell about the level of knowledge of Indian tribes regarding the plants. Though, till date there are several articles on ethnobotany have been published from different regions of the country, on different aspects, *viz.*, food plants; fodder plants; fibre plants; religious plants, plants in ritual uses, medicinal plants, etc., yet a huge amount of research must be done in this field (Dutta et al. 2014; Sharma and Alam 2018).

Ethnobotany has emerged as one of the most important sciences, on the front of social welfare. Seeing this facet of the subject, studies and intensive research, extensive plant surveys and field studies are being conducted in full swing in numerous universities, colleges, Government and non-governmental autonomous institutions, along with research institutes of higher level.

The wild plants in Indian folklore always have been a valuable reservoir and are being used to fulfill the various needs of the tribal people. Generally, it is projected that almost 15,000 species of plant are used by Indian tribes and other poor people. These plants provide almost everything to the tribes, *viz.*, food, fodder, clothing, shelter, drugs, medicine, agricultural implements, hunting, tranquilizers, toxin, masticatories, resins and dyes, fuels and insecticides etc.

In this manner it can be also said that the studies about human ecology and ethnobotany has attained a prime importance: it has become a critical need of modern ages, because ethnobotany deals with the direct, traditional, and natural relationships between plant wealth and human societies Ethnobotanical studies enable us to keep the records, and prepare proper documentation of the age old knowledge and wisdom of the tribal and rural people about the miraculous and useful properties of different plant species. In other words, ethnobotanical studies are considered as an important tool for the utilization of floristic diversity for the sake of human welfare (Jain 1994).

The enhancing interest of present-day workers in the field of ethnobiology, has resulted in the fact that biodiversity is now emerging as holistic segment of ecology. Therefore, studies related to ethnobotany undertake prodigious significance in enhancing our understanding about the floral wealth used by the tribal or native or rural people, the rich plant diversity assembled by these rural people or natives for their sustenance and the various methods followed by those people for the preservation and conservation of this information. Since the dawn of olden Indus Valley civilizations evidence proved the existence of a great dearth of ethnobotanical knowledge. Even the written records about the utilization of plant resources for the treatment and prevention of human or animal diseases can be traced back to the Vedic ages, i.e. the earliest from 4500 to 1600 B.C from the conventional scriptures of Hindu religion such as Rig Veda (Sharma and Alam 2018).

The traditional Indian indigenous structure of medicine popularly recognized as Ayurveda was splendidly established in the later parts of the vedic ages from 1500 to 800 B.C. (Harshberger 1896; Buckingham 1994). Even now it is well known as integral and inseparable part of tribal culture. This depicts that Aryans of Vedic ages had a thorough knowledge of medicinal plants. Similarly, in Atharava- Veda description of several plants was found. This illustrated narrative of plants was later on practiced by monumental ancient treatise on the subject like 'Charak Samhita' (1000–800 B.C), 'Sushutra Samhita' (800–700 B.C), and Vighatta's Astanga Hridasja. These three classical documentations can be considered as milestones in the traditional Indian indigenous curative system (Chaudhury and Pal 1975; Trivedi 2007).

Looking upon this inextricable link between indigenous ethnic culture and botanical upkeep, the decade beginning from 1 January 1995 was observed as year of ethno the "International decade for the World's Indigenous and Ethnic People". All over the world the ethnic people have engaged in a pivotal role for the safety of floral and faunal wealth with which they have emotional and interdependent relationship.

In the agenda 21 of Rio Earth Summit which was held in 1992, it is clearly stated that Indigenous (i.e. tribal or ethnic societies) folks and their societies and other local groups have a vivacious role in the environmental supervision and sustainable development as they have sound knowledge and traditional practices for the same. Hence, it is necessary that every nation and states should identify the areas to protect and duly support their ethnic identity, culture and welfares and enable their actual participation in the achievement of supportable development (Trivedi 2007; Alam 2020).

Since tribal communities have naturally educated to live in the most hostile conditions from Polar Regions to equator, from the arid deserts to the wet tropical rain forests, therefore these ethnic people have established, various sophisticated techniques to survive in their surroundings and to make the circumstances congenial or advantageous for leading their life rather with ease and comfort. Generally, tribes or the ethnic people have nurtured the areas of high biodiversity in their community lands and in their vicinity. In most of the cases, higher proportion of diversified species has been recorded in ethnic community areas, and in their water bodies (Jain 1991, 1994).

5.2 Ethnobotany in Indian Subcontinents

The plants mentioned in Indian literature and in the religious books of Hindus (e.g. Rigveda, Atharvaveda, Upanishads, and Mahabharata) provided the base for Indian ethnobotanists. The Indian subcontinent depicts a unique richness in floristic diversity. They have estimated that out of these 15,000 species, approximately 7500 species have medicinal importance; about 3900 species of plants are edible, while 700 species are utilized in traditional rituals, cultural purposes, and in the important functions of tribal societies (Jain 1991). Similarly, the other plant species are also used for diversified purposes such as fibres, fodder, gum, resins and dye, pesticides and insecticides, as well as for incense and perfume. The floristic wealth in India is spread in natural habitats, in the different types of forests and other plant communities. However, it is also quite interesting to note that in such areas where native communities dominate, they use native plant wealth and practice customary agriculture. In this manner these tribal or primitive societies maintain intact their own lifestyle customs, rituals and beliefs. Jain and Mitra (1997) combined all information on the uses of medicinal plants from English literature in form of catalogues, dispensaries, pharmacopoeias of plants. The researchers of Botanical Survey of India (B.S.I) roofed ethnobotanical studies which covered more than 30 diverse ethnic societies of 16 states of India (Sharma and Alam 2018).

According to 'All India Coordinated Research Project on Ethnobiology' (AICRPE) which had inputs from Botanical Survey of India (B.S.I), National Botanical Research Institute (N.B.R.I), Central Drug Research Institute (C.D.R.I), Tropical Botanical Garden and Research Institute, collected valuable information according to which about 10,000 species of wild plants have been used by ethnic people to satisfy their all requirements.

So, much information is already available by the great efforts of ethnobotanists of the country nevertheless there is always a scope to update the existing knowledge for the future. Keeping this in view it was found that no consolidated account about the aromatic and medicinal plants that are used by Indian tribes is available till date. Therefore, this article is an attempt to provide an all-inclusive account on this important aspect of ethnobotany. In this article, with the exhaustive study of available literature, a compilation of Indian tribes setting has been done and all the aromatic plants having medicinal value have been placed together (Mittre 1981; Sasikala et al. 2019).

5.3 Outline of Indian Tribes

To begin it must be noted that the list of Scheduled Tribes is State/Union Territory explicit and a community acknowledged as a Scheduled Tribe (ST) in a state need not be ST in another state. The insertion of a tribe as a Scheduled Tribe is a dynamic

process. The indispensable features first laid down in 1965 as the Lokur Committee which had a purpose to identify a community to as Scheduled Tribes. Those important features are:

- (a) Indications of primitive traits;
- (b) Distinctive culture;
- (c) Extremely low literacy; and
- (d) Subsistence level of economy.

5.3.1 Distribution of Tribes

The Scheduled Tribes are notified in 30 States/UTs and the number of individual ethnic groups, etc. notified as Scheduled Tribes is 705.

The tribal populace of India, as per 2011 census, was 10.43 crore, sharing 8.6% of the total population. About 89.97% of them are living in rural areas and 10.03% in urban areas.

The decadal population growth of the tribes from Census 2001 to 2011 was 23.66% against the 17.69% of the entire population.

The trend in ST population since Census 1961 is illustrated in Table 5.1. From 30.1 million in 1961, the ST population has increased to 104.3 million in 2011 (Alam 2020).

It can be observed from maps that more than 2/3 of the ST population is residing only in the 7 major states, *viz.*, Madhya Pradesh, Chhattisgarh, Jharkhand, Maharashtra, Odisha, Rajasthan, and Gujarat. Surprisingly, there is no ST inhabitants in 3 States (Delhi-NCR, Punjab and Haryana) and 2 UTs (Pondicherry and Chandigarh).

Table 5.1 Trends in proportion of scheduled tribe population

Census year	Total population (in millions)	Scheduled tribes population (in millions)	Proportion of STs population
1961	439.2	30.1	6.9
1971	547.9	38.0	6.9
1981#	665.3	58.1	7.8
1991@	838.6	67.6	8.1
2001\$	1028.6	84.3	8.2
2011	1210.8	104.3	8.6

Excludes Assam in 1981@ Excludes Jammu & Kashmir in 1991 (The figures exclude Mao-Maram, Paomata and Purul sub-divisions of Senapati district of Manipur, census 2001)

Source: www.census2011.co.in

5.3.2 Major Tribes of India

It is noticed that every 20th person on the earth belongs to tribal societies. Tribal culture occupies a significant role in our sociological society. Tribal can be called as the sons of the soil, as they are artistic of their place and also familiarize with living in absolute synchronization with nature. Tribal are dispersed in about more than 70 countries all over the world. More or less 150 million tribal found in Asia; out of which in China and India about two third of earliest people are lived.

In case of distribution of tribes on the globe, India possesses the highest traditional populations in comparison to other countries (Fig. 5.1).

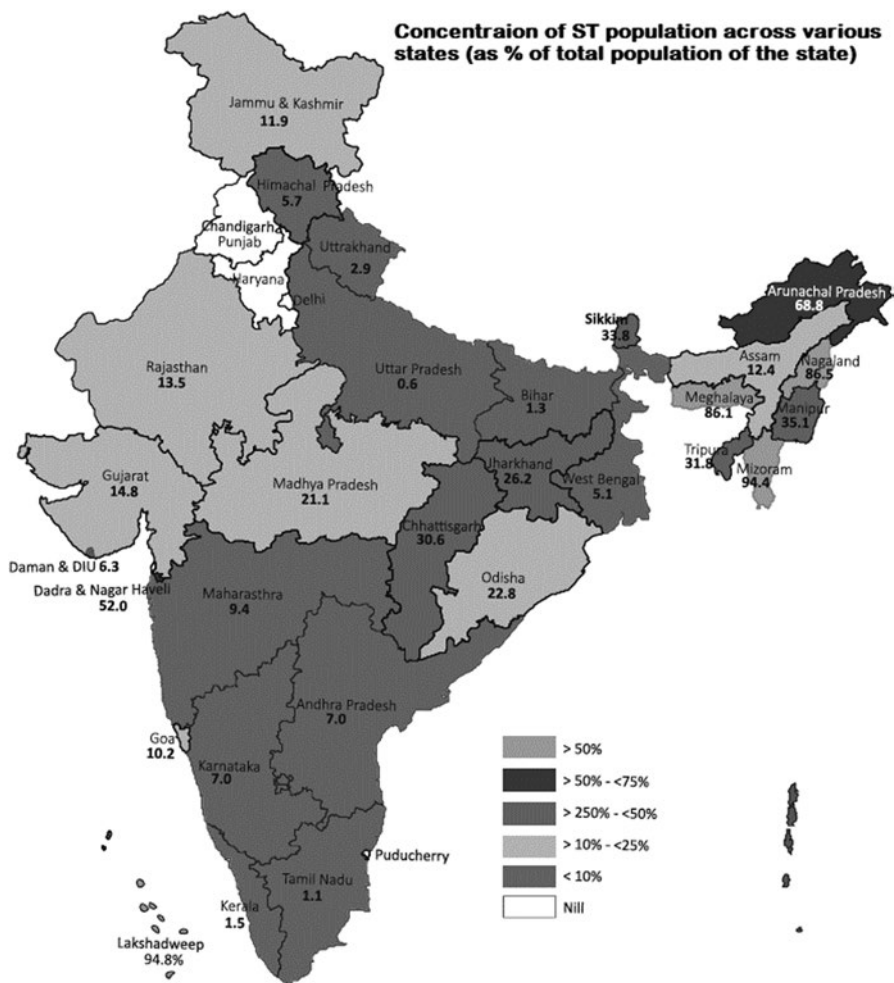


Fig. 5.1 Percentage of ST population in India. (Source: Census 2011 by Registrar General of India)

Different anthropologists called them by various names in different regions of India. e.g., Martin and A. V. Thackkar (Uprety et al. 2010) called them as 'Adivasis or Aborigines', termed them as 'Forest tribes' or 'Folk' (Kirtikar and Basu 1935, 1980; Jain 1991, 1994).

As designated by numerical data available the Indian subcontinent is populated by 53 million tribes occurring to over 650–700 tribal culture of 227 traditional groups.

Tribes add up to 7.7% of India's population. Out of which few are prehistoric tribes such as Lepchas and Rajis, which are facing problem of disappearance. So, for safeguard of tribes the constitution of India establishes some special concern under Article 46.

The importance of tribal cannot be estimated by their amplification and distribution. For example, "Toda" has possessed very small population, so it was ignored by ethnobotanists for a longer time period.

There are some tribal cultures in India which are called as earliest human population on this earth which are totally restricted in forest areas for their survival. Such societies include Jarawas, Sentineles and Shompens of Andaman and Nicobar Islands.

These tribes are totally dependent on hunting, food gathering and fishing. These tribes are behaving as massive protector of biodiversity as they follow various taboos and totems for securing the regeneration of wild plant species (Jain 1994).

There are several major tribes like – Khasi, Garo and Nagas from North East (Moa et al. 2009), Khonds from Odisha, Santhals from Bihar, Gonds from Central India, Bhotia, Khus, Boksa from Uttar Pradesh and Uttarakhand, Dabla from Gujarat, Kinnaur from Himachal Pradesh, Irula from South India, Bhils; Damors; Garasias; Kathodias; Meenas from Rajasthan (Table 5.1).

According to 1951 census, 5.6% of the total Indian population was tribal. While in accordance to the year 2011-Census, the number of scheduled tribes was '10, 42, 81,034'. It was 8.6% of the total population of India at that time. A total of '9, 38, 19,162' people belonging to scheduled tribes were residing in rural areas whereas 1, 04, 61,872 people shifted to urban areas.

According to census-2011, there were about 550 schedule tribes in India, constituted 11.3% of the total population of rural areas and 2.8% of urban areas.

5.4 State Wise Representation of Tribes and Major Uses of Plants

5.4.1 Andhra Pradesh and Telangana

Andhra Pradesh is considered as a home for 33 tribal communities, *viz.*, Lambada, Yarukala, Yanadi, Valmiki, Porja, Kondh, Bagata, Konda Kapu, Konda Reddy, Koya Dora, Konda Kammara etc.), which possess 6.59% of the state's population (Reddy 2008). They have knowledge of different drugs used for their ailments (Ratnam and

Raju 2008; Naidu 2011; Nadakarni 2013). These tribes tend to use the ambient plant wealth for the cure of skin diseases, burns, cuts, diabetes, backache, leucorrhoea, veterinary diseases, dysentery, scorpion sting, tumors, anthrax, malaria, worm infestation etc. (Kapoor and Kapoor 1980; Pullaiah 2007; Reddy et al. 2010). There is chance for cultivation of various native medicinal plants (which are produced by Pharmaceutical Companies of Andhra Pradesh and other states of India) by Government and Private Firms for establishing crude drug extracts of drug plants. This can increase the quality of Ayurvedic medicines and thus trust of tribal on native systems of medicine.

5.4.2 Arunachal Pradesh

Arunachal Pradesh is the only state in North East region of India having 28 major tribes along with 110 subtribes (Nath and Saikia 2002; Tag et al. 2005). The rich floral diversity of the state has given the immense benefit to its residents to understand, use, and conserved their natural resources and traditional knowledge base. There are over 500 plant species which has been mentioned from Arunachal Pradesh. The folks of Arunachal Pradesh find their way to survive by using natively accessible medicinal plants (Namsa et al. 2011).

5.4.3 Assam

There are some major tribes like – Rabha, Bodo, Garo, Hajong which found in Assam state. Beyond Assam, these tribes are also present in Meghalaya, Manipur, West Bengal. They utilize many plant species in their daily life (Tamuli and Ghosal 2017).

The Thengal-Kacharis tribe of Assam has the indigenous acquaintance on diseases as well as their remedies with the outmoded use of diverse parts of natural herbals, viz., roots, leaves and shoots etc. From which they treat patients suffering from different serious ailments. Thus, Thengal society has sturdy confidence on herbals to cure many human diseases (Borah 2014).

5.4.4 Bihar and Jharkhand

There are many prominent tribes exist in Bihar and Jharkhand states. Santhal tribe is considered as the second larger most tribe after Gond and it is present in Bihar, Bengal and Odisha states. In Bihar, this tribe is found in Dhanbad, Hajaribag, Singhbhum, Purnia, Bhagalpur and Saharsa (Prasad and Singh 2014; Dey and Mukherjee 2015).

The Oraon tribe is also commonly called as Dhangar and present near Raigarh and Surguja districts of Madhya Pradesh. They use various herbal drugs, tubers, bulbs, flowers and fruits of various species to cure many diseases. They believe in use of folk medicines in the treatment of their diseases.

5.4.5 Chhattisgarh

Chhattisgarh is gorgeous in terms of its forest resources; approximately 44% area of the state is under forest cover. Kanwar, Oraon, Korva and Gond tribes of this state use many medicinal plants as traditional knowledge (Dubey and Bhadur 1996; Patel 2012).

5.4.6 Gujarat

In this state of west about 5 million tribal populaces is living. Among all the Bhil tribe account for over 50% of the total Gujrat's tribal population. Most of the tribes claim descent from few clans, viz., the Solanki, Rathod, Chauhan, Makwana and Parmar. The main tribes of this state are Bhil (Taviyad/Garasia/Dholi/Dungri/Mewasi), Bhilala, Bhagalia, Vasava, Pawra, Vasave, etc. (Patel et al. 2018).

5.4.7 Himachal Pradesh

Due to varied altitudinal gradients and climatic conditions, this state possesses rich plant diversity, which includes around 3400 plant species (Chowdhery 1999). The state of Himachal Pradesh consists of various tribal like- Gaddi, Gujjar, Kinnura, Bhot or Bhotia, Swangla, Lahaula and Pangwal (Sharma and Lal 2005).

5.4.8 Jammu and Kashmir

Jammu and Kashmir can be authentically stated as the most sublime and out-of-this-world locale amidst the Himalayan splendor. Jammu and Kashmir are an abode to quite several tribal communities, who have settled down in every corner of this hilly region. The tribal and their places, the tribal and their customs, their cultures, their means of communication, or simply their culinary arts, makes the tribes of J&K stand out from the rest of Indian tribesmen. Gujjar and Bakarwal tribes of Jammu and Kashmir interpreted various medicinal and aromatic plants (Gupta et al. 1982; Sharma and Singh 1989).

5.4.9 Karnataka

These tribes of this southern state have built their settlements in some hilly and hilly areas. These tribes of Karnataka state interact with each other in dissimilar languages, however Kananda is their main language. However, they possess their separate tradition and ethnicity. Some of them are also supposed as being originated from the ancient warrior race (Bhandary et al. 1995; Narayanan et al. 2011).

5.4.10 Kerala

In Kerala the prominent tribes are Panyan, Irula, Pulaya and Kadar. These tribe have a rich heritage of adapting the profession of forests product collection and some of them are the practitioners of rituals and medicines. A tribe, Malaipandaram settled in the mountainous forest near to Sabarimala (Prakash et al. 2008; Xavier et al. 2014).

5.4.11 Madhya Pradesh

Central India also hosts rich flora of medicinal significance. Regions like Vindhyaachal, Aravali ranges, Bailadilla Hills, Satpuda, Abhujmar, Khurchel valleys, Kanger reserve, Amarkantak, Pachmarhi and Patalkot areas major reservoirs (Wagh and Jain 2010a, b). As observed earlier, total 500 species of medicinal plants found in which some are economically important medicinal plants that are now on the verge of extinction. The major tribes are Pardhi, Pawara and Bhil tribes who used the traditional medical practices (Sharma et al. 2010; Kumar et al. 2004).

5.4.12 Maharashtra

Tribes of Maharashtra are the primitive people of this region and are dispersed in different parts of the state. Mostly they are the inhabitants of the hilly areas. Some of the tribes are of primitive and nomadic character. Tribes like Warli Tribe, Bhil Tribe, Koli Tribe and Halba Tribe are some of the tribes that inhabit in the land of Maharashtra. They have their characteristic language, clothes, folklores, rites and (Natarajan and Paulsen 2000).

5.4.13 *Manipur*

The state has a central valley (Imphal Valley) populated by the Meitei and Meitei pangal whereas the hilly areas are settled by 30 different tribes of Naga and Kuki tribes (Yumnam and Tripathi 2012). The North – Eastern region of India including Manipur is part of both Himalaya as well as Indo-Burma biodiversity hotspots in the world supporting about 50% of the total India's biodiversity but represent only 8% of the total geographical area of India (Moa et al. 2009). In Manipur, the Meitei tribe inhabiting in the valley regions have the traditional knowledge of eating minor and underutilized fruit crops as medicine from time immemorial to treat different ailments and are associated with various folklore and rituals, which are performed by traditional herbal healers of medicinal men and women locally known as “Maibas” and “Maibis” (Singh et al. 2003).

5.4.14 *Meghalaya*

Meghalaya is a climatic gift of nature that gives rushes of rain to this region, which inclined by the Southwestern monsoon due to the Bay of Bengal. The Meghalaya state is divided into three mountainous regions, viz., Garo Hills (Western part), the Khasi Hills (Central part) and the Jaintia Hills (Eastern part). The tribes of this state are also residing well in these parts and enjoying the nature's gifts (Rao 1981; Kayang 2007).

5.4.15 *Mizoram*

Medicinal plants are the second most resources of Mizoram after water resources. Medicinal plants such as *Swertia* spp., *Neopicrorhizasp.*, *Podophyllum* sp., *Taxus wallichiana*, *Podocarpus* sp. are highly demanded and globally important. Thus, around 500 to 1000 ethnomedicinal and aromatic plants have been mentioned (Lalramnghinglova 1999). But one plant sp. i.e., *Parisipolyphylla*, locally called Khambal of (family Liliaceae) has not been documented. High valuable medicinal plants constitute basis for advanced allopathic drugs development and the use of aboriginal drugs from plant origin which form major part of complementary medicine (Joshi et al. 2004).

5.4.16 Nagaland

This state has 16 major tribes – Ao, Angami, Chakhesang, Chang, Khiamniungan, Konyak, Lotha, Phom, Pochury, Rengma, Sangtam, Sumi, Yimchunger, Zeliang, Kuki, and Kachari. Each tribe is exclusive in character in its linguistic, custom. Nagaland has basically an agricultural economy. With its different agroclimatic conditions has several types of forest and is covered with coniferous trees, broad varieties of flora, medicinal plants, bamboos, and thus has huge potential to utilize and cultivate various types of medicinal and aromatic plants. Phom tribe of Longleng district constitutes a great role in use of medicinal plants. Angamis tribe (Khonoma village) of Nagaland is needed to make careful use of the resources, so that the integrity and traditional knowledge is not lost (Rao and Jamir 1982a, b).

5.4.17 Odisha

Tribes of Odisha comprise a great variety with exclusive and vibrant socio-cultural life. The total population of tribes living in the state of Odisha is much more than other places in India. Their distribution in different districts however varies. Kalahandi, Koraput, Rayagada District, Naurangpur and Malkangiri are some of the districts of Odisha where more than 50% of the total population is tribal. The name of Saora tribe (or Sabar) is mentioned in the great Hindu epic of Mahabharata. The tribes of Odisha belong to three linguistic divisions, namely Dravidian, Indo-Aryan and Austric. These tribes follow the trend of building their houses with bamboo and thatched roofs (Sahu et al. 2011).

5.4.18 Rajasthan

The total area covered by tribes in Rajasthan is 5,474,881 which is 12.44% of the total population of this state. The tribes of Rajasthan constitute 8.07% of the total tribal population of India. Several tribes inhabited in the state of Rajasthan, namely – ‘Bhil’, ‘Bhil-Meena’, ‘Garasia’, ‘Damor’, ‘Dhanaka’, ‘Kathodia’, ‘Meena’, ‘Patelia’ and ‘Saharia’. Out of which, there are some nomadic, semi-nomadic tribes. Nomadic tribes are ‘Banjara’, ‘Gadia-Lohar’ and ‘Kalbelia’, whereas semi-nomadic tribes are ‘Rebari’, ‘Jogi’ and ‘Masani’. ‘Bori’, ‘Kanjer’, ‘Sansi’, ‘Bhat’ are included in de-notified tribes. The most common plants recorded are *Curcuma longa*, *Vitex negundo*, *Ocimum sanctum*, *Sesamum indicum* and flour of *Cicer aritinum* which used as ethnomedicinal purposes (Sebastian and Bhandari 1984; Trivedi 2002; Jain et al. 2005; Sharma and Kumar 2011; Sharma and Kumar 2012).

5.4.19 Sikkim

It is also a beautiful Indian state with great floral wealth. This small state is also a home to the common ethnic Gorkhali folks which comprise tribes via., Limbu (Subba), Gurung, Chhetri, Newar, Thakuri, Sherpa, Tamang, Magar (Manger), Shresthas, Kami, Sunuwar, Kirat Rai, Sarki, Hyolmo and Damai (Jha et al. 2016).

5.4.20 Tamil Nadu

The tribes of Tamil Nadu encompass noteworthy population ranging from diminishing to immense. A prominent fact about these Tamil tribes is sharing of enormously opposing and accompanying relationship among them. For instance, a precise tribe is involved in rational activity like tea/coffee cultivation, or dairy, and on the other tribe is involved in yet-primordial activities like witchcraft or occult. The Todas community inhabit as the dominant amongst the other tribes. The Kotas and the Irulas are also notable in the tribal section of Tamil Nadu, excelling mainly in handicrafts work and agriculture (Sharma and Alam 2018). The valuable contributions of ethnobotanical interests on Tamil Nadu given by Ramachandran and Nair (1982), Ansari and Dwarakan (1993), Kaushik (1988), and Kaushik and Dhiman (2000).

5.4.21 Tripura

Tripura tribes viz., Jamatia, Chakma, Halam, Kuki, Santhal, Murasing, Chaimal, Uchoi, Magh, Garo, Lushai, Oraon, Mog, Bhutia, Lepcha, Bhil, Munda, Reang, Tripuri possess great familiarity on the medicinal plants and their utilization. So, their traditional knowledge can enhance the potential of these medicinal plants to other societies as well and it can lead to be helped in conservation of these plants for further use (Dipankar et al. 2012; Debbarma et al. 2017).

The Ochoi possesses rich knowledge about treatment of minor diseases and perform many enchanted rites and worships for cure of diseases (Majumdar and Datta 2007). But with the time, their knowledge is vanishing rapidly due to lack of documentation and loss of interest.

5.4.22 Uttar Pradesh and Uttarakhand

The major tribal populace of Uttarparadesh and Uttarakhand are Tharu, Raji, BhotiaJaunsari, and Bhoksa. They are mainly residing in the Uttarakhand encompassing areas like Uttar-kashi, Tehri Garhwal, Pauri Garhwal, Champawat, Almora,

Chamoli, Bageswar, Haridwar, Nainital, Pithoragarh, Dehra Dun, Udham Singh Nagar and Rudraprayag. The tribal populace of Uttar Pradesh mostly hails from few hilly regions of U.P. and belongs to the Jat or the Gujjar race. These tribal people are mainly inhabiting in those districts which are adjacent to Nepal and be indebted for their lineage to the Indo-Aryan and Indo-Scythian tribes (Sharma et al. 2010; Singh and Singh 2009; Sharma et al. 2017).

5.4.23 West Bengal

The state of West Bengal has a mixture of tribes from Odisha, and Jharkhand. The diversity of state is evident from its population, individual count and of course the intellectual stamina of the people residing in this region. This state includes tribes like - Santal, Lodha, Munda, Oraon (Maji and Sikdar 1982; Dey and De 2012).

5.4.24 Andaman and Nicobar

Tribes of this particular state are - Nicobarese, Shompens, Jarawas, Sentinels, Onges and Great Andamanese and Karen tribes. The traditional knowledge of these tribes is disappearing along with its natural resources due to lack of support and recognition. Due to existence of wide biodiversity, there are about 71 medicinal plants in the island which are now endemic (Dagar and Dagar 1991).

States like Delhi, Haryana, Panjab, Goa are not known for their tribal communities.

5.5 Discussion

The present study reveals the great diversity in Indian tribes inhabiting in different phyto-geographical regions of the country with mutual understanding with their floral wealth. Based on this review it can be said that because of tribal communities, the modern researchers got valuable information regarding plant uses including medicinal and aromatic plants. The tribal communities are conserving and protecting this floral wealth across India. India contains two hot spots of biodiversity because of these tribes, as one can correlate the populations of tribal communities in these two hot spots of biodiversity, i.e. Eastern Himalayas and Western Ghats.

This review reveals the intimate relationships among the tribes and floral wealth and through light on this neglected relationship in the conservation of biodiversity. It can be said that all these tribal populaces have great knowledge of medicinal and aromatic plants, and surprisingly the use of particular plant of plant parts are more or less same among these tribes, though they have their uniqueness in other aspects

of life. So, the floral wealth of the country is successful in binding the diverse cultures by their exclusive significance. Although the plants have different names across the country, but they have somewhat same medicinal uses. This review is about the aromatic plants only and based on all available literature total 134 aromatic plants (Table 5.2) with medicinal properties have been listed along with their uses.

5.6 Conclusions

As we know medicinal plants play a vital role in ancillary wellbeing in India. From previous studies, it is estimated that around 75% of the population occupying in pastoral or rural areas are adjacent to the natural wealth and have vast knowledge about traditional use of medicinal and aromatic plants presented among prehistoric peoples for ages in India.

Medicinal plants continue to provide health security to millions not only in India but also all over the world. As far as India is concerned around 17,000 angiosperm taxa of the designed 25 hotspots in the world and due to which this constitutes 550 tribes having rich knowledge of traditional uses of medicinal and aromatic plants (Singh and Panda 2005).

Although a variety of studies has been conducted all over India, this attempt finds that the traditional knowledge of medicinal and aromatic plants among population of tribes is still under-documented because only 134 valid plant species have been listed here on the basis of previous work done by the researchers. However, this number can increase further in future because now the ethnobotanical research is regaining attention of botanists and herbalist after a dark phase.

Thus, by this review article an attempt was made to find out the aromatic plants that were mentioned in ethnobotanical text to provide a base for ongoing and future work in this direction.

5.7 Future Prospects

Traditional knowledge of the use of plants has always been transmitted from one generation to another generation. But now, the continuation of this knowledge is in threatened because of lack of transmission between younger and older generations. The young generations of these tribes are willing to settle in urban areas of the country hence causing a lacuna in the information transmission. Therefore, there is an urgent need to inventory and record all ethnobiological information of all tribes not only in India but all over the world. Thus, it is a duty of government, Non-Government Organizations (NGOs), and all of us to protect their habitats and culture and encourage them to stay at their native places and continue to traditions in

Table 5.2 List of medicinal and aromatic plants used by different tribes for various medications India

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
1.	<i>Alnus japonica</i> (Thunb.) Steud.	Betulaceae	Maibau	Tribes of North East India, Odisha and South India	The plant is well known for its traditional uses in the treatment of various diseases like stomachache, diarrhea rheumatism, dysentery, fever, etc. Nowadays this plant is also used to treat Cancer.	Kaur et al. (2001), Sati et al. (2011).
2.	<i>Allium hookeri</i> (Thw.) Enum	Liliaceae	Kanda	Tribes of Manipur	Bulbs and leaves are used to treat respiratory and skin problems.	Ayam (2011)
3.	<i>Aloe vera</i> (L.) Burm. f.	Asphodelaceae (Liliaceae)	Cherukattazha, Gheekumari; Kathalai, Kattar Vazha, Khorpad	All tribes of India	Extract of leaves is used as skin healer. It helps to calm skin injuries due to burning, skin irritations, wounds and insect bites. It also has bactericidal properties hence relieves itching and swellings in skin.	Jani et al. (2007), Salehi et al. (2018)
4.	<i>Alstonia scholaris</i> (L.) R.Br.	Apocynaceae	Sapthaparna	Tribes of both Eastern and Western Himalayas	It is used a mordant herb for treating skin disorders, malarial fever, chronic dysentery, diarrhea and in cases of snake bite	Jain et al. (2009)

5.	<i>Ampelocissus latifolia</i> (Roxb.) Planch.	Vitaceae	Dhodo	Tribes of Central India, Maharashtra, Uttar Pradesh, Bihar, and Western Himalayas	The root paste is used on old wounds. The ripe fruits are eaten as a good source of vitamins	Pednekar et al. (2014)
6.	<i>Andrographis paniculata</i> (Burm. f.) Wall. ex Nees	Acanthaceae	Bhui-neem, Kalmegh	Tribes of Odisha, Tribes of Tripura (Jamatia, Chakma, Halam, Kuki, Chaimal, Uchoi, Magh) and Chattisgarh (Kanwar)	This plant is one of the most popular medicinal plants with traditional uses in the treatment of various diseases like tumors, diabetes, hypertension, ulcer, leprosy, respiratory problems, influenza, malaria, skin diseases, flatulence, colic, dysentery, dyspepsia, etc. Also use to treat dog bite with some mantra and they used powder rice.	Hossain et al. (2014)
7.	<i>Angelica glauca</i> Edgew.	Apiaceae	Chora	Tribes of H.P. and Uttarakhand (Gaddi, Gujjar, Kinnura, Bhot/Bhotia, Swangla, Lahaula and Pangwal, etc.)	The young roots and root powder are used in treatment of digestive system related problems like dyspepsia and stomachache.	Dhar et al. (2000)

(continued)

Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
8.	<i>Annona muricata</i> L.	Annonaceae	Ata-phal	Tribes of North East India	It is a traditional medicine for the treatment of malaria in which the decoction of bark, root, seed and leaves is used. Besides malaria it is also used to treat the stomachache, parasitic infections, diabetes, and tumors.	Abdul Wahab et al. 2018
9.	<i>Annona squamosa</i> L.	Annonaceae	Sitaphal, Ata phol, Ganda gathram	Tribes of North Eastern India (except Sikkim), South India, Rajasthan and Central India	It has been used Traditionally it is used to treat the conditions of Diarrhea, Dysentery, common cold and cough, and as an Insecticidal.	Pandya (2011)
10.	<i>Argemone mexicana</i> L.	Papaveraceae	Satyanashi, Kateli, satyanashi, Darudi	Tribes of Assam (Oraoon tribe, Chakma, Dimasa, Garo, Hajong, Hmar, Khasi), Rajasthan and Gujarat	The whole plant is effectively used in the cases of guinea-worm infestations, as purgative and diuretic agent. Seeds are used as an antidote in snake bite and also as an expectorant, emetic, demulcent and also as a laxative. Seed oil is used to treat skin diseases leprosy and inflammations. Roots are used as anthelmintic.	Priya and Rao (2012)

11.	<i>Asparagus racemosus</i> Willd.	Liliaceae (Asparagaceae)	Utro, Satamul, Satmuli, Karangi	Tribes of Assam (Chakma, Dimasa, Garo, Hajong, Hmar, Khasi), Tribes of West Bengal, Korwa Tribe	Invariably root powder is used to treat malaria fever. Leaves and roots are given orally to treat bloody dysentery, bloody urine, Epilepsy, Filarial fever, Nocturnal emission, Biliary Colic, Hematemesis, etc. Root extract used as health tonic.	Bopana and Saxena (2007)
12.	<i>Azadirachta indica</i> A. Juss.	Meliaceae	Neem	All tribes of India	Whole plant is widely used in traditional medicine systems to treat various skin disorders and diabetes, as insecticides/pesticides Leaves are used to cure snake and scorpion sting.	Kumar and Navaratnam (2013)

(continued)

Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
13.	<i>Boswellia carteri</i> Birdw.	Burseraceae	Salai, Salai guggul	Tribes of Andhra Pradesh, Gujarat, Madhya Pradesh, Jharkhand and Chhattisgarh	In traditional medicinal texts it is registered as an effective remedy for diarrhea, dysentery, ringworm, boils, fevers, skin and blood diseases, mouth sores, bad throat, cardiovascular diseases, jaundice, hemorrhoids, cough, bronchitis, asthma, vaginal discharges, hair-loss, syphilitic diseases, stimulation of liver and irregular menses.	Siddiqui (2011)
14.	<i>Calendula officinalis</i> L.	Asteraceae	Genaduk, Jaragul	Tribes of South India, Odisha and Assam	The plant possesses antiviral, antifungal, antibacterial, antimutagenic, reno-protective, hepatoprotective, free radical scavenger, anti-inflammatory properties and used to treat problems related to central nervous system.	Shivasharan et al. (2013)
15.	<i>Cayratia carnososa</i> L.	Vitaceae	Kalitripanni	Tribes of Assam and adjacent regions (Chakma, Dimasa, Garo, Hajong, Hmar, Khasi)	This plant is used as diuretic, to cure tumors, neuralgia and spleno-pathic situations.	Kumar et al. (2011)

16.	<i>Celastrus paniculatus</i> Willd.	Celastraceae	Jayotismoti, Kujri, Malkangni, Kujari	Tribes of West Bengal (Snatali)	Paste of complete young and healthy plant is used to cure constipation and also as an abortifacient. The seed oil used to heal the wounds	Kulkarni et al. (2015)
17.	<i>Centella asiatica</i> (L.) Urban	Apiaceae	Brahmibuti, Ngyarikor, Beng Sag, Brahmibuti, Gotu Kola	Tribes of Assam (Chakma, Dimasa, Garo, Hajong, Hmar, Khasi) and Arunachal Pradesh (Apatami, Mongpa, Singpho and Tangsa tribes) and Oraon tribe	The plant extract mixed with water is taken as tonic against various neurological disorders, tuberculosis, leprosy and dysentery. Whole plant is used to increase appetite.	Gohil et al. (2010)
18.	<i>Chlorophytum tuberosum</i> Baker	Liliaceae	Safed Musli	Tribes of North east India, Central India and South India	This plant is well known as traditional herbal medicine mainly for treating various illnesses viz., diabetes, sexual disorders, kidney stones, diarrhoea, cholera, leucorrhoea, general debility, etc.	Patil and Deokule (2010)
19.	<i>Chloroxylon swietenia</i> (Roxb.) DC.	Rutaceae	Bhathi, Dhoura, Girya, Hurihulli,	Tribes of Odisha, Madhya Pradesh, Andhra Pradesh, Karnataka, Kerala and Tamil Nadu	The leaf paste is used to cure the swelling in legs.	Srivastava et al. (1998)

(continued)

Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
20.	<i>Cissampelos pareira</i> L.	Menispermaceae	Peepra mool, Heir, Jaljajmi, Korya-padbin, Padh, Bhatve	Tribes Madhya Pradesh, Chattisgarh, Bihar, West Bengal and Assam (Chakma, Dimasa, Garo, Hajong, Hmar, Khasi)	Roots are used to treat fever, leprosy, prolonged cough, chronic skin diseases like blisters.	Sood et al. (2015)
21.	<i>Cissus quadrangularis</i> L.	Vitaceae	Harjora lota, Adamant creeper, Kynbat-harjora	Tribes of Meghalaya, Manipur, West Bengal (Rabha, Bodo, Garo and Khasi)	Plant paste is applied topically in the cases of fractures/dislocation of bones. Leaf juice is used as an alternative of ear drop to treat otorrhea. Powder mixed with mustard oil is used in treating rheumatoid arthritis.	Brahmkshatriya et al. (2015)
22.	<i>Citrus maxima</i> (Burm.) Mitt.	Rutaceae	Obakotru, Pamparamasam	Tribes of North eastern India, South India, Rajasthan, Maharashtra and Gujarat	This plant has varied medicinal uses; traditionally used for the treatment of convulsive cough, epilepsy, and hemorrhagic Also used as sedative by some tribes.	Singh et al. (2018)

23.	<i>Cleome gynandra</i> L.	Capparaceae	Hulhul, Bagro	Tribes of North Eastern India and south India	Roots of the plant are used in chest pain; leaves used for the treatment of diarrhea. The decoction of seeds is used in fever and leaf decoction in headache. Traditionally the plant is uses as rubefacient and vesicant therefore beneficial in case of rheumatism, applied externally as well as orally taken.	Singh et al. (2009)
24.	<i>Cleome viscosa</i> L.	Capparidaceae (Cleomaceae)	Nayivelai	Tribes of South India, Central India, North India, Rajasthan and Gujarat	In cases of scorpion sting and snake bite, paste of leaves is applied on the affected portions. Also used for the treatment of rheumatic arthritis, malaria, neurasthenia, and as a common wound healer.	Mali (2010)

(continued)

Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
25.	<i>Cocculus hirsutus</i> (L.) Diels	Menispermaceae	Kattukkodai	Tribes of Tamil Nadu	Various plant parts have different uses in local medicinal system viz., the leaf juice to treat eczema. Roots decoction is used as an antipyretic and stomach related problems. The leaves are known to treat skin infections and itchy skin, rheumatism and gonorrhea. Root juice is taken as a tonic and alterative and as a diuretic and laxative.	Dhirajal et al. (2019)
26.	<i>Codiocarpus and amaniticus</i> (Kurz) R.A.Howard.	Stemonuraceae	Lansot	Tribes of Andaman and Nicobar	Leaves are used for the treatment of urinary tract infections, kidney problems, constipation and other stomach related problems.	Sharma et al. (2018)
27.	<i>Colchium luteum</i> L.	Colchicaceae (Liliaceae)	Suranjantalkh	Tribes of Jammu And Kashmir and Himachal Pradesh	As a traditional medicine the plant parts like corms and seeds are used for treatment of gout, rheumatism, liver and spleen related ailments. The extract of seed used as health tonic.	Kapur and Singh (1996)

28.	<i>Coleus barbatus</i> Andr.	Lamiaceae	PashanBheda, Pathar Chur, Fiwain	Tribes of Odisha and Uttarakhand	Roots are used to destroy out kidney stone/calculi. In traditional medicine, plant has also been used to treat spasmodic pain, heart diseases, painful urination and convulsions.	Sharma and Vasundhara (2011)
29.	<i>Commiphora myrrha</i> (Nees) Engl.	Bursaceae	Bola, Bol, Hirabol	Tribes of Himalayan regions	It has been used in traditional medicines against a many disease such as ulcerative colitis, fever, skin infections, problems of gall bladder, tumors, dysmenorrhea, amenorrhea, chest pain and burning sensation.	Hashim et al. (2016)
30.	<i>Commiphora wightii</i> (A.) Bhandari	Bursaceae	Guggal, kungliyam, gukkal,	Tribes of Assam (Chakma, Dimasa, Garo, Hajong, Hmar, Khasi); Tribes of Gujarat and Rajasthan	Plant parts are used for the treatment of Arthritis, Hemorrhoids, joint pain, etc. Gum resin is used to treat rheumatized arthritis, paralytic conditions and as laxative.	Qureshi and Chahar (2013)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
31.	<i>Costus spectiosus</i> (J. Koenig) Sm.	Costaceae	Jamlakhuti, Ketki	Thengal Kachari tribes of North Eastern India (Apatami, Mongpa, Singpho and Tangsa tribes)	Fresh roots of the plant are taken to clear the respiratory blockage and young stems are eaten to treat burning sensation during urination.	El-Far et al. (2018)
32.	<i>Crinum defixum</i> Ker-Gawl	Amaryllidaceae	Sukhdarshan, Bon-naharu	Tribes of Assam and adjacent states (Chakma, Dimasa, Garo, Hajong, Hmar, Khasi)	Leaf extract is commonly applied for the treatment of pimples, body-ache and dropsy, the bulbs have emollient, nauseate, emetic and diaphoretic properties hence used in various conditions of inflammation.	Trivedi (2007)
33.	<i>Croton roxburghii</i> Balak.	Euphorbiaceae	Putri	Tribes of Odisha and West Bengal	The young plant parts are used in the cases of snake bite, to treat pain and inflammation, stomach trouble, indigestion, liver disorders like jaundice; as cardio tonic and purgative, etc. The root and stem bark paste are beneficial in dysentery, while the poultice is used in muscular pain.	Panda et al. (2010)

34.	<i>Cryptolepis buchanani</i> Roem. & Schult.	Asclepiadaceae	Kankrashringi, Nedashringi, Karilata, Utri dudhi, Ananta mul	Tribes of West Bengal Meghalaya and, Manipur (Rabha, Bodo, Santali)	Root has demulcent nature, used as tonic in the cases of appetite loss, fever and skin diseases. It is having blood purification property and extensively used in leprosy and other skin diseases. Leaves are useful in the cases of rickets.	Dutta et al. (1978)
35.	<i>Cuphea aequipetala</i> Cav.	Lythraceae	Pani Jetuka	Tribes of Arunachal Pradesh, Assam, Nagaland and West Bengal	The plant is used as blood purifier, diuretic, purgative, emmenagogue, laxative and hypotensive agent. Also used to treat the cardiovascular diseases and menstruation problems.	Das et al. (2018)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
36.	<i>Curculigo orchitoides</i> Gaertn.	Amaryllidaceae	Talmule or Hookakand	Tribes of Eastern Himalayan region	The plant is an important ingredient of many medicinal properties like aphrodisiac, antioxidant, hepatoprotective, immunostimulant, antitumor and antidiabetic activities. The root paste is mixed with castor oil and rubbed on skull in lunacy. The root decoction is used in cases of stomach trouble and dysentery	Joy et al. (2004)
37.	<i>Curcuma amada</i> Roxb.	Zingiberaceae	Am-ada, Phacheng, Amo-haldi	All tribes of India	The rhizome is used to treat various diseases of skin, pain and digestion.	Policegoudra et al. (2011)
38.	<i>Curcuma angustifolia</i> Roxb.	Zingiberaceae	Tikhur	Gond and Bhil tribes	Tubers are useful in the treatment of digestive problems. The root mixed with milk is used to cure weakness and rheumatism.	Prajapati et al. (2003)

39.	<i>Curcuma aromatica</i> Salisb.	Zingiberaceae	Haridra, Ban Haridra	Tribes of Kerala	Fresh rhizome applies to treat skin irritation and as a cure for pimples. Also used as a carminative, antidote to snake bites. It is used for bruises, corn, sprains, joint pains and is a well-known for enhancing complexion.	Sikha et al. (2015)
40.	<i>Curcuma longa</i> L.	Zingiberaceae	Haldi, Haridra	All tribes of India	Dried and fresh rhizome crushed along with kodasheri and the resultant paste is applied in the cases of insect's bite. Fresh rhizome crushed along with leaves of <i>Lawsonia</i> and neem and applied to the infected nail. Rhizome paste is also useful in the cure of pimples and to recover skin color and for nourishment.	Prasad and Aggarwal (2011)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
41.	<i>Curcuma zanthorrhiza</i> Roxb.	Zingiberaceae	Adavikachola, Manjakoova, Pulakizhangu, Kasthurimanjal	Tribes of Kerala	The powder with milk is useful in the treatment of diabetes and blood pressure patients. Conditions of liver damage, hypertension and cancer can also be cure by this plant.	Salleh et al. (2016)
42.	<i>Cuscuta hyaline</i> Boiss. ex Engelm.	Convolvulaceae	Hrvatski	Tribes of Assam, Central India, Soth India and Rajasthan	The stem is very useful in the treatment of bilious disorders. The whole plant has a purgative property. It is used in the treatment of protracted fevers, and externally to treat body pains and itchy skin. Also, helpful to treat trouble in urinating, conditions of jaundice, chest pain, muscle pain and coughs.	Ahmad et al. (2017)

43.	<i>Cuscuta reflexa</i> Roxb.	Convolvulaceae	Akasbel, Amarble, Verillakothan, Zarbut, Moodillathali, Akashbel	Thegal Kachari tribes of NE India	This miraculous plant is well known for its purgative nature, used internally in the treatment of protracted fevers, and externally in the treatment of various body pains and itchy skin. The vapors of boiled plants are used daily to treat body swellings and fever.	Laichand et al. (2018)
44.	<i>Cydonia oblonga</i> Mill.	Rosaceae	Beech, Bom Chunth	Tribes of Jammu and Kashmir, Himachal Pradesh and Uttarakhand	The plant is used to treat several ailments viz., cancer, diabetes, hepatitis, ulcer, respiratory, and urinary infections, to cure sore throat, the seeds are chewed raw. Leaves, buds and bark have astringent property. Fruits are well known cardiac stimulant, tonic and expectorant.	Ashraf et al. (2016)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
45.	<i>Cymbopogon martini</i> (Roxb.) Watson	Poaceae	Rosha grass, Rauns, Thisankah	Tribes of Uttarakhhand, Himachal Pradesh	The paste of the leaf and stem of is applied in the cases of scabies and discoloration of the skin. The oil extract is mixed with hot water and used for hot steam inhalation in condition of asthma and common cold.	Promila (2018)
46.	<i>Cynodon dactylon</i> (L.) Pers.	Poaceae	Dhoobghas, Durba, Garike, Phaitualhnmim, Ambatehullu	Tribes of North eastern India like Mizoram, Manipur (Chakma, Dimasa, Garo, Hajong, Hmar, Khasi, Korva tribes), and Karnataka	This grass is used as a coolant, laxative, expectorant, carminative and as a heart and brain tonic. Decoction of leaves is used to treat Leucorrhoea.	Nagori and Solanki (2011).

47.	<i>Dendrophthoe falcata</i> (L. f.) Ettiingsh	Loranthaceae	Bandha	Tribes of Odisha, NE India, South India and Central India	Plant is invariably used in folklore medicine to treat diseases including ulcers, asthma, impotency, paralysis, skin diseases, menstrual troubles, pulmonary tuberculosis and wounds. The plant parts have diuretic, wound healing, anti-helminthic, anti-microbial, anti-fertility, anti-cancer, anti-diabetic, anti-hyperlipidemic, anti-hypertensive activities. The boiled flowers are used for regulating menstrual cycle.	Guptha et al. (2011), Gupta et al. (2020)
48.	<i>Dioscorea bulbifera</i> L.	Dioscoreaceae	Paharikan, Suarkand, Ganthe	Tribes of Assam, Odisha and Andhra Pradesh	Plant and plant parts are used to treat constipation, piles, dysentery and syphilis. The young tuberous roots are usually eaten during the onset of the rainy season. Also used against snake bite	Kundu et al. (2020)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
49.	<i>Dioscorea veltans</i> Prain & Burkill	Dioscoreaceae	Getti	Tribes of Andaman and Nicobar	Tubers are used for the treatment of asthma, arthritis, chronic cough, eczema, diarrhea, diabetes, regular metabolic activity.	Sharma and Bastakoti (2009)
50.	<i>Diospyros melanoxylon</i> Roxb.	Ebebeaceae	Tendu, Tendu-Seeta	Tribes of M.P., Chhattisgarh, Andhra Pradesh, Odisha	The ripened fruits are eaten by tribal people. However, both unripe, as well as ripe fruits have been used in folk-medicine system, especially by the by the Oraon tribe in the incidence of syphilis.	Tiwari (2018)
51.	<i>Echinops grjijisii</i> Hance	Asteraceae	Kantela	Tribes of Uttarakhand, Himachal Pradesh and Jammu & Kashmir	This plant is used to treat variety of pains, inflammation conditions, respiratory diseases, diseases due to exposure of different microorganisms. The plant has aphrodisiac nature hence used to fasten expulsion of placenta, and also for removal of kidney stones.	Dangwal et al. (2011)

52.	<i>Ehretia laevis</i> Roxb.	Boraginaceae	Tamboli, Pushipan, Khandu Chakka	Tribes of West Bengal; Maharashtra, Uttarakhand	Juice of leaves given to treat dysentery, to kill intestinal worms, and also applied externally on wounds. Paste of leaves used on joint and minor fractures to relieve pain.	Sharma et al. (2014)
53.	<i>Elettaria cardamomum</i> (L.) Maton	Zingiberaceae	Elakkai	Tribes of Tamil Nadu, Kerala and Karnataka.	Power of seeds with honey is taken to cure vomiting, while seed powder mixed with coconut water is used to cure urinary problems. The plant is also known to control asthma, to prevent teeth and gum infections, to treat cataracts, nausea, diarrhea, also to cure digestive, cardiac, and kidney disorders	Kaliyaperumal et al. (2020).
54.	<i>Elytraria acaulis</i> Lindau	Acanthaceae	Patharchatta	Tribes of Assam and adjacent regions	Plant is used to treat the abscess of mammary glands, boils, burns. Also useful in the treatment of asthma, migraine, leucorrhoea, and snake bite	Babu et al. (2015)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
55.	<i>Embelia tsjeriam-cottam</i> A. DC.	Primulaceae	Bai birangi, Joladhanna, Watwarung, Rahsen	Tribes of Malabar, South India, Mizoram and Maharashtra	Leaves are used to treat diarrhea and chest related problems. Seeds are used as vermifuge. The bark of the root is useful in toothache. Decoction of leaves used as a gargle in case of sore throat.	Sharma et al. (2011)
56.	<i>Emblita officinalis</i> L.	Phyllanthaceae	Amla, Awla, Aonla	Tribes of Central India, North Eastern India, Rajasthan, Gujarat, Uttar Pradesh and Madhya Pradesh	The plant parts have multiple uses in traditional medicine system. It possesses immunomodulatory, anti-inflammatory, antiulcer, hepatoprotective, and anticancer properties. The juice is used to cure asthma and digestive problems. The decoction of bark and fruits mixed with the fruits of <i>Terminalia chebula</i> and used to prevent continuous vomiting. The fruit powder and bark juice is useful in the treatment of stomach troubles.	Variya et al. (2016)

57.	<i>Epipremnum pinnatum</i> (Linden & André) G.S. Bunting	Araceae	Money Plant/Paisaped	Tribes of Gujarat, Maharashtra, Rajasthan, Andaman and Assam	Decoction of leaves and young shoots is used for gargle and as mouth wash in the situations of gum inflammations and tooth abscesses. Paste of leaves is a remedy for skin diseases.	Sasikala et al. (2019)
58.	<i>Erigeron annuus</i> (L.) Pers.	Asteraceae	Phuntha	Tribes of Himalayan regions of India	The plant is known for its astringent properties and used in the treatment of diarrhea, bleeding hemorrhoids rheumatic pain. Plant is also useful as diuretic agent and used in the cases of emmenagogue and styptic.	Nazaruk and Kalemba (2009)
59.	<i>Eulophia nuda</i> Lindl.	Orchidaceae	Ban singara, Amarkand, Balakand, Manakand, Munjatak, Amrita, Ambarkand, Salam, Budbar, Salab, Amarkand, and Salibmisiri.	Tribes Madhya Pradesh, Gujarat, Maharashtra, Madhya Pradesh, Uttar Pradesh	Tubers are useful in the treatment of chest problems and for snake bite. Also used as expectorant, diuretic, astringent, digestive, and soft purgative. It is useful for the cure of ear discharge, joint edema, blood clotting, and debility	Narkhede et al. (2016)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
60.	<i>Euonymus alatus</i> (Thunb.) Siebold.	Celastraceae	Bicchu-Jhad	Tribes of North Eastern Himalayas	Used in the treatment of cold, headache, body aches, irregular menstruation, and other gynecological diseases	Sharma et al. (2012)
61.	<i>Euphorbia hirta</i> L.	Euphorbiaceae	Dudhi, Bichujari, Dudhi, Dudhika, Nagarjuni, Vikshirini	Tribes of Assam and Uttarakhnad	Specially used in the treatment of female related disorders. Besides this the plant parts have been used to cure respiratory ailments, dysentery, worm infestations, jaundice, pimples, and tumors.	Kumar et al. (2010)
62.	<i>Euphorbia nerifolia</i> L.	Euphorbiaceae	Hathlo thubar, Danda-thor, Sehund, Elakkalli, Akujamudu	Tribes of south India, West Bengal and Odisha	Latex is acrid, laxative. The latex is given with <i>Piper betel</i> leaf to cure asthma. Latex is pungent and good for the treatment of tumors, abdominal troubles and leukoderma. It is also used as to cure enlargement of the spleen, colic and gall bladder stones. It is frequently used to eradicate warts and cutaneous eruptions.	Mali and Panchal (2017)

63.	<i>Fissistigma oldhamii</i> (Hemsl.) Merr.	Ammonaceae	Oldhamii	Tribes of Assam, Manipur, Madhya Pradesh and Chhattisgarh	In the traditional medicinal system this plant is used as an analgesic, astringent, and to treat various ailments like snakebite, diarrhea, dysentery, arthritis, rheumatic pain, neuralgia, and to enhance weight loss.	Atiq et al. (2017)
64.	<i>Fritillaria roylei</i> Hook.	Liliaceae	Shethkar	Tribes of Jammu and Kashmir	The extract of the bulbs is used as a potent anti-pyretic. The roots are used for healing wounds, corns. Also used for the treatment of asthma, rheumatism, tuberculosis.	Bisht et al. (2016)
65.	<i>Gardenia jasminoides</i> J. Ellis	Rubiaceae	Midola, Gandroya, Kaboklei, Gulchand	Tribes of Assam, Manipur, Assam, Maharashtra, Uttar Pradesh	Fruits are invariably used to cure swelling, diabetes and liver problems. Flowers are used for abortion.	Muthu et al. (2006)
66.	<i>Gardenia latifolia</i> Aiton	Rubiaceae	Ghas patti, Perunkambi, Kambi, Kattu marikalam, Papra	Tribes of Tamil Nadu and Himachal Pradesh	Leaves are useful remedy to treat irregular menstruation	Natarajan et al. (2000)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
67.	<i>Ceriscoides turgida</i> (Roxb.) Tirveng.	Rubiaceae	Khadarghar, Kha Vellakara, Karkar, Malankara	Tribes of south India, Chhattisgarh and Madhya Pradesh	The root powder mixed with sugary milk is used to treat spermatorrhoea, and indigestion in children. Pulp of fruits used to treat eye ailments of cattle. The pulp also applied to forehead in case of fever and used to treat abdominal, colic, and mammary gland related ailments.	Asolkar et al. (2005)
68.	<i>Glinus lotoides</i> L.	Molluginaceae	Ciru-Ceruppadai, Duserasag, Chandrasikoora, Kodak	Tribes of South India, Assam, Gujarat (Chakma, Dimasa, Garo, Hajong, Hmar, Khasi)	It is used as an antiseptic, an anthelmintic, as a treatment for diarrhea and bilious attacks, and as a purgative for curing boils, wounds and pain in general. Urinary troubles	Bhavani (2015)
69.	<i>Glochidion zeylanicum</i> (Gaertn.) A. Juss.	Phyllanthaceae	Neervetti, Pannimutti, Kumbalm, Keotomi, Keoura, Paniatori, Pannyaturi	Tribes of South India, Assam and West Bengal	The leaves have anti-inflammatory compounds with anti-tumor properties.	Sharma et al. (2011)

70.	<i>Gloriosa superba</i> L.	Colchicaceae	Kalihari	Tribes of Tamil Nadu and Kerala	Multiple uses of this plant are known such as it has been used in the treatment of gout, infertility, snakebite wounds, kidney problems, cholera, ulcers, arthritis,, colic, itching, leprosy, sprains, hemorrhoids, cancer, impotency, nocturnal discharge, smallpox, sexual diseases.	Bhide and Acharya (2012)
71.	<i>Heterostemma brownia</i> Hayata.	Apocynaceae	Not known	Tribes of North East India	Plant parts are used to cure digestive ailments, malarial fever, diabetes, body pain.	Bhadane et al. (2018)
72.	<i>Himatanthus sucuiba</i> (Spruce ex Müll. Arg.) Woodson	Apocynaceae	Dodo-ni	Tribes of Kerala and Tamil Nadu	Latex is used to treat abscesses/boils	Dwivedi et al. (2016)
73.	<i>Holoptelea integrifolia</i> Planch	Ulmaceae	Papri, Bastedun, Chirabilva	Tribes of North Eastern India (Chakma, Dimasa, Garo, Hajong, Hmar, Khasi)	Leaf paste is used to get relief from localized swelling, skin problems. Powder of tender plant parts is used for the treatment of nausea, piles, diabetes, stomach disorders, and also used to purify blood.	Srivastava et al. (2013)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
74.	<i>Homalomena aromatica</i> Schott.	Araceae	Sugandhmantri	Tribes of Tripura (Jamatia, Chakma, Halam, Kuki, Chaimal, Uchoi, Magh, etc.)	The rhizomes are used as anti-inflammatory, antiseptic, analgesic, sedative, antidepressant, antispasmodic, and to treat joint pain and skin problems.	Raomai et al. (2013)
75.	<i>Hyssopus officinalis</i> L.	Lamiaceae	Jufa	Tribes of Western Himalayas (Gaddi, Gujjar, Kinnura, Bhot/Bhotia, Swangla, Lahaula and Pangwal)	Decoction of leaves is considered a stimulant, expectorant and carminative. Also used in the treatment of congestion, colds, cough, and other lung problems. Leaves are used to make a herbal tea like drink to get rid of toothache. Also operative in pulmonary, digestive, and urinary problems. For the treatment of asthma and other respiratory problems the decoction of young leaves is given.	Fathiazad et al. (2011)

76.	<i>Jatropha curcas</i> L.	Euphorbiaceae	Mukhrandhe, Rataniyot	Tribal of Madhya Pradesh and Chattisgarh	The massage of seed oil is useful in muscular pain and swellings. The young twigs are used as an alternative of toothbrush in gum diseases. Root bark decoction is used to treat the conditions of diarrhea and dysentery.	Maravi et al. (2015)
77.	<i>Justicia adharoda</i> L.	Acanthaceae	Barsikhe, Aadalodakam, Vasaka, Baska tita, Boga-bahak	Thengal Kachar tribes of North East India, Assam, Madhya Pradesh, Odisha	The aerial plant parts and bark of have been used to treat respiratory problems like asthma, bronchitis, bronchial catarrh, tuberculosis, common cough and colds, also used as an expectorant and bronchodilator.	Khan et al. (2018)
78.	<i>Justicia gendarussa</i> Burm F.	Acanthaceae	Titabahak	Tribes of Meghalaya, Manipur, West Bengal	Paste of leaves issued as an anti-inflammatory agent. While decoction of shoot and bark has antibacterial properties.	Paval et al. (2009)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
79	<i>Kigelia africana</i> (L.) Benth.	Bignoniaceae	Balamkheera	Tribes of Karnataka and Kerala	The whole plant is used for the treatment of syphilis, dysentery, elephantiasis, piles, constipation, rheumatic swellings, and as a vermifuge	Saini et al. (2009)
80.	<i>Lagerstrœmia parviflora</i> Roxb.	Lythraceae	Sidha, Bot Dhaiyanro, Bondga, Bondara, Ajhar	Tribes of Himalayas, Assam and Nilgiri hills	Tender plant parts are used to cure inflammatory, and as analgesic. The decoction is used to treat gastrointestinal infection.	Choudhury (2015)
81.	<i>Leea grandifolia</i> (L.) Pers.	Vitaceae	Takteyu, Agasti	Tribes of Andaman and Nicobar	Leaves are used to treat intestinal disorders and abdominal pain.	Yadav et al. (2010)
82.	<i>Leucas aspera</i> (Willd.) Link.	Lamiaceae	Gumma, gomo, Thumbai, Comba or Cuma	Tribes of Assam (Chakma, Dimasa, Garo, Hajong, Hmar) and Tamil Nadu (Thoda)	Decoction of young shoots is used as antipyretic to lessen fever. Decoction of the leaf with hot milk is given to the patient with high fever. The paste of young leaves is used for the relief from headache.	Kundu et al. (2018)

83.	<i>Litsea monopetala</i> (Roxb. ex Baker) Pers.	Lauraceae	Meda, Chiru maamidi, Meda, Bon-khuwalu, Khuwalu	Kanwar tribe (Chhattisgarh)	Bark has anti-inflammatory properties and hence used to treat swelling in bones and muscles. The seed-oil massage is useful for the treatment of rheumatism. However, the oil extraction in this case is very difficult and the yield is low.	Biswas et al. (2017)
84.	<i>Lygodium flexpsum</i> (L.) Sw.	Lygodiaceae	Sorgajal	Chakma, Dimasa, Garo, Hajong, Hmar, Khasi (Assam)	Decoction of roots is used for earache.	Yadav et al. (2012)
85.	<i>Madhuca longifolia</i> (L.) J. F. Macbr.	Sapotaceae	Mahua, Mahva, Mohva, Mohua, Erappe, Ippa, Iluppai, Madhukah, Irippa	Tribes of North Eastern India.	The oral doses of bark juice are given to pregnant females for easy delivery. The extracted oil from seeds is used for the treatment of epilepsy, diabetes.	Rangari (2009)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
86	<i>Mallotus philippinensis</i> Muell. Arg.	Euphorbiaceae	Rohini, Kampillaka, Shendri	Tribes of Outer Himalayas (Oraon tribe)	The leaves are bitter in taste but have cooling effect and decoction is used as an appetizer. Fruits have purgative in nature and used as anthelmintic, carminative and useful in treatment of abdominal diseases, bronchitis, spleen enlargement, etc. Paste of bark is applied topically for pain relief.	Gangwar et al. (2014)
87.	<i>Manilkara zapota</i> (L.) Van Royan	Sapotaceae	Sapota, Chikoo	Oraon tribe	Paste of leaves is used for skin treatment and as anti-inflammatory remedy. Fruits are used to get rid from constipation.	Kulkarni et al. (2007)
88.	<i>Marsdenia tenacissima</i> (Roxb.) Wight et. Am.	Apocynaceae	Murva or Moorva, Jartor, Safed Nishoth, Chindhaur, Chagaveru, Perunkurinjhan, Chumhar	Tribes of South India, Central India and Odisha (Bhuiyans, Gonds, Kharias, Kisans, Mundas and Oraons)	Powder of roots is taken orally with water to treat postnatal problems.	Girach et al. (1998)

89.	<i>Martynia annua</i> L.	Martyniaceae	Caimokhe, kakanasika, Hathajori	Tribes of South India, Maharashtra and Odisha	Traditionally the fruits and seeds are used for the treatment of epilepsy, cardiac problems, dysentery, dysuria, hemorrhage, fever, constipation, worm infestation, bacterial infection, ulcer and tuberculosis.	Dhingra et al. (2013)
90.	<i>Maytenus emarginata</i> (Willd.) Ding Hou	Celastraceae	Thandisamaram, Mulmaram, Baikal	Tribes of Karnataka and Kerala	Plant is useful to treat the various stomach complaints, tumors, rheumatoid arthritis and fever.	Sangwan et al. (2011)
91.	<i>Melhania futeyporensis</i> Munro ex Mast.	Sterculiaceae	Basni	Tribes of Assam and adjacent states (Chakma, Dimasa, Garo, Hajong, Hmar, Khasi); tribes of Andhra Pradesh and Telangan (Yanadi tribe)	The paste of leaves has anti-inflammatory and wound healing properties.	Savithramma et al. (2016)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
92.	<i>Melia azadirachta</i> L.	Meliaceae	Bakain, Bevu, Malaveppu, Bakan-nimb	Tribes of South India, Maharashtra, Central India	Decoction of tender shoots and leaves is used as an anthelmintic, diuretic, expectorant, vermifuge, emmenagogue, and also used in case of piles. In south India the tribes used this plant as an astringent, to treat hysteria and leprosy.	Kumar et al. (2003)
93.	<i>Mollugo cerviana</i> (L.) Ser.	Molluginaceae	Chiriyaro khet, Parpataka, Kaagepurulegida, Citam	Tribes of Assam, Odisha and tribes of south India	Flowers and young roots are used as blood purifier and stimulating the lochial discharge.	Parvathamma and Shanthamma (2000)
94.	<i>Nandina domestica</i> Thunb.	Berberidaceae	Manna	Tribes of Assam and Meghalaya	the plant is toxic to dogs, cats, horses and grazing animals	Khare (2007a, b)
95.	<i>Neurada procumbens</i> L.	Neuradaceae	Ya-hom	Tribes of Assam, Odisha and West Bengal	Seed extract is used as energy drink and health tonic.	Chen et al. (2004)
96.	<i>Ocimum basilicum</i> L.	Lamiaceae	Safed Bhabdi	All tribes of India	Decoction of aerial parts is used to treat headaches, diarrhea, constipation, coughs, worms, warts, and kidney failures. Fresh leaf juice is applied in eye to cure the infection.	Joshi (2014)

97.	<i>Ocimum sanctum</i> L.	Lamiaceae	Tulsi, Tulasi	All tribes of India	<p>This is a revered plant for most of the tribes and has an exceptional amalgamation of actions viz., antimicrobial, anti-cataract, anti-inflammatory, antimalarial, analgesic, anthelmintic, anti-diarrheal, anti-diabetic, anti-hypercholesterolemia, anti-hypertensive properties. Also known for anti-oxidant, chemo-; radio-; hepato-, neuro-, cardio-protective, anti-carcinogenic, anti-pyretic, anti-allergic, immunomodulator, anti-depressant, diaphoretic, memory enhancer, anti-asthmatic, anti-tussive, anti-spasmodic, anti-arthritic, anti-stress, anti-leukoderma and anti-coagulant properties.</p>	Singh et al. (2010)
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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
98.	<i>Panax ginseng</i> C.A. Mey	Araliaceae	Ginseng	Tribes of higher Himalayan range	Tribal use this plant to improve the conditions of fatigue, insomnia, and depression. The plant is also used to reduce cholesterol levels hence cardio-protective.	Seenivasagam et al. (2011)
99.	<i>Petiveria alliacea</i> L.	Phytolaccaceae	Anamu	Tribes of Kerala	Plant is used for the treatment of tumors, skin diseases, diabetes, muscular pain, central nervous system disorders, respiratory and pulmonary infections, and malarial fever.	Sathiyabalan et al. (2017)
100.	<i>Hebanthe eriantha</i> (Poir.) Pedersen	Amaranthaceae	Gadrya, Garke	Tribes of higher Himalayan range	Powder/decoction of plant is used to arouse sexual aspiration and to enhance the pleasure during sexual act.	Chauthan et al. (2014)
101.	<i>Phoenix acaulis</i> Roxb.	Arecaceae	Chhindi or Khajoor	Tribes of NE India and south India	The paste of the aerial parts of the plant paste is rubbed on nipple of the tribal female to get relaxed lactation.	Khare (2007a, b)

102.	<i>Physalis peruviana</i> L.	Solanaceae	Ras bhari	Tribes of Uttar Pradesh, Madhya Pradesh	The plant is used as antispasmodic, sedative, diuretic, antiseptic and analgesic.	Singh et al. (2019)
103.	<i>Pimpinella bracteata</i> Haines	Apiaceae	Tiryo	Tribes of Uttarakhand and Himachal Pradesh	The root decoction is used twice daily for few days to get rid of frequent constipation and dysentery.	Arya (2017)
104.	<i>Plectranthus mollis</i> (Aiton) Spreng.	Lamiaceae	Bhosare	Tribes of Kerala	The root juice is used to cure usual fever, while the paste is used to get relief from headache.	Arumugam et al. (2016)
105.	<i>Plumbago zeylanica</i> L.	Plumbaginaceae	Chituar	Tribes of Kerala	The decoction of root and leaf is used to treat various stomach ailments.	Rajakrishnan et al. (2017)
106.	<i>Portulaca oleracea</i> L.	Portulacaceae	Lunar, Leeshakh	Tribes of Jammu and Kashmir	Leaf extract with mustard oil is used as boost for hair growth. Leaf extract is also massaged on forehead to get rid of headache. The bitter taste roots are effective for the treatment of prolonged muscular pain, intestinal pain and rheumatism.	Rahimi et al. (2019)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
107.	<i>Prunus persica</i> (L.) Batsch	Rosaceae	Satalu	Tribes of Himachal Pradesh	The seeds have antitussive, emollient, antiasthmatic, haemolytic, laxative and sedative and properties. It is taken for the treatment of constipation in the old-aged peoples. Also useful to cure asthma, coughs and menstrual disorders. The bark is used as diuretic, expectorant and sedative.	Kant et al. (2018)
108.	<i>Rhaphidophora korthalsii</i> Schott	Araceae	Not known	Tribes of Arunachal Pradesh	Paste of leaves is used for the treatment of skin infections.	Sasikala et al. (2019)
109.	<i>Rhus javanica</i> L.	Simaroubaceae	Balaniog	Tribes of Jammu and Kashmir	The decoction of aerial parts of the plant has anti-tumor, anti-malarial, and anti-inflammatory properties.	Sasikala et al. (2019)
110.	<i>Toxicodendron vernicifluum</i> (Stokes) F.A. Barkley	Anacardiaceae	Not known	Tribes of North East India	The young plant parts are used to treat tumors, inflammation, viral infections, and for its anti-rheumatic action.	Sasikala et al. (2019)

111.	<i>Ricinus communis</i> L.	Euphorbiaceae	Erand/ Jada/Rendi	Chakma, Dimasa, Garo, Hajong, Hmar, Khasi (Assam), Gond tribe of Central and Northern India	Powder of seeds is used as an anti-fertility drug for birth control. Decoction of leaves is used to treat jaundice. The oil used as a protection against sun stroke	Majumder et al. (2019)
112.	<i>Rivea hypocrateriformis</i> Choisy	Convolvulaceae	Phanji	Kandha tribes of Odisha	The leaf paste is used in skin diseases	Borkar et al. (2015)
113.	<i>Rollinia mucosa</i> (Jacq.) Baill.	Annonaceae	Biriba	Tribes of South India	Paste of tender shoots is used as a medicine for rheumatism.	Pathak et al. (2010)
114.	<i>Ruellia tuberosa</i> L.	Acanthaceae	Ranughare, Ruwel, Tapas kaaya	Tribes of North East India	This plant has been used as anti-diabetic, diuretic, antipyretic, gastro-protective, thirst-quencher, analgesic, anti-hypersensitive, and antidotal agent.	Ananthkrishnan and Doss (2013)
115.	<i>Rumex nepalensis</i> Spreng.	Polygonaceae	Hobul	Tribes of Jammu and Kashmir	Roots of the plant are used to counter the insect bites and wounds. The paste of roots is used as a cure for hair.	Ghosh et al. (2003)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
116.	<i>Salvia aegyptiaca</i> L.	Lamiaceae	Asvagola	Tribes of Eastern Himalayas and South India	Decoction of aerial parts of the plant is used to treat diarrhea	Bose et al. (2007)
117.	<i>Sarcandra glabra</i> (Thunb.) Nakai	Chloranthaceae	Kari-kari	Tribes of South India	Oil extracted from the leaves is used to treat stress related diseases and high blood pressure.	Sasikala et al. (2019)
118.	<i>Scoparia dulcis</i> L.	Plantaginaceae	Cranghae/Bhuidhania/Bundighas	Tribes of Uttar Pradesh and Uttarakhnad	The decoction of entire plant is used for the treatment of dysentery in children and menstrual disorders in women. The paste of leaves is applied to cure from skin diseases.	Ahmed et al. (2001), Singh and Navneet (2016)
119.	<i>Scutellaria baicalensis</i> Georgi	Lamiaceae	Baikal	Tribes of South India and North East India	The decoction of tender aerial plant parts is used for the treatment of hypertension, insomnia, diarrhea, hemorrhaging, dysentery, inflammations and few respiratory ailments.	Tiwari et al. (2008)

120.	<i>Smilax zeylanica</i> L.	Smilacaceae	Ramdattoon or Sarmukhare, Kumarika, JanglAushbah, Kaltamara, Ayad	Tribes of South India, Tripura, Andaman and Nicobar	The young twigs of the plant are used as tooth-brush. The decoction of roots is used in general weakness and spermatirrhoea. The paste of leaves is used to treat skin diseases, piles, toothache, rheumatism, arthritis, dysentery, venereal diseases, and urinary problems.	Kekuda et al. (2018)
121.	<i>Solanum torvum</i> Swartz.	Solanaceae	Turkey Berry	Tribes of Madhya Pradesh and Chhattisgarh	Traditionally this plant is used to treat diabetes and kidney dysfunctions.	Gandhi et al. (2011)
122.	<i>Soyimida febrifuga</i> (Roxb.) A. Juss	Miliaceae	Rohan, Rakh-rohan, Ruhina, SomidaChettu	Tribes of South India, Maharashtra, Madhya Pradesh	The decoction of bark is used for the treatment of fever and cough.	Reddy et al. (2008)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
123.	<i>Tabebuia rosea</i> (Bertol.) Bertero ex A. DC.	Bignoniaceae	Basant Rani, Tikoma	Tribes of South India	Decoction of bark is taken orally to get rid of intestinal parasites. Also used against malaria and uterine cancer. The decoction is suggested for anemia and to cure from constipation. A decoction of the floral parts, young leaves and tender roots has been used to lessen fevers and pain, tonsil inflammation.	Sasikala et al. (2019)
124.	<i>Terminalia alata</i> Roth.	Combretaceae	Saja	Tribes of south India, Madhya Pradesh, Rajasthan and Uttar Pradesh	The bark decoction is used for the treatment of high fever.	Saraswathi et al. (2012)
125.	<i>Terminalia arjuna</i> (Roxb.) Wight and Arn.	Combretaceae	Arjuna	Tribes of South India, Central India, Rajasthan, Uttar Pradesh and Maharashtra.	The decoction of bark has been used to cure ulcers, leucorrhoea, anemia, diabetes, fracture, cirrhosis and heart related problems.	Dwivedi and Chopra (2014), Alam et al. (2019)
126.	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Combretaceae	Bahera, Baheda, Bahuvirya	Almost all tribes of India	The extracted oil from the seed kernel is used to get rid of pain and inflammations.	Gupta et al. (2020)
127.	<i>Terminalia chebula</i> Retz.	Combretaceae	Harra	Kanwar tribe of Chhattisgarh	Bark decoction and unripe fruits are used to cure intermittent cough.	Bag et al. (2003)

128.	<i>Tripterygium wilfordii</i> Hook. f.	Celastraceae	Peeli bel	Tribes of Central India	The paste of aerial parts is used in the treatment of rheumatoid arthritis and muscular pains.	Rathore et al. (2007)
129.	<i>Tulipa clusiana</i> DC.	Liliaceae	Cur Posh	Tribes of Jammu and Kashmir	Bulbs are eaten raw as worthy heart tonic.	Rivera et al. (2005)
130.	<i>Urginea indica</i> (Roxb.) Kunth	Asparagaceae	Jangalpiyaz	Tribes of Odisha, Assam, West Bengal and south India	Bulbs are consumed to get rid of bronchial troubles. The paste of bulbs mixed with Mahua oil is used for the cure of ulcers.	Aswal et al. (2019)
131.	<i>Withania somnifera</i> (L.) Dunal.	Solanaceae	Ashwagandha, Asgand	All tribes of India	Powder of roots is used in curing numerous ailments including asthma, diabetes, stress, hypertension, arthritis and cancer.	Rayees and Malik (2017)

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Table 5.2 (continued)

Sr. No.	Botanical name of the medicinal plants	Family	Local name	Tribes and their states using the plant	Uses	References
Plants used by the Indian tribes for medicinal purposes						
132.	<i>Wrightia tinctoria</i> R. Br.	Apocynaceae	Kueda, Indrajao, Pala, dhudi	Tribes of Rajasthan, Madhya Pradesh, Gujarat	The decoction prepared from leaves and bark is used as febrifuge, to get rid of toothache, stomach spasms and to cure bowel problems. The decoction of bark is used as an anti-dysenteric, particularly beneficial in piles, also used to treat skin infections and biliousness. The latex is given with water in case of malarial fever.	Srivastava (2014)
133.	<i>Zingiber officinale</i> Roscoe	Zingiberaceae	Sonth	Tribes of North Eastern states	Powder of dried rhizome is used to treat several digestive and respiratory problems.	Rahmani et al. (2014)
134.	<i>Ziziphus xylopyrus</i> (Retz.) Willd.	Rhamnaceae	Chhotaber	Tribes of central and south India	The fruits are used for making dye to protect skin from sun stroke.	Gandagule et al. (2013)

eco-friendly way. They are the hidden protectors of India's natural way but now there is great need to protect them and make them comfortable in their livelihoods.

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Chapter 6

Opportunities and Challenges in Ethnobotanical Studies of Indian Medicinal Plants



Sagarika Damle, Sharon Kadirvelu, and Mayuresh Joshi

Abstract Since the dawn of Ayurveda, the Indian healthcare system has been closely intertwined with plant-based medicines. Diverse Indian tribes and ethnic groups have knowledge of medicinal plants that yield pharmaceutically important biomolecules. Ethnobotanical study begins with understanding of the complex interaction patterns of both the biotic and abiotic factors of a habitat and moves on to Ethnopharmacology, the study of indigenous medicinal systems and aligning them with anthropological activities. Ethnobotanical studies focus on plant resource utilization for food, medicines, art, construction, music, aesthetics, rituals, etc. and play a pivotal role in Bio-prospecting of novel compounds, potent biomarkers, new crop foods, timber and non-timber product utilization, etc.

Thus, the scientific management of the Ethnobotanical database becomes a primary goal in amalgamating traditional and ethnobotanical medicinal knowledge with main stream medicine. This review discusses key points regarding the interrelationship between the biotic and abiotic factors with reference to medicinal plants and their management. Further, it also discusses the complex role of traditions, beliefs, and cohesive existence of stakeholders in plant conservation leading to the preservation of traditional and ethnic knowledge.

The article discourses a five-year database (2015–2020), compiling published literature about important ethnobotanical medicinal plants, listing of new plant species and plants utilized for ethnobotanical purpose with their conservation status and strategies. Based on the compilation, possible strategies and road map for effective conservation has been suggested. As an end-note, opportunities are mentioned that could serve governmental and non-governmental organizations to develop sustainable conservation practices for ethnobotanically important medicinal plants.

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6.1 Introduction

Ethnobotany, by definition, applies to the study of practical utilization relationship, including medicinal applications, between plant environment and humans in a natural setting (Harshberger 1896; Soejarto et al. 2005). The socio-economic-ecological interaction between nature and humans has evolved over time with plants governing a critical position. The knowledge of ethnobotany has been crucial for the survival of the species (Pathak and Bharati 2020; Albuquerque et al. 2017).

India, as a country, is not just rich in its culture and heritage, but is also home to the world's finest and rare flora and fauna. Over the centuries, the indigenous tribes of India have learnt to make these plants yield for them, and over the last 70–80 years science has played a strong role in studying these plants to understand their values and bringing them to crores of Indians who could benefit from it. India's large source of medicinal plants is used for traditional medicinal treatments. Therefore, documenting traditional and folklore knowledge of ethnobotanical plant resources plays an important role in compiling an inventory of newer and hitherto unknown sources of phytoconstituents and therapeutic usage (Chauhan 2020).

In recent times, the term ethnopharmacology has expanded the reaches of ethnobotanical knowledge to encompass a broader multidisciplinary approach (Soejarto et al. 2005). At the same time, with the rise and prominence of bio-prospecting, chemo-prospecting, and with the projected compound annual growth rate (CAGR) of 6.1%, i.e. from \$29.4 Bn in 2017 to approximately \$39.6 Bn by 2022 for the period of 2016–2024 of global market value for botanicals and plant derived drugs (Lawson 2017) (Nasr 2019), over-harvesting, degradation, loss of indigenous and traditional knowledge from local communities, non-acceptance of traditional systems of medicine in mainstream healthcare services and uncontrolled trans-boundary trading has become a grievous issue (Chauhan 2020).

The current chapter reviews the various aspects of ethnobotany in India with emphasis on studies on indigenous and traditional habitats, interaction of biotic factors, advantages of conservation and plant – human interactions. Further, threats and challenges to ethnobotanical resources due to anthropogenic influences, and roadmaps for better conservation have been discussed.

6.2 Status of Ethnobotany and Traditional Knowledge in India

Numerically speaking, the quantitative amount of published research in the field of ethnobotany in India has seen a constant incline despite the advent of newer avenues in research and a reported loss in the available pool of traditional and indigenous

knowledge (Pathak and Bharati 2020). On a quantitative basis, a review article by Pathak and Bharati in 2020 reports that around 2123 research articles have been published during 2007–2018 in the field of ethnobotany. Around 5458 authors affiliated to 1927 organisations have been contributors to this work (Pathak and Bharati 2020).

The current population of India is reported to be 1.388 billion (Worldometers 2020), out of which 104 million are tribal populace spread across approximately 705 communities with unique cultural diversification (Kumar et al. 2020). The reported botanical strength of the country is estimated at approximately 18,386 angiosperms, 79 gymnosperms, 1289 pteridophytes, 2748 bryophytes, 2511 lichens, 15,115 fungi, and 7357 algae (Dash et al. 2019) (Pathak and Bharati 2020).

It is interesting to note that the study of Ethnobotany in India began to see light in the early 1940s and since then, has seen a sustained growth in the number of studies being conducted. Knowledge about plants exists in both recorded and unrecorded formats; unrecorded knowledge usually gets handed down from one generation to another in sects, families, and tribes. According to a paper by Jain SK in 1994, there were 45,000 plant species that were recorded and many of these had medicinal values. India's knowledge of plants can be traced back to ancient Indian doctors; according to a study (Murthy et al. 2008), at least 8000 plants were known to have been used for treating various ailments without any known side-effects. A recent publication by Ministry of Environment of Forests (MoEF), Government of India, under All India Coordinated Research Project on Ethnobiology (AICRPE), reports that the ethnic communities of India use more than ten thousand wild plants for various therapeutic, edible, and miscellaneous uses (Gopal 2019).

However, there has been a severe loss observed in the knowledge about traditional methods of plant cultivation, caretaking, and medicinal properties and also the traditional knowledge about flora. Most of this knowledge is part of the oral methods of information transfer. Hence, as healers and village elders grow old and succumb to the ravages of time, knowledge dies with them (Pandey and Tripathi 2017).

Currently, ethnobotany is predominantly concerned about traditional facts and has a multidisciplinary approach focussing mainly on plant resource utilization. Studies focussing on ecological evaluations and climate analysis, plant habitats and distribution, agricultural and cultivated design studies are slowly gaining fame among ethnobotanists (Ijaz et al. 2017). Thus, even in a modernized approach to scientific discoveries and innovations, the field of ethnobotany shall provide better results when viewed from the stand-point of the traditional healers and ethnic communities.

6.2.1 Relevance of Ethnobotanical Studies

Ethnobotany is reported to be a flourishing segment of research which appeals to the interests of a wide range of researchers from pure science and academic backgrounds to anthropological studies. Being predominantly linked to economic aspect

of botany, ethnobotany has also been pursued by many in order to benefit from the potential economic and resource benefits obtained from plant and plant-related products. The cross-cultural relevance and trade exchanges pertaining to plants and plant resources between communities, societies, and across nations, add significance to ethnobotanical studies and discoveries. Understanding the relation between plants, their medicinal potencies, their commercial values and human interactions and evolution has been a common focus for a wide variety of research endeavours in the interdisciplinary fields concerning ethnomedicine, ethnopharmacology, pharmacognosy, etc. (Leonti et al. 2020).

It is known that the ancient Indian medical system and medicines have been beneficial to treat thousands of chronic illnesses. According to Indian Ayurvedic monographs, reserpine (*Rauwolfia serpentina*) was used to treat high blood pressure (Vicker and Zollman 1999). *Decalepis arayalpathra*, more commonly known as 'Amritha Palam' in the regions of Tamil Nadu, is used for peptic ulcer, cancer-like afflictions, stomach ache, and other similar ailments (Mishra et al. 2015). Similarly, Sunthi (Ginger – *Zingiber officinale* Rosc), Ashwagandha (*Withania somnifera* (L.) Dunal), Guduchi (*Tinospora cordifolia* Miers) are commonly used for treating chronic illnesses.

The assimilation of a traditional or indigenous system is challenging not only because of the difficulties faced in transposition of unwritten and oral evidences into written scientifically valid studies but also due to the decontextualization of information that occurs in a scientific representation of ethnic data. This dissociates the ethnic knowledge from the socio-cultural background, which is necessary in order to understand the plant – human – society interactions (Berkes 2018; Albuquerque et al. 2019). Though inclusion of indigenous populace and community members as co-authors has been appreciated, it certainly does not guarantee legitimate representation of ethnobotanical, indigenous, and traditional knowledge (Zenderland et al. 2019).

6.2.2 Interdependence of Biotic and Abiotic Factors Influencing Human and Forest Relationship

One of the baseline results of an ethnobotanical study is the understanding of the complex interaction patterns of both the biotic and abiotic factors of a habitat. The interdependence is not limited only to other living things but extends to non-living factors too. Though such habitats and interactions have been reported by many to share similarities at a biosphere scale such as average productivity, nutrition turnover rate, soil parameters, etc., these micromanagement capacities of plant communities and interactions with abiotic factors can be held accountable for the wide variations with respect to the composition of plant community (Fujii et al. 2018).

With just tropical forest communities in reference, articles present a comparative scope of analysis between tree species community composition such as prominence of Leguminosae members vs. Dipterocarpaceae members or Combretaceae members, against soil types and other abiotic factors as a function of biodiversity.

Nevertheless, not all community interaction is competitive of nature, positive interactions like facilitation has also been well documented in plant communities (Ma et al. 2019).

Forests are currently the most threatened ecosystems all over the globe. The alterations to this ecosystem have been observed to damage it in ways which are at the core of the planet's atmospheric and climatic cycles (Roberts et al. 2017). The reported decreasing emotional and physical attachment to nature and natural elements post urbanization has further increased the risk of disconnect from nature and inability to understand the importance of ecosystem services that the current urban population benefits from (Palliwoda et al. 2017). Tribal and village population on the other hand are intricately involved with forest communities at a psychosociological level at times. Such a relation enables them a deeper and at times clearer understanding of the interactions among the forest species. Thus, *in-situ* conservation, if undertaken with the help and involvement of forest-based human communities, might be beneficial on both fronts i.e., conservation at a species level and indigenous knowledge level.

Further, as many of the social and ecological changes that affect a forest community occur on a much larger spatial and temporal dimension, with their effects visible after the observation of larger phytogeographical regions and accumulating in the order of decades, a longer, deeper, and community-based study using traditional and indigenous knowledge sources is most important to bridge the gap between reality and theories (Fischer 2018). Hence, study of forest and human interactions becomes a valid stepping stone in understanding ethnobotany and ethnic knowledge systems.

6.2.3 Tribes and Sacred Groves

A mystic approach to the concept of human – forest relation in the realms of conservation comes from the concept of sacred groves (Panda and Mund 2019). With the restrictions in place, sacred groves have been reported to act as treasure houses for rare species. Such a common property-based resource system embroidered with religious sentiments is a successful model for culture-based mode of biodiversity conservation in India (Parthasarathy and Babu 2019). Moreover, sacred groves further assist conservation in the form of ecological functions, unhindered progression of biological and cultural diversity and progress in renewal of ecosystem services (Parthasarathy and Babu 2019).

With the total number of sacred grooves in India being vague at best ranging from as low as 13,720 reported in some publications (Parthasarathy and Babu 2019) to as high as 1,00,000 – 1,50,000 (CPREEC 2016a, b). Governmental databases further suggest a total of 2820 sacred grooves being documented in the state of Maharashtra (CPREEC 2016a, b). The pivotal backbone of traditions and beliefs based on ancestral spirits, myths, rituals, and taboos around these grooves have long preserved the sanctity and existence of sacred grooves, which has in consequence played its part in conserving flora and fauna as if a natural museum of massive trees

and safe havens for medicinal species and fauna alike. For the tribes associated with such groves, these forests are an integral part of the life and, at times, livelihood. These indigenous tribal communities have a deep and intimate relationship with these forests and the conservation and maintenance of such an area is most often managed via voluntary cooperation and selfless communal efforts (Rath and Ormsby 2020).

In the state of Maharashtra, such sacred forests are called as ‘Devrai’ or ‘Devgudi’ or the forest of the God (Amirthalingam 2016). But with urbanization and western influences, these important traditional conservational strategies are being neglected, undermined, and at times terminated. Reports suggest that many such villages have sold off their lands to speculators because of urbanization and an enormous rise in land property prices. This has indeed triggered a cascade of breakdowns of socio-economic beliefs and cultural and traditional systems, which have led to significant losses to the protective sentiments towards sacred groves (Vipat and Bharucha 2014).

Some research articles present a substantial evidence regarding the relation of sacred groves and efforts of conservation. A 1998 study by Singh et al., around the Nagoni sacred forest in Himachal Pradesh reports a higher degree of species richness in comparison to non – sacred forest areas. Furthermore, density of medicinal plant species was reported to be twice in comparison to reserve forest areas wherein close to 40% medicinally important species were observed to be unique to sacred groves (Singh et al. 1998; Parthasarathy and Babu 2019). Similar reports of higher species richness have also been published from studies on sacred groves in Karnataka.

It is worth mentioning that, such reports are not confined to India; a similar study on sacred groves in Tanzania reported a higher woody species richness in comparison to state-managed forest (Mgumia and Oba 2003). Onyekwelu and Olusola (2014) report higher species richness and better conservation of endangered species in sacred groves of Nigerian forests (Onyekwelu and Olusola 2014). Many such comparative studies between sacred groves and non-sacred or state-managed forest land put forth evidence related to the success of the common property resource model of sacred groves in the conservation of forests. Thus, the need for promotion of community management forest schemes like social forestry, community forestry, and commercial forestry from the government is essential to reap the benefits of sacred groves in the modern setting.

6.2.4 Ethnobotanical Products and their Importance in Human Life

Numerous ethnobotanical studies focus on the discovery and utilization of plant resources of various applied fields like pharmacognosy, pharmaceuticals, cosmeceutics, etc. In this reference, sacred groves not only provide plant resource but the never-ending strings of stories associated with these groves provide the much needed holistic in-sight required in designing an ethnobotanical product.

Many of the medicinally potent drugs used in society today have been developed from medicinally important plants utilized in tribal communities under traditional systems of medicine (Heinrich 2003). Bio-prospecting for the discovery and utilization of novel compounds, potent biomarkers, new crop foods, non-timber product utilizations, etc. has always classically been dependant on ethnobotany. Because of the knowledge banks from traditional and indigenous sources behind ethnobotanical information, ethnobotanically directed bio prospecting has become a sought-after avenue in comparison to random assaying of plants in search of bio-active phytoconstituents (Garnatje et al. 2017). Numerous potent medical drugs such as Aspirin, Codeine, Colchicine, Vincristine and Vinblastine, Digoxin and Digitoxin are all examples of the same procedure. Though the practitioners of tradition schools of medicine may not be aware of the chemical structures and properties of individual phytocompounds, these same natural products do form the basis of numerous traditional treatment regimens. In the Indian setting, Ayurveda, other traditional schools of medicine and associated ethnomedicinal and indigenous knowledge is valuable components for workable bio prospecting and value addition processes. Equal benefit sharing among the prospector and the ethnic source of information as part of short-term processing, and in event of a discovery or commercialization of the product as long-term benefits with protection under Indigenous Intellectual Property Rights needs to be highlighted (Noorunnisa et al. 2020). The next section discusses the various strategies used by researchers towards achieving the goal of conservation of medicinal plant. The data presented in the tables namely Tables 6.1, 6.2, and 6.3 provided below is collected from the past 5 years of published work (2016–2020).

6.3 Roadmap for Conservation

Though over the past few years there is a focused attempt at promoting the sustainable use and conservation by the government, for example UNDP in partnership with the Ministry of Environment and Forests and the Global Environment Facility, the urban set up that utilize medicinal plants and their products are heavily dependent on their rural counterparts for providing more than 60% of the raw material that is forest-based. Some of the challenges faced by the wealth of knowledge regarding medicinal plants are manmade or natural calamities such as undocumented, unorganized or inappropriate cultivation practices and usage leading to over foraging and extinction of species, herbal health practitioners working in isolation and passing on their traditional knowledge only by word of mouth or on to family members. Also, resistance of the established mainstream medicinal practices to include herbal medicine, the rural-urban divide for the knowledge exchange and dissemination, the vagaries of fragile ecosystems resulting in habitat loss for the medicinal plants are also some of the challenges. Therefore, it is imperative for India to devise effective strategies for sustainable use and conservation of these medicinal plants. The value chain would include collection of germplasm, newer techniques of propagation, characterization and evaluation, disease resistance, effective storage and distribution to the manufacturer or the end user, with minimum post-harvest losses (IUCN 2011).

Table 6.1 Scientific basis for conservation of select ethnobotanical plants

Sr. No	Botanical name	Reason for conservation	Assays/screening	References
1.	<i>Decalepis hamiltonii</i> Wight & Arn.	It is being exploited for its nutraceutical and medicinal properties; root specific flavor metabolite 2-hydroxy-4-methoxy benzaldehyde	<i>In-vitro</i> propagation, bioactive potential, <i>in-vitro</i> production of flavour metabolite	Pradeep et al. (2016)
2.	A total of 108 plants belonging to 51 families have been identified,	Needs to be conserved for commercial benefit of tribal populace	Soliga tribal community residing at Biligiriranga Swamy Temple Tiger Reserve (BRTTR) uses plants for curing various ailments.	Nautiyal et al. (2016)
3.	The present study reported 51 medicinal plants belonging to 37 families.	Needs to be conserved for commercial benefit of tribal populace	Survey on medicinal plants in southern Western Ghats of Virudhunagar district, Tamil Nadu,	Suresh et al. (2016)
4.	Calamus vattayila Renuka (A study of 3 populations)	Needs to be conserved for commercial benefit of tribal populace	Genetic differentiation and total gene diversity among the population was significantly high, therefore conservation of each population is required as a representative. Possibility of in breeding is indicated.	Priya et al. (2016)
5.	<i>Saraca asoca</i> (Roxb.) Willd	Medicinally important tree and hence needs to be conserved	To understand genetic variation, ISSR markers were used and RP-HPLC of selected phytocompounds were analysed. No significant trends indicating in-situ and ex-situ conservation is required.	Hegde et al. (2018)
6.	<i>Saraca asoca</i> (Roxb.) De Wilde	Commercial herbal preparations, traditional medicine.	RAPD employed to understand genetic diversity; results reveal good genetic diversity; therefore, gene pool is not under immediate threat.	Saini et al. (2018)
7.	<i>Arenga wightii</i> Griff	Commercial applications such as food, fiber, medicinal properties etc.	Genetic variability was assessed in 32 natural populations using ISSR markers that showed similarity more than diversity; this data will help in determining the conservation strategies for the future.	Madar et al. (2019)

(continued)

Table 6.1 (continued)

Sr. No	Botanical name	Reason for conservation	Assays/screening	References
8.	<i>Ensete superbum</i> (Roxb.) Cheesman	The endosperm is used for various human disorders	Exomorphic characters were examined by SEM. This indirectly unveils the genetic diversity of the plants as the size and phenotype varied across the latitudes	(Kumar et al. (2019))
9.	<i>Garcinia indica</i> (Thouars) Choisy	High value medicinal plant.	Determine genetic diversity using ISSR markers. IT revealed genetic diversity; this information will help conservation of potential germplasm	Palkar and Sellappan (2019)
10.	<i>Saraca asoca</i> (Roxb.) Willd	Medicinally important plant.	Determining genetic diversity using ISSR markers and metabolic studies using HPLC. This was done to help develop conservation strategies.	Hegde et al. (2019)
11.	<i>Gnetum ula</i> Brongn	Medicinally important plant of western ghats	Its phytochemical profile is reported as a review; it is not considered classical drug; therefore, these studies are required for implementing proper conservation policies	Irfan et al. (2020)
12.	<i>Decalepis salicifolia</i> (Bedd. Ex Hook.f.) venter	Steno-endemic and critically endangered species	Genetic diversity assessed using ISSR markers- 62% variance detected. GC analysis of 2 HMBA showed significant variation. This allows for planning of in situ conservation strategies for maximum preservation of genetic resources	Gokul et al. (2020)
13.	Genus <i>Calamus</i>	Economically important	Genetic diversity assessed using 26 microsatellite markers	(Kurian et al. (2020))
14.	<i>Garcinia imberti</i> Bourd	Endemic species	Genetic diversity assessed using ISSR markers; revealed less or moderate genetic diversity but all has its own characteristic which should be conserved	(Anto et al. 2020)

Table 6.2 Identification of new species and their conservation status

Sr. No	Species of plants/ethnobotanical surveys	Conservation status	References
1	Endemic riparian angiosperm	Floral diversity of Netravati River system in Western ghats; threatened status. In-situ and ex-situ conservation was proposed	Korse (2017)
2	<i>Henckelia lyrate</i> (Wight) A.Weber & B.L.Burt	Enumeration and conservation assessment has been reported as critically endangered.	Geethakumary et al. (2016)
3	<i>Phyllagathis indica</i> J.Mathew, Yohannan & Kad.V.George	Conservation status is updated as critically endangered	Mathew et al. (2016a, b)
4	<i>Strobilanthes malabarica</i> Josekutty, P.Biju & Augustine	Large population is found in the windward side of Paithalmala along the slopes in the evergreen forests and bordering the grasslands, but not protected from human interactions.	Josekutty et al. (2016)
5	A total of 132 plant species (included Pteridopytes) belonging to 101 genera under 45 families; the present study listed 52- plant species of medicinally important plants utilized by the ethnic people to address their daily healthcare needs	Biodiversity of the Sathuragiri hills in the southern Western Ghats of Tamil Nadu, India	Gurusamy et al. (2016)
6	<i>Andrographis megamalayana</i> Gnanasek, Karupp. & G.V.S.Murthy.	It is a new species from western ghats. It is evaluated as vulnerable using IUCN red list categories and criteria version 3.1	Gnanasekaran et al. (2016)
7	<i>Ceropegia ravikumariana</i> Kambale & Gnanasek.	Data deficient and explorations from similar habitats are required to determine its exact IUCN threat status	Kambale and Gnanasekaran (2016)
8	<i>Miliusa sahyadrica</i> G.Rajkumar, Alister, Nazarudeen & Pandur. a Paleotropical genus	A new species in western ghats. A total of 23 species and 1 genus is recorded in India. 15 species and one variety are reported from western ghats. Except 4 species, all are endemic to that region. It has been treated as critically endangered.	Rajkumar et al. (2016)
9	Medicinal Flora and Related traditional knowledge of Western Ghats	An article reporting the plants for community-based malaria management	Prakash et al. (2016)
10	<i>Piper rukshgandhum</i> J.Mathew	A new species from Achankovil forest, Kerala section. Categorized as critically endangered.	Mathew et al. (2016a, b)

(continued)

Table 6.2 (continued)

Sr. No	Species of plants/ethnobotanical surveys	Conservation status	References
11	<i>Pseudoglochidion anamalayanum</i> gamble	One of the few collections of Anamalais, Coimbatore district. ITS taxonomic position using matK and ITS markers reveals it to be nested among the <i>Phyllanthus</i> species of subgenus <i>Isocladus</i> .	Pagare et al. (2016)
12	A total of 3896 individuals comprising 97 species, 79 genera and 45 families were reported to be present in sholas in the Nilgiri Mountains	This study aimed at providing descriptive information on the floristic composition of the sholas in the Nilgiri Mountains	Mohandass et al. (2016)
13	Two hundred and eighty-five genera of 41 families of climbers were identified in southern western ghats of Tamil Nadu	These are listed as rare, endangered and threatened species (RET). Conservation strategies are required for the same.	Sarvalingam and Rajendran (2016)
14	163 species of plants were reported to be used as ethnomedicinal plants by local traditional healers of Irulas tribes	Ethnobotanical survey among the Irulas tribes in Maruthamalai hills	Tamilselvi et al. (2016)
15	Ethnobotanical survey in Karnataka along the western ghats	A book chapter dedicated to the uses of plants by the indigenous community along the western ghats	Somashekhar (2016)
16	Ethnoveterinary medicines and practices of western ghats	A chapter describing the ethnoveterinary practices and the medicinal plants used along the western ghats.	Nair and Punniamurthy (2016)
17	Tropical reeds: Bamboo genus <i>Ochlandra</i> (endemic to Western ghats)	Ecological function, its unscientific usage, demands and a need for conservation is mentioned in this review	Siji Mol et al. (2016)
18	<i>Litsea floribunda</i> (Blume) gamble	Ratio of male trees are lower and needs conservation of the same.	Srinivas and Krishnamurthy (2016)
19	Ethnomedicinal assessment of riparian vegetation of Bhavani river in Pillur beat, Karamadai range, Western Ghats,	A total of 112 plants were recorded and leaves were the most frequently used part for disease treatment.	Dhivya and Kalaichelvi (2017)
20	<i>I. mankulamensis</i> sp. nov. and <i>I. panduranganii</i> sp. nov.	New taxa of impatiens identified in southern parts of western ghats; classified as critically endangered	Mambetta Prabhukumar et al. (2017)

(continued)

Table 6.2 (continued)

Sr. No	Species of plants/ethnobotanical surveys	Conservation status	References
21	88 species of medicinal plants identified from Ratnagiri of which 5 plants were found to be endemic	This study is an ethnobotanical survey of selected sample villages in Ratnagiri. Conservation of biodiversity of study area is suggested.	Patil and Satyawani (2017)
22	<i>Liparis sanamalabarica</i> P.M.Salim	A new species found in the forests of Wayanad district in Kerala; conservation status is vulnerable	Salim (2017)
23	<i>Anisochilus petraeus</i> Mathew & Yohannan	A new species collected from Achankovil Forests of southern Western Ghats, India. Conservation status assigned as "Critically endangered"	Mathew et al. (2017)
24	<i>Eriocaulon govindiana</i> Nov.	A new species from from marshy areas in the Wayanad wildlife Sanctuary, Kerala. It is categorized as "Data deficient"	Sunil et al. (2017)
25	<i>Dendrocalamus stocksii</i> (Munro) M.Kumar, Remesh & Unnikrishnan	Preservation, sociocultural aspects of this species was studied from Sindhudurg district, south Konkan region of Maharashtra	Digambar Patil (2017)
26	<i>Cucumis silentvalleyi</i> (Manilal, T. Sabu & P.Mathew) Ghebret. & Thulin and <i>Cucumis indicus</i> Ghebret. & Thulin	Both are rare, narrow endemics of western ghats. Both are reported to be highly vulnerable and needs in situ as well as ex situ strategies of conservation	Kattukunnel et al. (2017)
27	A total of 1142 angiospermic taxa was reported at Bhimashankar wildlife sanctuary, northern Western Ghats	Of these 53 taxa are under different threat categories according to IUCN.	Rahangdale and Rahangdale (2017)
28	A total of 99 orchids were reported in a survey in western ghats of Kerala	The survey was carried out to identify the orchis with horticultural and commercial importance, thereby proposing its conservation strategies to protect the gene pool	Ajithkumar et al. (2017)
29	<i>Hopea glabra</i> Wight & Arn. And <i>Hopea utilis</i> (Bedd.) bole	They were located in Silent Valley National Park and Shankili forests in Kulathupuza range respectively for the first time. They are reported to be threatened species	Sreekumar et al. (2017)
30	<i>Strobilanthes sainthomiana</i> Augustine, Josekutty & P.Biju	A new species reported from Paithalmala hills, Kannur District. Population quite large but now protected from anthropogenic disturbances	Augustine et al. (2017)

(continued)

Table 6.2 (continued)

Sr. No	Species of plants/ethnobotanical surveys	Conservation status	References
31	31 plants were found to be reported as ethnomedicinal which were used by Sholaga tribes of Kathri hills.	Ethnobotanical survey of Sholaga tribes	Yogeshwari and Kumudha (2018)
32	<i>Cinnamomum goaense</i> Kosterm.	Rediscovery at Idukki District of Kerala after a lapse of 57 years, and termed as data deficient to determine the conservation status	Geethakumary et al. (2018)
33	<i>Hedyotis beddomei</i> Hook. f.	Rediscovery after 144 years from Elivalmala of Muthikulam forests, Palghat district; assessed as 'critically endangered'	Mambetta Prabhukumar et al. (2018)
34	<i>Distimake rhynchorhiza</i> (Dalzell) Simões & Staples	Was found to be widely distributed in western ghats; also, it is proposed that the status be decreased from "endangered" to "vulnerable"	Rita Simões and More (2018)
35	<i>Kingiodendron pinnatum</i> (DC.) harms	17 populations mapped to 13 forest location in Kerala; based on their economic and medicinal values, isolated and fragmented population, irregularities in flowering and fruiting period, in situ conservation strategies are proposed	Jose et al. (2018)
36	<i>Peperomia ekakesara</i> (Piperaceae) Syam Radh & Nampy	A new species identified from Mathikettan shola National Park in southern Western Ghats; conservation status is "near threatened"	Syam Radh and Nampy (2018)
37	<i>Strobilanthes orbiculata</i> Sinj. Thomas B.Mani & Britto	A new species was found in southern parts of the Western Ghats, India	Thomas et al. (2018)
38	<i>Memecylon travancorense</i> Sivu, N. S. Pradeep, Pandur. & Ratheesh	A new species from Agastyamala Biosphere reserve; it is categorized as "data deficient"	Raghavanpillai Sivu et al. (2018)
39	<i>Crotalaria suffruticosa</i> S.Subraman. & A.K.Pandey and <i>C. multibracteata</i> S.A.Rather & A.K.Pandey	Two new species were found in the Karul Ghat and Panhala region of Maharashtra respectively. matK and ITS markers were used to assess the phylogenetic relationship. Both are considered under "endangered" category	Rather et al. (2018)
40	<i>Micromitrium vazhanicum</i> sp. C. N. Manju, V. K. Chandini, and K. P. Rajesh	It was identified in Peechi-Vazhani wildlife sanctuary and its conservation status is discussed	Manju et al. (2019)

(continued)

Table 6.2 (continued)

Sr. No	Species of plants/ethnobotanical surveys	Conservation status	References
41	<i>Humboldtia bourdillonii</i> Prain	A new population was discovered in Vagamon Hills of Kottayam District which is quite distant from original location. The new location witnesses many environmental calamities therefore conservation measures are required	Balan et al. (2019)
42	33 species were documented as ethno – botanical plants from Salher and Mulher and adjoining areas in western ghats	The list was generated as a result of ethnobotanical survey. Bhil, Kokana and Mahadeo koli tribes were interviewed for the same.	Sonawane (2019)
43	<i>Capillipedium parviflorum</i> (R. Br.) Stapf.	Occurrence reported for first time in Chitramoola, Karnataka	Abhijit and Krishnamurthy (2019)
44	<i>Trichopus zeylanicus ssp. travancoricus</i> Burkill ex K. Narayanan	Ethnomedicinal plant. Fragmented population was found in Agasthyamalai Hills. Has been included under endangered category, therefore conservation and propagation techniques are encouraged	Sasikala and Ramasubbu (2019)
45	<i>Eugenia velliangiriana</i> Murug., V. Ravich., Murugan & Arum.	New species reported from Velliangiri hills, Coimbatore. Designated as data deficient	Maruthakkutti et al. (2019)
46	<i>Strobilanthes tricostata</i> Sinj. Thomas, B.Mani, Britto & Pradeep	New species is reported in Megamalai hills, Tamil Nadu. It is termed as critically endangered	Thomas et al. (2019)
47	<i>Boswellia serrata</i> Roxb.	Ethnobotanical plant of Soliga tribes in the Western Ghats. Gum-resin extraction is carried out by these tribes. The cultural practices, beliefs of these tribes can help conservation plans of these trees in its natural habitat	Kori et al. (2019a, b)
48	<i>Boswellia serrata</i> Roxb.	Gum resin harvest in the western ghats. Used for religious practices. Currently threatened by <i>L. camara</i> invasion and also the harvesting, therefore management is required for viability of this tree.	Kori et al. (2019a, b)
49	Eighty-one climbing plant species and 12 species are threatened in	Distribution of climber in Courtallam hills was carried out. In situ conservation and protection by involving local community is proposed.	Elumalai and Perumal (2020)

(continued)

Table 6.2 (continued)

Sr. No	Species of plants/ethnobotanical surveys	Conservation status	References
50	<i>Desmodium velutinum</i> (Willd.) DC	A review which lists the ethnobotanical uses and pharmacological properties of this plant	Aswathi et al. (2020)
51	Genus <i>anemone</i> L.	One species reported in western ghats. Has medicinal properties; needs assessment of conservation status therefore.	Rajput and Agnihotri (2020)
52	<i>Wendlandia angustifolia</i> Wight ex. Hook.f.	Presumed to be extinct but should be assigned to endangered based on new data from western ghats	Muthumperumal et al. (2020)
53	<i>Impatiens sauliereae</i> B.Mani, S. Thomas & Britto and <i>I. josephia</i> Sinj.Thomas, B.Mani & Britto	New species from Idukki district in Kerala. Classified as endangered	Mani et al. (2020)
54	<i>Cryptocarya sheikelmudiyana</i> A.K.H. Bachan & P.K. Fasila, sp. nov.	New species from Kerala; endangered status	Fasila et al. (2020)
55	<i>Impatiens nidholapathra</i> Vishnu & Nampy, sp. nov and <i>I. grandispora</i> <i>Impatiens grandispora</i> Nampy & Vishnu, sp. nov.	New scapigerous species found in Idukki district in Kerala; assessed critically endangered	Mohan et al. (2020)
56	<i>Goniothalamus sericeus</i> Sujana & Vadhyar, sp. nov	New species found in Western Ghats of Tamil Nadu; provisionally termed critically endangered	Sujana and Vadhyar (2020)
57	Genus <i>Salacia</i>	Reported as endangered and this study assesses the chromosome number of these species.	Kamat et al. (2020)
58	138 species representing ethnomedicinal plants used by Kani tribe	Ethnobotanical survey was carried out amongst the Kani tribe of Pechiparai hills of Kanyakumari wildlife sanctuary, Western Ghats	Sukumaran et al. (2020)

Table 6.3 Opportunities via conservation practices

Sr. No.	Botanical nomenclature	Plant and metabolite/ property of interest	Other reasons of interest	Conservation strategy	References
1	Fruits of <i>Syzygium travaccouricum</i> gamble.	Economic importance	Critically endangered species of southern western ghats. Infested with insect pests	Application of pheromone (methyl eugenol) during the time of fruit setting in the natural habitat of the plants.	Hussain and Anilkumar (2016)
2	<i>Dysoxylum malabaricum</i> Bedd. Ex C. DC. (<i>white cedar</i>)	Economically important endemic tree; genetic diversity in the trees from northern and southern western ghats	–	Variation at ten nuclear simple sequence repeat loci; reduced genetic diversity observed; forest conservation especially in the northern region is required	Bodare et al. (2017)
3	Rattans, or canes	NTFP – Supports many forest dwelling communities	–	High species richness of rattans in western ghats detected using niche-modelling tools; conservation values for 21 economically important endemic rattans identified	Joshi et al. (2017)
4	<i>Impatiens naimudica</i> <i>Impatiens anaimudica</i> C. E. C. Fisch., <i>I. elegans</i> Bedd., <i>I. disotis</i> Hook. f. and <i>I. phoenicea</i> Bedd.	Endemic and rare balsams	–	Ex situ conservation by vegetative propagation using stem cuttings	Prasad et al. (2017)
5	<i>Ceropegia karulensis</i> Punekar, Tamhankar, Lakshmin., Kumaran, Raut, S.K.Srivast. & Kavade	Exploitation of tubers and poor regeneration from seeds	Endemic, endangered	Callus induction, somatic embryogenesis and microtuberization as one of the conservation strategies as the secondary metabolites produced by <i>in-vitro</i> callus tissues and native wild plants varied slightly.	Meena et al. (2017)

(continued)

Table 6.3 (continued)

Sr. No.	Botanical nomenclature	Plant and metabolite/ property of interest	Other reasons of interest	Conservation strategy	References
6	<i>Anoectochilus elatus</i> Lindl	Economic importance	Endangered	Micropropagation and genetic stability assessment by ISSR molecular markers as conservation strategy	Sherif et al. (2017)
7	<i>Spathoglottis plicata</i> Blume.	To protect plant genetic resources by in situ conservation	–	<i>In-vitro</i> asymbiotic seed germination.	Aswathi et al. (2018)
8	<i>Nothapodytes nimmoniana</i> (J. Graham) Mabb.	Camptothecin (CPT)	Red listed species	Assessed CPT content by HPTLC from five different ecotypes. This leads to identification of “chemical hot spots” that ultimately leads to monoculture of these species, as a step towards conservation	Hannah et al. (2018)
9	<i>Garcinia gummi-gutta</i> (L.) N.Robson	Medicinally important	Declining populations	Reproductive biology studies done for proper conservation strategies; highest percentage of fruit set was found in hand cross pollination than natural which is wind pollination.	Aswathi et al. (2018)
10	Endangered anticancer medicinal plants of western Ghats	Therapeutic and medicinal properties	–	A chapter reviewing the plant conservation strategies using key biotechnological tools	Swamy et al. (2018)
11	Threatened medicinal plants of western Ghats	Therapeutic and medicinal properties	–	This chapter addresses the <i>in-vitro</i> multiplication and conservation strategies	Radha (2020)
12	<i>Ceropegia media</i> (Huber) M. Y. Ansari	Medicinal value	Difficulty in germination, slow growth	<i>In-vitro</i> propagation protocol and subsequent phytochemical profile, for conservation of this RED listed plant	Pandey et al. (2020)

Although the Convention of Biological Diversity had set a goal for all participant countries, including India, to reduce the rate of biodiversity deterioration by 2010, along with additions such as the Nagoya Protocol and COP – 10 strategic plans for biodiversity 2011–2020 i.e., Aichi Targets, it should be accepted with stable all around evidences that the targets have not been met (CBD, UN). The inter-governmental Science policy platform on Biodiversity and Ecosystem Services warns us about the unprecedented degradation and decline of natural equilibrium with accelerated rates of species extinction (Dash et al. 2019).

One of the major rationales behind promotion of In-situ conservation is to enable continuous evolution of the ethnobotanical target species and ethnomedicinal plant resources in the face of changing selection pressures, both natural of origin due to climatic changes and induced effects of human interventions (Bellon et al. 2017). As, notwithstanding their indubitable value, ex-situ conservation strategies have an elementary drawback of essentially being a ‘frozen snapshot’ of the phenological and genetic make-up of species at collection.

Taking into account the bio-diverse nature of the medicinal plant species found in India, there are huge opportunities for developing strategies for the creation of above value chain by the Government in close association with rural communities. Ministry of Environment and Forests and the Global Environment Facility, Ministry of AYUSH, National Medicinal Plant Board (NMPB), CSIR – Central Institute of Medicinal and Aromatic Plants (CIMAP), Indian Institute of Integrative Medicine, ICAR- Directorate of Medicinal Aromatic Plant Research and many more agencies are involved in research and development of medicinal plants.

The possible conservation strategies have been illustrated in the Fig. 6.1:

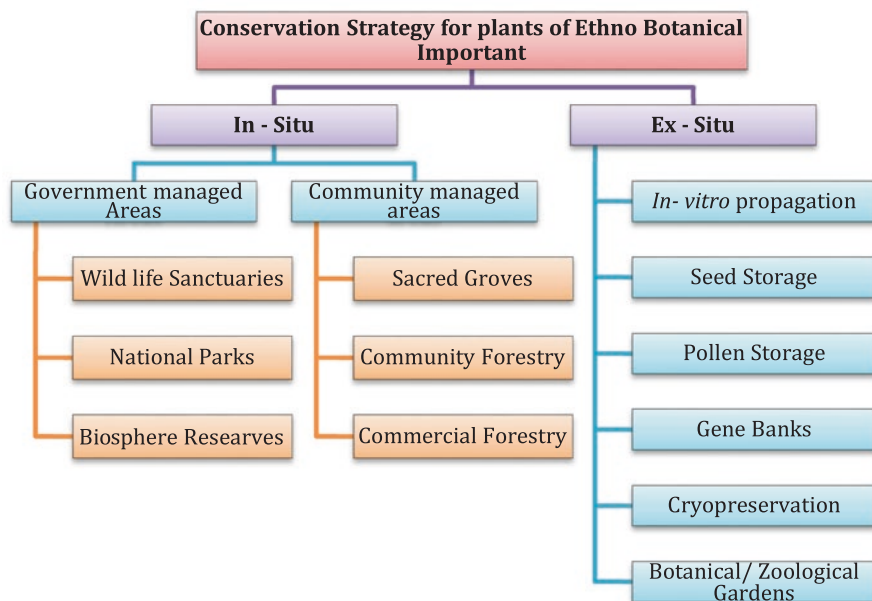


Fig. 6.1 Conservations strategies of medicinal plants

Most of the conservation strategies implemented by NMPB involve capacity building through trainings, raising awareness through promotional activities like the creation of Home/School herbal gardens, support programs for quality assurance and standardization through development of Good Agricultural and Collection Practices (GACPs), development of monographs laying down standards of quality, safety and efficacy; development of agro-techniques and credible institution a mechanism for certification of quality of raw drugs, seeds and planting material (Joshi 2008). The community level efforts of educating the rural farmers regarding sustainable harvesting techniques, discouraging cutting down of native and medicinally important trees, celebrating National Tree planting day, have improved the quality of the produce and increased the incomes of the villagers.

Another effective strategy is the setting up of Medicinal Plant Conservation Areas (MPCAs) which are natural forest areas established and managed by the State Forest Departments in collaboration with local communities to conserve threatened medicinal plants. Technological advances in Biotechnology as well as GIS mapping system have played a pivotal role in conservational success stories, especially ethnobotanical regions. Foundation for Revitalization of Local Health Traditions (FRLHT) has been working on Medicinal Plants knowledge documentation and conservation efforts since 1993 (Utkarsh 2006).

According to a latest report by United Nations Developmental Program (UNDP), 16 Biodiversity Management Committees have been created; and close to 500 women have been trained to document the biological resources found in the forests and local knowledge associated with it (UNDP 2021). If these efforts continue to develop confidence in the traditional healers of safeguarding their knowledge against misappropriation and bio-piracy and ensure their share in the profit incurred by technology driven value addition to the final product, it will add further in conserving the ethnobotanical wealth of our country.

6.4 Conclusion

In conclusion, it can be stated that, there are adequate opportunities for developing the sound practice of sustainable conservation of ethnobotanically important medicinal plants. Successful attempts have already been made by government agencies in some states and an effective public private partnership model can further boost the cultivation and exports of Indian medicinal plants, thus making India the number one country in medicinal plant exports. The following strategies can be adopted for sustainable development of medicinally and economically important plant species:

- Updatable Geo mapping for identification of sacred groves, unprotected forests and other ecotypes for evaluation of actual and factual data.
- Developing tribal leaders and sacred grove managers as mentors to promote sacred groves for younger populations.

- Linking forest or tribal communities with Scientific Community and development of a common nationwide repository for newly discovered tribal and indigenous knowledge.
- Providing adequate funding and infrastructure for promoting Research and Development for Ethnobotanical based bio-prospecting in India.
- The evaluation of Tribal Knowledge and implementation of the same to main stream medicine after scientific evaluation of data.
- Establishment of a value chain from protector/grower to consumers for ecological and economical sustainability.
- Establishment of a regulatory authority to develop norms to prevent misuse and bio-piracy and compensation to all legitimate stake holders.

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Chapter 7

Breeding and Conservation of Medicinal Plants in India



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Abstract India is richly endowed with the plant wealth of medicinal plants, over 8500 species of ethno-botanical interest have been recorded. The medicinal plants are cultivated in commercial scale based on their demand in the industry. To meet demands of both industry and traditional usage, the development of resistant, high yielding, good quality varieties is of paramount importance. Breeding is the way to achieve the desirable traits in plants: to initiate any breeding or crop improvement programs, germplasm collection is essential. Prevailing conventional breeding methods in medicinal plants include selection, hybridization, induced mutation and polyploid breeding. Use of biotechnological tools and research on genes controlling the formation of secondary metabolites and on methods for their transmission are in fancy stage. Therefore, breeding can become one of the key factors for advancing the phytopharmaceutical sector in the future. Keeping this in view, the breeding methods and germplasm conservation details pertaining to commercially important medicinal crops *viz.*, Aloe, Ashwagandha, Glory lily, Isabgol, Medicinal coleus, Medicinal yam, Medicinal solanum, Opium poppy, Periwinkle and Senna are discussed in this chapter.

Keywords Aloe · Ashwagandha · Glory lily · Isabgol · Medicinal coleus · Medicinal yam · Medicinal solanum · Opium poppy · Periwinkle and senna · Selection · Hybridization · Induced mutation and polyploid breeding · Germplasm conservation

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7.1 Introduction

India is one among the 12 mega-diversity countries of the world. As per the study made by Foundation for Revitalization of Local Health Traditions (FRLHT), of the 960 traded medicinal plant species, 178 species are consumed in volumes exceeding 100 MT per year, with their consolidated consumption accounting for about 80% of the total industrial demand of all botanicals in the country. An analysis of these species by their major source of supply revealed that 36 species (20%) from cultivation medicinal plants are the source of many important pharmaceutical preparations, especially plants rich in secondary metabolites are of interest. Considerable effort has been done to generate such metabolites in plant cell or tissue culture. Nevertheless, collection from wild and agricultural production of medical plants still remains the most important supply for plant-derived pharmaceuticals. However, harvesting from wild, especially in species with a high demand, can cause loss of genetic diversity and habitat destruction due to over harvesting.

In this context, breeding of new cultivars is a key factor allowing us for increased yield of valuable compounds, for elimination of unwanted compounds, for tolerance against abiotic and biotic stresses, and for better homogeneity of the cultivars. When compared with traditional food crops, breeding of medicinal plants is now in the initial stages, with the advantage that breeders can exploit a high available natural variability within one species. The generally high natural variability within medicinal plant species is one of the reasons that classical breeding approaches were mainly used till now. Knowledge of reproductive biology of medicinal plants is crucial for improvement, effective conservation and management plans to evolve genetically superior varieties.

7.2 Breeding Methods of Selected MAP Species

In this chapter, breeding of aloe, ashwagandha, glory lily, Isabgol, medicinal coleus, medicinal yam, medicinal solanum, opium poppy, periwinkle and senna, with their botanical description, floral biology and breeding methods, are discussed. Varieties developed in these crops are presented in Table 7.1.

7.2.1 *Aloe*

The genus *Aloe* is indigenous to African continent and Mediterranean countries. It is naturalized over arid tracts of all over India. Out of 275 species, 42 belongs to Madagascar region (Africa). 12–15 belong to Arabian Peninsula and rest of the species is distributed in South Africa. In India only four species are reported to be occurring and of these *Aloe barbadensis* is the most widely naturalized species.

Table 7.1 Varieties of medicinal plants

Crop/Variety	Character
Aloe	
CIM – Sheetal	Developed through clonal selection during 2005 High leaf and sap yielding suited to rainfed conditions
Aswagantha	
CIM – Poshita	Developed by CSIR-CIMAP during 2011 Developed through half-sib selection Dry root yield of 14 q/ha Alkaloids – 1.3 kg/ha Withaferin – 0.538% in dry leaves
CIM – Chetak	Developed through half-sib selection during 2011 Dry root yield of 11.77 q/ha Withanolide – 0.40% Withaferine – 1.22% in dry leaves
CIM – Pratap	Developed through half-sib selection during 2011 Long tap root with less fiber Suitable for drought Dry root yield of 34.95 q/ha
Rakshita	Developed from CIMAP – Lucknow 8–10 q/ha dry roots with alkaloid content High yielding variety
WS 20	Developed through selection High dry yield
Jawahar Asgandh-20	Single plant selection during 1989 High dry root yield
NMITLI-118	Developed jointly by CSIR-CIMAP and NBRI and was released in September 2009 The variety has uniform crop canopy, non-spreading plant architecture (more plant/unit area), withanolide A and withanone in roots and high content of withaferin A (up to 2) Dry root yield of about 15 q/ha.
NMITLI-101	Developed jointly by CSIR-CIMAP and NBRI Potential to yield up to 23 q dry roots under optimal agronomic conditions
Isabgol	
Niharika	Developed through mutation in 1998 Swelling index/mucilage: 442 Seed yield 10–11 q/ha
Mayuri	Developed by mutation breeding during 2003 Early maturing, higher seed and husk yielding variety Distinct pigment marker of the panicles relatable to the maturing Seed yield 11 q/ha
Nimisha	Developed by mutation breeding during 1999 Leaves dark green and medium, long panicles Seed yield 10 q/ha
Haryana Isabgol-5	Selection from GI-2 Duration 140–145 days Yield – 1000 -1200 kg/ha Husk seed ratio – 25:75

(continued)

Table 7.1 (continued)

Crop/Variety	Character
Jawahar Isabgol 4 (JI-4)	Selection from germplasm Boat shaped seeds Violet pink ovary Yield – 1300-1500 kg/ha
Gujarat Isabgol 1 (GI-1)	Introduction Dark green leaves Moderate tillers and medium spike length (4–4.5 cm)
Gujarat Isabgol 2 (GI-2)	Mutation (mutant from GI -1) Medium broad and pale green leaves Matured in 110–115 days
Gujarat Isabgol 3 (GI-3)	Selection from local germplasm collections Long, thin and dark green leaves Profuse tillers and long spike (4.5–5.1 cm)
DOP-14	Mutation breeding during 2010 Early maturing High harvesting index
Medicinal coleus	
Suphala	High yielding (15.93 t/ha) Year round cultivable variety
K-8	Selection from Karnataka (IIHR – Bangalore) 0.5% forskolin and a higher tuber yield
CO 1	Clonal selection from Theni local Moderately resistant to root rot and wilt diseases, field tolerance to nematode and mealy bug infestation. Duration 160–180 days Dry tuber yields 2 t/ha, 33% yield increase over local Forskolin content 23%
Medicinal solanum	
Arka Sanjeevani	Spineless variety
RRL-20-2	A reduced spine mutant developed by RRL (Jammu & Kashmir) through chemical mutagenesis of seeds. Occurrence of spines is reduced to 2–4 per leaf and it is absent on stem. It is photo insensitive Solasodine yield: 42–45 kg/ha
RRL-GL-6	A spineless mutant developed by RRL (Jammu & Kashmir) through chemical mutagenesis of seeds Spines are totally absent in stem, branches, leaf surfaces and pedicel. It is marginally poor in solasodine content.
BARC mutant	A curved spine mutant developed through irradiation of dry seeds with 10 Kr gamma rays High berry yield and glycoalkaloid content
GLAXO mutant	A less spiny mutant evolved by mutation breeding using wild <i>solanum</i> This mutant is characterized by presence of well-developed straight spines on the laminar surface while the stem is devoid of spines Yield of fresh berries – 11.92 t/ha

(continued)

Table 7.1 (continued)

Crop/Variety	Character
Arka Sanjeevani	Inter-varietal hybrid between Glaxo mutant and BARC mutant developed from IIHR, Bangalore Least spiny hybrid with curved spines suitable for high density planting Three fold increases in berry yield and Solasodine content
ArkaMahima	Developed from IIHR, Bangalore Induced tetraploid recording higher solasodine content (2.88%) than diploid counterpart Arka Sanjeevani (1.99%) This variety is devoid of spines
IIHR 2n – 11	Completely devoid of spines Suitable for high density planting 2.5–3% solasodine content
Opium poppy	
Shweta	Developed through half-sib selection in 1984 Tall variety with fringed leaves, white peduncle and flowers Very big and bold capsules
Shyama	Developed through half-sib selection in 1984 Tall variety with black peduncle and white flowers Medium size capsules
Vivek	Developed through induced mutation breeding in 1984 Tall variety with white petal Big size capsule
Sapna	Developed through half-sib selection in 1987 Early maturing for extraction of latex White peduncle and flowers Small size capsules
Dola chota gotia	Dwarf cultivar 85–90 cm height
Sanchita	Developed through mass selection in 1990 Black peduncle and white flower
Rakshit	Developed through selection in intra-specific hybrids for diverse resistance Highly resistant to downy mildew disease caused by <i>Peronospora arborescens</i> High seed and straw yielding variety released in 2001
Shubhra	Developed through mass selection in 2001 Tall, medium fringed leaves, white peduncle and flower Concentrated poppy straw variety Medium size capsule
Sampada	Developed through induced mutation in 2002 Black peduncle
CIM – Ajay	Developed through hybridization followed by selection in 2010 White peduncle
Chetak	Developed through selection
Single dark red	Earliness (80–81 days)
Dhaturia	Individual plant selection
Soma	Spontaneous mutant variety in India

(continued)

Table 7.1 (continued)

Crop/Variety	Character
BROP 1	Crossing selections from Kali Dandi, Suryapankhi and Safed Dandi Highly adaptable to varied agro-climatic conditions Moderately resistant to diseases Morphine content – 13% A synthetic variety, with high morphine, opium yield and seed yield
Kek Duma	Hybrid between <i>P. somniferum</i> var. Havan and <i>P. orientale</i> . 0.65–0.75% morphine in the capsule as compared to low morphine content in <i>P. somniferum</i> var. Havan.
Sujata	Developed through mutation breeding in 2002 World's first opiumless and alkaloid free seed poppy cultivar
Jawahar Aphim 16 (JA-16)	Pure line selection from MOP 539 Flowers – White serrated petal Peduncle – Non hairy Capsule – Spherical, flat at the top and surface grooved
MOP – 16	Beats white flowers Serrated petals and round flat topped capsules Lancing start 120 days after planting Drought tolerant
Periwinkle	
Dhawal	Developed from an induced mutant with high content of leaf alkaloids in 1997 Resistant to die-back Leaf yield 2.0 t/ha and total leaf alkaloids content: 1.3–1.7%
Prabhal	Developed through pure line selection in 2001 Winter hardiness
Nirmal	Developed as a pure line variety from a single plant in 1989 Possess high level of resistant in die-back and collar and root rot White colour flowers
Senna	
Sona	Dry leaves up to 8–10 q/ha and that of dry pods as 4–5 q/ha. Sennoside content – 3.51%
KKM 1	Selection from thenkalam local Sennoside – 2.5%
AFLT 2	Developed through selection Leaf purpose variety, late flowering

Aloes are in considerable demand because of laxative property of Aloin and its gel for cosmetic industry. Barbaloin is the main bitter components of aloe juice which on drying forms semi opaque, dark brown substance called Mussabar in trade. Pharmacopoeia recognized three grades of aloe drug

- (a) Curacao aloe – obtained from *A. barbadensis*
- (b) Socotrine aloe – from *A. perryi*
- (c) Cape aloe – from *A. ferox*

Aloe barbadensis, a member of Liliaceae, is a small, stemless, herbaceous perennial with shallow root system. It bears fleshy leaves in rosette to give a distinct

appearance. The fully grown mature leaves are greyish green, round at the base with broad flat upper surface. It bears bright yellow flowers which are arranged in axillary spike. The flower is actinomorphic, its perianth is arranged in two whorls of three tepals each. It has six stamens in two whorls, the outer whorl has longer filaments than the inner whorl. Ovary is superior. Anthesis period is of 5–10 days within raceme, start from 7.00 am and continued up to 3.00 pm. The receptivity of stigma was observed high at anthesis. The peak period of dehiscence observed from 10.00 to 12.00. The fruits *Aloe vera* matures within 60–67 days (Rathod et al. 2014).

Chromosome number of *A. barbadensis* is $2n = 14$, a triploid plant ($2n = 21$) have been reported from monazite region and Kanyakumari (Abraham and Nagendra Prasad 1979). Morphometric analysis for different species of *Aloe* was carried out by Nayanakantha et al. (2010). As the species does not produce many viable seeds and plants are propagated by vegetative means like suckers. Anusree Das et al. (2015) evaluated morphological and genetic characterization of micro-propagated field grown plants of *Aloe vera* L. and identified three seed setting plants, designated as somaclones. Seeds were viable and germinated (70.58%) *in vitro*. Although chromosome number and morphology of somaclones were identical with the donor plants their RAPD profiles and ITS-1 sequences were different from donor plant. This study reports seed setting somaclones in *Aloe vera*, for the first time which may serve as new genetic resource for biotechnological improvement.

Selection is the method of breeding attempted in *Aloe vera*. CIMAP has released one variety CIM -Sheetal through clonal selection.

7.2.2 *Ashwagandha*

Ashwagandha or Asgandh (*Withania somnifera* Dunal) is an important medicinal plant belonging to Solanaceae family, mainly cultivated in Madhya Pradesh and Rajasthan. The total alkaloid content of the root varies between 0.13 and 0.31%. Roots are used in treating rheumatism, stomach and lung inflammation. It is an erect, stellate-tomentose, greyish, under shrub with 30–75 cm in height having long tuberous roots. Leaves are sub-opposite, broadly ovate to oblong, alternate, petiole, entire and sub-acute with lamina (5–10 × 2.5–7) cm. Flowers are bisexual, greenish, small, solitary, axillary, or in few-flowered cymes. Fruit is a globose/berry, orange-red in colour when ripen and covered in the enlarged calyx. Seeds are many, discoid, yellow and reniform. The chromosome number is $2n = 48$. Only two species, *Withania somnifera* Dunal) and *Withania coagulans* Dunal are found in India. *Withania coagulans*, a rigid, ashy-grey undershrub, 60–120 cm high is found occurring wild in Punjab.

Studies conducted at Indian Institute of Integrative Medicine, Jammu by Mira et al. (2012) revealed that flowering (peak) takes place during April–July and anthesis occurs between 08:00 and 11:00 h. The period of stigma receptivity coincides with anther dehiscence. Fruit set on pollination treatments ranged from 90.8% (passive autogamy), 72% (assisted autogamy), 30.30% (xenogamy), and 56.50%

(geitonogamy) through 50.40% (open pollination). Xenogamy brings about very low fruit set, seed-set and seed germination percentages. It is inferred that *W. ashwagandha* is predominantly an autogamous and self-compatible species. Self-compatibility is mainly accomplished due to close proximity of stigma and anthers.

Synchrony in the flowering periods of the wild and cultivated accessions, monoecious sex expression and an amenability to emasculation and crossing further enhance the possibility of genetic improvement of this amphimictic species through hybridization (Mira et al. 2012). Crossing is done during evening hours 4–6 p.m. Flowers are selected which will open in the next morning. Remove the rest of flowers and buds in the bunch. Petals along with the stamens of selected buds are removed and emasculated flowers are bagged. Pollination is done in morning. Opened or about to open flowers, anthers are collected. Corolla tube having attached anthers is separated by forceps from such flowers. Anthers are allowed to burst on stigma naturally. The flowers are bagged. Seed set is observed in 3–5 days.

Breeding objectives in ashwagandha includes increased root yield and alkaloid content besides resistant to biotic and abiotic factors. Half-Sib Selection with Progeny Testing is followed for development of varieties in ashwagandha. Selections are made based on progeny test performance instead of phenotypic appearance of the parental plants. Seed from selected half-sibs, which have been pollinated by random pollen from the population is grown in un replicated progeny rows for the purpose of selection. A part of the seed is planted to determine the yielding ability, or breeding value, for any character of each plant. The seed from the most productive rows or remnant seed from the outstanding half-sibs is bulked to complete one cycle of selection.

Pure lines form an important genetic resource for improvement of yield and quality. A set of 327 (DWS1-DWS327) pure lines were developed from JA134 out crossed population through individual plant selection, selfing and generation advancement for the first time in Ashwagandha. Variation for qualitative and quantitative traits was observed between pure lines and lines with distinct morphological traits were obtained. Heritability and genetic divergence among a set of 48 pure lines with JA134 and JA30 was assessed based on 20 root yield and its component traits. Based on the inter cluster distance and *per se* performance, the pure lines DWS84 and DWS85 were selected which could be intercrossed to obtain high heterosis and also to recover transgressive segregants for the improvement of root yield and its quality. Pure lines developed in the present study form important genetic resources for the improvement of yield and quality of Ashwagandha (Manivel et al. 2017).

Iqbal and Datta (2006) studied mutation breeding, mutagenic effectiveness and efficiency of gamma-rays, hydroxylamine (HA) and ethyl methane sulphonate (EMS) in *W. somnifera* based on M_1 biological damages (lethality, injury and sterility) and viable mutation frequency (M_2 generation) and suggested that HA and EMS were effective than gamma-rays; while lower doses of gamma-rays along with all the doses of chemical mutagens administered were found to be efficient.

Iqbal and Datta (2007) also reported a selection line possessing broad leaf trait ($2n = 48$) with high root and seed yield. Das et al. (2010) reported eight macro

mutants in 'Poshita' and 'Jawahar 22' of *W. somnifera* following EMS treatments (0.25, 0.50 and 1.00% for 2 h and 4 h durations) to dry seeds. Viable mutation frequency was recorded to be 0.00–3.00% in 'Poshita' and 0.00–2.31% in 'Jawahar 22'.

In Ashwagandha, availability of less variability in nature for total alkaloid content and root yield, it would be advantageous to develop a variety through ploidy breeding, having enhanced root alkaloid with higher root yield beyond the present existing level. In this regard, Chinapolaiah (2008) induced polyploidy in three genotypes of ashwagandha (Poshita, JA-20 and KRC-11) using different concentrations of colchicine solution (0.25, 0.5 and 0.75%) and found variety Poshita was more responsive for the induction of autotetraploids. Autotetraploids of ashwagandha has a potential in getting high alkaloid yield per unit area (Vidya et al. 2013).

7.2.3 *Glory Lilly*

Gloriosa superba L. (Colchicaceae) also known as Malabar glory lily is a perennial tuberous climbing herb, extensively scattered in the tropical and sub-tropical parts of the India, including the foothills of Himalayas. It is a beautiful perennial climber with hollow stem of about 6 m, which emerges per year from the tuberous underground stem in rainy season. The leaves are etiolated, alternate, sessile, lanceolate, and spear shaped with curved end, which helps them to climb and creep. Flowers are large, solitary at ends of branches, greenish at first, then yellow, passing through orange, and scarlet to crimson. The peculiar structures of the large flowers with six perianth lobes bent backwards, six radiating anthers and the style bent almost 90° at the point of attachment to the ovary does not make them suitable for pollination by small insects. Fruits are oblong, ellipsoid capsule. Seeds are numerous and rounded.

Gloriosa superba is considered as a single highly variable species. *Gloriosa* is monobasic with a genetic base $x = 11$. Out of the 10 elemental species, *Gloriosa superba*, *G. lutea* and *G. plantii* are diploids ($2n = 22$), *G. carsonii*, *G. virescens* and *G. richmondensis* are tetraploids ($2n = 44$) and *G. rothschildiana*, *G. latifolia* and *G. magnifica* are octoploids ($2n = 88$). In general, octoploid species are comparatively short statured and constitute a medium group of plants.

Five stages of flower development viz., bud initiation, bud opening, pre-anthesis, anthesis, post pollination stage was reported. The flower colour changed during each stage of flower development. The perianth lobes at the bud opening stage were light greenish in colour followed by the stigma receptive stage which was characterized by perianth lobes that were scarlet red at the tip, yellow in the middle and greenish towards the base. Post pollination stage was characterized by the upper half of perianth lobes being scarlet red and the lower portion being yellow coloured. Lastly, the perianth lobes turned entirely into scarlet red.

Anthesis was observed to occur earlier than 7.30 am to 9.30 am with 40% of the flower opening by 7.30 am, 50% by 8.30 am and rest 10% by 9.30 am (Farooqi et al. 1993). According to Mamtha (1989), the peak period of anthesis in *Gloriosa superba* was between 8.30 and 10.30 A.M. It was observed that on the day of

anthesis, there was no anther dehiscence. One day after anthesis, the anther started dehiscing earlier than 7.30 am to 9.30 am. On an average, 5% of the anthers dehiscenced before 7.30 am, 70% before 8.30 am and another 25% by 9.30 am (Nagajothi 2008). According to Anandhi and Rajamani (2012), the anther dehiscence started from 6.30 am and reached the peak at 9.30 am and thereafter started declining and reached the minimum at 10.30 am. This indicated that glory lily is photosensitive and anthesis corresponded to the intensity of sunlight falling on the plants.

In *Gloriosa superba*, 97.50% pod set was observed in flowers which were pollinated on the day of anthesis, indicating the maximum receptiveness of stigma during anthesis. In general, the percentage of pod set was higher in the early morning hours (7.00–11.00 am) irrespective of the pollination done on different days. In general, the stigma remains receptive for 3 days *viz.*, 1 day prior to anthesis, on the day of anthesis, 1 day after anthesis. These receptive periods coincided with pre-anthesis, anthesis and post pollination stage of flower development. The loss of stigma receptivity can be identified from the change in stigma colour from green to red. The mean percentage of fertile pollen in *G. superba* was maximum on the day of anther dehiscence and declined gradually as the age of pollen increased (Anandhi and Rajamani 2012). Vigneshwari and Renugadevi (2006) reported that flowering in *Gloriosa superba* occurs during the month of November to March, but peak flowering is in the month of December to January. The duration of flowering phase was 21.10 days in *G. superba*.

Gloriosa superba is both self- and cross-pollinated (Gupta and Raina 2001), seed set is dependent upon both pollinator activity and the time of pollination. Although there are no self or cross-incompatibility barriers, the herkogamous nature and attractively coloured flowers, favours cross pollination. Nagajothi (2008) stated that hand pollination recorded the highest pod set per cent of about 70.93% followed by air blowing pollination using power sprayer (65.52%). Maximum pod set was observed in artificial cross pollination within the species followed by self-pollination.

Breeding objective in glory lily includes increase in seed yield and alkaloid content with resistant to biotic and abiotic factors.

Mamtha et al. (1993) studied the relationship between vegetative growth and yield in glory lily who revealed that seed number was high in medium sized fruits and dry seed yield increased with increase in number of branches and leaf area. Seemanti Ghosh et al. (2007) attempted studies on polymorphism in five different populations (Amtala (AM), Baruipur (BR), Siliguri (SG), Darjeeling (DJ) and Sikkim (SK)) of *Gloriosa superba* L. The colchicine content in tubers ranged from 0.06% in AM to 0.37% in BR population. Chitra and Rajamani (2010) evaluated 18 ecotypes of glory lily at Coimbatore, Tamil Nadu. The ecotype GS15 exhibited superior performance for most of the morphological and yield characters, followed by GS06.

In *Gloriosa superba*, the genetic variability is low owing to the continued vegetative propagation through tubers. Wide hybridization enables the interspecific gene transfer, which may lead to the additional source of variation for desirable characters. Attempts were made by Anandhi et al. (2013) to investigate the possibilities for

developing variability in this species with varying flower colour, shelf life, high seed yield and improved colchicine content through interspecific hybridization involving *G. superba* with *G. rothschildiana*. Five ecotypes of *G. superba* were crossed with *G. rothschildiana* in both directions.

Varying percentage of pod set was observed with pods of 2.00 cm length within 25 days of pollination and thereafter shrunk and died irrespective of the cross combination under study. None of the pods reached the harvestable stage. Post fertilization barrier was observed in both direct and reciprocal crosses. This may be due to embryo abortion and degeneration during embryogenesis.

Induction of variability in glory lily through mutagenic treatments is of paramount importance for crop improvement. Chandra and Tarar (1991) worked on development of mutants using Co-60 gamma rays, EMS and DES on *G. superba* and obtained various morphological changes in height, structure of the plant, flower and capsules under gamma treatments. Multi armed tubers and furcated stem mutants under EMS and flower size mutants under DES treatment was obtained. Rajadurai and Vadivel (2001) concluded that colchicine content of leaves was higher on treatment with gamma rays @ 1.00 kR and also the yield attributes was greater in the treatment DES @ 0.75%. Anandhi et al. (2013) made an investigation to induce mutants in *G. superba* L. possessing high content of alkaloids viz., colchicine and colchicoside. VM₂ generation was raised from EMS, DES and gamma ray treated VM₁ tubers of glory lily. The maximum colchicine content of 0.70% was observed in T₇P₃ (2% EMS), followed by 0.702% in T₈P₂ (1.00% DES).

7.2.4 *Isabgol*

Plantago ovata is commonly called as Psyllium. India is the largest producer and exporter of this crop in the world. It is grown as a cash crop in Gujarat, Punjab and Uttar Pradesh. The seed husk is used to cure inflammation of the mucus membrane of gastrointestinal and genito-urinary tracts, chronic constipation, dysentery, gonorrhoea and piles. It is native to Mediterranean region and distributed from Canary Islands across Southern Spain, North Africa, Middle East and North Western Asia. Although *P. ovate* is cultivated widely throughout the world, India dominates the world market in its production and export. In India, it is cultivated as a 'rabi' or post rainy season crop in the western regions. The main states in which it is cultivated on commercial scale are Gujarat, Rajasthan and Madhya Pradesh, more particularly in North Gujarat and its adjoining part in Madhya Pradesh and Rajasthan.

Plantago is a large genus of herbs of sub-shrubs distributed mostly in the temperate regions and a few in the tropics. The important species are *P. psyllium*, *P. indica*, *P. ovata* and *P. psyllium*. *P. psyllium* is glossy deep brown seeds with boat shape, outline elongated ovate, 2.0–3.0 mm in length with 100 seed weight of 0.09–0.10 g. *P. indica* has dull blackish brown with boat shaped seed, outline elliptical, 2.0–2.5 mm with 100 seed weight of 0.12–0.14 g. *P. ovata* has dull pinkish

grey – brown with boat-shaped: outline ovate, 1.8–3.3 mm with 100 seed weight of 0.15–0.19 g.

Isabgol is a sub-caulescent or stem less, soft, soft, hairy annual herb which grows to a height of 30–45 cm. Leaves – 6.0–25.0 cm long, 0.5–1.0 cm broad, narrowly linear or linear lanceolate, strap like, recurved, finely acuminate, entire or distantly toothed, attenuated at the base, sessile Stem – pseudo petiole. Inflorescence-cylindrical terminal spikes (0.6–5.6 cm). Small, white or colourless, sessile, bisexual, tetramerous and actinomorphic flowers crowded on a main axis, sepals are 4, free, ovate, obtuse and glabrous. Petals are 4, fused, forming corolla tube, lobes ovate or orbicular and glabrous. Stamens are 4, epipetalous. Gynoecium is bicarpellary, syncarpous, ovary superior, bilocular, containing one ovule per locule. Style is filiform. Fruit is capsule covered by a persistent calyx. Seeds are hard, cymbiform (boat shaped), outline ovate, acute at one end, smooth surfaced and dull pinkish, pinkish grey or pinkish brown in colour.

The inflorescence spike with about 60 florets crowded at the top of the fragile peduncle in about 3 cm length. The flowers are protogynous with floral maturity occurring in acropetal succession. Thus, the gynoecium of the bottom most flower mature first protruding its stigma through the tip of the unopened flower studies on stigma-pollen maturation schedule and their interrelationship have revealed that anthesis occurred during early morning hours, but stigma maturity was distributed to both morning and evening hours with the lowest frequency at noon (Patel et al. 1980) also reported that 14 florets in a spike born matured stigmata before anthesis started in that spike. In a spike, minimum time gap between stigma receptivity and anthesis in the same floret was observed to be 14 h with variation up to 120 hours. Pawar (1981) concluded that stigma receptivity, as judged by stigma elongation, continued to occur in acropetal succession in florets of a spike throughout the day and probably at night. Pollen grains collected in the morning and stored at 0°C without silica gel till evening were most viable as judged by percent florets which set seed. Pollen grain could, however, remain viable for about 50 hours if stored at a temperature range of –3 to 7°C. The stigma remained highly receptive for 1 day (seed set 92.18%), moderately receptive for next 5 days and significantly lost the receptivity in subsequent days. After the ninth day, the receptivity was completely lost.

Breeding objectives in Isabgol should be to increase in the seed size and yield, compact and non-shattering spikes, synchronous maturity, dwarf size, seeds with higher swelling factor and genotypes resistant to drought and frost.

Crop improvement in Isabgol is difficult as *P. ovata* has a narrow genetic base and lack of variability on account of low chromosome number ($2n = 8$), small chromosome size, presence of high heterochromatin in the chromosomes, low chiasma frequency and low recombination index and high selfing rate with the genome size of 500 Mb (Sareen and Koul 1999; Dhar et al. 2005).

The prospects of evolving better varieties through selection alone are limited since the species has a narrow genetic base and lacks variability.

Autotetraploid using colchicines @ 0.5% was found to be best for induction of tetraploid in the variety GI-2. Their autotetraploid status was confirmed by

conducting chromosome counts on dividing pollen mother cells (PMCs). Tetraploids showed superiority over their diploid counterparts except number of seeds per spike. Tetraploids were late in 50% flowering by 13.30% number of days than diploids. Pollen size was larger (31.55%) than that of diploids, but pollen viability decreased by 16.53%. Size of stomata was bigger in tetraploids.

Mutation breeding in Isabgol resulted in identification of an early mutant DOP 14. It has early maturing with desirable traits such as high seed yield, early & uniform seed maturation and high harvest index. Single plant with earliness (80 DAS) was identified and isolated in M2 generation of 0.4% DES treated Isabgol cultivar GI-2. It was self-pollinated and advanced to next generation. In M3 generation, the mutant bred true and all the plants showed the early maturity. DOP-14 mutant started flowering at 34 DAS and seed matured at 85 DAS at DMAPR, Anand, Gujarat which is 35 days early compared to its parent GI-2 (120 DAS). The harvest index was 22.8% which was 31% higher than parent GI-2. It can be an important source for developing early maturing high yielding Isabgol varieties with desirable quality (Manivel and Saravanan 2010).

7.2.5 Medicinal Coleus

Coleus forskohlii Briq. (syn. *C. barbatus* Benth.), an important indigenous medicinal plant in India is a member of the mint family, Lamiaceae. The diterpenoid, forskolin (syn. Coleonol), is very important among the secondary metabolites and is used for the treatment of various diseases viz., eczema, asthma, psoriasis, cardiovascular disorders and hypertension. It is a herbaceous, pubescent, aromatic species with annual stems and perennial rootstock, growing 45–60 cm tall. Tuberous roots are succulent but hard, tortuous or straight, short and stout or long and slender up to 56 cm in length. The cultivated types are fleshy and succulent, spindle shaped or fusiform, generally long, several and radically spread. Root tubers in both the kinds have white or orangish pink flesh with bitter after taste and are aromatic. Tuber skin is papery, yellowish brown, brownish or brownish black based on the soil substratum. Leaves are 7.5–12.5 cm in length and 3–5 cm in width *Coleus forskohlii* Briq. (Syn. *Plectranthus barbatus* Andr.). In India, the major medicinal *Coleus* plant species are the tuberous *C. forskohlii*, *C. amobonicus*, *C. blumei* and *C. malabaricus*.

Reddy (1952) reported that *C. forskohlii* is diploid with $n = 14$. However, Riley and Hoff (1961) from their studies on chromosome numbers in South African dicotyledons reported that *C. forskohlii* is diploid with basic chromosome number $n = 16$. Bir and Saggoo (1982, 1985) reported that Central Indian collections have basic number of $n = 17$, while South Indian collections have $n = 15$ and concluded that variability in base number of various members of the family could be due to aneuploidy at generic level which ultimately leads to morphological variations. Shah (1989) reported that populations of *C. forskohlii* from different ecogeographic areas vary greatly in their morphology.

Breeding objectives of medicinal coleus include increase in tuber yield and forskolin content. To improve varieties for resistant to biotic and abiotic stress. Selection and mutation breeding were attempted in medicinal coleus and varieties were released.

7.2.6 Medicinal *Solanum*

The genus *Solanum* comprises of about 2000 species which can be broadly grouped as tuberous group and non-tuberous group. *Solanum viarum* belongs to the non-tuberous group. It occurs naturally in Sikkim, West Bengal and Orissa and in Western Ghats up to 1600 m MSL. *Solanum viarum* is a natural source of glycol alkaloid solasodine which is Nitrogen analogue of diosgenin. Solasodine content is extracted from berries of Medicinal solanum and the content is about 1.60 to 1.75%, Solasodine acts as a substitute for diosgenin in the synthesis of steroid hormones.

S. viarum is an erect perennial, 50–150 cm high, with shortly pubescent stems and branches with recurved prickles up to 5 mm long, pubescent at their base. There are also longer, straight spines up to 2 cm long on the petioles and the veins of upper and lower surfaces of the leaves. The leaves are broadly ovate up to 20 cm long and 15 cm wide, bluntly lobed with markedly undulate edges, generally dark green and glossy above, duller below. The flowers are white, borne in racemes with 1–4 flowers per cluster. The fruit is a globose in shape, berry is mottled green when young, maturing yellow, 2–3 cm across. It contains up to 400 brown, flattened, discoid seeds, 2–3 mm in diameter. Roots have buds which will regenerate new shoots. The root system can be extensive, with feeder roots 1–2 cm in diameter located a few cm below ground extending 1–2 m from the crown of the plant.

Flowers are hermaphrodite, white and appear in axillary racemes, 14–16 mm in diameter, complete, actinomorphic, bisexual, hypogynous, pentamerous in nature. Anthesis during early morning (4.00–6.00 h), anther dehiscence just after flower opening, mode of anther dehiscence is by apical pores. Pedicels are 6.8–7.5 mm in length. Sepals are five in number, spiny, persistent, green in colour. Petals are five in number, white in colour, recurved. Stamens are five in number, filament is short (1.0–1.5 mm), stout, slightly swollen at the base, whitish, anthers more or less oblong, 2-celled, basifixed, dehisces by apical pores, creamish white. Carpels are 2 in number, syncarpous in nature. in two different forms: long style (9.0–9.5 mm), and short style (2.0–2.5 mm), long style has high fruit setting percentage. Stigma is green in colour, wet, partially lobed (0.5–1.0 mm). Ovary is superior, 2 chambered with many ovules. Each berries are globose in shape with a persistent calyx, 20–30 mm wide, green with dark speckled, when immature, dull yellow when ripe. Somatic chromosome number is $2n = 24$ (Krishnappa and Chennaveeraiah 1976).

Anthesis was found to be occur throughout the day with 84% flowers opening between 8.00 and 16.30 h. Anther dehiscence commenced an hour after anthesis. Stigma receptivity was noticed from 4 to 64 h after anthesis (Krishnan 1995).

Inter varietal hybridization was reported by Nandha Kumar (1983). Biparental matings were carried out adopting six parameter model using two morphologically distinguishable mutants-Glaxco and BARC varieties and the wild type. Differences in the shape and distribution of spines are the only characters that distinguished these three varieties. In the F₂ generation of the cross involving BARC and Glaxo varieties, a new recombinant type for spine character was recovered. This recombinant was similar to the less spiny Glaxo parent in the restricted presence of spines in the lamina, but the spines are vestigial a curved resembling the BARC parent. Three advanced generation lines tested for 3 years were found to be on par with parents and other released varieties both for berry and solasodine yields (Krishnan et al. 1988). It indicates the narrow genetic variability and inadequacy of varietal hybridization for achieving enhancement of solasodine content and berry yield.

Interspecific hybridization of *Solanum viarum* was attempted with non-steroid bearing *Solanum* species but the crosses have resulted either in parthenocarpic fruits with aborted seeds and no fruit set (Krishnappa and Chennaveeraiah 1976).

As genetic variation in *S. viarum* is limited, mutation breeding is the option to achieve target characters for such as removal of spines, enhancement of berry yield and solasodine content. Bhatt (1972) reported the isolation of curved spine mutant (BARC variety) having higher berry yield and glycoalkaloid content following irradiation of dry seeds with 10 kr gamma ray dose. A less spiny mutant viz., Glaxo was evolved by mutation breeding using the wild type as the base material (Gadwal 1977). Two mutants viz., Glaxo and BARC were used as parents in the inter-varietal hybridization program which led to the development of IIHR-2n variety, Arka sanjeevini (Krishnan et al. 1988).

The seeds of diploid, Arka sanjeevini ($2n = 24$) and tetraploid, Arka mahima ($2n = 48$) varieties of *Solanum viarum* were used for obtaining explants for *in vitro* mutagenic study. Frequency of variant progenies in F₂ (Tissue cultured and mutagen treated) was more than SC2 for many characters both quantitative and qualitative (Maruthi Kumar and Tejavathi 2011).

In-vitro mutagenesis using stem explants of diploid and tetraploid varieties of *S. viarum* with both chemical and physical mutagens and cultured on MS+IAA (17.13 μ M)+BAP (8.87 μ M) to obtain large number of multiple shoots. That obtained shoots were made to root on MS+IBA (4.90 μ M) and transferred to land after a brief period of acclimatization. Berries were harvested and second generation progenies were raised from the seeds of untreated and treated first generation progenies. Harvested berries from eight samples including treated and untreated diploid and tetraploid varieties along with seed control progenies were subjected to HPLC analysis to evaluate the effect of mutagens on the solasodine content. Berries of tetraploid progenies obtained through physical mutagen treated cultures were found to contain more percent of solasodine compare to other progenies.

7.2.7 Medicinal Yam

Yam belongs to the genus *Dioscorea* of family Dioscoreaceae. The tubers of some species of *Dioscorea* are important sources of diosgenin, a chemical used for the commercial synthesis of sex hormones and corticosteroids, which are widely used for anti-inflammatory, androgenic and contraceptive drugs. Some specious twin clockwise and some anti-clockwise, all are dioecious and rhizomatous. Monocotyledonous, it shares several features with dicots. These include presence of cordate and acuminate leaves with 5–9 basal nerves and in some of its species, embryo with two cotyledons.

The genus contains some 600 species with more than 10 species cultivated for food and pharmaceutical use.

D. deltoidea Wall is an indigenous species to north western Himalayas, 1000–3000 msl, Chromosome number $2n = 20$ m The petioles are 5–12 cm long, 4–12 cm wide cordate. It is a hairless vine, twinning clockwise. Flowers are borne on auxiliary spike, male spike 8–40 cm long and stamens 6, female spike 15c long 3.5 cm board, 4–6 seed, seeds are winged and round, rhizome is lodged in soil, superficial, horizontal, tuberous, digital and chestnut brown in colour. It produces very slender vines and is very weak. Rhizomes are ligneous, irregular. Regeneration of tuber is slow takes about 7–10 years – commercially not attractive.

D. composita Hemsl., is native to Mexico (Central America), Robust climber, right twining, nearly glabrous, alternate leaves, long petioles, membranaceous or coriaceous lamina. The fasciculate, glomerate inflorescence, single or branched 2–3 sessile male flowering having fertile stamens. Female flowers bifid stigma tuber large, white, deep rooted upto 45 cm.

D. floribunda Mart. & Gal. is also a Central American introduction. Grown in Karnataka, Magalaya, Andhaman and Goa. Glabrous and left twining. Leaves alternate, boardly ovate or triangular ovate. Shallow or deeply cordate, coriaceous lamina with 9 nerves, petioles 5-7 cm long thick. Male flowers solitary rarely in pairs, female flowers divaricated stigma which is bifidat apex, capsule obovate, seed winged, tuners thick-yellow in colour and grows up to 35 cm.

The major breeding objectives in steroid bearing *Dioscorea* species are higher diosgenin content, high tuber yield, compact tuber growth, resistant to diseases and wide adaptability.

Under Banglore conditions, flowering in *D. composita*, *D. floribunda* occurred during March–October in both male and female parents and peak periods in March and April (Bammi et al. 1979). Anthesis occurred in the early morning hours between 4 and 6 am. Anther dehiscence occurred after or about an hour of anthesis. Stigma receptive for 12 h in *D. deltoidea*, 30–35 h in *D. floribunda* and *D. composita*. Under open pollinated conditions, fruits matured in 90, 110 and 120 days in *D. deltoidea*, *D. floribunda* and *D. composita* respectively. In *D. floribunda* and *D. composita*, physical proximity of male and female flowers (to lesser than 2 ft) was found to be necessary for pollination. Ants are pollinating agent. Intertwining of male and female inflorescence gives good seed set.

Selection in introduced materials of *D. floribunda* and *D. composita* was documented (Bammi and Randhawa 1975). Variability for tuber yield is 0.32–3.05 kg/plant and for diosgenin content it ranged from 1 to 3 kg/plant. In *D. deltoidea*, studies conducted at IIHR, Bangalore indicated wide clonal variations among collections drawn from Himachal Pradesh and Jammu & Kashmir states for diosgenin content. Positive association was found between flesh colour of tuber and diosgenin content with white colour associated with high diosgenin content (Bammi et al. 1972).

Intra and inter specific hybridization was attempted in the three species. In *D. floribunda* 10 intra specific crosses were compared for germination percentage and seedling vigour. Character inter-relationships indicated that heavier seeds from large leaved vines with lesser number of fruits per panicle is likely to produce more vigorous seedlings in greater frequencies.

Inter specific hybridization was attempted in the steroid bearing *Dioscorea* species. Among the three species, the old world *D. deltoidea* was successful parent only in crosses with *D. sylvatica* but failed to cross with *D. composita*, *D. floribunda*, *D. alata* and *D. friedrichsthallii* (Rama Rao et al. 1973). In *D. deltoidea* and *D. sylvatica* cross both F1 and F2 generations were reported to combine the characters of parents viz., high diosgenin content of *D. deltoidea* and compact tubers of *D. Sylvatica* parent.

D. composita and *D. floribunda* have been successfully crossed with each other (Martin and Cabanillas 1966; Rama Rao et al. 1973) and reciprocal crosses were also successful. The F1 hybrid of *D. floribunda* and *D. composita* cross manifested heterosis for leaf size, leaf number, stem diameter, side branching, flower size and seed size. The hybrid combined the early sprouting vigour and high tuber yield of *D. floribunda* parent. It had less marked dry season dormancy and high drought tolerance as the *D. composita* parent (Bammi and Randhawa 1975). Both *D. Composite* and *D. floribunda* have been successfully crossed with *D. friedrichsthallii* (Rama Rao et al. 1973). Reciprocal crosses of *D. floribunda* and *D. friedrichsthallii* resulted in the production of viable seeds while seed set and germination was obtained only in *D. friedrichsthallii* x *D. composita* but not reciprocal cross. The hybrids were low in tuber yields and not found promising.

Rama Rao et al. (1973) recognized four levels of crossability based on comprehensive study of hybridization involving *D. deltoidea*, *D. floribunda*, *D. composita*, *D. friedrichsthallii* and *D. alata*. In group A, represented by *D. deltoidea* as male parent in crosses with *D. floribunda*, *D. composita* and *D. friedrichsthallii* and *D. composita* and *D. friedrichsthallii*, hybrid embryo abortion occurred at four or eight celled stage resulting in post fertilization ovular breakdown. In group B, pre fertilization ovular breakdown following failure of fertilization ascribable to either non germination of pollen or slow pollen tube growth have been implicated for failure of crosses. The successful combinations falling in this group included reciprocal crosses of *D. alata* with *D. deltoidea*, *D. composita*, *D. floribunda* and *D. friedrichsthallii*, *D. deltoidea* as female parent in crosses with *D. floribunda*, *D. composita* and *D. friedrichsthallii*. In-group poor seed germination was noticed in *D. friedrichsthallii* x *D. composita* belonged to this group. The successful crosses with readily

produced interspecific hybrids were grouped in D: these consisted of four crosses involving three new world species, viz., *D. floribunda*, *D. composite*, *D. floribunda* x *D. friedrichsthallii*, *D. composite* x *D. floribunda* and *D. friedrichsthallii* x *D. floribunda*.

In mutation breeding of *Dioscorea*, chemical mutagens, especially (N-nitroso methyl urea-NMU and diethyl sulphate) stimulated germination and seedling vigour. Treatment with 0.05% EMS, resulted in higher tuber yielding mutant (88.63 t/ha as against 62.01 t/ha) with higher diosgenin content in 0.15% EMS treatment and compact tuber mutant (Sahoo et al. 1986).

Induction of autotetraploids have been reported in *D. deltoidea* (Janaki Ammal and Singh 1962) and *D. floribunda* (Murthy 1977). For induction of autotetraploids in *D. deltoidea*, aqueous colchicines solution (0.4–1%) was injected into dormant tubers, shoots with tetraploid chromosome number of $2n = 40$ were identified and shoots of these autotetraploid were characterized by large and thicker leaves and bigger stomata than diploid. Rhizomes of tetraploid were found to be slow growing.

7.2.8 *Opium Poppy*

Opium poppy (*Papaver somniferum*) is an important medicinal plant belongs to family Papaveraceae. It is grown as a dual-purpose crop for both seeds and alkaloids. Its seeds are very nutritive and contain high percentage of linoleic acid which is lower blood cholesterol in human system. The opium latex is extracted from green but fully grown capsule which contains several alkaloids used as analgesic antitussive and antispasmodic in present day medicine. The opium is considered an oldest and perhaps the best known pain killer from times immemorial. Opium distributed in temperate and subtropical regions of world extending from 60°N in North West Soviet Union whereas the southern limit reaching almost the tropics. India is one of the largest producers of opium alkaloids in the world. To cultivate Opium poppy license has to be obtained from office of Narcotics department, Government of India. At present, cultivation is confined to UP, Rajasthan and MP. The major alkaloids present in *Opium poppy* are Morphine:7–17%, Codeine: 2.1–4.4%, Thebaine: 1.0–3%, Nacrotics: 3–10%, Papverine: 0.5–3%.

The plant is an annual herb, erect, commonly 30–150 cm long with 0.5–1.5 cm thick stem. Root is much branched, tapering and yellow. Stem is glabrous with thick waxy coating. Leaves are numerous, alternate, sessile, spreading horizontally. In Indian poppy wide variation of leaf serration was noticed by Nigam et al. (1989). Flowers few, solitary on 10–15 cm long peduncle. Buds are ovate – ovoid drooping hermaphrodite, regular with two caducous sepals, smooth. Petals are green, very large, poly petalous white in colour. “Malwa” forms like “Lukka” petals are large rose lilac or purple colour with fringed margin. Stamens are numerous, hypogynous arranged in several whorls. Anthers are linear attached with filament, cream coloured becoming pale brown and twisted after dehiscence. Ovary large depressed, globular, smooth pale green, one celled with large spongy parietal placenta. Stigma is sessile,

capitates. Fruit is a capsule which vary in colour, shape and stigmatic rays. Mature capsule may be globose or roundish. Seeds are numerous, very small, whitish grey in colour.

In India, six species of the genus *Papaver* are described by Husain and Sharma (1983) viz, *P. somniferum*, *P. orientale*, *P. nudicaule*, *P. rhoeas*, *P. argemone*, *P. dubium*. Among these, only *P. somniferum* is commercially cultivated and pharmaceutically useful due to the presence of rich sources of alkaloids. *P. somniferum* has two subspecies viz., *P. somniferum* var. *hartense* and *P. somniferum* var. *somniferum*. The subspecies *hartense* has dehiscent capsules with no opium but is useful for seed purpose. The subspecies *somniferum* bears indehiscent capsule containing latex (opium) when immature and white seeds are formed when matured. This is further divided into two groups viz., var *glabra* grown commercially for seeds and oil mostly in European countries. var. *album* which is cultivated mainly in India for opium production.

Chromosome number varies from diploid (2n) to octoploid (8n) in different species of *somniferum*. *P. somniferum* has chromosome number of $2n = 22$. Self-pollinating crop, 9% cross pollination is reported and the percentage can be expected more due to major role of insect and wind in out crossing (between plants with low and normal alkaloid content). Anther dehiscence occurs before anthesis but stigma is not receptive and contribute for cross pollination.

Male sterility encourages out crossing in *Opium poppy*. As occurrence of spontaneous mutation is rare, induced mutation can be generated either by mutagenic treatment or due to distant hybridizations. Treatment of poppy seeds with 10 and 20 kr gamma rays produce male sterility in M_1 generation (Khanna and Singh 1975). Male sterility was noticed in F_1 generation of Interspecific hybridization of *P. somniferum* and *P. setigerum* (Hrishi 1960). Khanna and Shukla (1989) reported triploid F_1 from *P. somniferum* and *P. setigerum* which was partially sterile and such type of sterility also promotes outcrossing. However, occurrence of spontaneous or induced male sterility is not enough for exploitation under heterosis breeding program. The nature and genetics of male sterility must be determined because only Cytoplasmic genetic male sterility is of commercial significance for hybrid seed production.

Breeding objective in Opium poppy includes development of variety with high seed yield and alkaloid content besides resistant to biotic and abiotic factors.

Introduction of exotics in Opium poppy revealed that cultivars introduced from European required long photoperiod and unsuitable for Indian condition, however some of them set seed, thus can be exploited in hybridization program to transfer specific genes to genetic background of Indian stocks. The Iranian race cultivated in India was by Introduction only (Khanna 1975).

Selection is a process by which individual plants or group of plants are sorted out from heterogenous population. The efficacy of selection is based upon the presence of variability in *Opium poppy*. Three different selection methods are followed in *Opium poppy*.

Mass selection is a simple method to improve the general level of local land races. A group of similarity appearing plant is selected and harvested seeds are mixed for commercial cultivation or for further selection. Khanna (1979) reported

indigenous land races manifested as high degree of intra population variation. He detected early types “Ornamental Red” and Aphundi” having 80–83 days flowering period. Similarly “MOP-2” and “Jawahar Ahim-16” from Madhya Pradesh and I.C.42 from New Delhi were isolated from open pollinated cultivars. Recently, the “Kirtiman” and “Chetak” were also developed through selections made in local races of Faziabad and Rajasthan, respectively (ICAR 1989).

Individual Plant Selection is practiced in populations where much variability is available in local land races. Generally, it is practiced where the particular character requires improvement. Khanna (1979) made individual selection for earliness (80–81 days) in variety ‘Single Dark Red’. A high yielding from “Dhaturia” yielded 54 kg opium/ha on dry weight basis. Individual plant selection may also practice where spontaneous mutation occur. Nyman (1978) isolated and released mutant “Soma” from spontaneous mutation variety in India.

Pure line selection is also practiced in opium poppy. In self-pollinated crops, all the plants are homozygous because of continued self-fertilization. It consists of the progeny of self-fertilized plants and is used for developing variety. The variety developed is genetically pure and more stable. Khanna (Gupta et al. 1978) developed a number of pure line by several generations of selfing. Kumar (1981) and Shukla (1985) produced several pure lines and tested them for combining ability through line x tester and diallel analysis, respectively. Consequently, NBRI developed and released “BROP 1”, a synthetic variety (ICAR 1989; Shukla et al. 1992) with high morphine, opium yield and seed yield. Earlier, Kopp et al. (1961), and Heltman and Silva (1978) also developed synthetics in opium poppy using suitable pure lines.

Pedigree method of breeding was followed in Opium poppy. Taranich (1974) isolated high yielding recombinants with good morphine content lodging resistant from inter-varietal hybrids. Lőrincz (1978) developed ‘Mayak’ and ‘Vaskhod’ varieties of opium poppy and noticed 25–30% high morphine content than standards. Khanna and Shukla (1989) developed high opium and seed yielding genotypes from Interspecific crosses between *P. somniferum* and *P. setigerum*.

Hybridization is very easy in poppy as compared to other self-pollinated one. (Khanna and Shukla 1983), the handling of hybrids depend upon better recombinant/transgressive segregants. Extent of heterosis and combining effects of parents.

Inter-varietal/interspecific hybridization involving parents of same species/strains, varieties or races of the same species were attempted in Opium poppy. Earlier Singh and Khanna (1975) observed 37.33% heterosis for capsule/plant. 46.62% for opium yield and 10.26% for morphine content over superior parent. Khanna and Gupta (1981) studied heterosis for crosses between ‘Aphuri’ (not cultivated one), a grey seeded, violet flowered and dehiscent variety, and 15 major Indian cultivars and noticed 46.34% for opium yield and 37.14% for morphine.

Interspecific hybridization by crossing two different species of plant which leads to transfer of some genes from one species to another species was attempted in Opium poppy. Lorincz and Tetenyi (1970) developed ‘Kek Duma’ from the hybrid progenies of *P. somniferum* var. Havan and *P. orientale*. The new varieties had 0.65–0.75% morphine in the capsule as compared to low morphine content in

P. somniferum var. Havan. To explore *P. setigerum* for genes of economic values which may be transferred in *P. somniferum*. Khanna and Shukla (1989) crossed these species and produced triploid hybrids with viable seed. They investigated the potential of F₁ and subsequent generation. The F₁s are tall and bear 9–23 capsules/plant. The capsules are larger than *P. setigerum* and an average opium yield was 290 mg/plant.

A mutation breeding experiment was carried out using physical and chemical mutagens to develop non-narcotic opium poppy from narcotic crop. They isolated two families containing 12 latex less/opium-less and 12 partial latex bearing plants in M₁ generation which gave similar observations in M₂ generations also. The best mutant genotype, LL-34 of family C¹-Comb-113-2 with 5.66 g seeds/capsule had 52.6% oil was designated as cv. 'Sujata'. This was the world's first opium-less and alkaloid free seed poppy cultivar, offers a cheap and permanent (fundamental) solution to the global problem of opium-linked social abuse. Simultaneously, it serves as a food grade crop with proteinaceous seeds along with healthy unsaturated seed oil.

NBRI-1 was more sensitive than NBRI-5 and that the mutagen EMS was most potent in creating chromosomal abnormalities. Two doses i.e. kR 10 + 0.2% EMS possessed high chiasms frequency while 0.2% EMS in combinations with all doses of gamma was effective in enhancing the total alkaloid as well as specific alkaloids. The dose kR30 and kR10 + 0.4% EMS gave highest positive results for genotypic coefficient of variability, heritability and genetic advance (%) for seven traits in NBRI-1 and ten traits in NBRI-5 respectively.

A mutant variety known as 'TOP 1' ('thebaine oripavine poppy 1') in opium poppy (*Papaver somniferum*) was developed by Tasmania Company. In this mutant the morphinan pathway is blocked at thebaine results in absence of codeine and morphine. The major loss of this blockage is on the end product i.e. morphine which is absent in this mutant. The Tasmania drug industry has been using TOP 1 mutants since 1998 for production of various analgesic drugs viz. buprenorphine, oxycodone, naloxone and naltrexone.

Induced polyploidy to enhance total alkaloid content along with specific alkaloid using colchicines was attempted in opium poppy. The induced auto-tetraploidy did not show any significant differences in phenotypic level while stomatal and chromosomal studies confirmed the tetraploidy. They also noticed differential gene expression of the diploids and auto-tetraploids which led to the elucidation of dosage regulated gene expression leading significant enhancement in morphine content in tetraploid plants. Their study in auto-tetraploids opens avenues towards the development of hexaploids and amphidiploids which can give multifold increase in specific alkaloids. This study also opens a new vista towards understanding of ploidy level changes in term of phenotypic, genetic and genomic and a better understanding of the complex mechanism involved in polyploidization.

7.2.9 *Periwinkle*

Periwinkle (*Catharanthus roseus*) is considered to be a native of the West Indies but was originally described from Madagascar (Ross 1999). It is commonly grown as an ornamental plant throughout tropical and subtropical regions of the world by virtue of its wide adaptability, ever blooming nature and variously coloured flowers. The genus *Catharanthus* consists of eight species, seven of them viz., *C. roseus* (L.) G. Don, *C. ovalis* Markgraf, *C. trichophyllus* (Baker) Pichon, *C. longifolius* (Pichon) Pichon, *C. coriaceus* Markgraf, *C. lanceus* (Bojerex A. DC.) Pichon, *C. scitulus* (Pichon) Pichon, indigenous to Madagascar and one viz., *C. pusillus* (Murray) G. Don, indigenous to India (Stearn 1975).

Periwinkle is an annual or perennial semi-shrub growing to a height of 75 cm–1 m, sub-woody at the base and profusely branched. Stem colour – yellowish green, light pink or dark purple. Leaves, petioles and twigs contain milky latex. Leaves – oblong or ovate, opposite, short-petioled, smooth or pubescent with entire margin. Flowers, borne in pairs axils, pedicellate, bracteate, hermaphrodite, actinomorphic, complete, hypogynous and pentamerous. Calyx, five parted, the sepals free almost to the base. Corolla, five lobed, small to large, salver shaped, rose or white; tube cylindrical, throat bearded, slender, externally swollen at the insertion of the stamens but contracted at the mouth; lobes free or overlapping, aestivation convolute. Stamens – five, attached to the middle of the corolla tube or just below the mouth, conniving over the stigma; filaments very short, not geniculate; anthers free from the stigma, dorsifixed, the connective not prolonged into an apical appendage, anther 2.5 mm long and the filament about 0.3 mm long, at anthesis. Carpels – two, distinct narrowly triangular glands present at the base of the carpels; ovules, numerous (about 10–30) in two series in each carpel; style long, slender; clavuncle shortly cylindrical, truncate at base. Carpels united only by the style at the apex. Stigma capitate, bearded at the top and furnished with a cup-shaped/‘skirt’ like membrane below, which sheaths the upper part of the style. Fruit consists of two long cylindrical pointed follicles (mericarps) diverging or parallel, containing 10–30 seeds, dehiscent at maturity along the length. Seeds – numerous, small (1.5–3.0 mm long), oblong, cylindrical, not arillate, with the hilum in a longitudinal depression on one side, blackish, muriculate, the surface minutely reticulate (Kulkarni 2016).

A knowledge of the morphology of the flower, its development, anthesis, pollination mechanism, mode of pollination, presence or absence of incompatibility, fruit and seed set of the plant is an essential prerequisite for understanding the breeding behaviour of the plant species. Information on these aspects is necessary for developing artificial selfing and hybridization techniques, maintenance of varietal purity and for choosing and executing appropriate breeding methodology. Flowering in periwinkle begins when plants are about 10–15 cm tall or about 10 weeks old and continue to flower as long as plants live. Flowers appear in pairs in the alternate leaf axils and the two flowers are never in the same stage of development, with one flower opening approximately 2–3 d before the other. Under South Indian conditions, anthesis generally starts from 15.00 to 16.00 h and continues until the next

day. Anther dehiscence occurs just before anthesis and the pollen is shed as a sticky mass. The stigma was found to be receptive between 06.00 and 10.00 h, and between 15.00 and 17.00 h (Sreevalli 2002).

A characteristic feature of *Apocynaceae*, is the disc-like or otherwise shaped enlargement of the stigma-head with a sticky secretion and a brush of hairs on which pollen collects as it is shed. The receptive portion of the stigma is at the base of the stigma head, and owing to the position of the anthers, self-pollination is rendered almost impossible, and insect-visits are necessitated (Rendle 1971).

Experimental studies have also shown that automatic self-pollination does not occur in periwinkle and pollinators are necessary to bring about pollination (Kulkarni 1999; Sreevalli et al. 2000). Two pollinating butterflies *Pachliopta Hector* and *Catopsilia pyranthae* have been found to exhibit flower colour constancy during their flower visits and cause about phenotypic assortative mating for flower colour resulting in greater number of intra-flower colour matings than inter flower colour matings (Kulkarni 1999). Some self-pollinating strains have also been found in periwinkle. In these strains self-pollination occurs due to continuous elongation of either ovaries or styles until pollination by overcoming spatial separation of stigma and stamen. Thus, both type of pollination occur in periwinkle (Kulkarni et al. 2001, 2005).

Periwinkle is a diploid plant species with a chromosome number of $2n = 16$ (Janaki Ammal and Bezbaruah 1963). Florry (1944) explained flower colour differences in three phenotypes. Genotypes carrying dominant alleles R and W bears pink flowers, only R pink eyed flowers and recessive alleles rr produce white flowers. Artificial hybridization and selfing techniques have been employed for carrying out genetic studies. Flower buds have been emasculated 1 d before anthesis (Kulkarni et al. 2001) or 2–3 d before anthesis (Levy et al. 1983; Sevestre-Rigouzzo et al. 1993) by making a partial cut, about 1 mm, above the base of the throat of the corolla tube. The top portion of the flower bud was then removed long with the undehisced anthers. (Kulkarni et al. 2001). The per cent fruit set ranged from 90 to 100% (Sevestre-Rigouzzo et al. 1993; Kulkarni 1999).

Levy et al. (1983) reported marked differences for yields of leaves and roots and for contents of ajmalicine in roots of three un related pure lines representing three flower colour types: pink corolla, white corolla and white corolla with red eye. The differences between lines varied according to developmental stage of the plant. They also observed 29 and 24% significant and positive heterosis over better parent for leaf and root dry yields per plant, respectively, in the F_1 hybrid involving the parental lines, pink corolla, and white corolla with red eye. However, no heterosis was observed for ajmalicine content in roots.

Mishra et al. (2001) evaluated 32 accessions collected from wide geographical areas such as different regions of the Indian sub-continent, Sri Lanka, Madagascar, Singapore and Malaysia for 53 growth, development and alkaloid yield related characters over two seasons. Large differences were observed between accessions for six morphological and 14 agronomic traits; the differences were 3, 80 and 15-fold for the main alkaloid yield components viz., leaf dry matter yield, VLB and VCR contents, respectively.

Information on inter-trait correlations is essential to know the effect of selection for one trait of interest on other unselected traits, and to know the possibility of carrying out indirect selection for characters of interest which are difficult or time consuming to measure, or are less heritable. In periwinkle, estimation of contents of total alkaloids and specific alkaloids viz., VLB, VCR, vindoline, catharanthine and ajmalicine is time consuming and limits the number plants that can be evaluated in a breeding programme. Any trait with high heritability and a strong correlation with contents of these alkaloids could be useful for preliminary screening for content of alkaloids as well as for indirect selection for these important traits.

Leaf yield and root yield, leaf yield and leaf alkaloid yield, root yield and root alkaloid yield were found to be positively correlated suggesting that simultaneous improvement for these pairs of traits should be possible through selection (Mishra et al. 2001; Sharma et al. 2012). Leaf yield and root yield were not correlated with leaf alkaloid concentration and root alkaloid concentration, respectively (Mishra et al. 2001). Therefore, it should be possible to combine high yield of these two plant parts with high concentrations of alkaloids in them.

No relationship was found between flower colour and contents of vindoline and atharanthinein 50 horticultural cultivars which had been bred for flower colour. However, one of the cultivars had low content of vindoline and ten times lower tabersonine-16-hydroxylase activity as compared with *C. roseus* cv. Little Delicata (Magnotta et al. 2006).

Catharanthus roseus is considered to be incompatible with other *Catharanthus* species, except *C. longifolius*. Reciprocal differences were observed in crossability between *C. roseus* and *C. trichophyllus*. *Catharanthus roseus* as female parent failed to form fruits and therefore, no introgressions were found from *C. trichophyllus* to *C. roseus* (Sevestre-Rigouzzo et al. 1993). In a reciprocal cross, however, upto 100% seed set with good germinability was found. Alkaloid profiles of *C. trichophyllus* and *C. roseus* differed with absence of serpentine in leaves of *C. trichophyllus* and catharanthine in roots of *C. roseus*. Hybrids contained serpentine in leaves such as leaves of *C. roseus* and catharanthine in roots such as roots of *C. trichophyllus*. Further, significant heterosis was found for the contents of ajmalicine, catharanthine and serpentine both in leaves and roots and for the content of vindoline in leaves. The hybrids also had higher leaf and root yields than the parental species. Therefore, they suggested development of hybrids coupled with micro propagation to exploit observed heterosis for alkaloid production.

Induced auto polyploidy is a rapid method to increase the yield of vegetative parts in plant species and the method was experimented in periwinkle. Auto-tetraploids had significantly higher leaf yield, root yield and content of total alkaloids than diploids, while in other studies (Kulkarni et al. 1984; Krishnan et al. 1985) they were found to be on par with diploids for leaf yield and root yield but had lower ajmalicine content and harvest index. Goswami et al. (1996), The contents of vindoline, catharanthine and VLB were found to be higher in tetraploid lines than in diploids (Xing et al. 2011).

In industrial crops, such as medicinal plants, the content of the economically important metabolite is more important than the yield of the plant parts containing

the metabolite because it determines the cost of extraction of the metabolite. Mutation breeding is one of the most promising approaches for the development of 'ideochemovars' (Swaminathan 1972; Levy 1983).

Mutation breeding has been adopted more frequently in self-pollinating crops than in cross-pollinating ones, due to failure of recessive mutations to express in cross fertilizing systems without manual selfing or sib-mating. Periwinkle, although a herkogamous species, was earlier considered to be a self-pollinating species because of geitonogamy and the need for artificial selfing was not realized. Nevertheless, periwinkle has been subjected to induced mutagenesis and several mutants affecting different traits including contents of alkaloids, with direct or indirect utility through hybridization, have been isolated. Estimation of contents of alkaloids is time consuming.

In the absence of rapid methods for screening plants for their alkaloid contents, macro-mutants with altered morphology have been evaluated for identifying mutants for altered alkaloid contents. Induced macro-mutants in periwinkle include those with altered plant height, leaf morphology, floral traits, reproductive traits, and those with tolerance to salt, heat and water stress.

Plant height is an important trait which along with shoot branching and inflorescence morphology determines plant architecture and crop yield (Wang and Li 2006). Three distinct reduced plant height mutants, 'dwarf', 'semi-dwarf' and 'bushy', respectively, about 60, 40 and 30% shorter than their parental variety, Nirmal have been reported in periwinkle (Kulkarni et al. 1999a, b, 2009). The 'dwarf' and 'semi-dwarf' mutants were due to monogenic recessive genes (dw_1 and dw_2 , respectively) which were allelic to each other and had significantly higher content of root alkaloids than parental variety. The 'bushy' mutant which was governed by an independently inherited non-allelic recessive gene (by), however, had similar contents of leaf as well as root alkaloids as the parental variety, Nirmal. The double-mutant recombinant ($bydw_1$) was 30% shorter than the shorter of the parental mutants and exhibited 20% higher content of root alkaloids than the better parent. All the three mutants and the double-mutant recombinant ($bydw_1$) had similar contents of leaf alkaloids. Higher root alkaloids content has been found to be related to thin root morphology in hairy root cultures of periwinkle (Palazon et al. 1998).

As economically important alkaloids are present in the leaves, altered leaf morphology may suggest altered alkaloid contents. Three leaf mutants, viz. wavy leaf margin, 'necroticleaf' (a lesion mimic mutant) and 'neriumleaf' (resembling leaf lamina of another Apocynaceous plant, *Nerium oleander*) exhibited higher contents of leaf alkaloids than their respective parents (Kulkarni et al. 1999a, b; Baskaran et al. 2013). Further, enhanced contents of leaf alkaloids of 'necrotic leaf' and 'nerium leaf' mutants over their parental variety were found to be due to recessive alleles at different loci, and 13 out of 14 double mutant recombinants for parental mutant traits 'necroticleaf' and 'nerium leaf' developed by crossing the two mutants had significantly higher content of leaf alkaloids than parental mutants (Kulkarni and Baskaran 2014). No studies have been carried out on linkage between leaf alkaloids content and these morphological mutant traits. However, it appears that 'necrotic leaf' trait could be used as a seedling marker trait for enhanced content of

leaf alkaloids. Extract of *Pythium* (a soilborne pathogen of periwinkle) is well known to be an elicitor of alkaloid production in cell and tissue cultures of *C. roseus* (Nef et al. 1991). Therefore, constitutive expression of self-defence reactions in the 'necrotic leaf' mutant may have induced enhanced production of alkaloids similar to that elicited by *Pythium*. For commercial exploitation of heterosis, male sterility is required for large scale production of hybrid seeds. A patent has been granted for the method for developing hybrids in *Catharanthus* using male sterility (Bowman 2000). Streptomycin was used to develop genetic male sterile line '13,861-1' with msGS gene, which does not set selfed seed. The gene did not show any undesirable pleiotropic effect.

Two other types of male sterile mutants viz., indehiscent anthers (functional male sterility) and pollen-less anthers governed by a single recessive allele and duplicate recessive alleles, respectively, have also been reported (Sreevalli et al. 2003; Kulkarni and Baskaran 2008). The mutant with indehiscent anthers had relatively smaller anthers and about 30% lesser number of pollen grains but was otherwise phenotypically normal, had high pollen fertility and showed high seed set on artificial selfing.

Mutations affecting the gynoecium have also been reported. In periwinkle flower, the stigma is about 0.5 mm below the anthers, typical of reverse herkogamy. Mutants with short style (about one-third length of normal style) and long style with stigma 2.5 mm above the tip of the cone of anthers, with partial and high pollen sterility, respectively, have been reported (Mishra and Kumar 2003; Kulkarni and Baskaran 2008). The short-styled mutant trait was inherited as a recessive trait, while the long-styled mutant constituting 'pin' flower in contrast to 'thrum' flower in normal plants appeared to be under the control of inhibitory, epistatic interaction between two independently inherited genes *P* and *T*, with gene *T* being inhibitory to gene *P*. Accordingly, genotypes *P-T*, *T-pp*, or *pptt* produce normal 'thrum' flowers whereas *P-tt* produce mutant 'pin' flowers.

A recessive mutant producing heterocarpous flowers, with one (3%), two (82%) and three (15%) carpels and high fertility has been reported with a possibility of genetic engineering for fruit size, carpel number and seed number per plant (Rai and Kumar 2001).

As periwinkle is also valued as an important garden plant because of its variously coloured flowers, mutants affecting flower colour, flower density, floral persistence etc., would also be of interest. A mutant described as 'leafless inflorescence', in which flowers are borne on nodes without leaves, has been reported and the locus '*lli*' has been mapped (Chaudhary et al. 2011). The mutant produced more number of flowers per plant than its parent and further enhancing its horticultural value. New improved horticultural genotypes were developed by crossing this mutant with other genotypes with different flower colours and plant habit (Kumar et al. 2012).

Another novel mutant with caduceous closed corolla corolla abscising before anthesis) inherited as a monogenic recessive, was isolated after mutagenesis with EMS. The trait was used for development of cleistogamy, a new trait, in periwinkle (Kulkarni and Baskaran 2013a, b).

Screening of plants treated with EMS 3600 resulted in identification one plant with high ajmalicine, and low catharanthine and vindoline contents was identified (Thamm 2014).

Eight mutants (*gsr1* to *gsr8*), tolerant to salinity (250 mM NaCl) or high-temperature (45 °C) stress have been isolated (Rai et al. 2001, 2003; Kumari et al. 2013). These mutants (*gsr 1* to *gsr 6*) accumulated more proline and glycine betaine constitutively as well as under water stress, and transpired lower amounts of water under water stress than their parental variety. The contents of catharanthine, VLB, VCR and serpentinein two of these *gsr* mutants (*gsr3* and *gsr6*) were found to be higher than those in their parental variety.

Detection of mutations is the first and most critical step in mutation breeding. Historically, mutants have been identified phenotypically, in large mutagenized populations, for easily recognizable characters such as, altered plant height and architecture, early or late flowering and maturity, altered flower, fruit and seed characteristics, resistance to diseases that can be screened easily in natural or artificial epiphytotics, and for biochemical quality traits for which low-cost, high-throughput and rapid evaluation methods are available. To increase efficiency of mutation breeding, high-through put DNA technologies for mutation screening such as TILLING (Targeting Induced Limited Lesions INGenomes), ECOTILLING, and high-resolution melt analysis (HRM), have been developed and used in crop plants; (Xin et al. 2008). These reverse genetics techniques can be used for discovering allelic variation in natural or mutagenized populations using large number of mutants isolated and TI A pathway genes already cloned in periwinkle.

7.2.10 *Senna*

Cassia angustifolia, a small leguminous shrub, is exclusively grown for its leaves and pods which contains glycosides having usefulness in a variety of ailments, such as liver complications and abdominal distress. It is also used in modern medicine as a laxative because of its glycosides-sennoside A and B. Leaves and pods from *Cassia angustifolia* Vahl and *Cassia acutifolia* Del are the commercial senna drug of the Unani system of medicine. Both species, *Cassia angustifolia* (native of South Arabia, West Asia) and *Cassia acutifolia* (Sudan, East Africa), are exotic to India. In their native lands, these species grow on arid tracts as perennial bushes.

Senna (*Cassia angustifolia* Vahl.) is a small perennial under shrub, native of Saudi Araia. It attains 0.7–1.0 m height under cultivation but grows taller (1.5 m) when left uncoppiced. It bears compound pinnate pale green leaves, having 5–8 pairs of shortly stalked leaflets. It bears axillary or subterminal racemes earing many large, brilliant, yellow coloured showy flowers. Pods are flat, thin, 3.5 × 6.5 cm × 1.5 cm, pale green in the beginning which change to greenish brown and dark brown on maturity and after drying. Each pod contains 5–8 obovate yellow to of cream coloured flat seeds. Alexandrian senna ears 4–5 pairs of leaflets which are shorter and narrow in dimension but dark green and thicker. Pods are short

(2.75–5.6 cm long and 1.5–2.5 cm broad) with 5–7 pairs of obovate seeds (Rajendra Gupta and Pareek 1995). Surface markings on the seed coat and stomatal index of leaves are distinct and remarkably consistent in the two species to maintain their separate identity (Fairbairn and Shesta 1967).

Senna ($2n = 26,28$) is predominantly a self-pollinated species but percentage of outcrossing is fairly large and pollination is done by beetles. Flowers are dichogamous with 7 stamens and 3 staminodes. Flowers open in the morning hour and anthers commence dehiscing after 3 h of opening of flowers, dehiscence is apical and the pollen is continued to be released for 24 h. Stigma become receptive soon after opening of flowers. Pollen grains are triangular, highly fertile (98%). The life of the flowering axis is around 35 days and flowering continues till the close of the season (Mohan Rao et al. 1976).

In India, 24 species of Cassia are distributed in different parts of India, Kapur and Atal (1982), tabulated the presence of various anthracene derivatives in different parts of 25 Cassia species from literatures.

Seeds of Tinnevely senna were irradiated with gamma ray doses @ 350 Gy, 450 Gy, 550 Gy and 650 Gy. Single plant selection done on the basis of phenotypics from M1 were raised to get M2 generation. In both M1 and M2 there was in general reduction in percent germination with increase in the dose. Lowest germination percentage was observed for 650 Gy and highest for 350 Gy. Micromutants for quantitative traits viz., days to 50% flowering, days to maturity, plant height, number of branches, pod length, pod width, number of seeds per pod, dry leaf weight/pod, pod weight/plant, seed yield/plant, 100 seed weight). Gamma rays affected the expression of quantitative traits except plant height and number of branches per plant. Gamma ray treatments 350 Gy and 450 Gy were found to be promising for inducing variability for yield and yield contributing characters.

Macro mutants for qualitative traits viz., leaf mutant, pod mutant, dwarf mutant, sterile mutant and synchronous maturity mutants were observed. Highest mutant frequency was deducted in 650 Gy gamma ray dose for all the macromutants. Among the leaf mutants, frequency of tiny leaf mutant was highest. Chlorophyll mutations were not observed for any of four doses. Among pod mutant's frequency of constricted pod mutants were highest. Sterile mutants and dwarf mutants were observed in all four doses but frequency was negligible. Synchronous maturity mutant was observed except 350 Gy (Yadawrao, S.V. 2012).

7.3 Conservation of Medicinal Plants

Loss of biodiversity of medicinal plants occur due to environmental factors, deforestation, developmental activities. Hence, conservation of medicinal plants is of very much important. The primary goals of biodiversity conservation is maintenance of essential ecological processes and life support systems on which human survival and economic activities. Preservation of species and genetic diversity and sustainable use of species and ecosystems which support millions of rural

communities as well as major industries. The conservation of the wild medicinal plants or any other such threatened species can be tackled by scientific techniques as well as social actions. Three methods of conservation are legislation, *in-situ* conservation and *ex-situ* conservation. The legislation is covered under existing laws pertaining to forestry which include Forest Act, 1927, Wildlife (Protection) Act 1972 and Wildlife (Protection) Amendment Act 1991. Forest (Conservation) Act, 1980, Environment Protection Act, 1986, National Forest Policy, 1988, National Biodiversity Act, 2002 and The scheduled tribes and other traditional forest dwellers act, 2006 De (2016).

7.3.1 In-Situ Conservation

Conservation of a given species in its natural habitat or in the area where it grows naturally is known as *in-situ* conservation. It includes Gene bank/Gene sanction, Biosphere reserves, national parks, sacred sites, Sacred grooves etc. It is only in nature that plant diversity at the genetic, species and eco-system level can be conserved on long-term basis. It is necessary to conserve in distinct, representative biogeographic zones inter and intra-specific genetic variation.

On-farm conservation: On-Farm Conservation involves the maintenance of traditional crop cultivars (land races) or farming systems by farmers within the traditional agricultural system. Traditional farmers use land races, which are developed by the farmer and well adapted to the local environment. This method of conservation has been gaining importance in recent years, though farmers have used it for centuries.

Home gardens: Home garden conservation is very similar to on-farm conservation; however scale is much smaller.

7.3.2 Ex-Situ Conservation

Conservation of medicinal plants can be accomplished by the *ex-situ* (i.e. outside natural habitat) by cultivating and maintaining plants in botanic gardens, parks, other suitable sites, and through long term preservation of plant propagules in gene banks (seed bank, pollen bank, DNA libraries, etc.) and in plant tissue culture repositories and by cryopreservation).

7.3.3 Field Gene Bank (Field Repository/Clonal Repository)

Gene Bank: Storage in the form of seed (Base collection at -20°C ; Active collection at $+4^{\circ}\text{C}$ to 10°C). The three national gene banks have been established in India for *ex situ* conservation of medicinal and aromatic plants are National Bureau of Plants Genetic Resources (NBPGR), New Delhi, Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow and Tropical Botanical Gardens Research Institute, (TBGRI), Palode, Thiruvananthapuram (Kerala).

7.3.4 Seed Gene Bank

Germplasm conservation in Seed Gene Bank is more economical. The NBPGR, New Delhi, houses National Gene Bank (NGB) which is primarily responsible for conservation of germplasm. These are referred to as “Base Collection” stored in modules maintained at -20°C . The seeds are dried to attain 4–6% moisture content and hermetically sealed in moisture proof aluminium foil packets. These stored seeds remain viable for 50–100 years. In most crops, seeds samples with more than 85% seed viability are only processed. The seeds in gene bank are stored preferably as per the gene bank standards recommended by FAO/IPGRI.

7.3.5 National Active Germplasm Sites

The National Active Germplasm Sites (NAGS) are the integral component of the network. There are presently 40 NAGS, which are based at ICAR institutes, (crop-based institutes for a specific crop or a group of crops) and SAUs. These are integral part of national plant biodiversity conservation network. The NAGS are entrusted with the responsibility of multiplication, evaluation, maintenance and the conservation of active collection and their distribution to bonafide users both at the national and international levels. These active/ working collections are stored in modules maintained at $+4^{\circ}\text{C}$ and 35–40% relative humidity (RH). Under these temperatures, seeds are expected to remain viable for 15–50 years. For medium term storage, seed moisture content is brought down to 8–10%.

The NBPGR has a network of II regional stations located in different agroclimatic zones of the country to support the active germplasm conservation activities of the regions.

7.3.6 Cryopreservation

The cryopreservation of *in-vitro* cultures of medicinal plants is a useful technique. Cryopreservation is long-term conservation method in liquid nitrogen ($-196\text{ }^{\circ}\text{C}$) in which cell division and metabolic and biochemical processes are arrested. A large number of cultured materials can be stored in liquid nitrogen. Since whole plants can regenerate from frozen culture, cryopreservation provides an opportunity for conservation of endangered medicinal plants. For example, low temperature storage has been reported to be effective for cell cultures of medicinal and alkaloid-producing plants such as *Rauwolfia serpentina*, *Digitalis lanata*, *Atropa belladonna*, *Hyoscyamus* spp. When plants are regenerated and no abnormality is seen either in fertility or in alkaloid content, the materials can be stored using cryopreservation methods. Cryopreservation has been used successfully to store a range of tissue types, including meristems, anthers/pollens, embryos, calli and even protoplasts. However, the system will depend on the availability of liquid nitrogen methods (Tripathi and Tripathi 2005).

7.4 Conclusions

Breeding opens up the avenues to adapt plants to the particular demands of the stakeholders in the production chain. Conventional breeding methods that prevail in MAP breeding include selection in natural populations, combination and hybrid breeding, breeding synthetic varieties, induced mutation and clone breeding. Contemporary biotechnological breeding methods are highly expensive. Furthermore, consumers prefer natural products due to safety and reject herbal drugs that originate from genetically modified plants because they do not have their natural constitution. Nevertheless, the use of biotechnological tools and research on genes controlling the formation of secondary metabolites and on methods for their transmission are in fancy stage. At present, the exploitation of the genetic potential of medicinal plants by breeding is yet to be underutilized. Therefore, breeding can become one of the key factors for advancing the phytopharmaceutical sector in the future. Species specific characteristics influencing the success of postharvest processing should be well defined and considered in future improvement programs.

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Chapter 8

Expanding Horizons: Role of Biotechnology in MAP Research, Production and Utilization



Nupur Mehrotra and Sara Anees Khan

Abstract Plant tissue culture (PTC) plays a vital role in selection, multiplication, and conservation of the critical genotypes of medicinal plants. These techniques hold immense potential for enhancing the production of high-quality secondary metabolites which form the basis of plant-based medicines. Rapid Biotechnology Based Breeding Methods (BBBMs) have led a revolution in Medicinal Aromatic Plant (MAP) research. Immense contribution has been made through the use of PTC based BBM's as *Agrobacterium* mediated gene transformation and induction of polyploidy. Metabolic pathway engineering has received a boost using hairy root cultures. The identification of the genes and enzymes mediating the biosynthesis of secondary metabolites, through specific transcriptome detailing, through RNA-sequence analysis, has facilitated better yield of secondary metabolites. Using Next-Generation Sequencing (NGS) techniques like sequence specific nucleases, namely Zinc-Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9) are used for genome editing which can mediate the production of designer MAPs. The application of such genome editing tools have a high potential in MAP research.

Keywords Medicinal aromatic plants · Biotechnology · Next-generation sequencing · Plant cell tissue and organ culture · Secondary metabolites · Micropropagation · Bioreactor

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Abbreviations

2,4-D	2,4-dichlorophenoxyacetic acid
BA	6-benzyladenine
BAP	6-Benzylaminopurine
BBBMs	Biotechnology based breeding methods
Cas	Caspases
COSTREL	Combinatorial super transformation of transplastomic recipient lines
CRISPR	Clustered regularly interspaced short palindromic repeats
DArTTM	Diversity array technology
EST	Expressed sequence tags
GA3	Gibberellic acid
GDA	Gene-driven array
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
IMPPAT	Indian Medicinal Plants, Phytochemistry and Therapeutics Kyoto Encyclopedia of Genes and Genomes
Kn	Kinetin
LS	Linsmaier and Skoog
MAPs	Medicinal Aromatic Plants
MS	Murashige and Skoog
NAA	1-naphthaleneacetic acid
NGS	Next-generation sequencing
PCTOC	Plant cell tissue and organ culture
PGRs	Plant growth regulators
PTC	Plant tissue culture
QTL	Quantitative trait loci
RAPD	Random amplified polymorphic DNA
SDA	Subtracted diversity array
SH	Schenk and Hildebrandt
SNP	Single nucleotide polymorphism
TALE	Transcriptional activator-like effector
TDZ	Thiadiazuron
WPM	Woody plant medium
ZFNs	Zinc-finger nucleases

8.1 Introduction

Traditional systems of medicine practiced globally rely on plants and their products for maintaining health through the repertoire of biologically active constituents present in them. India is amongst the most ancient civilizations and one of the richest repositories for developing knowledge on medical sciences. Shushruta, the

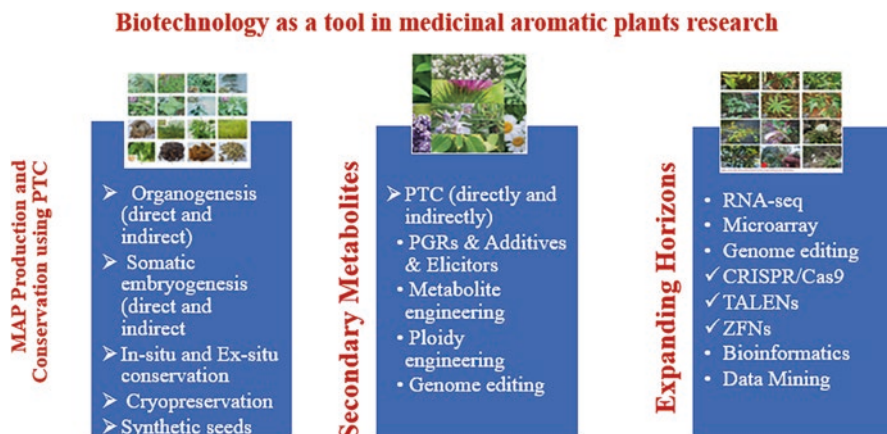


Fig. 8.1 Biotechnology as a tool in Medicinal Aromatic Plants research

Indian physician, referred across the globe as ‘Father of surgery’ and many alike have established a strong foundation to use medicinal plants for preserving and rectifying good health.

Over the years MAPs have attracted not only researchers but farmers, traders, economists as well as health professionals, as they are natural biological resources for multiple secondary metabolites with potential uses ranging from cosmetics, fragrances, insecticides dye etc.

Though traditionally these plants were grown in wild, the over-exploitation of many species has today lead to close to 4000 MAPs being listed as endangered species (El Meskaoui 2013). Thus, the need of the hour is MAP selection and cultivation using cutting edge biotechnological tools to cater to the increased demand (Canter et al. 2005) (Fig.8.1).

8.2 Plant Cell Tissue and Organ Culture

Use of plant cell tissue and organ culture (PCTOC) technology has reported success as an efficient tool in selection of MAPs as typically MAPs, offer less yield and they are highly sensitive to biotic stress (Isah et al. 2018). This is also an efficient tool for preservation of rare MAP species (Kayser and Wim 2007) and also for production of phytochemicals and secondary metabolites (Nagata and Ebizuka 2002).

The pragmatic approach towards successful PCTOC techniques is dependent on choice of explant, supplementation of medium with phytohormones along with the physical environment. Biotechnological approaches have facilitated *in-vitro* propagation and growth of MAP’s through micropropagation, callogenesis, organogenesis, embryo or anther culture, somatic and asexual embryogenesis. The choice of the technique is dependent on plant species and determines the rate of success (Bhojwani and Razdan 1996).

8.2.1 Explants Used

Nodal, internodal, apical segments, leaves and its segments, rhizomes, seeds and shoot tips are the types of explants generally used. For *Acorus calamus*, using rhizome as explant, 73% shoot organogenesis was observed along with IAA-BAP treatment (Bhagat 2011). Tejovathi et al. (2011), successfully used media supplemented with BAP, IBA and GA3 with shoot tips as explant for *Commiphora wightii*. The very useful *Rauvolfia serpentina* responded well to *in-vitro* regeneration with juvenile leaf explants (Singh et al. 2009). Similarly maximum proliferation was achieved using nodal explants in *Thymus hyemalis* (Nordine et al. 2013); apical meristem as explants for *Psoralea corylifolia* Linn (Pandey et al. 2013) and stem explants in *Scrophularia striata* (Lalabadi et al. 2014). Trimmed shoot segments bearing two nodes as explants of *Thymus bleicherianus* Pomel (Aicha and Abdelmalek 2014) and internode explants of *Thymus persicus* cultured on MS medium also gave maximum callus induction (Bakhtiar et al. 2016).

8.2.2 Media Requirements

The most popular media used is Murashige and Skoog (MS) supplemented with vital nutrients. The medium should provide a source for carbon, both macro- as well as micro-nutrients, along with a constant source for growth regulators and vitamins. Phytohormones facilitate regulation of plant physiological as well as morphological processes and are commonly termed Plant Growth Regulators (PGRs). The quantity of PGRs to be supplemented in the media is determined by the capability of explant to itself provide the same. Some species growing successfully in PCTOC without external medium supplements have also been reported. (Murthy et al. 2014).

A high-frequency clonal propagation procedure was developed for *Curcuma angustifolia* Roxb, resulting in a yield of 14.1 ± 0.55 shoots per explants by Jena et al. (2018) using $13.3 \mu\text{M}$ 6-benzyladenine (BA) fortified MS medium along with $5.7 \mu\text{M}$ IAA and $135.7 \mu\text{M}$ adenine sulphate (Ads) within 60 days of inoculation. Thus, a variation in culture media facilitates effective mass propagation thereby enriching commercial application. The effect of thidiazuron (TDZ) on multiple shoot induction from nodal segments of *Allamanda cathartica*, was noted by Khanam and Anis (2018). For shoot proliferation accompanied by shoot elongation, the TDZ exposed cultures were further cultured on MS medium containing different concentrations of 6-benzyladenine (BA) and Kinetin (Kn) but without TDZ. Thus, changing the constituents of culture medium can facilitate rapid clonal propagation of MAPs.

PCTOC is also potent tool for preservation of endangered species. Somatic embryogenesis of an endangered native of Iran, MAP - *Kelussia odorotissima* Mozaff, was facilitated by Ebrahimi et al. (2018). Embryogenic callus induction of

overcotyledonary leaves was observed in a MS media supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.25 mg/l Kinetin. 100% improvement in the conversion rate of the cotyledonary-stage embryos was observed while maintaining genetic stability during *in vitro* multiplication was also assessed with no polymorphic band observed by amplification fragment length polymorphism.

Palmer and Keller (2011) studied the regeneration of plants from the petal explants of *Hypericum perforatum* L. The formation of callus and shoot was induced at 1:10::Cytokinin: auxin concentration using indole-3-acetic acid (IAA), 1-naphthaleneacetic acid (NAA) and indole-3-butyric acid (IBA), though with 2,4-dichlorophenoxyacetic acid (2,4-D) only callus induction resulted in 100% shoot regeneration frequency with 57.4 and 53.4 shoots per explant were obtained with IAA and IBA, respectively, at 1.0 mg/l auxin and 0.1 mg/l kinetin concentration. Kinetin levels of 0.1 and 0.25 mg/l, in absence of auxins lead to low frequency of callus and shoot formation. Rooting and successively flowering with initial use of media without exogenously added auxin was successfully achieved in the greenhouse, suggestive of auxin type being an important governing factor using petal explants.

8.2.3 Environmental Factors

Some desired characteristics, achieved through plant tissue culture (PTC) would be the overexpression of genes leading to synthesis of important secondary metabolites, alterations in the regulatory genes mediating better production of these metabolites along with modifying genes providing better adaptability to stress conditions.

Drought stress tolerance in *Salvia miltiorrhiza* transgenic plants was demonstrated by Liu et al. (2017) through expression of AtEDT1 transcription factor. Another study on the same plant by Zhou et al. (2018), showed an increase in 3,4-dihydroxyphenyllactic acid resulting in better quality of rosmarinic acid produced via rosmarinic acid synthase gene suppression by CRISPR/Cas9-mediated mutagenesis. Kumar et al. (2018a, b) studied over-expression of geraniol synthase genes and geranyl diphosphate synthase genes in *Catharanthus roseus* and obtained significant increase in vinblastine and vincristine yields which hold immense utility for cancer therapy. In *Raphanus sativus* L., higher biosynthetic capacity for quercetin and flavonoid was found in *Agrobacterium rhizogenes*-induced hairy roots (Balasubramanian et al. 2018). Another example of similar use of hairy root cultures in *Sphagneticola calendulacea*, reported better yield of flavonoid, phenolic acid and wedelolactone (Kundu et al. 2018). *Agrobacterium rhizogenes*-derived hairy roots have facilitated industrial production of secondary metabolites mediated through induction of polyploidy artificially (Dehghan et al. 2012), bioreactors (Patra and Srivastava 2017), elicitors (Kastell et al. 2018), and CRISPR/Cas9- a method to edit genome (Li et al. 2017).

8.3 *In Vitro* Micropropagation of Medicinal Plants

Micropropagation is the most developed of all PCTOC techniques. It can be accomplished by somatic embryogenesis and organogenesis, through mainly two paths, direct and indirect. The former is a preferred mode due to induction of bipolar structures while the latter requires various PGR's for induction of shoot and root through a two-step method (Hesami and Daneshvar 2018). Multiple factors, viz. explant-its type, size and age, culture media composition, gelling agent, pH and concentration of PGR's along with external conditions as temperature, photoperiod and light intensity, govern the efficiency of the process (Nalawade and Tsay 2004). A chapter in Vol. 1. of the series Medicinal and Aromatic Plants of the World by Máthé et al. (2015) has dealt with this subject in detail.

8.3.1 *Explants Used*

Khamushi et al. (2019) developed an efficient method to micro propagate and further achieved effective plantlet regeneration of the cypress of Abarkuh, known to be 4000 years old plant. The secondary shoots proliferated on the woody plant medium (WPM) supplemented with sucrose, agar, benzyladenine (BA) and indolebutyric acid (IBA). After elongation in *in-vitro* conditions, roots were induced by pulse treatment and the plantlets successfully adapted to develop into mature plants in outdoor conditions. The effect of age of explant was investigated by Niazian et al. (2017) on hypocotyl segments of Ajowan (*Carum copticum* L.) which were 5, 10 and 15 day old. The media used was MS supplemented with different concentrations of Kin PGRs and 2,4-D and the best results for somatic embryogenesis were obtained with 15-day-old hypocotyls. Generation of embryogenic calli with combination of Kin PGRs and 2,4-D also showed success in *Sapindus trifoliatus* though use of media free from PGR helped develop induction of somatic embryos into mature plantlets in some MAP's (Asthana et al. 2017).

8.3.2 *Organogenesis*

Fadel et al. (2010) in *Mentha spicata* L investigated the effect of changing concentration of inorganic salts used in MS media of quarter, half and full strength via *in vitro* organogenesis using nodal segments as explants. The induction of highest number of shoots and the maximum average shoot length was detected in media of half strength, while in full strength highest leaf number and root length was observed.

Using leaf explants in *Coleus forskohlii*, briq direct organogenesis was attempted by Krishna et al. (2010). An MS medium with BAP 5 mg/l and cytokinins, showed

multiple shoot regeneration, followed by use of MS media fortified with cytokinin and combination of BAP 0.1 mg/l and IAA.

Multiple shoot induction in *Angelica glauca* through direct organogenesis, using rhizomes as explant was conducted by Janhvi et al. (2018). MS medium with 6-Benzylaminopurine and IAA depicted maximum shoots. Roots (average 4.2 roots per shoot) appeared within 14 days in IAA and NAA supplemented medium. These rooted plantlets, in a greenhouse hardened successfully and recorded survival rate of 72% after 45 days when established in field.

Recent research suggests that additives as casein hydrolysate, nanoparticles, glutamine and picloram, in the culture media augment the efficiency of micropropagation. In cultured *Gloriosa superba* L. rhizome explants, silver nanoparticles of *Ulva lactuca* extracts (ULAgNPs) were used along with 0.5 mg/L silver nitrate (AgNO_3), 0.5 mg/L ABA, 2 mg/L BAP and 20% *Ulva lactuca* extracts in MS medium, leading to high percent of embryo maturation (Mahendran et al. 2018).

8.3.3 Somatic Embryogenesis

Somatic embryogenesis is an efficient way for regeneration of plants and scores higher than organogenesis, as the shoots and buds formed from a somatic embryo will obligatory form from a single cell which ensures superior genetic stability. Its applications are diverse especially for large-scale multiplication of MAP's and mass production of artificial seeds.

Propagation via somatic embryogenesis using *O. basilicum* leaves as explants was worked upon by Gopi and Ponnuragan (2006). Initial induction of globular embryos was achieved with BA (1 mg/L) and 2, 4-D (0.5 mg/L) while embryo maturation was in NAA (1 mg/L), KN (0.5 mg/L), BA (1 mg/L).

In *Portulaca oleracea* L., using stem and leaves as explants induction of callus, somatic embryogenesis, as well as regeneration of plant at varying concentrations of 6-Benzylaminopurine (BAP) and kinetin (Kin) along with auxins as IAA, NAA 2,4-D were investigated. Good transformation was achieved with leaf explants which were pre-cultured for 7 days, then co-cultivated at 25 ± 2 °C for 4 days (Sedaghati et al. 2019).

Somatic embryogenesis of an endangered medicinal plant *Kelussia odoratissima* Mozaff was achieved by Ebrahimi et al. (2018). On the cotyledonary leaves, embryogenic callus was induced in MS medium supplemented with 2,4-D and Kinetin. The development and proliferation of somatic embryos showed significant differences under variable sources of carbon supplemented in media along with different light treatments. In absence of polymorphic bands between mother plant and in-vitro plantlets, using the amplification fragment length polymorphism, the results indicate genetic stability was maintained.

8.4 PTC for Conservation of Medicinal Plants

Plant Tissue Culture, in addition to the aforementioned uses in the plant propagation, has acquired an important role in the conservation of medicinal plants, too. *Ex-situ* conservation, Cryopreservation and Somatic embryogenesis are important domains that will be briefly outlined and illustrated with medicinal and aromatic plant examples.

8.4.1 Ex-Situ Conservation

MAPs used to grow wild, but were indiscriminately harvested, once their utility was realized. Further, the effect of manmade factors as pollution, afforestation, industrialization and other anthropogenic factors has now put the MAPs, under risk. To safeguard these versatile plants *ex-situ* conservation is required. It forms a substitute for preserving plant germplasm which is likely to be lost through *in vitro* slow growth cultures which facilitates storage of cloned plants through regular sub-culturing over a period of 1–15 years (Rao 2004). The sub-culturing procedure itself, if not conducted carefully, is the cause of the germplasm getting contaminated and hence the method has a short life. The alternative to long term preservation is cryopreservation.

Capparis spinosa L, *Lavandula dentata* L. and *Rhazya stricta* Decne, plants were grown and the medium time storage was studied. Using axillary buds as explants, *in vitro* propagation of *C. spinosa* L and *L. dentata* L. plants in MS medium with varying concentration as well as combination of auxins and cytokinins was successful. Further, the shoot tips and nodal buds thus developed, were used in the next stage of *in vitro* conservation. 91.1% and 93.33% survival rates, post a year of conservation were observed by *R. stricta* Decne and *C. spinosa* L, respectively, though it was 90% for *L. dentata* at higher supplementation of sucrose. High genetic stability was observed using Random amplified polymorphic DNA (RAPD), suggestive that the technique is successful for the plants under study in MS media with carbon source being sucrose and sorbitol as osmotic agent (Attia et al. 2017).

8.4.2 Cryopreservation

Long term storage by preserving material like seeds, buds, cuttings, roots, rhizomes, at very low temperatures like -196°C , is termed as cryopreservation. The techniques aim mainly at preservation along with capacity to retain biosynthetic potential, conservation of germplasm and maintaining genetic stability of the clones. For MAPs, the technique should also lead to good survival rate and biochemical

stability for secondary metabolite production. Long term storage using this technique has been successfully implemented for *Eruca sativa* Mill (Xue et al. 2008), *Hypericum perforatum* (Urbanová et al. 2006) and *Dendrobium candidum* (Yin and Hong 2009).

The conventionally used technique for cryopreservation is vitrification. The plant material for short periods is exposed to glycerol-based cryoprotectants, known as plant vitrification solution (PVS). The procedure involves steps as pretreatment, preconditioning, preculture, osmoprotection, dehydration, cooling, warming, dilution, and finally regrowth. Another tool is encapsulation–dehydration wherein the plant is partially dehydrated in calcium alginate beads. In this technique, simplification of the rewarming step is facilitated as the alginate beads once dehydrated does not lead to formation of ice crystals.

In *Dioscorea floribunda*, cryopreservation of shoot tips provided genetic stability, with high survival rate (87%) though the success rate of plant regeneration was 30%. using vitrification technique (Ahuja et al. 2002). Another study using vitrification along with encapsulation–dehydration technique, in the same plant proved that the content of diosgenin remained unchanged in cryopreserved shoots as compared to control plants, thereby suggestive of maintaining biochemical stability (Dixit-Sharma et al. 2005).

The commonly used techniques for cryopreservation, viz. desiccation, vitrification, and encapsulation–dehydration were investigated by Ghaffarzadeh-Namazi et al. (2017) in the young leaves in the callus of *Satureja spicigera*. The regrowth of callus was studied using agents as PVS2, PVS3, DMSO used in vitrification and highest regrowth of 98.7% was observed when vitrification was done with PVS3.

8.4.3 Synthetic Seeds

With the advantages of genetic stability, handling ease and effectiveness of space, time, cost and labour, another new technique is alginate encapsulated seeds or synthetic seeds. Synthetic seeds besides being a method for conservation, is an efficient method to screen as well as maintain a selected genotype with the potential of producing high yield of secondary metabolites, one of the many reasons MAPs are economically important (Lata et al. 2009). Gantait et al. (2015) evaluated the factors which need optimization for success of this technique and suggested that the important ones are selection of explants and the choice of matrix used for encapsulation. The synthetic seed of *Capparis decidua* were employed for *in vitro* generated shoot tips and nodal segments by encapsulation and complexation was done in 3% alginate solution and 100 mM calcium chloride. Generally, micro-cuttings, unipolar or bipolar propagules of vegetative parts, differentiating aggregates, somatic embryos, ranging from 3–5 mm, lead to successful attempts at producing artificial seeds in various medicinal plants (Atanasov et al. 2019).

Al-Qurainy et al. (2014a, b) produced synseeds from *in vitro* cultured shoots and the encapsulated buds were stored at 4 °C for 60 days. Both dry and non-dry synseeds over the period showed growth though 100% conversion was observed upto 30 days of storage. Thereafter, month old plantlets were analyzed for genetic fidelity with no anomaly in comparison to the mother plant was observed either in morphology or molecular profiles of plantlets.

The conversion response of encapsulated and nonencapsulated nodal segments of *Althaea officinalis*, a medicinal plant, post 6 weeks storage at 6 °C, was significantly higher in encapsulated than non-encapsulated nodal segments (Naz et al. 2018).

8.5 Metabolic Pathway Engineering and Hairy Roots

Currently, gene transformation is being mediated by either direct or *Agrobacterium*-mediated indirect approaches, with the latter being most efficient. *Agrobacterium*-mediated gene transformation methods are largely independent of tissue culture and termed *in planta* gene transformations and bear advantage of being free from somaclonal variation, require less time and are simpler than tissue culture based transformation methods. Transformation of *Bacopa monnieri* with *Catharanthus roseus*, strictosidine synthase and tryptophan decarboxylase genes (engaged in pathway associated with terpenoid indole alkaloid), using LBA1119 strain of *Agrobacterium tumefaciens*, resulted in an increase of 25-fold in the tryptophan content in transgenic tissues in comparison to non-transformants. However, intricate developmental regulation involving different organelles, cells and tissues, in the synthesis of industrially used products, using only tissue culture, possess limitations (Sharma et al. 2018a, b).

Ray of light is in use of metabolite engineering for the medicinal plant wherein the technology helps in manipulating overexpression of genes for biosynthesis of secondary metabolites, or inhibition of desired pathways, utilization of regulators of transcription and preventing catabolic activities leading to denaturation of product (Matveeva and Sokornova 2018). The technique utilizes species of *Agrobacterium* as *A. tumefaciens* and *A. rhizogenes*, generally in conjugation with biolistic transformation methods.

This gene transformation technique faces some technical limitations as recalcitrant response, dependency on genotype and the normal problems associated with tissue cultures. Efficiency of *Agrobacterium*-mediated transformation is affected by concentration of *Agrobacterium* measured through optical density, immersion time of inoculation, *Agrobacterium* elimination of antibiotic, concentration of chemical stimulants as acetosyringone as additives and PGR's used (Niazian 2019).

Various explant types have been used for regeneration of MAP's using *Agrobacterium*. Active shoot regeneration in absence of PGR being supplemented

in media using leaf explants of *Nicotiana tabacum* and *Nicotiana benthamiana* were transformed with *A. tumefaciens* GV3101 (Han et al. 2013). *A. tumefaciens* transformation was used to incorporate Cry3A gene of Bt in embryogenic tissue of Norway spruce (*Picea abies*) (Briza et al. 2013).

The LBA1119 *Agrobacterium tumefaciens* strain was used to transfer to *Catharanthus roseus*, the genes for tryptophan decarboxylase and strictosidine synthase, and an increase in terpenoid indole alkaloid metabolite was confirmed using HPLC. The same was attributed to transitory overexpression of *CrTDC* and *CrSTR* genes (Sharma et al. 2018a).

Use of *A. rhizogenes* has facilitated the enhancement of secondary metabolite production via induction of hairy roots in MAPs, a major advantage, which can further be used via metabolite engineering. The hairy root cultures possess immense genetic and metabolic stability *in-vitro*, in comparison to culturing of cells or callus (Grzegorzczak-Karolak et al. 2018).

8.6 Techniques to Enhance Secondary Metabolite Production

Plant-based medicines have been employed by *Homo sapiens* since time immemorial, with Weyrich et al. (2017), reporting that Neanderthal's used anti-bacterial natural products for therapy. Medicine is one of the most lucrative prospects of biotechnology. According to Iannicellia et al. (2020) MAPs are treasured natural assets as they can produce different types of secondary metabolites (Fig. 8.2).

With increased commercialization, there has been an increased focus on secondary metabolites (VijayaSree et al. 2010). The advent of advanced biotechnological techniques has paved the way for genetic enhancement of medicinal plants (Tripathi and Tripathi 2003). Kutchan et al. (2015) reported the presence of more than 200,000 known secondary metabolites. Terpenes have been found to be the most abundant closely followed by alkaloids. The synthesis of majority of these secondary metabolites involves defined metabolic pathways and mechanisms (Kutchan et al. 2015). The genes that code for the synthesis of secondary metabolites have been elucidated to be derived from the ones that code for primary cellular metabolites (Ober 2010). The synthesis of many of the secondary metabolites, like flavonoids, involves a synergistic approach between the different metabolic pathways, shikimic and malonic acid, in plant metabolism (Fig. 8.3). Biotechnological advances contribute in the synthesis of MAP production by: (i) development of plant seeds and plantlets on a large scale with desired traits (ii) opportunities available for modifying important phytochemicals to more valued molecules in an *in vitro* environment which is difficult to attain by synthetic processes (iii) serves as a means of *in-vitro* conservation (Chatterjee 2002).



Fig. 8.2 Techniques to enhance secondary metabolite production

8.6.1 In Vitro Regeneration

In vitro propagation of plants from excised tissue under controlled conditions is referred to as micropropagation. This technique utilizes the inherent genetic reproductive ability of the donor plant (Tasheva and Kosturkova 2013). *In-vitro* propagation and/or regeneration of MAPs hold remarkable possibilities for the production of high-quality plant-based medicines (Murch et al. 2000). By controlling nutritional and hormonal parameters, *in vitro* techniques, are reported to have improved considerably the yield of secondary metabolites.

Ramachandra Rao and Ravishankar (2002) have stated two primary pathways that can be effectively considered for micropropagation: shoot organogenesis and somatic embryogenesis. Successful organogenesis can be attained by maintaining the right physical environment, ideal growth medium composition and choice of appropriate explant (Brown and Thorpe 1995). Somatic and zygotic embryos develop and differentiate identically. Both types of embryos have similar

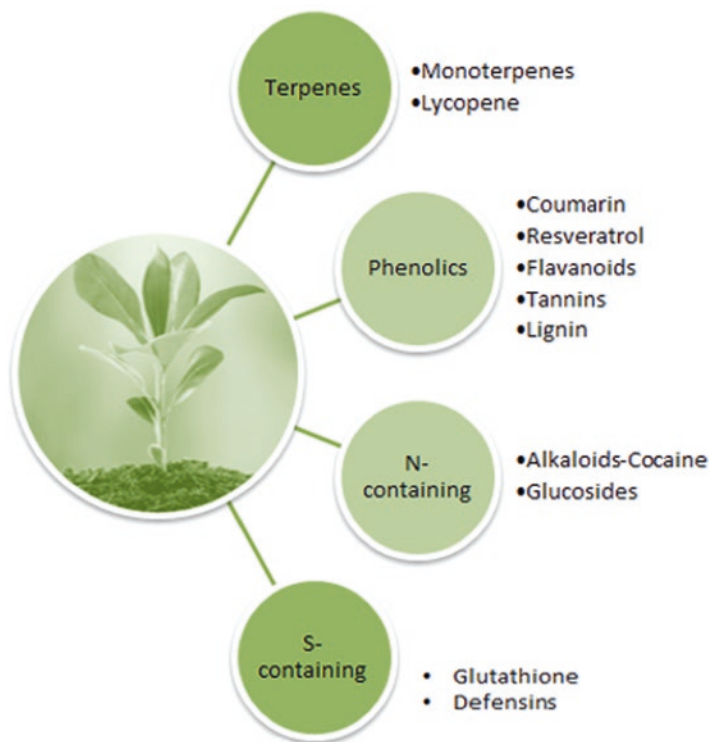


Fig. 8.3 Types of secondary metabolites

developmental stages-proembryo, globular, torpedo (or scutellar stage in monocots), and cotyledonary stages (Bajaj 1995).

8.6.2 Hairy Root Culture

Plant tissue culture facilitates cultivation of medicinal plants and for low-yielding plants and those susceptible to biotic stresses, it is a boon. Grzegorzycy-Karolak et al. (2018) have appropriately stated that PTC leads to successful implementation of both *in situ* and *ex situ* conservation as well as *in vitro* propagation, polyploidy induction, genetic engineering, and bioreactor applications. PTC has also been employed for successful culture of hairy roots, thus leading to the higher production of secondary metabolites that exhibit enhanced stability in the genetic as well as metabolic set-up when compared with traditional *in vitro* cultures (Roychowdhury et al. 2016). Hairy root culture is involved in large scale production of various secondary metabolites (Kundu et al. 2018). *Agrobacterium rhizogenes* undergoes nucleic acid modifications that induce differentiation of hairy roots. Hairy root

culture has several advantages over the traditional cultivation techniques such as genotypic stability over a long duration, high growth rate and higher yield of secondary metabolite (Srivastava and Srivastava 2007).

The LBA1119 *Agrobacterium tumefaciens* strain was used to transfer to *Catharanthus roseus*, the genes for tryptophan decarboxylase and strictosidine synthase, and an increase in terpenoid indole alkaloid metabolite was confirmed using HPLC. The same was attributed to transitory overexpression of *CrTDC* and *CrSTR* genes (Sharma et al. 2018a).

8.6.3 Process Optimization for Producing Secondary Metabolites

Culture systems, which include cell and organ, have wide possibilities for the commercial production of important secondary metabolites. According to Murthy et al. (2014), strain improvement methodologies for maintaining optimum medium and culture conditions, introduction of elicitors for the enhanced synthesis of secondary metabolites, are strategies that have been developed over the years, for the efficient synthesis of secondary metabolites. It is imperative to select a precursor plant that has a high content of secondary metabolites of interest as this would then lead to desirable cell and organ cultures. The genetic makeup of the plant determines the synthesis of secondary metabolites. Recent advanced techniques such as High Pressure Liquid Chromatography and radio-immunoassay can be effectively employed for selection and screening of cell lines that provide a high yield (Matsumoto et al. 1980).

Nutrient medium optimization remains one of the most critical approaches for increased secondary metabolite production by cell/organ culture. The medium composition in turn is affected by several other factors that range from nutrient concentration such as carbon, nitrogen concentration to maintaining the optimum physical as well as fermentation conditions. Since the advent of the science of tissue/cell/organ culture, various media formulations have been introduced, starting from the classic MS (Murshige and Skoog 1962) media to Gamborg's (B5) (Gamborg et al. 1968), Schenk and Hildebrandt (SH) (Schenk and Hildebrandt 1972), Linsmaier and Skoog (LS) (Linsmaier and Skoog 1965), with different media being found to be more suitable for a particular cell/tissue/organ culture. According to the study of Nagella and Murthy (2011) a high concentration MS medium was found to be appropriate for the accumulation of gymnemic acid in culture of *Gymnema sylvestri*, however a 0.75 strength MS medium was found to be better for ginseng adventitious root cultures (Sivakumar et al. 2005a, b). The right composition of medium constituents is vital for the culture of isolated cells/organs/tissues (Murthy et al. 2014). A source of carbon, nitrogen and phosphate is also needed for the proper growth. Among the different carbon sources, sucrose was found to be the most productive in *Gymnema sylvestri* cell cultures.

Other important nutrient, nitrogen, is known to influence metabolite synthesis and accumulation in *in vitro* culture system. Nitrogen is largely available to the plant in the form of either ammonium ion or as nitrate salts. The ratio of the two has been found to markedly affect both biomass as well as secondary metabolite production. Zhang et al. (1996) studied the overall effect of the ratio of these two important nitrogen metabolites on plant cell development as well as production of secondary metabolite, the ginseng saponin, in cell cultures of *Panax notoginseng*. It was reported that saponin biosynthesis was more susceptible to the $\text{NO}_3^- / \text{NH}_4^+$ ratio than that of polysaccharides. Also, ammonium ions were observed to be ameliorative for saponin production.

Phosphate concentration in the media has been found to elicit a positive effect on the biosynthesis of secondary metabolites in cell culture systems. Hagimori et al. (1982) have reported that an elevated phosphate concentration promotes the synthesis of digitoxin in *Digitalis purpurea*. According to the research of Liu and Zhong (1998), a high saponin production can be directly correlated to a high phosphate concentration in *Panax ginseng* and in *Panax quinquefolium*.

Weathers et al. (2005) have dealt in detail on the positive effects of phytohormones as growth regulators for cell/organ culture. The external application of these growth regulators have shown to impact growth and metabolite accumulation in certain hairy root cultures. 2, 4-Dichlorophenoxyacetic acid (2, 4-D), indole acetic acid (IAA) and naphthalene acetic acid (NAA) have exhibited stimulatory effects on the production of anthocyanins and carotenoids in suspension cultures (Seitz and Hinderer 1988; Sahai and Shuler 1984). Another factor that affects secondary metabolite production by cell culture is the size/density of the inoculum as below a minimum size of the inoculum, the growth is impaired. For every culture type, there will be an optimum inoculum size that would promote cell growth and metabolite accumulation.

Along with the biochemical requirements, physical environment-temperature, hydrogen ion concentration, light intensity, aeration, agitation, also significantly influences the production of secondary metabolites. Usually, a temperature ranges 17–25 °C is considered optimum. However, different plant cultures exhibit different temperature requirements, for example, as per Morris (1986). *Catharanthus roseus* cell line C87 was reported to show enhanced rate of growth at 35 °C. Light intensity has been known to affect plant growth as well as that of plant cell cultures (Zhong 1986). The effect of light intensity, quality and duration has been studied by different scientists on different plant cell cultures which includes studies on the effect of light on anthocyanin pigment production in cultures of *Perilla frutescens*, (Zhong et al. 1991) and *Melastoma malabathricum* (Chan et al. 2010).

For any biochemical process, be it growth or synthesis, pH of the medium ensues to be an important consideration. Generally, extremes of pH are avoided and are usually need to be maintained between pH 5 and 6. Sivakumar et al. (2005a, b) reported a pH of 6–6.5 as ideal for the growth of *Panax ginseng* root cultures. In experiments with low pH, roots failed to thrive.

8.6.4 *Elicitors in Tissue Cultures*

Various biotic and abiotic stress conditions are known to affect the rate of production of secondary metabolites. These are referred to as elicitors and have been extensively employed in plant cell/tissue/organ culture for the enhanced production of secondary metabolites. (Ramakrishna and Ravishankar 2011). Scientists have been using structural modification studies for developing new elicitors that are crop specific (Qian et al. 2004, 2005). The addition of elicitors promotes the efflux of intracellular products along with easy isolation and purification of the metabolite. Elicitors also promote enhanced production of secondary metabolites per unit mass of biomass. (Halder et al. 2019).

Enhancement in production of ginsenoside in *Panax quinquefolius* was studied with abiotic elicitors as nickel sulphate, cobalt nitrate, hydrogen peroxide, nickel sulphate and sodium nitroprusside, and with biotic ones as filtrates of cultures of *Bacillus circularans*, *Pseudomonas monteili*, *Trichoderma harzianum*, and *Trichoderma atroviridae*. The ginsenosides content doubled with cobalt nitrate in 5 days along with Rc synthesis being induced in plantlets, as against controls wherein same was lacking. Amongst the elicitors studied, *P. monteili* lead to 2.4 times enhancement in yield of saponin, while with *T. atroviridae* or hydrogen peroxide, Rg3 and Rh2 synthesis was induced and highest ginsenosides efficiency of 3.2 times that of control was noticed at a *T. atroviridae* dose of 1.25%v/v dose for 5 days. Thus, it was observed that an increase in panaxadiols was observed with abiotic elicitors while upregulation in the panaxatriol synthesis was observed with biotic elicitors.

Because of these reasons, various plant growth regulators are used to alleviate the biotic and abiotic stress. Different plant growth regulators like auxins, cytokinins, salicylic acid, jasmonic acid, methyl jasmonate, indole acetic acid, gibberellic acid, NAA, brassinosteroids, ethylene etc. were used. Many of the abiotic elicitors can be used for the production of secondary metabolites of the *Withania somnifera* like Plant growth hormones or regulators; Methyl jasmonate, Salicylic acid, Jasmonic acid, Calcium, Polyamines, Nitric oxide, Serotonin, Abscisic acid, Melatonin, Brassinosteroids, Metal ions, Nitrogen source, Carbon source; sucrose, glucose, maltose, fructose etc., Climatic changes; light, temperature, cold stress, drought stress, salinity stress, nutrient stress, chemical stress etc. (Akula and Ravishankar 2011).

8.6.5 *Ploidy Engineering*

To envisage an effective breeding program for successful production of secondary metabolites, it is important to be aware about the genetic variations prevalent in plants (Niazian et al. 2017). Stability of genome is vital for *in vitro* plant conservation (da Silva et al. 2016). Development of cell lines that give a high yield with

genes of interest have paved the way for increased synthesis of secondary metabolites (Pathak and Abido 2014). Presence of more than one complete genome, polyploidy, is believed to have an evolutionary significance for plants (Madlung 2013). Polyploidy provides an advantage in genetic selection (Levin and Soltis 2018) and is an important tool employed for plant cultivation with the aim to develop genetically improved varieties. One of the most visible effects of polyploidization is enlargement in plant cell size, with a concurrent increase in DNA content as well (Rauf et al. 2006). Lavania (2013) has established a strong correlation between polyploidy induced increase in plant cell size and secondary metabolite production. Plants are susceptible to both biotic as well as abiotic stresses. A polyploid plant species is said to be better suited to adapt to these environmental factors, as it has been found to possess a higher content of antioxidant enzymes (Zhang et al. 2010).

Several researchers have suggested that the quantity of secondary metabolites produced in polyploidy plant species is higher as compared to their diploid counterparts (Pradhan et al. 2018). Thus, artificial induction of polyploidy can be effectively utilized as it not only improves the general health of the plant but also the chemical composition of the medicinal plant (Salma et al. 2017).

Corrêa et al. (2016), induced polyploidy in *Pfafala glomerata* which led to an increase of approximately 31% in the amount of 20-hydroxyecdysone as compared to that in a diploid specie. The most employed inhibitors of mitosis include trifluralin, colchicine and oryzalin, of which colchine has been observed to be the most effective on medicinal plants. Several variable factors such as the type of anti-mitotic agent, concentration applied, and duration of exposure, ascertain the induction of polyploidy (Salma et al. 2017). Pan-pan et al. (2018) explored that in *Bletilla striata* 0.2% colchicine application for 36 h was found to be most effective for tetraploid development. Biotechnological engineering mechanisms are also being used for inducing haploidy. Different techniques have been explored for haploidy induction in plant tissue culture systems which include androgenesis (Kasha 2005), gynogenesis (Piosik et al. 2016), and wide hybridization-chromosome elimination (Forster et al. 2007). One of the most suitable pathway for introduction of haploidy in medicinal plants has been the androgenesis pathway. (Sharma et al. 2018b). Iannicellia et al. (2016) developed a method for *in vitro* polyploidy development in *Lippia integrifolia* wherein the polyploids exhibited enlarged organ sizes and enhanced production of essential oils. In *Centella asiatica* (L.) Urban, Kaensaksiri et al. (2011) studied morphological changes in the polyploidy plant which were evident by larger stomata and a higher stomatal index in contrast with the normal diploid genotype. Also, there was a visible positive increase in triterpenoid synthesis in the tetraploid species. Zahedi et al. (2014) worked with *Dracocephalum kotschy* Boiss., an endangered medicinal plant species known to be localized in Iran. Flavanoid content was found to increase approximately 1.2% in the tetraploid species of this plant.

8.6.6 Bioreactors

For enhanced production of secondary metabolites via plant/cell culture, the traditional techniques of cultivation can be further improvised as the cultures have varied physical and chemical requirements with a low rate of germination (Canter et al. 2005). Bioreactor is an eco-sustainable alternative to produce valuable secondary metabolites of medicinal plants in large scale (Werner et al. 2018). A bioreactor is a preferred option for the mass scale production of secondary metabolites, which is also environment friendly. They function as cell culture systems that are continuous and act like biological industries that provide isolation of secondary metabolites in high quality and quantity (Máthé et al. 2015).

Selection of suitable bioreactors plays a crucial role in secondary metabolite production by plant cell cultures. Certain important points need to be considered when designing a bioreactor which include, emphasis on low shear mixing for efficient nutrient transport without sedimentation or clumping of cells, conditions for required aeration, maintenance of sterilization process and optimum light conditions. Choice of bioreactor remains an important consideration. In both suspension cell and hairy root culture, different variants of bioreactors like fluidized bed reactors, stirred reactor, rotating drum reactor, airlift reactor, etc. have been used (Rizvi 2012). For plant cell suspensions, mechanically driven bioreactors are the systems of choice (Eibl et al. 2018). In these bioreactors, an antifoam agent is usually not added as foam is constantly incorporated into the culture broth.

Different strategies have been employed to enhance the production of secondary metabolites in different bioreactor systems. One such strategy is elicitation and co-culture. Wu et al. (2007) studied co-cultivation of adventitious root cultures of *Panax ginseng* and *Echinacea purpurea* in an airlift bioreactor which resulted in increased production of their secondary metabolites like, ginsenosides, chichoric acid, chlorogenic acid and caffeic acid derivatives (Wu et al. 2008). Shin et al. (2002) employed different variants of airlift bioreactors for cultivation of hairy root cultures of *Beta vulgaris* L and a particular type of bioreactor, the cone type, yielded the highest rate of formation of betacyanin.

8.7 Genome Editing

Genetic manipulations have helped in the cheaper production of novel secondary metabolites on a large scale with minimum wastage. The field of genetics with biotechnology, a specialized arm has helped in precise revelation of the biosynthetic pathway followed for secondary metabolite synthesis, identification of the genes involved in the same, as well as the enzymes catalysing the different metabolic steps (Gandhi et al. 2015). Traditional genetic breeding methods have fallen out of practice because of the ambiguity associated with DNA integration, and the appearance of undesirable phenotypic effects (Fig. 8.4). Naqvi et al. (2010) have elucidated that these techniques are not very effective as the changes incorporated are in a few metabolic steps and not in the complete pathway.



Fig. 8.4 New Generation Sequencing Techniques

The technique of knock-out gene has been utilized to reveal the role of genes in the biosynthesis of secondary metabolites. Several plant-based cancer chemotherapeutic medicines such as the *Vinca* alkaloids have been characterized through this technique (Risчер et al. 2006). Also, research undertaken to assess the type of traits that are transmitted from one generation to the other generation, suggest that these traits could be either qualitative or quantitative. Traditional biometrical methods have been employed to elucidate the inheritance of these traits, as many of the traits that determine the chemical composition of the secondary metabolites have been found to be quantitative in nature (Kumar and Gupta 2008). Scientists have extensively employed the quantitative trait loci (QTL) analysis for elucidation of genes that control these quantitative traits by using DNA-based molecular markers (Mary et al. 2013; Collard et al. 2005).

New arenas of molecular biology, like metabolomics, proteomics, transcriptomics and genomics have made it possible to identify genes involved in the synthesis of secondary metabolites. RNA sequencing analysis is frequently employed to identify genes coding for selected secondary metabolites in different MAPs (Tripathi et al. 2016). On elucidation of the complete biosynthetic pathway, genetic biology approaches are adopted and applied for the increased production of the secondary metabolite of interest. Such methodology has been successfully implemented on *Saccharomyces cerevisiae* (DiCarlo et al. 2013) and *E. coli* (Mami et al. 2018). In yeast, the earliest successful attempts on employing synthetic biology approach, was for the production of artemisinic acid, a precursor of anti-malarial artemisinin (Paddon et al. 2013).

8.7.1 *Bioreactors*

To have enhanced production of secondary metabolites via plant/cell culture, the traditional techniques of cultivation are not the right alternative. The cultures have varied physical and chemical requirements and have a low rate of germination (Canter et al. 2005). Bioreactor is an eco-sustainable alternative to produce valuable secondary metabolites of medicinal plants in large scale (Werner et al. 2018). According to Werner et al. (2018), bioreactor is a preferred option for the mass scale production of secondary metabolites, which is also environment friendly. They function as cell culture systems that are continuous and act like biological industries that provide isolation of secondary metabolites in high quality and quantity (Máthé et al. 2015).

8.8 The Future Belongs to the World of ‘Omics’

The study of ‘Omics’ is aimed at analyzing the structure, function, and dynamics of organism/s, through the characterization and quantification of biomolecules, viz. genes, proteins and metabolites. The current perspective and the near future in MAP research is focused on integration of genomics, proteomics, transcriptomics, and metabolomics, broadly categorized as functional genomics (Oksman-Caldentey et al. 2004). The focus thus is to reveal the association of different cellular elements, viz. proteins, genes and metabolites and their function (Rai et al. 2017).

With the cost of sequencing dropping along with the easy availability of ground-breaking procedures, as linked read sequencing, long reads sequencing, optical genome mapping and Hi-C genome linked sequencing, along with the availability of smart sequence assembling, informatics have hastened *de novo* whole genome analysis at the molecular level (Fig. 8.5).

8.8.1 *DNA Profiling*

A potent tool for detection of genomic DNA and mRNA to further study protein expression is use of DNA microarray. This technique holds significance for MAP’s as it can be used for comparative evaluation of the expression of secondary metabolites under different growing conditions. This can be used to hybridize genome fragments and can be a measure for expressed genes. Modifications of this technique are the subtracted diversity array (SDA) and diversity array technology (DArTTM) and are applicable in plants whose sequence information is lacking.

The technique uses an array of DNA probes depicting DNA–DNA and DNA–RNA selective binding for studying profiling of gene expression as well as for comparative genomic (Allen et al. 2010). Various reference sequences are paired to

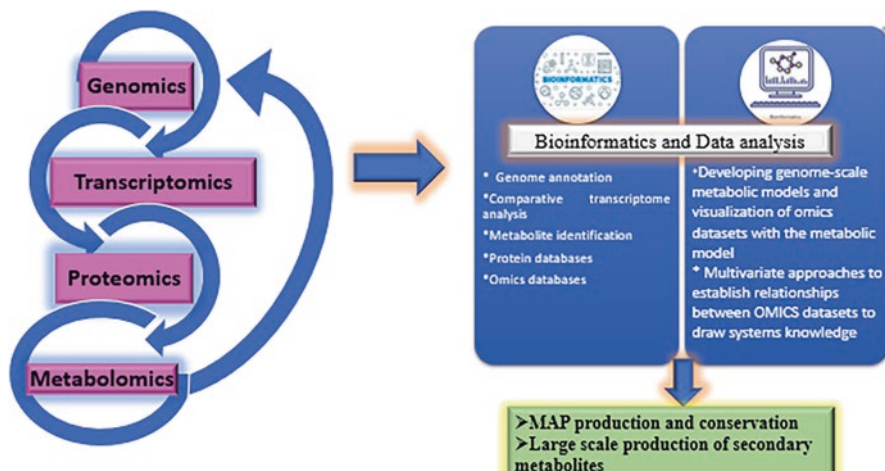


Fig. 8.5 Integrative studies using 'Omics' and Bioinformatics

oligonucleotide probes and the non-overlapping ones are carefully chosen involving 200–300 nucleotide bases of the gene or the cDNA. Another tool for comparative genomics includes expressed sequence tags (EST) (Haq et al. 2014). Such sequence analysis has been accomplished for *C. roseus*, for monoterpene indole alkaloid (MIA) pathway with 3655 unique ESTs reported (Murata et al. 2008) while for *Salvia miltiorrhiza* (Yan et al. 2010), 10,288 ESTs were assembled.

8.8.2 MAP Transcriptomes

For MAP's, the interest is to identify genes coding for proteins associated with the biosynthesis of secondary metabolites (Han et al. 2016). This leads to interest in studying the structural and functional aspects of the genome including the sequencing of mRNAs and *de novo* transcriptome assemblies and analysis (Tripathi et al. 2016).

In *Zanthoxylum planispinum*, an East Asian herb, transcriptome centered studies were conducted by Kim et al. (2019). From the early and maturing fruit stage as also from leaf tissues sequencing of the entire mRNA, was completed and an isoform of the transcriptome was also identified. In the isoforms taken cumulatively, 51,402 unique genes leading to proteins associate with various metabolic pathways and especially ones for secondary metabolite synthesis were short listed.

Using *de novo* transcriptome sequencing, a comparative study of *Ocimum sanctum* and *O. basilicum* was conducted at CIMAP, Lucknow. A Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis of total mRNA- transcripts included 105,470 and 59,648 genes from *O. basilicum* and *O. sanctum*, respectively. The study revealed that 952 transcripts in *O. basilicum* and 501 transcripts in *O. sanctum*

were associated with terpenoids and phenylpropanoids synthesis in the two respective species and the genes associated with these pathways were also found to be linked (Rastogi et al. 2014).

Twenty-seven genes for polyoxypregnane glycosides were distinguished in the transcriptomic studies in *Gymnema sylvestre* using protein databases. Two hundred and thirty five CDSs (coding DNA sequences) were identified through KEGG analysis, of which 19 CDS corresponding to 10 significant enzymes of polyoxypregnane glycoside biosynthesis were analysed (Kalariya et al. 2018).

For *Azadiracta indica*, a comparative study of genome and transcriptomes (four) disclosed that the key steps leading to the secondary metabolite production are similar (Krishnan et al. 2012). Rajakani et al. (2014) established the role of individual cytochrome P450's, involved in isoprenoid synthesis. An analysis of ESTs generated through suppression subtractive hybridization attempts resulted in first full length gene, viz. hydroxyl methyl glutaryl CoA enzyme A reductase (HMGR), the key regulatory for numerous isoprenoids biosynthesis like limonoids (Narnoliya et al. 2014).

Secondary metabolites as essential oil is often stored in trichomes, which play a role in protecting the plant against predators as in *Artemisia annua* L. (Yadav et al. 2014). *A. annua* synthesizes monoterpene oil along with a number of non-volatile sesquiterpenes like artemisinin (anti-malarial) and the biosynthesis of terpenoids and flavonoids in the glandular trichomes, holds significance in plant protection (McKerrow 2015).

Transcriptomic approaches also find utility in comparative profiling of gene expression for different tissue of a plant or between species. In *W. somnifera*, the metabolites in the leaf and root are and the same was analyzed using transcriptomic data (Gupta et al. 2013). A study of different *Panax* species, revealed 19,226 distinctive sequences of *P. notoginseng*, held similarity with *P. quinquefolius* but 11,626 transcripts were distinctive only for *P. notoginseng* while with *P. ginseng*, 19,479 sequences were similar to in *P. notoginseng* and 11,373 sequences were unique to *P. ginseng*.

8.8.3 Molecular Markers and Transcription Factors

Using the study of transcriptomes is a cost-effectual method for molecular markers identification as compared to the conventional methods of plant breeding. With transcriptome data, molecular markers as Single nucleotide polymorphism (SNP) and Single sequence repeat (SSR) can be identified and then used for quantitative trait loci, genetic diversity analysis, gene flow, marker assisted selection, parental analysis and also for evolutionary studies (Zheng et al. 2013). This approach has been utilized in transcriptome analysis of *Withania somnifera* (Gupta et al. 2013) and *Centella asiatica* (Sangwan et al. 2013). In *W. somnifera*, it was found that the dinucleotide SSRs are maximum (2489), with trinucleotide (1681) being next followed by tetra- (92) and finally penta- (20)-nucleotides.

The transcriptional factors (TFs) are DNA binding proteins and are cis regulatory elements. DNA binding domain permits binding with the trans-regulatory region on DNA and thus facilitates regulation of the gene expression resulting in switching the gene on or off under condition thereby enabling the gene expression in a defined and controlled manner. Thus, in MAP's the TF(s) play a vital role and can control the secondary metabolic pathways as often one TF may regulate the entire metabolic pathway. Thus, the identification and isolation of TFs through transcriptome analysis is desirable. TFs as "Zinc finger family", WRKY, F-Box Homeobox, MYB, WD40 repeat family besides others have been worked upon a lot. Such a transcriptome analysis in *C. asiatica*, revealed identification of TFs from 71 different families with "Zinc finger family" being most prominent (Sangwan et al. 2013). Other classes found were F-box Homeobox, AP2, bHLH, GATA, GRAS, MYB, WD40, etc. and for jasmonic acid-treated trichomes, a quantitative expression database was obtained (Spyropoulou et al. 2014).

8.8.4 Combinatorial Supertransformation of Transplastomic Recipient Lines (COSTREL)

Metabolic engineering has influenced the overproduction of useful metabolic products from MAPs. Nuclear transformation has facilitated the same, though recently it has been observed that chloroplast transformation has better advantages. Plasmid or chloroplast transformation provides the potential for multigene transformation and enriched expression of transformed genes. Further, as chloroplast depicts maternal inheritance, the containment of transformed gene is higher. Fuentes et al. (2016, 2018), developed new technique involving both nuclear as well as chloroplast transformation, viz. Combinatorial supertransformation of transplastomic recipient lines (COSTREL).

Industrial scale production of secondary metabolites can be achieved by the transfer from the MAP the entire biosynthetic pathway to another plant which has high biomass. Most researched secondary plant that has been used as the recipient is the tobacco plant. The technique involves conduct of nucleus transformation of an earlier plastid-transformed plant/ cell line. COSTREL was first used to enhance the yield of artemisinin, in *Artemisia annua*. The methodology involved the transfer of genes of artemisinin biosynthetic pathway to the chloroplast of tobacco via "gene-gun" transformation, followed by transformation in transplastomic lines for accessory genes that had the potential to enhance artemisinin production. Such a supertransformation increased the artemisinin production to >120 mg/kg fresh weight in tobacco.

8.8.5 RNA Interference

RNA interference involves manipulating genes for silencing the expression of genes thereby regulating enzymes especially of biosynthetic pathways. Small ribonucleic acid molecules with 20–22 nucleotide, present endogenously termed micro-RNA (mi RNA) along with small interfering RNA (siRNA) are involved in regulation post-transcription, thereby affecting gene expression and the secondary metabolite production can be regulated in MAP's.

In-vitro yield of vincristine and vinblastine from *C. roseus.*, which is antineoplastic is low. Pani and Mahapatra (2013), in an *in silico* study projected the role of 2 prospective miRNAs and 12 mRNA targets encoding metabolic enzymes regulating terpenoid indole alkaloids (TIA) pathways and signaling, cell growth and development, and depict perfect complementarity to each other.

In *Podophyllum hexandrum* (Himalayan Mayapple), which produces podophyllotoxin with multiple medicinal properties, Biswas et al. (2016) studied miRNA mediated management of biosynthesis of this secondary metabolite. In the study 60 mature miRNAs and 6 pre-miRNAs were found through pyrosequencing. Validation by quantifiable real-time PCR suggested that the expression of podophyllotoxin was enriched.

Turmeric is a multiversatile herb. Eighteen families of miRNA were identified by Singh and Sharma (2017) of which 16 families revealed their role in regulation of 238 transcripts. These were associated with regulation of rhizome development, biosynthesis of terpenoid backbone, isoquinoline and curcumin along with growth and developmental process of turmeric.

8.8.6 Engineered Sequence-Specific Nucleases

Currently, the engineered sequence-specific nucleases being researched are TALENs (Transcription activator-like effector nucleases), Zinc-finger nucleases (ZFNs), and CRIPR/Cas. These have the potential to generate in the DNA sequence, site-specific double-strand, thereby leading to modification in characteristics of plants. The above mentioned nucleases are fusion proteins and contain two domains namely a sequence-specific DNA-binding domain which is programmable and a nonspecific DNA-cleavage domain, and thus facilitate genetic and metabolic engineering. Such an approach in MAP's can lead to new varieties with the potential to biosynthesize desired secondary metabolites as well as new bio-products of commercial importance (Pouvreau et al. 2018). These techniques mark notable site-specific DNA modification, with an additional advantage that the modification is restricted to gene disruption only and thus the modified plants under regulatory standards do not fall under transgenic plants.

8.8.7 *Zinc Finger Nucleases (ZFNs)*

The ZFN's are dimers composed of monomers which are a fusion protein of a FokI nuclease domain and DNA binding domain zinc fingers. Recurring cysteine and histidine residues comprise the zinc finger and they generally identify 3 nucleotides. 3/4 zinc fingers comprise a ZFN monomer recognizing 9–12 nucleotides. The possibility to recognize a long section of DNA by incorporating multiple zinc fingers together has been successful as they are modular, though they have elevated toxicity and poor activity.

Construction of desired ZFPs can be achieved from earlier characterized zinc fingers through modular assembly (Kim et al. 2010) and such methods considering framework dependence between nearby zinc fingers can yield functional ZFNs (Bhakta et al. 2013). Modification of wild-type FokI domain to yield obligatory heterodimeric FokI domain has proved to improve specificity by reducing other undesirable effects (Miller et al. 2007).

The advantage of ZFNs as a genome editing techniques is mainly its high specificity and efficiency. The limitations include the complications associated with technical challenges to design the ZFNs, especially for substitution of larger fragments for knockout development. Moreover, the procedure is expensive and has limited target availability.

8.8.8 *Transcription Activator-Like Effector Nucleases (TALENs)*

Like ZFNs, TALENS belong to the class of chimeric nucleases obtained through the coupling of FokI endonuclease possessing a cleavage domain, with 13–28 transcriptional activator-like effector (TALE) repeats which are virulence factors developed from *Xanthomonas* plant pathogenic bacteria. Unlike ZFNs, in TALENs, every TALE repeat, targets only one nucleotide providing for a flexible target strategy, thereby increasing potential target sites. Type III secretion system facilitates incorporation of TALEs into the host and these then trigger transcription of target genes thereby manipulating the normal cellular functions. The TALENS via set of tandem repeats identify and activate DNA sequences upstream to the site of transcription initiation and thus lead to an efficient but selective manipulation of the target DNA (Bogdanove and Voytas 2011).

The limitations associated are that the cDNA encoding TALEN has a size of about 3 kb which makes delivery and expression of TALENs in a cell, difficult. Another bottleneck is the composition of TALE repeat and the effectiveness with which TALENs targets a specific gene, is variable. Additionally, being highly repetitive in nature their compatibility with certain viral vectors, also possess a limitation.

8.8.9 CRISPR/Cas

CRISPR Cas-9 (CRISPR is short for Clustered Regularly Interspaced Short Palindromic Repeats; Cas-9 is a protein in the body) is one of the fastest and most efficient ways to edit genes (Elias 2016). CRISPR-Cas9 is a more robust and simpler tool for targeted genetic editing in agro-based research. It involves a reverse-genetics approach. The endonuclease mechanism, CRISPR, is a useful RNA-guided genome editing tool (Bortesi and Fischer 2015). The Cas9 protein is the endonuclease machinery of the system and comprises of around 1400 amino acids (Song et al. 2019). This mechanism has been extensively studied in various plant species aimed at directed genomic manipulation. The CRISPR-Cas system was for the first time elucidated to function as a defense mechanism in *S. thermophiles* (Barrangou 2007).

Yagiz et al. (2016) have successfully used this approach in *Papaver somniferum* L. in which the biosynthesis of morphine, thebaine etc., was reported to be considerably reduced. Nielsen et al. (2017) utilized this technology to genetically engineer *T. atrovirens* for studying secondary metabolism in this fungus and identified a novel gene that encodes for the production of ZG-1494 α , an innovative platelet-activating factor. CRISPR-Cas9 has also been utilised for the manipulation of *P. chrysogenum* genome. CRISPR based techniques of control of gene expression will not only aid in metabolic engineering and secondary metabolite biosynthesis but also enable the development of manipulated genomes (Tong et al. 2019). Based on proteins and accessory RNA, CRISPR/Cas9 genome editing technique is basically categorized into three types (type I, II and III) (Makarova and Koonin 2015). In nature, CRISPR/Cas9 provides protection against viruses in bacteria and archaea. This immunity is attained by integrating short fragments of foreign DNA (called spacer) between two adjoining repeats of the CRISPR locus (Iqbal et al. 2020).

The advantage associated with CRISPR/Cas is that it utilizes a single targeting molecule (gRNA) for genetic sequence manipulation. The recognition sites in ZFNs and TALENs are composed of proteins whereas that in CRISPR are composed of nucleic acid. This allows easy development of CRISPR plasmids. The disadvantage associated with the same is the high incidence of non-specific DNA breakdown.

In *Candida. albicans*, a CRISPR-Cas9-based gene-driven array (GDA) platform by developed by Shapiro et al. (2018) for inserting two different gRNA in the middle of homologous arms to perform genome editing on the adenine biosynthesis gene ADE2. Nødvig et al. (2015) utilized the orthogonal three-function CRISPR system, which combines transcription activation, transcription interference and gene deletion. A three-fold increase in the production of β -carotene was observed with the CRISPR technology (Pan et al. 2016).

Cho et al. (2017), experimented with the CRISPR tool in *Corynebacterium glutamicum*, an important industrial microorganism involved in the production of amino acids, specifically that of γ -aminobutyric acid which is a chemical of high significance. Feng et al. (2018) developed a CRISPR/Cas9 assisted multiplex

genome editing (CMGE) technique in *Escherichia coli* to dissociate transformation from editing, thus leading to an overall increase in the efficiency of the editing process. In this technique, the desired genomic sequences are assembled into replicative plasmids, and Cas9 gene expression is controlled by stringent inducible expression system. The CRISPR/Cas genome editing tool has opened a new era in plant breeding and secondary metabolite production (Malzahn and Lowder 2017). The CRISPR genome editing system has superseded the other genome editing techniques and has countless unexplored applications.

In recent years, there has been increased application of the CRISPR/Cas9 approach in medicinal plants. This system has been successfully employed on the hairy roots of *Salvia miltiorrhiza* to edit a vital gene that is involved in the biosynthesis of tanshinone (Liu et al. 2017). Various research studies have highlighted the application of CRISPR system for altering the chemical composition of certain important metabolites of medicinal plants (Noman et al. 2016).

8.8.10 Bioinformatics and Data Mining

The benefits of metabolomics for metabolic modeling using functional genomics, is a boon due to the advancement via bioinformatics, which has made available many resources and databases. Insight into the metabolome has been facilitated due to the availability of automated tools for analysis of immense high-resolution datasets. Resource databases available for MAP's include Medicinal Plant Metabolomics Resources (MPMRAtMetExpress) (Saito and Matsuda 2010), Metabolome Express (Carroll et al. 2010), Plant Metabolite Network (PMN) (Dreher 2014) and KNApSACK database (Afendi et al. 2012), the Kyoto Encyclopedia of Genes and Genomes (KEGG) and KEGG-plant, METLIN (Smith et al. 2005) and MassBank (Horai et al. 2010).

Immense information on chemical constituents especially essential oils, their GC-MS profile, agro-morphological effect on yield variations, type of fragrance, and bioactivity details of MAPs is found on AromaDb (<http://bioinfo.cimap.res.in/aromadb/>). The database houses data on 1321 aroma chemical structures and the bioactivities of essential oil/s or the aroma compounds and 357 fragrance types from 166 commercially used plants and 148 high yielding varieties/chemotype. The data base also provides information on the cheminformatics properties as identification, properties as physico-chemical and toxicological and pharmacokinetics (Kumar et al. 2018a, b).

Indian Medicinal Plants, Phytochemistry And Therapeutics (IMPPAT) is a comprehensive online manually curated database of 1742 Indian MAPs mentioning 9596 phytochemicals and 1124 therapeutic utility. The database bridges 27,074 and 11,514 plant-phytochemical and plant-therapeutic associations respectively. The data base comprises of an *in-silico* library of 9596 phytochemicals stating information on chemical structure and identification. The pharmacokinetic, toxicity and similarities in pharmacological properties of the phytochemicals in IMPPAT were

computed using cheminformatic approach (Mohanraj et al. 2018). The IMPPAT database is publicly accessible at: <https://cb.imsc.res.in/imppat>.

A catalogue of transcriptomic information on MAPs is the EGENES database - a platform for effective plant Expressed Sequence Tags (ESTs) analysis through linking of genomic information with information on functionality (Masoudi-Nejad et al. 2007). The cataloging is done via a process involving sequence cleaning, masking of repeats and vectors, sequence assembling followed by KEGG annotation. Though EGENES lists only few plants, it has attempted to capture on basis of EST information, the reactions and pathways in the plants. Medicinal Plants Genomics Resource (MPGR): <http://medicinalplantgenomics.msu.edu/> is another such database, currently for 11 species, with the objective to make accessible information on transcriptome and metabolome of the plant species.

8.9 Conclusions

With increasing concern about mental, as well as physical health, along with the ideology to prevent adverse effects due to long-term use of synthetic drugs, MAPs are being researched as a feasible and viable option. Wild MAPs have been used indiscriminately and thus many of them have become endangered species. Further, to making the cultivation of MAPs profitable, the conventional methods of MAP-breeding need to be upgraded to biotechnology-based breeding methods (Sinha et al. 2019).

PCTOC facilitates not only the faster propagation of these valued plants but also the conservation of the same. Via direct and indirect methods, it can also promote MAP chemical profiles. Important contributions towards the improvement of yields through tissue culture have been achieved through the induction of polyploidy and the use of advanced bioreactors. *Agrobacterium mediated transformation*, also needs a special mention, as it has been reported to facilitate the overexpression of the key-genes of secondary metabolite biosynthesis and to assist in the down-regulation of genes adversely affecting the pathway.

Recently developed techniques like the induction of hairy root cultures and the use of genome editing constructs as ZFNs, TALENs, and CRISPR/Cas9 have been used to improvise the production and utilization of many MAPs. CRISPR/Cas9 has the potential of being developed as a robust method for the alteration of MAP biochemical profile mediated via induction of targeted mutation in the genome. COSTREL has also facilitated enrichment of secondary metabolism production for commercial activities.

The world of 'omics' is the way ahead for MAP's research. A simplified strategy is being shared which can lead to expanding the horizons in MAP research, production and utilization through tools of biotechnology (Fig. 8.6).

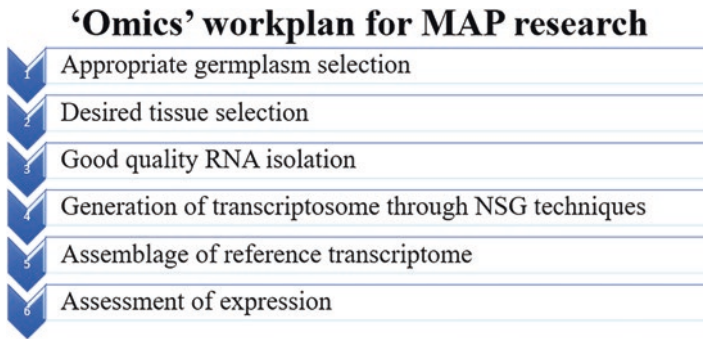


Fig. 8.6 ‘Omics’ workplan for MAP research

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Chapter 9

Demand and Sustainable Use of Medicinal Trees



Rekha R. Warriier

Abstract The growing demand on medicinal trees paralleled with the increasing population and resulting greater demand, has led to the over-exploitation of natural resources, especially in the case of destructively harvested tree species. To conserve the wild tree resources, and to meet the demand of raw materials by the herbal industries, strategies are to be developed in the areas of genetic-conservation, cultivation, post-harvest and marketing. Cultivation looks like an easy option for the supply of raw materials and conservation of existing genetic stocks; however, due to the perennial nature of trees, it is not very imminent. Applying biotechnological tools has tremendous potential both in the propagation of hard-to-multiply arboreal species and in the production of important secondary metabolites of medicinal tree species. Research advancements along these lines could greatly contribute to the industrial-scale production of both crude drug supplies and pharmaceuticals.

Keywords Medicinal plants · Destructive harvest · Trees · Buy-back · *In vitro* production

9.1 Introduction

In India, the National Medicinal Plants Board (NMPB) estimated the annual demand of botanical raw drugs at around 300,000 MT during 2005–06, amounting to 130 million USD. World Health Organization (WHO) projects a value of five billion USD for herbal products in 2050. The threat to medicinal plants, among them medicinal trees, due to destructive harvesting has been highlighted.

Terminalia chebula, commonly known as Myrobalan, dried fruits have high medicinal values. They are used in Ayurveda, due to their purported antitussive,

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cardiotonic, homeostatic, diuretic, and laxative properties. The fruits are harvested by lopping the branches. This, frequently, leads to the removal of immature fruits, too, and the increased mortality of the trees. Due to the careless lopping, the fruit yield decreases year by year. Regeneration is also difficult as the percentage of seed germination is low.

In species such as *Saraca asoca*, where the bark is majorly used, destructive harvesting causes damage not only to the trees but also to the habitats (habitat-loss) bringing about a decline in natural populations. In order to produce the potent magical concoction of “Dashamoola”, a powerful health rejuvenator in Ayurvedic medicine, dried roots of ten different plants are used. It is estimated that annually about 8000 metric tonnes of Dashamoola roots are required. This is achieved by extracting the roots, leading to the loss of the whole plants.

In India, 66% of the species sourced from the wild are reported to be harvested destructively (Ved and Goraya 2008). The primary source of these botanicals is the forest; about 90% is from wild-crafting (Kala and Sajwan 2007).

The ever-rising demand has led to unscientific and unprecedented harvesting from medicinal trees, causing extinction risk to many. So, cultivation of these plants is urgently needed to ensure their prospective availability. Effective steps for conservation, cultivation and mass propagation should be taken to retain the wild stock.

9.2 Demand for Medicinal Trees in India

NMPB has carried out a national study on demand and supply of medicinal plants (Ved and Goraya 2008). The tree species heavily traded in the herbal industry, based on the report, are listed in Table 9.1.

The study has revealed that a long term, integrated, scientific action plan needs to be adopted for the conservation and sustainable utilization of medicinal trees. This should address issues of protection, preservation, conservation, maintenance, exploitation and sustainable utilization. A holistic and systematic approach encompassing social, economic and ecological systems is desirable.

A cost-effective method of protecting existing biological and genetic diversity is *in situ* conservation, which focuses on the ecosystem rather than individual species (Arvind Kumar 2000). *In situ* approaches alone cannot meet the industry demand. Adopting appropriate propagation and cultural practices, using elite planting material, organized cultivation to ensure the supply of raw material at the grower's end (Joy et al. 1998) would help tide over the problem.

Ex-situ conservation is a desirable goal with medicinal trees. It can be adopted for several species in high demand and trade. These germplasms can be preserved in botanical gardens, field-gene banks, seed banks, pollen banks, DNA libraries, *in vitro*, and by cryopreservation (Krishnakumar et al. 2011).

A holistic and integrated approach for important tree species through molecular biology, tissue culture, rationale and method of practice of traditional systems,

Table 9.1 Tree species heavily traded in the herbal industry

Species	Source	Parts used	Estimated consumption (MT)	Cat A & B	Cat C & D
<i>Abies spectabilis</i>	W	Leaf	571	1	99
<i>Acacia catechu</i>	W	Bark (stem), wood (heartwood)	411	32	68
<i>Acacia nilotica</i>	W/C	Gum, bark (stem)	454	40	60
<i>Aegle marmelos</i>	C/W	Leaf, fruit, bark (root, stem)	2939	33	67
<i>Albizia amara</i>	W	Leaf	270	100	0
<i>Alstonia scholaris</i>	W	Bark (stem)	181	1	99
<i>Aquilaria agallocha</i>	I/W	Bark (stem), wood (heartwood)	129	88	12
<i>Azadirachta indica</i>	C/W	Fruit (fruit, seed), flower, leaf, bark (stem)	2255	21	79
<i>Bombax ceiba</i>	W	Exudate of bark (stem), flower, root	166	17	83
<i>Boswellia serrata</i>	W	Oleo-gum resin	762	19	81
<i>Butea monosperma</i>	W	Bark (stem), flower, root, fruit (seed), wood, gum	463	39	61
<i>Caesalpinia sappan</i>	C	Wood (heartwood)	419	10	90
<i>Cassia fistula</i>	W/C	Flower, fruit (seed), bark (stem)	471	21	79
<i>Cedrus deodara</i>	W	Wood	930	16	84
<i>Embllica officinalis</i>	W/C	Fruit (seed)	16820	58	42
<i>Ficus benghalensis</i>	W/C	Bark (stem)	313	2	98
<i>Ficus religiosa</i>	W/C	Bark (stem), leaf	287	1	99
<i>Garcinia indica</i>	W/C	Fruit (fruit, peel)	493	100	0
<i>Gmelina arborea</i>	W/C	Bark (root)	1439	24	76
<i>Holoptelea integrifolia</i>	W	Bark (stem)	113	44	56
<i>Myristica fragrans</i>	C/W	Fruit	180	12	88
<i>Oroxylum indicum</i>	W	Bark (stem, root)	1201	29	71
<i>Pongamia pinnata</i>	C/W	Bark (stem), fruit (seed), leaf, root	277	21	79
<i>Premna latifolia</i>	W	Root, root bark	1003	22	78
<i>Pterocarpus marsupium</i>	W	Wood (heartwood), bark (stem), resin, fruit	522	9	91
<i>Pterocarpus santalinus</i>	W	Wood (heartwood)	442	14	86
<i>Santalum album</i>	W	Wood (heartwood)	291	13	87
<i>Sapindus emarginatus</i>	W/C	Bark (stem), fruit, leaf	182	36	64
<i>Saraca asoca</i>	W	Bark (stem)	2041	26	74
<i>Stereospermum chelonoides</i>	W	Root	1322	25	75

(continued)

Table 9.1 (continued)

Species	Source	Parts used	Estimated consumption (MT)	Cat A & B	Cat C & D
<i>Strychnos nux-vomica</i>	W	Fruit (seed), stem or bark	2891	97	3
<i>Symplocos racemosa</i>	W	Bark (stem)	629	30	70
<i>Terminalia arjuna</i>	W/C	Fruit (seed), bark (stem)	2355	10	90
<i>Terminalia bellirica</i>	W/C	Fruit	3424	31	69
<i>Terminalia chebula</i>	W	Fruit, galls	8158	23	77

C cultivated, I imported, W wild, C/W cultivated and wild, W/C wild and cultivated, I/W imported and wild, Cat A&B medium to large industries (~50 units), Cat C&D small to very small industries (~9000 units)

isolation and validation of active constituents, and other related aspects is needed (Kumar et al. 1997).

Presently, *Caesalpinia sappan* is the only medicinal tree species in high trade demand that is sourced from cultivation, in India. The leaves of *Aquilaria agallocha* (agar), a tropical forest tree species are also collected in sizeable quantities from the wild. Due to heavy demand and rapid decline from the forests, it has been included in Schedule II of the CITES. More than half the species indicated in the list are sourced from the wild, implying their total dependence on the natural forests (Fig. 9.1). The estimated volume of collection from the wild ranges between 113 and 8158 MT.

About 60% of the collections constitute the bark, roots and wood. Eighty percent of the collections are from the wild (Fig. 9.2). In view of the high levels of consumption, it is pertinent to prioritize these species in conservation and cultivation programs. These species are found in scant numbers and/or small patches in the tropical forests; hence the need for effective conservation and efficient utilization of medicinal tree genetic resources is imminent.

Successful long-term utilization and conservation require a thorough investigation of the distribution, genetic status and anthropological use of species (Shaanker et al. 2006).

9.3 Production and Supply of Quality Planting Material (QPM) of Medicinal Trees

Most of the medicinal trees are difficult to propagate. They are slow growing and require specific growth conditions. Further, the process of domesticating and cultivating an identified tree resource is time-consuming (Warriar et al. 2020). Once this is achieved, the species can be propagated (Palevich 1991; Uniyal 2000). While designing cultivation strategies, one should promote 'area projects' wherein in a compact area, suitable species are promoted via low-risk cultivation, involving thousands of farmers who could earn a supplementary income; the risk of causing

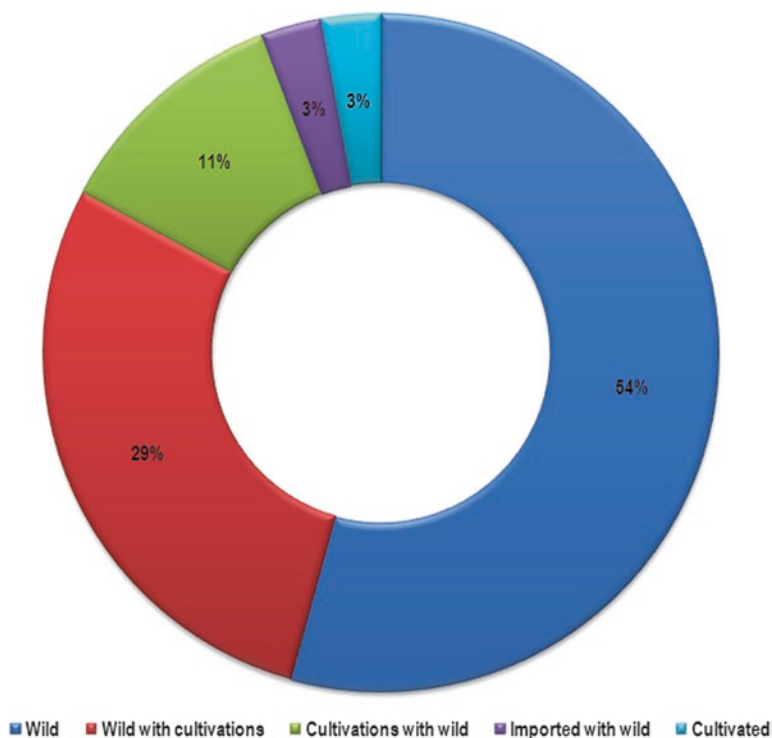


Fig. 9.1 Sources of collection of tree species used in Indian herbal industries

damage to the plant resources is also relatively meagre. There would be an income spread in such strategies, and as such, this kind of cultivation strategy would provide benefits to a large number of small and marginal farmers. Cultivation, however, cannot prevent extinction. It is necessary to bear in mind that even if a particular variety is under active cultivation, the species can still go extinct in the wild, if its wild populations with all the inherent intra-specific diversity are not conserved.

Domestication of the wild resources is not possible in all cases. Most of the species mentioned in Table 9.1 are difficult to propagate, slow growing; and require specific growth conditions for optimal metabolite production. Such cases result in time lag in the production of sufficient volumes of the raw material. This drives the users to resort to wild harvesting, where the same materials are available at a lesser price (Warrier et al. 2013). We need to ensure that at least species with an annual market demand >1000 MT are cultivated and marketed at a minimum support price so that the trend of cultivation of these tree genetic resources is encouraged. The period of domestication process of an identified tree resource could vary from a few years to several decades.

Research requires small quantities of plant material, which is normally harvested from the wild. The damage done is minimal and the wild resources remain intact. Once the potential is established, and the product “catches the eye of the

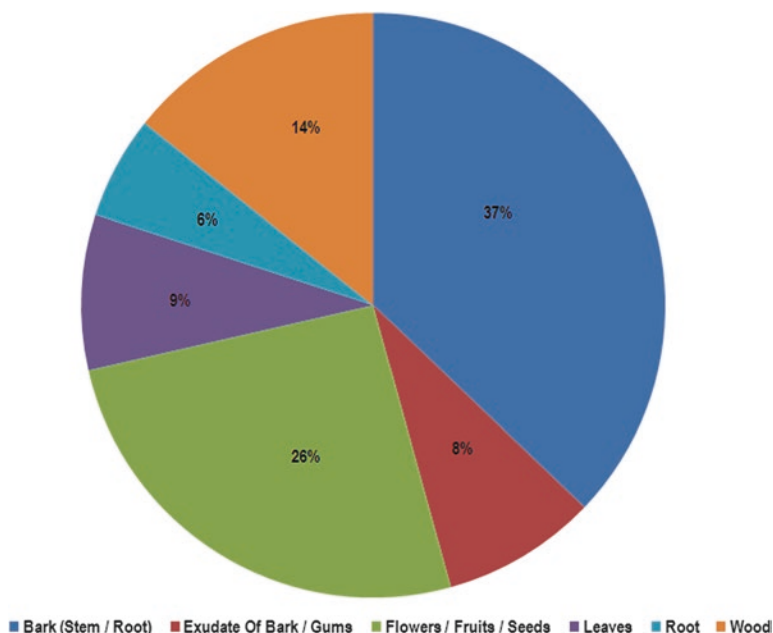


Fig. 9.2 Different parts of the tree species used in Indian herbal industries

consumer”, the demand increases, especially if it aims at the international markets. Species with sparse distribution, or rare occurrence, most likely get affected due to this commercial extraction. With a reduction in the raw material supply from the forests, and the need to sustain the market for the product, there is a tendency to encourage cultivation of the species. At this point, the prices for cultivation are not attractive enough and the wild-harvested resources are sustainable. This is the period of balance in all the three realms, the cultivator, the industry and the natural resources.

Yet, this is also the best time to encourage cultivation with an increase in price, which can support the next phase. The next phase witnesses a sharp decline in the raw material with an associated price rise due to transport costs, search time and long-distance trade. At this point, cultivation is the only way out. The balance maintained in the earlier phase gets disrupted. The danger of losing the genetic resources of slow growing species is very high, in this phase, if control on collection is not strictly enforced. The last phase resorts to cultivation either as a small-scale enterprise or large-scale plantations. Genetic selection, cloning, breeding and biotechnology is applied in order to increase raw material production. The forest areas become hands-off for the species, and resilient species may recover in their wild populations (Warriar and Krishnakumar 2010; Warriar et al. 2011). The described process with the implied status of the resource and the price fluctuations are presented in the Figs. 9.3 and 9.4, respectively.

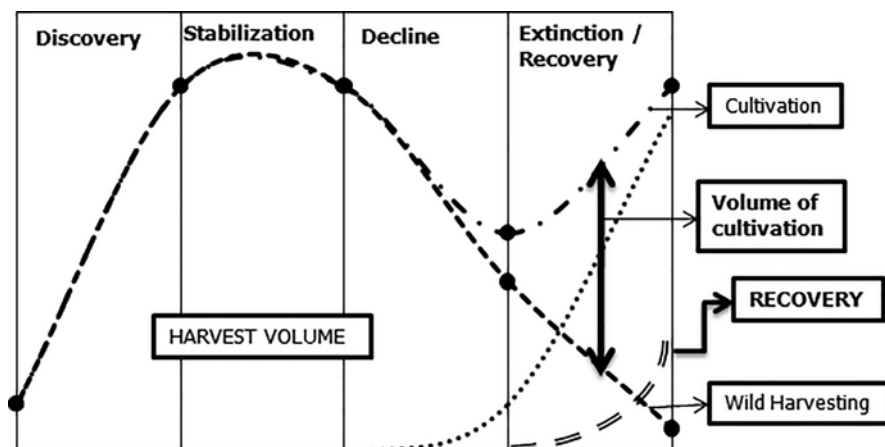


Fig. 9.3 Harvest volume – transition phases from wild collection to cultivation: Wild populations decline before cultivation when there is heavy demand for the species. Realizing the prospects, when cultivation is taken up, there is scope for the natural recovery of resilient species in the wild. (Modified from Schippmann et al. 2002)

For medicinal trees with limited distribution and slow growth, destructive harvesting results in resource depletion leading to species extinction. So, the sustainable use of medicinal trees should be promulgated, and good harvesting practices must be formulated.

A case study of medicinal plant cultivation and marketing to large scale consumers is the Labour Cooperative Society for Local Development and Social Welfare Ltd. (LCS) in Kerala. A Medicinal plant cultivation project with the support of State Medicinal Plants Board and National Medicinal Plants Board ‘Grameenam’ Project was initiated in 2013. Starting with herbaceous species, they are now moving on to different tree species also. Presently MOUs have been signed with 15 leading Ayurvedic Drug Manufacturers in Kerala for a buy-back which has helped retain price stability for farmers.

9.4 Future Prospects of Medicinal Tree Production and Utilization

Science based propagation and cultivation of medicinal plants is crucial to prevent their indiscriminate exploitation. Cultivation ensures the use of botanical identity of plant material, guaranteed quality, and enhances prospects for genetic improvement (Warrier et al. 2011).

Cultivation/domestication can provide alternative supplies for medicinal species in high market demand. However, relatively few medicinal and aromatic species have been brought into cultivation worldwide and most of these species

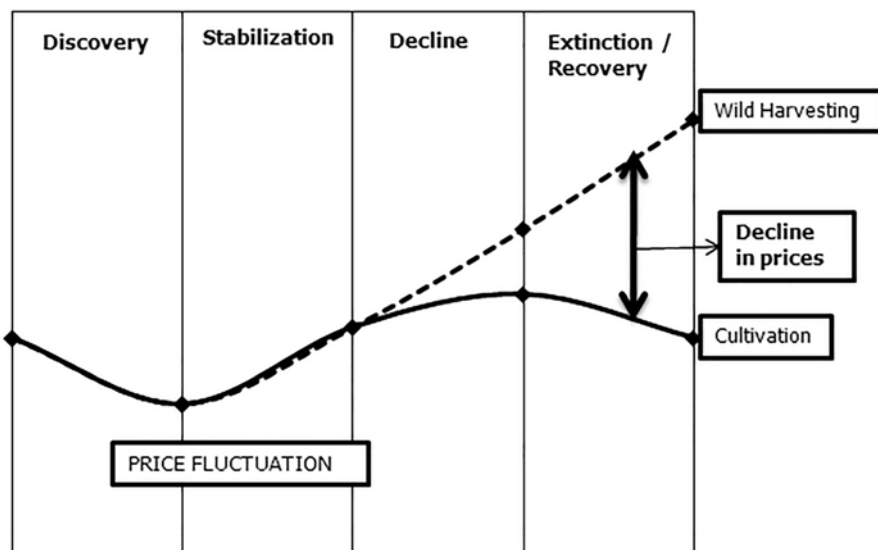


Fig. 9.4 Price fluctuation – transition phases from wild collection to cultivation: genetic stocks decline with over-harvesting. Therefore, raw material prices increase. When cultivation is adopted, the price levels decline making the process economically feasible. (Modified from Schippmann et al. 2002)

continue to be harvested from their native habitats (Warriar et al. 2020). Botanic gardens have undertaken *ex situ* conservation of medicinal plants, focusing more on interspecific diversity. Genetic material for research and conservation held in gene-banks is meagre and for a handful of species only. Most of the collections are with the private sector, and such collections' genetic diversity status is largely unknown. One of the major constraints in the cultivation of medicinal trees is the non-availability of QPM. Although attention given to the development of propagation methods has increased in recent years, very little understanding exists of how these methods and collections can support conservation objectives (Rajasekharan and Ganeshan 2002).

9.4.1 Controlled Cultivation

Controlled or protected cultivation is widely used in the production of crops. Production of plants in greenhouses, growth chambers and/or “polyhouses” under controlled environment (temperature, relative humidity, nutrients and water) help in increasing yields, with consistent quality, efficiency and sustainability. It also ensures a year-round supply of QPM. The controlled conditions in the protected structures can favor the accumulation of secondary metabolites in the plant material and thus enhance the quality.

The Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, India, has taken initiatives to develop cultivation technology for selected medicinal trees. Ten commercially important medicinal tree species namely *Aegle marmelos*, *Gmelina arborea*, *Oroxylum indicum*, *Saraca asoca*, *Terminalia chebula*, *T. bellerica*, *Pterocarpus marsupium*, *P. santalinus*, *Santalum album* and *Embllica officinalis* were cultivated under greenhouse conditions.

Germplasm collected from different natural habitats has been assembled, and evaluated for genetic improvement aimed at augmenting both yield and quality. The concept of “seed orchards” is being implemented to serve as a source of quality seeds ensuring continuous supply. Seed orchards are intensively-managed plantations of specifically arranged trees for the mass production of genetically improved seeds to create plants, or seeds for the establishment of new forests. Since intensive cultivation of medicinal plants triggers pests and diseases, management practices for medicinal trees have been developed (Jacob and Warriier 2019).

Constraints: Long harvest cycles of medicinal trees and requirements in large quantities impede controlled cultivation. Since these raw materials are still readily and cheaply available from the wild, cultivation prospects are limited as it is difficult to justify the capital investment and operating costs for such ventures.

9.4.2 Seed Production Systems

Seed production systems or orchards of the medicinal trees can cater to supply of large quantity seed requirements for harvest / large scale planting. They are an efficient and scientific method that ensures continuous supply of quality seeds for further propagation of the species, in addition to germplasm conservation. The establishment of the orchards will serve as an interim arrangement that enables the availability of quality seeds until comprehensive genetic improvement programs are initiated. Five such medicinal seed orchards have been established in Kerala and Tamil Nadu by the IFGTB. Presently, these orchards yield seeds, used for further propagation of the species (Warriier et al. 2011, 2013, 2020).

Most of the resources exist in forest areas; hence, participatory approaches have to be drawn up. This type of venture can be pursued by Research Organisations in collaboration with State Forest Departments involving farmers as a Public-private partnership. A cluster approach could be adopted to collate information on farmers cultivating medicinal plants and providing them marketing linkage. It would enable the development of models with protocols for species specific conservation and sustainable use, based on research and practical experience. This would also encourage intercropping, pure crop of appropriate model in private lands /waste-land/common lands to cultivate medicinal trees in consultation with communities. A befitting example is the medicinal plant cultivation project of the LCS (detailed in Sect. 9.3 of this chapter) in Kerala. Initiated in 2013, the community initially started with cultivation of *Plumbago indica* species, *Momordica charantia*

(Bittergourd), *Trichosanthes cucumerina*, *Asparagus racemosus* and *Kaemferia galanga*. Now they are envisaging cultivation of tree species, as well.

The IFGTB, Coimbatore, has developed a trait-based selection criterion for the selection of medicinal trees for germplasm conservation. Selection intensity varies for each species. The number of trees for assessment of economic characters and number of locations/trees to be selected are also specified (Warriar et al. 2011).

9.5 Biotechnological Tools to Multiply and Conserve Medicinal Trees

Techniques such as *in vitro* culture adopted for the industrial-scale manufacture of pharmaceuticals include the production of azadirachtin (Thakore and Srivastava 2017; Dawkar et al. 2019), podophyllotoxin (Chattopadhyay et al. 2002a), withaferin (Shajahan et al. 2020), taxol (Sahai and Sinha 2021), camptothecin (Mohinudeen et al. 2021). Careful selection of genotypes and manipulation of culture conditions improves the secondary metabolite production to economically rewarding levels (Thakore and Srivastava 2017).

Plant Cell Culture Plant cell culture “holds the key” to produce complex secondary metabolites *in vitro* (Ravishankar and Venkataraman 1991). Cost-effective plant cell culture methods reduce the problems associated with the raw material’s supply, cost, and quality (Dicosmo and Misawa 1995). The chemical variability among different genotypes within a species, different parts of a plant individual is well documented (Constable et al. 1982; Evans et al. 1984). This is particularly important in developing cell culture systems for medicinal trees, as variations in secondary metabolite content are due to genetic variability and environmental factors (Sidhu and Behl 1996; Sidhu et al. 2003). Establishment of callus and suspension cultures from the various plant parts containing the highest amount of secondary metabolites (Chattopadhyay et al. 2002a, b) and the selection of high yielding genotypes, is desirable to maximise the production of specific compounds in cell culture systems (Zenk et al. 1977). The extent of metabolite production in tissue culture depends on the media and supplements or on the origin of plant material used. It is also influenced by origin, age and differentiation status of the tissue. Variability in production in callus and suspension cultures of medicinal trees is a problem. A classical example is the production of azadirachtin from neem (Prakash et al. 2005). The concentration of azadirachtin in callus cultures varied with source. Seed kernels with high aza content yielded higher, while low yielding genotypes produced lesser aza under *in vitro* conditions. Hence, it is essential to identify chemotypes prior to attempting cell culture.

Cell Suspension Cultures Cell suspension cultures are capable of producing high yields of secondary compounds. Prakash et al. (2005) reported azadirachtin production from different seed sources of neem varying in their azadirachtin content. Mitsui

Petrochemical Industry, Japan was the first to produce shikonin (a dye) and berberine on a commercial scale. Another industry, Samyang Genex Corporation, Korea commercially produced 20 kg paclitaxel annually (Chattopadhyay et al. 2002a, b).

Plant Cell Cultivation – in Certain Cases – Can Offer a Suitable Alternative to Conventional Cultivation for the production of desired compounds (Chattopadhyay et al. 2002a, b). Classic examples are the commercial production of taxol, shikonin, and berberine. Taxol production through cell culture was first illustrated in 1989 (Christen et al. 1989). Chemical synthesis of taxol was uneconomical due to its complex chemical structure (Nicolaou et al. 1994). Cell culture of *Taxus* provided a steady supply of taxol and its related derivatives (Slichenmyer and Von Horf 1991). Elite cell lines of *Taxus* in suspension cultures produced 200 mg taxol per litre following precursor feeding and incubation for 6 weeks (Mulabagal and Tsay 2004). This is against a century-old tree yielding an average 3 kg bark, corresponding to 300 mg of taxol, (a single dose in cancer treatment) (Cragg et al. 1993). *T. chinensis* cell culture with sucrose feeding in fed-batch cultures produced 274.4 mg/L taxol in the suspension medium (Wang et al. 2000; Wang and Zhong 2002). This was followed by modifications in culture conditions like media composition, use of elicitors to enhance taxol content (Howat et al. 2014), specific metals, light, pH, and temperature (Howat et al. 2014). Today, with four decades of research on its production, a yield of $\sim 610 \text{ mgL}^{-1}$ has been achieved (Sabzehzari and Naghavi 2019). Zeng et al. (2009) reported the production of condensed tannins from *Elaeagnus angustifolia*. Srividya et al. (1998) reported that in vitro production of azadirachtin and nimbin from shoots and roots of *Azadirachta indica*.

Production of anthraquinone from *Morinda citrifolia* (Zenk et al. 1977) is another example. Ginsenoside production, biomass and growth rate increased in ginseng cultures grown on low strength media (Sivakumar et al. 2005a, b). Gymnemic acid accumulation in *Gymnema sylvestre* culture (Nagella and Murthy 2011; Murthy et al. 2014) has been enhanced through the use of low strength medium and high sucrose concentrations. Elicitors are also used to enhance production of these metabolites (Jeong and Park 2005; Wang and Wu 2013)

Similar approaches are being tried for the production of various categories of secondary metabolites (e.g.: azadirachtin, vincristine, vinblastine and colchicine), in cell cultures. This would enable reducing pressures on the wild populations also.

9.6 Conclusion

With the heavy and unsustainable overexploitation of medicinal trees from the wild, there is a rapid erosion of their natural resources. To sustain a steady supply of medicinal trees, regular cultivation by farmers with an assured buy-back from industries needs to be implemented. Through conventional conservation methods this can be achieved only to a relatively limited extent. Adoption of non-conventional

methods can be a good strategy for both efficient conservation and sustainable production of medicinal tree genetic resources with the ultimate goal of their feasible utilization.

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Chapter 10

Major Diseases of Cultivated Indian Medicinal Plants: Overview and Management Strategies



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Abstract Medicinal plants are broadly cultivated in the fields with utmost care to meet the growing demand for health care drugs and products. Frequently, they are infected by pathogens and parasites when cultivated in commercial agroecosystems. Devastating diseases can result in huge yield losses increasing the importance of these pathogens in medicinal plants, especially in *Aegle marmelos*, *Aloe barbadensis*, *Andrographis paniculata*, *Atropa belladonna*, *Catharanthus roseus*, *Centella asiatica*, *Cinchona officinalis*, *Coleus forskohlii*, *Costus speciosus*, *Datura stramonium*, *Digitalis purpurea*, *Dioscorea alata*, *Gloriosa superba*, *Rauvolfia serpentina*, *Senna alexandrina*, *Senna occidentalis*, *Solanum nigrum*, *Solanum trilobatum*, *Solanum viarum*, *Stevia rebaudiana*, *Withania somnifera* etc. The sustainable management of diseases and nematodes requires a high level of consideration. Integrated disease management for sustainable development is a precondition for safe disease management and reliable drug outcome in the future. This chapter provides inclusive information about the symptoms of the major diseases, their pathogens, and adaptive management approaches for medicinal plants that are reported necessary in commercial cultivation.

Keywords Biopesticides · Disease management · Fungi · Fungicides · Medicinal plants · Oomycetes

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10.1 Introduction

Medicinal plants and their various organs are consumed as food enhancements or products that add additional flavor to food and feed additives in animal husbandry to boost the immune system. They are gaining worldwide importance because of their medical benefits and interest among growers as phytochemicals promise high revenues in the global market. For the safe use of herbal produce, medicinal and aromatic plants must be produced in impeccably good and healthy quality. In the current scenario, increasing threat lies before the production of medicinal crops due to changing climate and this could lead to rapid disease severity (Elad and Pertot 2014). Disease-affected leaves or plant parts must be discarded as they accumulate unwanted pathogen-driven molecules that interfere with the extraction of phytochemicals of pharmaceutical importance. In this context, owing to the increased economic importance and demand of medicinal plants, this chapter describes the significant symptoms of medicinal plants and the related pathogens. The ultimate aim is to enable the identification of diseases and their control (management) at the appropriate time to avoid huge losses.

10.2 Impact of Diseases in Medicinal Plants

To obtain a high quality, guaranteed yield of medicinal plants, pest and pathogen-free plants are mandatory. The rapid diminishing of cultivable and wild medicinal plants due to biotic and abiotic factors is aggravating at various levels (Carrubba et al. 2015). Apart from the economic losses incurred by diseases, the pathogens decrease the production of secondary metabolites of commercial and traditional value and accumulate harmful residues that ultimately affect the consumers. In nature, plants, including the medicinal plants and pathogens, coexist in the biological system and are prone to be infected by numerous pathogenic fungi, bacteria, phytoplasma, viruses, and nematodes resulting in crop losses all over the globe. Much research has been carried out on the etiology and management of diseases in food and fruit crops; however, significantly less attention is paid to medicinal plants.

All medicinal plants, including leaf, stem, underground parts (roots and tubers), flowers, and pods, can be affected by plant pathogens. Several reports showed the infection of Erisiphales on prominent medicinal plant families, among them Anacardiaceae, Apiaceae, Asteraceae, Lamiaceae, where mildew becomes severe with age and occasionally leads to the loss of foliage (Madia and Gaetán 2005). Symptomatically, mildews colonize on aerial leaves, inflorescence, and fresh stems and developing plant parts. Rust disease produces raised pustules on the surface of leaves, twigs, branches, and fruits and significantly affects yield. Soil-borne diseases *viz.*, wilt, root-rot, damping-off, and disease nematode complex are considered very important due to the prolonged survival of fungal propagules, which may eventually kill the host plant havoc to the commercial growers. Though leaf spots

are season-oriented, depending on climatic conditions, they cause severity (Bhandari et al. 2014), causing a significant hindrance in phytochemical extraction from herbals. Moreover, most medicinal plants are cultivated along with other agricultural and horticultural crops, the chances for disease severity increase.

10.3 Diseases of Medicinal Plants and their Management

10.3.1 Diseases of Wood Apple (*Aegle marmelos* Correa)

Aegle marmelos Correa, commonly known as Bilva or Sripthal or Shivadruma in Sanskrit, Bel or Bael in Hindi, and as wood apple, stone apple, Bengal quince, Indian quince, holy fruit, or golden apple in English, is an essential medicinal tree in India.

Foliar Diseases

Alternaria leaf blight of wood apple reported for the first time in Eastern Plateau and Hill region of India (Madaan and Gupta 1985), exhibits symptoms on leaves with lesions appearing pale to intense brown of the undefined area followed by brownish rings. In extreme cases, the entire foliage gets blighted, leading to tree death (Parthasarathy et al. 2021).

Soil-Borne Diseases

Fusarium semitectum var. *majus* earlier was observed with the symptoms of pitch-black on young shoots (Mitra 1935). Besides other rot caused by *Fusarium moniliformae* (Arya et al. 1986), *F. solani* causes stalk end rot in wood apple exhibiting the symptoms of the early dropping of immature fruits during the winter season. Under the severe case, the infected portions show dark brown rotting of rind and pulp (Bhargava et al. 1977).

Fruit Diseases

During harvesting, storage, and transportation due to damage, the internal rotting of fruit becomes a severe problem. Post-harvest rot caused by *Aspergillus awamori* exhibits whitish growth on the shell, later turns black sooty growth, and leads to softening and rotting (Singh et al. 2018). Shell soft rot caused by *Syncephalastrum racemosum* is a significant emerging disease in wood apple where the affected fruits rotted with unpalatable fruit pulp unfit for consumption. Lesions developed as rapidly expanding water-soaked light brown rot patches with dark brown margins and a gelatinous texture. The affected fruit produced an unpleasant odor typically associated with decay (Misra et al. 2016).

Disease Management

Management of the diseases is primarily based on preventive measures by adopting good agricultural practices to keep plants, away from the infections. Most registered fungicides, with the exception of the strobilurins, have mild effect against *Alternaria*

sp. (Grove et al. 2003). Soil-borne *Fusarium* spp. can be managed by prophylactic application of well-decomposed farm manures along with recommended doses of biocontrol agents such as *Trichoderma harzianum* and *Bacillus subtilis*. Fruits are to be stored in godowns with proper ventilation to avoid *Aspergillus* post harvest rot.

10.3.2 Diseases of Aloe (*Aloe barbadensis* Mill.)

Aloe vera is a succulent plant with about 550 species growing worldwide in different habitats and has occupied a key position in the herbal and cosmetic industry. This crop has succumbed to both foliar and root diseases that significantly affect the cultivation of *A. vera*.

Foliar Diseases

Earlier reports in India recorded the infections of *Colletotrichum gloeosporioides*, *Fusarium solani*, *Pestalotiopsis versicolor*, *Phoma sorghina* and *Uromyces aloes* on leaves of *Aloe* species (Mukerji and Bhasin 1986). Earlier, *A. alternata* has been reported as a primary leaf spot pathogen, which led to 30–40% crop loss (Bajwa et al. 2010). Later, in Nigeria, fungal pathogens viz., *Fusarium oxysporum*, *Pestalotia psidii*, *Cladosporium herbarum*, and *Heteropatella alpine* were found associated with tip-rot disease of *Aloe* species (Ilondu 2013). Presently, more than 15 fungal pathogens viz., *Alternaria alternata*, *Alternaria tenuissima*, *Alternaria brassicae*, *C. gloeosporioides*, *Curvularia sphaerospermum*, *Curvularia ovoidea*, *Curvularia lunata*, *Fusarium fusaroides*, *Fusarium proliferatum*, *F. solani*, *Fusarium phyllophilum*, *Penicillium purpurogenum*, *Phoma betae*, *Phoma eupyrena*, *Polystrata indica*, *Pythium aphanidermatum*, *Sphaeropsis sapinea*, *Helminthosporium* sp., and *Phomopsis* sp., were broadly reported from different parts of *A. vera* growing areas in India (Ghosh et al. 2018; Avasthi et al. 2019). Similarly, in Bangladesh, reports show *Alternaria pluriseptata*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium oxysporum*, *C. gloeosporioides*, *Curvularia brachyospora*, *Epicoccum purpurascens*, *Nigrospora oryzae*, *Pestalotiopsis guepinii* and *Sclerotium rolfsii* to be associated with diseased plants. Typical symptoms of *N. oryzae* infection in *Aloe* sp. are tiny, circular, brown to black spots surrounded by yellow halo (Begum et al. 2018). The foliar pathogen *C. gloeosporioides* causes irregular reddish-brown to brown color lesions on the leaves with a dark margin on *A. vera* leaves exhibiting anthracnose disease in India (Thiribhuvanamala et al. 2020b) (Fig. 10.1). Similarly, characteristic symptoms of *C. siamense* causing black leaf spots appeared as small, circular dark green lesions found on the upper surface of the leaves (Azad et al. 2020). Reports show the occurrence of *C. sphaerospermum*, *C. lunata*, and *C. ovoidea* causing leaf spot disease (Avasthi et al. 2016a) and *P. indica* infection appearing as small, circular, light maroon spots on tips and the middle portion of leaves, later becoming dry, necrotic and turned into brownish-black in colour in *A. vera* (Avasthi et al. 2017b). Aloe rust, caused by the fungi

Fig. 10.1 Symptoms of anthracnose in *Aloe barbadensis* caused by *Colletotrichum gloeosporioides*



Phakopsora pachyphiza and *P. meibomia*, fungal infections cause's symptoms with black or brown circular spots on the aloe leaves (Soni et al. 2011).

Soil-Borne Diseases

The soil-borne pathogen, *P. aphanidermatum* was isolated from rotting leaves of *A. vera* in India during the winter season (Shukla and Alam 2009), a secondary infection *A. vera* caused by *Fusarium* sp. and *F. proliferatum* is reported from India. Typically, *F. proliferatum* produces irregular, sunken, dark cream brown spots with reddish brown margin on both the surface of leaves in Aloe (Avasthi et al. 2018a) and reported to cause leaf spot and root rot (Avasthi et al. 2018b). Root rot with similar symptoms were caused by *F. solani* in China (Ji et al. 2007). *Phoma eupyrena* and *P. betae* were isolated from the dried leaves of *A. vera* (Avasthi et al. 2013; Avasthi et al. 2017a). *Penicillium purpurogenum* and *Phomopsis* sp. were reported to infect the collar and root portion, causing rot disease of Aloe in India (Avasthi et al. 2015, 2016b). Combined parasitism of root-knot nematode *Meloidogyne incognita* with other nematodes is reported in cultivated *A. vera* plants (Tariq et al. 2007).

Disease Management

Aloe leaf spot disease can be controlled by the field-level application of potential biocontrol agents such as *Beauveria bassiana*, *Burkholderia cenocepacia*, *Pseudomonas poae*, *Trichoderma asperellum*, *T. longibrachiatum*, *T. harzianum*, and *T. viride*, additionally blue copper and mancozeb is also found effective to combat the devastating leaf disease (Ghosh et al. 2018). Foliar application of the Plant Growth Promoting Rhizobacterial strain, *Acinetobacter radioresistens* minimizes the leaf rot disease on Aloe (Meena et al. 2019). To minimize the spread of soil-borne diseases, avoid over-watering, avoid malnutrition, timely remove and

eradicate the infected plants. Fusarium rot can be managed by soil drenching with mancozeb (0.25%) carbendazim (0.2%) (Mondal et al. 2018). Fungicidal treatment of cuttings with carbendazim 0.1% solution reduces the soil-borne diseases. Foliar rot diseases caused by necrotic fungal pathogens can be controlled by field spraying tebuconazole 0.1% (Sharma et al. 2010).

10.3.3 Diseases of Kalmegh (*Andrographis paniculata* (Burm. f.) Wall. ex Nees)

Andrographis paniculata (Burn F. Nees) is one of the most popular annual medicinal herbs belonging to the Acanthaceae family, widely planted in India to treat a wide range of human diseases. The crop is affected by a very few disorders; maybe the secondary metabolites of Kalmegh confer resistance.

Foliar Diseases

Leaf spot caused by *C. gloeosporioides* appears as brown or black water-soaked spots sometimes with a yellow halo, usually uniform in size (Rahman et al. 2019).

Soil-Borne Diseases

Currently, root rot caused by *Macrophomina phaseolina* is one of the significant constraints in kalmegh. Symptoms are observed as yellowing and drooping of leaves on one portion of the plants. As the disease progressed, the entire plant turns yellow, wilts and dries (Fig. 10.2) (Thiribhuvanamala et al. 2020a). Collar rot caused by *F. oxysporum*, showing typical rotting symptoms in all seasons at the plant's collar region, has been reported.

Disease Management

Culturally, the initial infection is reduced by removing infection sources and providing sufficient organic nutrients with microbial or natural immunomodulators. Avoiding monocropping is the best practice to eradicate the primary inoculum. Recently several biocontrol agents have been exploited against *Macrophomina phaseolina* in crop plants. The biocontrol agents, *T. harzianum* and *B. subtilis*, have been effective in reducing soil-borne fungi infection realising improved growth and productivity of MAPs. Soil drenching with liquid formulation of *B. subtilis* (Bs-1 of TNAU) @ 5% can manage the root rot infection followed by applying talc-based formulation of *B. subtilis* (mixed with FYM) @ 50 g/plant at 30 and 60 days after planting.

10.3.4 Diseases of Belladonna (*Atropa belladonna* L.)

Atropa belladonna is a perennial herb, and the drug belladonna is obtained from the flowering tops and roots.



Fig. 10.2 Symptoms of root rot in *Andrographis paniculata* caused by *Macrophomina phaseolina*

Foliar Diseases

The foliar pathogen, *Ascochyta atropae* produces greyish, white irregular spots with slight depression on the upper surface of the leaves. Under favourable conditions, these spots coalesced and cause necrosis resulting in defoliation and death of the plants. Leaf spot disease of belladonna caused by *Cercospora atropae* first reported from USA (Fenne 1942) and India (Singh and Singh 1984) exhibited typical symptoms of angular to round brown spots with chestnut colour on margins of the leaves.

Soil-Borne Diseases

Barker (1917–1918) from England gave the first record of root rot of *A. belladonna* to be caused by *Phytophthora* sp. Later, Westerdijk and van Luijk (1920) later described a root-rot of belladonna in the Netherlands and stated that *Phytophthora erythroseptica* is the causal agent. Alcock (1926) recorded a stem-rot and wilt of belladonna in Scotland and reported that *P. erythroseptica* var. *atropae* be the causal agent. Presently, *P. nicotianae* var. *nicotianae*, *P. erythroseptica*, *Rhizoctonia solani* are also known to cause root rot and associated with dying symptoms in *Atropa* worldwide. It is found that both fine and more extensive fibrous roots attacked by *Phytophthora* cause brown, discolored areas, but later sources become water-soaked and flaccid, and subsequently, the plant dies (Middleton 1943). Belladonna is affected by several leaf spot and necrosis diseases (Ganguly and Pandotra 1962; Singh and Singh 1984). Leaf necrosis was reported from India and also from Argentina. *Pythium butleri* root-rot is said to be one of serious diseases occurring in India, which causes browning and extensive disintegration of cortical tissues, sudden wilting of the aerial portions leading to drying and death of plants (Janardhanan and Husain 1974). Similar symptoms on the root and stem regions of infected plants with extensive disintegration of cortical tissue is reported to be caused by *F. solani* (Janardhanan 2002).

Disease Management

Diversified actions exhibited by microbial biocontrol control agents to combat the pathogens in soil and plants. Seed treatment with biological agents, *Trichoderma* spp. @ 4 g/kg seeds or drenching with copper oxy chloride or mancozeb @ 0.2%, providing elevated nursery beds and maintaining adequate plant population are recommended to minimize the soil-borne diseases. Foliar spraying of mancozeb 0.4% or copper oxy chloride 0.25% or carbendazim 0.1% can reduce the foliar disease incidence (Mondal et al. 2018).

10.3.5 Diseases of Periwinkle (*Catharanthus roseus* (L.) G. Don)

Catharanthus roseus (Apocynaceae family), referred to as the common periwinkle or Madagascar periwinkle, is a perennial evergreen shrub cultivated worldwide. Numerous diseases on periwinkle are reported from different parts of the world to be caused by fungi, bacteria, phytoplasma, nematodes, and viruses.

Foliar Diseases

Corynespora cassiicola leaf spot first appeared as small, necrotic lesions most often associated with margins of leaves of all ages, later turned to yellow and dropped prematurely (Mc Govern 1994). Severe leaf blight of *C. roseus* caused by *A. alternata* was reported in Lucknow (Goyal and Pathak 1982), followed by the report on *P. nicotianae* leaf blight disease of *C. roseus* from USA with enlarged necrotic spots resulting in typical blight symptoms (Gill et al. 1977). The leaf blight caused by *Myrothecium roridum*, *A. alternata*, *R. solani*, *Ophiobolus catharnathicala* and *Glomerella cingulata* also has been reported. The *Phytophthora* aerial blight is reported to induce wilting symptoms with grayish-green and brown discoloration leading to the death of plants. *R. solani* AG 1B causes leaf blight with typical symptoms of semicircular, water-soaked lesions developed on the leaf-petiole junction and later along the leaf margins on the leaves just above the soil line (Garibaldi et al. 2006). Similarly, in Iraq, nursery-grown rosy periwinkles showed severe leaf blight symptoms which increased severity of the disease in the rainy season (Lahuf 2019).

Soil-Borne Diseases

Stem rot disease caused by *Rhizopus stolonifer* appeared as pale green water-soaked lesions on the stem or at the collar region of the plant (Saini et al. 1996). Dieback or twig blight or top rot caused by *P. butleri*, *P. nicotianae*, *Pythium debaryanum*, *A. tenuissima*, *Collectotrichum dematium* and foliar blight caused by *Phytophthora parasitica*, *Phytophthora tropicalis*, *C. dematium* and *Botrytis cinerea* have been reported (Nejat et al. 2015). Foot rot disease by *S. rolfsii* is observed in West Bengal. The bark at the collar region rots accompanies white mycelial growth on the infected area (Chakravarty et al. 1976). *Choanephora cucurbitarum* causes flower blight, which induces the production of dark grey spots on the petals. The spots often

merged, leading to blight symptoms. The disease caused severe damage under high humid conditions (Holcomb 1998). Most of the soil-borne fungal infections recorded are root rot and damping-off incited by numerous fungi such as *F. oxysporum*, *Thielaviopsis* sp., *Sclerotinia sclerotiorum*, *S. rolfsii*, and *P. aphanidermatum*. Dieback is one of the most serious diseases caused by *C. dematium*. The symptoms of the disease consist of the withering of the terminal buds followed by wilting and drying is considered serious (Janardhanan and Husain 1967). Janardhanan (2002) noticed several pathogens, viz., *P. butleri*, *P. aphanidermatum* and *P. debaryanum* associated with dieback symptoms with the appearance of water-soaked spots on young leaves. Madagascar periwinkle cultivars are susceptible to *M. incognita* race 3 (Walker et al. 1994). Also, *C. roseus* is most vulnerable to rice-knot nematode *Meloidogyne graminicola* (Mac Gowan and Langdon 1989) and *Pratylenchus roseus* (Castillo and Vovlas 2007). *Hoplolaimus* sp. is also reported in *C. roseus* at Narendrapur medicinal plants garden in India (Haldar and Gupta 2015).

Disease Management

Kulkarni et al. (1992), managed dieback, collar and root rot diseases of periwinkle successfully by combining soil solarization and host resistance. Soil application of Plant Growth Promoting Rhizobacterial strains viz., *Pseudomonas* spp., or *Bacillus* spp., induced systemic resistance against major plant pathogens in field conditions. *M. graminicola* infestation can be managed by the application of *Pochonia chlamydosporia* (Swarnakumari et al. 2020).

10.3.6 Diseases of Indian Pennywort (*Centella asiatica* L.)

Centella asiatica is an important medicinal herb widely used as a leafy vegetable in several Asian countries. Production of *C. asiatica* has been severely affected by leaf spot diseases.

Foliar Diseases

Leaf spot caused by *Cercospora centellae* (Manoharachary et al. 2003), leaf blight caused by *Alternaria* sp., and *Cochliobolus geniculatus* is reported (Chowdhury et al. 2011). In Korea, *Septoria centellae* associated with leaf spot of *C. asiatica* is reported for the first time when the leaf lesions were irregular or angular (<5 mm to 10 mm in diameter), often delimited by veins. Later the spots coalesced with one another, appearing as pale brown to grayish-white in the center and surrounded by dark purplish halo (Park et al. 2011). Similar leaf lesion symptoms caused by *Lasiodiplodia* sp. are reported as irregular or angular spots with pale brown to greyish white center surrounded by a dark purplish margin (Keshiga et al. 2018). The white-rot caused by *Sclerotinia sclerotiorum* has been recorded for the first time in India (Mondal and Khatua 2015).

Disease Management

Collection and destruction of infected leaf debris eradicates inoculum levels. Foliar application with phyllosphere antagonistic bacteria prevents the infection of plant pathogen by the mechanism of saprophytic competitive ability. Spraying of *B. subtilis* or *B. amyloliquefaciens* @ 5% can combat the invading and existing fungal pathogens on the foliage.

10.3.7 Diseases of *Cinchona* (*Cinchona officinalis* L.)

A dozen species of the genus *Cinchona* are used in medicine as a source of cinchona alkaloids, namely quinine or quinidine.

Foliar Diseases

Pink disease caused by *Pellicularia salmonicolor* is first reported from Darjeeling (Mc Rae 1930). The leaves lost lustre, gradually turning yellow with branches withering rapidly and forming pinkish encrustation, which became ochraceous or white with aging. After the pink stage, the spore-forming stage, earlier known as the nectar stage of the disease appeared (Janardhanan 2002).

Soil-Borne Diseases

Seedling blight of *Cinchona* caused by *Phytophthora* species is considered a serious disease in many parts of Asia and Africa. Such discolored area symptoms in the seedlings' collar region were first observed in Darjeeling district of West Bengal and reported to be caused by *P. palmivora*. The discolored area exhibited rotting, and the seedlings gradually drooped downwards and eventually died. Seedlings of *C. officinalis* and some *Cinchona* hybrids are affected by tip blight disease or die-back caused by *P. parasitica*. *Cinchona*'s two distinct bark diseases, called stripe canker *Phytophthora cinnamomi* and girdle canker by *P. parasitica*, cause dead and dying of inner bark. The disease is characterized by necrosis and death of terminal portions of the branches and leaves of young seedlings (Crandall and Davis 1945). Another major soil-borne pathogen, *Pythium vexans* infected seedlings and One to three-year-old plants, exhibited rotting and water-soaked lesions at the base below soil level. Further, Ramakrishnan (1949) explained that the infected plants showed reddening leaves that drooped down and ultimately defoliated with many blackened roots where the cortex of the roots is easily sloughed off. The occurrence of brown root rot on *Cinchona* by *Fomes lamaoensis* was reported (Janardhanan 2002).

Disease Management

Removing the infected portions and treating the cut ends with copper-based fungicides or a mixture of fungal and bacterial biocontrol agents can manage pink disease and root rot. Adopting hygiene procedures in nursery and main field by applying well decomposed manures and prescribed nutrients, avoiding water logging, and providing proper drainage facility helps in managing the infection due to *Pythium*

and *Phytophthora*. Treating the seeds with mancozeb 0.2% and soil drenching of metalaxyl 0.2% offers initial protection.

10.3.8 Diseases of Medicinal Coleus (*Coleus forskohlii* L.)

Coleus forskohlii (Wild) Briq. medicinal plant is of Indian origin and very little explored. Recently, the crop gained importance due to the alkaloid colchicine useful in the treatment of gout disease. The productivity of coleus is challenged by root rot and wilt disease, and nematodes. The plant is affected by few significant diseases.

Foliar Diseases

Severe leaf infection of *C. forskohlii* by *Rhizoctonia solani* is reported with symptoms of water-soaked spots on leaves that increased rapidly in size and became light then turned to brown. Later, the spots merged, resulting in blight symptoms. Severe infection caused defoliation and death of the plants (Shukla et al. 1993). Stem blight caused *P. nicotianae* var. *nicotianae* caused blighting symptoms on the stem leading to the death of plants (Singh et al. 2011).

Soil-Borne Diseases

Rotting roots with consecutive yellowing and wilting symptoms, decaying of roots accompanied by putrefaction caused by *Fusarium chlamydosporum* is occurring in severe form in India (Boby and Bagyaraj 2003). Similar root-rot-like symptoms caused by *F. solani* reported in *C. forskohlii* (Anirban and Sabita 2008). Reports of Root-rot caused by *M. phaseolina* in *C. forskohlii* in Tamil Nadu show symptoms of yellowing and drooping, blackening and rotting of stem and roots is (Fig. 10.3) (Kamalakaran et al. 2006). Collar rot symptoms due to *F. chlamydosporum* associated with root rot nematode infection occurs to the tune of 12–25% incidence in major growing areas of Tamil Nadu, India. The plants exhibited wilting symptoms, and the collar region was fully decayed, which led to the collapse of the plants within 3–4 days. When given a longitudinal split, the roots showed pinkish discoloration (Thiribhuvanamala et al. 2020b). Reports have been made on the leaf spot of coleus caused by *C. cassicola* (Fig. 10.4) (Fernandes and Barreto 2003) and *Botryodiplodia theobromae* (Singh et al. 2011).

C. forskohlii is severely infested with root knot nematode, *M. incognita*, which causes significant loss in yield and quality (Rajendran and Vadivelu 1991). Symptoms of damage caused by *Meloidogyne javanica* and *M. incognita* appear with stunting and chlorosis of the aerial parts with galled root system (Senthamarai et al. 2008).

Disease Management

A combination of fungus *T. viride* + bacterium *P. fluorescens* reduced the *Fusarium* wilt in coleus compared to carbendazim (Paramasivan et al. 2007). Soil drenching of *T. viride* + *P. fluorescens* at 2.5 kg ha⁻¹ reduced root rot incidence on coleus plants (Senthamarai et al. 2008). Similarly, a combination of *T. viride* with neem-products

resulted in reduced disease incidence in the field condition (Kulkarni et al. 2007). Root rot by *F. chlamydosporum* is effectively managed with sprays of plant extracts such as *Allium schoenoprasum*, *Annona squamosa*, *Annona indica*, *Calendula officinalis*, *Cinnamomum verum*, *Eucalyptus sp.*, *Lawsonia inermis*, *Ocimum sanctum*, *Piper nigrum*, and *Zingiber officinale* (Mudalige et al. 2011). Root rot is controlled by dipping stem cuttings with carbendazim 0.1%, followed by soil drenching with carbendazim 0.1% 30 days after planting, which reduces the disease pressure and increases the yield (DMAPR 2012). Coleus intercropped with *Tagetes erecta* as trap crop, pre-planting soil drenching with neem cake 400 kg ha⁻¹, and dipping cuttings with *P. fluorescens* bacterial solution significantly controlled the nematode population in soil (Ramakrishnan and Deepa 2011). Among the bio-agents, soil application of *T. viride* + *P. fluorescens* at 2.5 kg ha⁻¹ minimized the galls of nematode, *M. incognita* on coleus (Senthamarai et al. 2008). Soil application of *Pochonia chlamydosporia* and vermicompost reduced root nematode population (Swarnakumari and Muthulakshmi 2020).

10.3.9 Diseases of *Costus* (*Costus speciosus* Koen ex. Retz)

Costus speciosus (Koen.) has been used in the various traditional and folk systems of medicine in India and China.

Fig. 10.3 Symptoms of root rot in *Coleus forskohlii* caused by *Macrophomina phaseolina*



Foliar Diseases

Meeta and Jindal (1994) reported leaf blight disease caused by *Curvularia paradissi*, and *Drechslera maydis* in India. In Hainan Province, China, a survey revealed that the *Nigrospora oryzae* caused severe damage (80–85% incidence) during the typhoon season. Early symptoms appeared yellow to brown irregular-shaped lesions on the leaf margin or tip. Under favorable conditions, lesions expanded along with the midvein until the entire leaf turned grayish-brown, and finally leading to defoliation (Sun et al. 2020).

Soil-Borne Diseases

Meeta and Jindal (1994) reported the rhizome rot (*F. solani*), and *Pythium* rot (*Pythium spirosum*). The wilt pathogen, *Fusarium semitectum* recorded from India, is considered most important, with varying infection levels between 70–90%.

Others reported are *B. cinerea*, *Cephalosporium maydis*, *F. solani*, *F. verticillioides*, *Lasiodiplodia theobromae*, *Melanospora zamiae*, *Myrothecium verrucaria* and *Phoma sorghina*. The *C. maydis* causes black bundle disease or late wilt in *C. speciosus* from India with 20% infection, internally and externally seed-borne pathogen (Akhtar et al. 2017).

Disease Management

Sustainable disease management practices in costus medicinal plant are to be practiced by adopting proper crop rotation with non-host crops, removing and destroying infected leaf debris elimination of alternate weed hosts around the field, and providing proper nutrients at the right time. Prophylactic soil application of FYM enriched with regional specific strains of beneficial PGPR's viz., *Pseudomonas* spp., or *Bacillus* spp., or *Actinomyces* spp., or fungal biocontrol agent, *Trichoderma* spp., confers induced resistance against diseases.



Fig. 10.4 Symptoms of leaf spot in *Coleus forskohlii* caused by *Corynespora cassicola*

10.3.10 Diseases of Thorn Apple (*Datura stramonium* L.)

Datura stramonium is a perennial herbaceous plant—primarily cultivated in Europe and South America on a small scale. Major constituents of *Datura* are hyoscyamine and hyoscine.

Foliar Diseases

Leaf spots on *D. metel* caused by *Alternaria tenuissima* are characteristically dark-brown, round to oval, or slightly irregular, merging and forming large necrotic areas (Aktaruzzaman et al. 2015). Woudenberg et al. (2014) reported seed borne infection of *A. crassa* on *D. stramonium*. The infection of *A. crassa* appears as small necrotic spots on leaves that spread to the fruit, finally resulting in fruit blight and rot leading to seed infection beneath the seed coat. Moreover, lesions are pale yellow to light brown with concentric rings, and under severe conditions, the spot coalesces, and the entire leaf turns yellow and falls off (Bessadat et al. 2019). Leaf blight and pod blight are reported on *D. metel* from Israel (Halfon-Meiri 1973). A similar disease is reported on *D. stramonium* in Lucknow, India (Sattar et al. 1986).

Nematode species *Meloidogyne ethiopica* on *D. stramonium* (O'Bannon 1975), *Ditylenchus destructor* in thorn apple weeds in South Africa (Waele et al. 1990) and *M. incognita*, *M. javanica* and *M. arenaria* in Khorasan Province (Iran) and South Carolina (Gharabadiyan et al. 2012) are also reported.

Disease Management

The foliar diseases can be minimized by removing and destroying infected plant parts, deep summer ploughing and soil solarization with polythene sheets, followed by the application of biocontrol agents. Prophylactic spraying of *Bacillus subtilis* or *Pseudomonas* spp. @ 2% can protect the crop against foliar diseases. Also, upon initial symptom expression, spraying of carbendazim 0.1% or mancozeb 0.4% is recommended (Mondal et al. 2018).

10.3.11 Diseases of Fox-Glove (*Digitalis purpurea* L.)

Digitalis was used in folk medicine in England as early as the tenth century. The plant is a native of British Island and Western Europe. *Digitalis purpurea* is a biennial (annual in subtropic) herbaceous plant grown in United States that has not been severely affected by the disease.

Foliar Diseases

Digitalis lanata is known to be infected with anthracnose disease incidence from the USA, UK, Japan, Czechoslovakia, other European countries, and Asia (Goto 1938). The anthracnose pathogen identified as *Colletotrichum fuscum* appeared on leaves with symptoms of purplish brown, circular, or angular spots, 1–4 mm in diameter with small, sunken, fusiform lesions on veins and petioles (Goto 1938). Leaf spots caused by *Phyllosticta digitalis* in *D. lanata* from UK (Spilsbury 1953)

and *Septoria digitalis* from Canada, Belgium, USA and UK are reported. Both *D. purpurea* and *D. lanata* is affected by several leaf spot diseases. *S. digitalis* appeared on leaves as circular spots with purple margins and later the spots became irregular and necrotic with numerous pycnidia in the stroma (Kowalski 1968).

Disease Management

Collection and destruction of infected leaf debris. Preventing the severity of foliar diseases by spraying with fungicides viz., carbendazim @ 0.2% or mancozeb @ 0.2% or chlorothalonil @ 0.2% either alone or in combination. The nematode infestation can be reduced by treating the plants with *Pochonia chlamydosporia* or *Pasteuria penetrans* (Swarnakumari and Sivakumar 2006; Sindhu et al. 2019).

10.3.12 Diseases of *Dioscorea* (*Dioscorea alata* L.)

Rhizomes of *Dioscorea* species are the source of steroidal sapogenin, diosgenin. There are more than 15 species of *Dioscorea*, are reported to contain an appreciable amount of diosgenin, a steroid saponin.

Foliar Diseases

The rust infection by *Puccinia dioscorea* is observed on *Dioscorea deltoidea* with symptoms consisting of minute brownish-orange scattered pustules (uredial) of the fungus on the leaf surface, which later turns black, representing telial stages of the fungus. The severe infection results in leaf fall (Ganguly and Pandotra 1962). *D. deltoidea* is affected by leaf spots caused by *Cercospora dioscorea* characterized by irregularly angular, dark brown spots indefinite margins. Under severe incidence defoliation takes place (Ganguly and Pandotra 1963). Leaf spots caused by *Helminthosporium* and *Drechslera sorokiniana* are also noticed in *Dioscorea floribunda*. A similar type of leaf spot incidence appears in *Dioscorea composita*. Leaf blight caused by *Glomerella cingulata* appeared initially as a small circular light brown, spot-on upper surface of leaves that tend to increase in size and coalesced, forming reddish or dark brown to black patches leading to blight (Boruah et al. 1992), later the disease was reclassified as anthracnose and considered as most prevalent fungal disease throughout tropical countries of *Dioscorea* plants (Kwodaga et al. 2020).

Disease Management

Collection and destruction of infected leaf debris. Spraying with biocontrol agents like *Bacillus subtilis* or *Pseudomonas* spp. @ 2% reduces disease intensity of leaf spot and leaf blight diseases.

10.3.13 Diseases of Glory Lily (*Gloriosa superba* L.)

Gloriosa superba is susceptible to many fungal pathogens, especially leaf blight (*C. gloeosporioides*, *C. lunata*, and *Alternaria* sp.) and tuber rots (*Sclerotium* sp.).

Foliar Diseases

Leaf blight caused by *A. alternata* appeared as small, circular, pale brown spots on the entire leaf surface, later develop as brownish-black concentric rings, enlarges and blighted (Maiti et al. 2007b). A new leaf blight caused by *Diaporthe masirevicii* exhibited symptoms with small (2–5 mm diameter), circular to oval, light brownish spots, surrounded by a yellow halo. These leaf spots occurred on the leaves' leaf tips, margins, and midribs, enlarging to form spots with black fruiting bodies of a fungus; the spots are surrounded by concentric rings (Naveen et al. 2018). Another leaf blight caused by *C. gloeosporioides* exhibits typical oval-shaped lesions on the entire leaf with acervuli at the blighted areas (Fig. 10.5) (Thiribhuvanamala et al. 2020b).

Soil-Borne Diseases

Root rot caused by *M. phaseolina* is observed with typical yellowing of leaves, dark lesions on the stems with black sclerotia, and expanded rotting of roots (Thiribhuvanamala et al. 2018; Meena et al. 2019). Tuber rot is marked by rapid yellowing of leaves and wilting of plants caused by *S. rolfsii* that cause whitish cottony growth at collar region, representing whitish mycelial strands and sclerotia leading to rotting of tubers (Fig. 10.6) (Thiribhuvanamala et al. 2020a). The post-harvest *Aspergillus* storage rot is recently observed in rhizomes of *Gloriosa*, particularly when there is high humidity under storage conditions (Thiribhuvanamala et al. 2020a) (Fig. 10.7).

Disease Management

The combination treatment of soil application of *T. viride* (2.5 kg ha⁻¹) along with mahua cake (150 kg ha⁻¹), dipping the tubers in *Pseudomonas fluorescens* 0.2% followed by spraying tebuconazole + trifloxystrobin 0.1% twice after planting, is effective in managing the root rot disease and increasing the seed yield in *G. superba* (Meena et al. 2019). Similarly, dipping tubers of Glory lily in talc based formulation of *B. subtilis* @ 0.2% followed by drenching with *B. subtilis* @ 0.2% on 30 DAP was effective in managing the root rot disease which recorded root rot incidence of 15.5% with 45.7% reduction in disease over control with significant increase in number of pods/plant (42.5 and 38.3) and number of seeds/pod (62.7 and 56.6) (Thiribhuvanamala et al. 2018).

10.3.14 Diseases of Indian Snakeroot (*Rauvolfia serpentina* L.)

Rauvolfia serpentina, the Indian snakeroot, devil pepper, or serpentine wood, is a flower species in the milkweed family Apocynaceae.

Foliar Diseases

Powdery mildew disease caused by *Leveillula taurica*, is prevalent on Indian snake-root in the plantations in India (Ganguly and Pandotra 1962). The characteristic symptoms include whitish growth of the fungus in patches on the lower surface of the leaves representing the conidia and conidiophores; later, rolling of leaves was observed. Leaf blight and bud rot caused by *Alternaria tenuis* are frequently observed in Indian snakeroot plants, reported as destructive in young plantations. The spots covered the entire leaf, resulting in blight symptoms and subsequently causing drying and defoliation. The leaf spot disease of Indian snakeroot identified as *Cercospora rauvolfiae* is identified with initial symptoms of minute yellow spots, which gradually increased in size and became dark brown patches, finally leading to drying and defoliation infected leaves (Mohanty and Addy 1957). Another leaf blight disease of Indian snake root caused by *Phomopsis sethii* is observed in 4-year-old plantations in Madhya Pradesh, India (Mehrotra 1976). The pathogen attacked the lower leaves initially. The disease progressed upward, primarily affecting the apex and occasionally margins, leading to curving and brittleness of infected parts at margins. The leaf blight symptoms covered a relatively larger leaf surface. The earliest symptom was a small dark roundish to an angular speck on the leaves.

The tiny spots, usually one or two, on the leaf at the apex and the margins widened rapidly, and as the disease advanced, the pathogen *M. phaseolina* affects a major part of the leaf (Janardhanan 2002). *Mycosphaerella rauvolfiae* leaf spots appeared as minute inconspicuous greyish black with pycnidia deeply seated in the infected tissue (Janardhanan 2002). Leaf anthracnose caused by *C. gloeosporioides*,



Fig. 10.5 Typical oval shaped spots of leaf blight in *Gloriosa superba* caused by *Colletotrichum gloeosporioides*

produce enlarged spots which coalesced into large circular patches. Later it completely invades the surrounding tissues resulting in drying and defoliation (Varadarajan 1964). *Corynespora cassiicola* and *C. lunata* leaf spot cause premature defoliation (Mohanty and Addy 1958; Chowdhury et al. 2011; Varadarajan 1966). *Rauvolfia* is reported to be affected by a dieback disease by *Colletotrichum dematium* (Lele and Ashram 1968), which produces smaller lesions that coalesce to form large and circular necrotic patches. *Rhizopus stolonifer* produces slimy wet rot symptoms on inflorescence and fruits, leading to the rotting of Indian snakeroot (Shukla et al. 2006).

Soil-Borne Diseases

The wilt disease of Indian snakeroot caused by *F. oxysporum* f. sp. *rauvolfii* was observed for the first time in India. This disease gained importance due to its considerable damage to the crop. Symptoms appear as wilting of branches initially followed by wilting the entire plant. The collar region and the root portion turned into dark color (Janardhanan 2002).

Disease Management

Seed-borne fungal spots were controlled by treating seeds with mancozeb 0.2% or propineb 0.2%. Soil drenching with neem cake (250 kg ha⁻¹) mixed with eucalyptus litter (2.5 t ha⁻¹) or pongame cake + mahua cake (10 g plant⁻¹) followed by foliar spraying of neem cake 3% or NSKE 5% 500 l ha⁻¹ reduced the incidence of cercospora leaf spot (Arumugam et al. 2010). Treating seeds of *R. serpentina* with mancozeb 0.4% or propineb 0.2% serves effective in managing seed-borne infection of *A. alternata* (Ramappa and Shivanna 2013). Likewise, target leaf spot can be managed by a mixture of *Pseudomonas* sp. 1×10^{-9} cfu ml⁻¹ + salicylic acid (1 ml l⁻¹) + 4% leaf powder of glory bower plant (DMAPR 2014). Spraying of mancozeb 0.4% reduces the foliar disease in Indian snakeroot (Mondal et al. 2018).



Fig. 10.6 Whitish mycelial strands and sclerotia in *Gloriosa superba* caused by *Sclerotium rolfsii*



Fig. 10.7 Black fungal growth on *Gloriosa superba* rhizome

10.3.15 Diseases of *Senna* (*Senna alexandrina* Mill.; *Senna occidentalis* (L) Link.)

Senna was used by Arab physicians as early as 900 A.D. The Arabs introduced this in South India by the traders. *Senna alexandrina* is indigenous to Somalia, Southern Arabia, Sind, and Kutch area in India. A perusal of literature shows that Alexandrian senna is susceptible to fungal diseases but resistant to virus diseases.

Foliar Diseases

Several species of *Cassia* in India and the USA are affected by seedling and leaf blights caused by *Alternaria* sp. Similarly, two pathogens *A. alternata* and *A. tenuissima* cause foliar blight of *C. fistula* and *C. tora* in Indian sub-continent (Lenne 1990). Leaf blight disease is reported to cause severe damage to senna crop by *A. alternata*. The spots were initially black in color minute. They later enlarged to brown irregular spots (3–8 mm) spreading over the entire leaf blade leading to drying and premature leaf fall (Fig. 10.8) (Thiribhuvanamala et al. 2020c). Tetarwal and Rai (2007) reported another leaf blight disease affecting *C. angustifolia* caused by *Alternaria cassia*. The symptoms consisted of small, pale to dark brown spots that became enlarged lesions leading to a blighted appearance in the field. Dark, irregular spots are also observed on the stem and pods. Infected pods showed irregular discoloration and affected stem often collapsed, thereby causing the death of the plants. A leaf spot caused by *C. gloeosporioides*, reported from India exhibits initial symptoms as tiny pinhead brown to dark brown spots on the leaf lamina. The spots enlarged towards the margins resulting in death and defoliation (Gupta et al. 1997). Several leaf spot and leaf blight diseases of minor importance affect senna. Leaf blights caused by *Phyllosticta* sp. and *Cercospora* sp. are observed in the later stage



Fig. 10.8 Symptoms of leaf blight in *Senna alexandrina* caused by *Alternaria alternata*

of growth of the plants (Pareek et al. 1980). The fungus, *Cercospora* spp. causing spots on leaf and pod are reported on *Cassia* sp. (Lenne 1990).

Soil-Borne Diseases

Damping-off caused by *M. phaseolina* showed blackening on the collar region extending upwards, leading to the death of seedlings under field conditions. An infection caused the death of plants in patches (Fig. 10.9) (Patel and Patel 1984). In Tanzania, wilt and dieback caused by *Phomopsis cassiae* in *C. alata* caused severe damage (Ebbels and Allen 1979). *F. oxysporum* causes vascular wilt diseases, where the affected leaves become yellow and droop. Later, the predominant symptom, blackening of the stem near the soil with discoloration on the root system is observed in *Cassia* sp. (Magar and Barhate 2013). Root-knot nematode (*M. incognita*), infected plants showed yellowing of leaves, with reduction in leaf size and small knots on the infected roots. Severe infection resulted in stunting and wilting of the plants in patches (Lenne 1990).

Disease Management

Seed treatment with carbendazim @ 0.1% reduces the disease incidence in the main field (Mondal et al. 2018). Treating seeds with talc based formulation of *B. subtilis* or *P. fluorescens* @ 10 g/kg followed by spraying with *B. subtilis* or *P. fluorescens* @ 2 g/l on 30 and 60 DAS is effective in managing the *Alternaria* leaf blight with the lowest disease intensity of 11.1 and 11.6 PDI with increased leaf yield (18.4 and 16.5% respectively) compared to control (Thiribhuvanamala et al. 2020c). Soil application of FYM enriched with talc-based formulation of *B. subtilis* @ 50 g/plant at 30 and 60 days after planting provides induced resistance against soil-borne diseases.



Fig. 10.9 Symptoms of root rot in *Senna alexandrina* caused by *Macrophomina phaseolina*

10.3.16 Diseases of Black Shade Night (*Solanum nigrum* L.)

Solanum nigrum is cultivated for medicinal and culinary purposes in many parts of the world.

Foliar Diseases

Fungal diseases pose a severe problem in these species, including late blight by *P. infestans* that cause seedling mortality in the nursery; the early blight by *A. solani* causing the appearance of circular spots on leaves (Fig. 10.10). The leaf spot caused by *Cercospora celosia* and leaf mold *C. oxysporum*, characterized by the appearance of light-green or yellowish coloration on the upper side, while a greenish-grey 'mould' develops on the undersides leads to drying of leaves (Epenhuijsen 1974). Rust is first reported in Columbia and recently observed as a devastating disease in Asia, caused by *Puccinia pittieriana*. The initial symptoms appear as minute, round, greenish-white spots that later turn to cream color, with reddish centers, then tomato-red, and finally, rusty-red to coffee-brown on the underside of leaves with corresponding chlorotic discoloration on the upper surface of leaves (Fig. 10.11) (Jeger et al. 2017). Powdery mildew caused by *L. taurica* is first reported in India (Sudha and Lakshmanan 2007) with leaves as white powdery growth that enlarges and colonizes leaves' whole leaf drying. Leaf blight caused by *A. solani* produces symptoms on the oldest leaves and starts as small, brownish to black lesions. These leaf spots enlarge in a characteristically concentric fashion with a yellow halo surrounding the spot. Under favorable conditions, significant defoliation of lower leaves may occur.



Fig. 10.10 Symptoms of concentric circular rings on upper leaf surface of *Solanum nigrum* caused by *Alternaria solani*



Fig. 10.11 Symptoms of chlorosis and necrosis on upper leaf surface of *Solanum nigrum* caused by *Puccinia pittieriana*

Disease Management

Biological management is sustainable for controlling the *Phytophthora* infections, especially by soil application with promising strains of *T. harzianum* reduces the disease incidence. Spraying with chemical fungicides viz., mancozeb 0.2% or chlorothalonil 0.2% can be leaf spot disease. The application of systemic fungicides as seed treatments may offer protective measure, however soil application of a copper oxy chloride 0.25% or Bordeaux mixture 1% nearer to the plants can inhibit *Phytophthora* infections. Powdery mildew disease can be managed by spraying of triadimefon or wettable sulphur fungicides at initial stage of infections.

10.3.17 *Diseases of Tropical Soda Apple (Solanum viarum Dunal)*

Solanum viarum Dunal (tropical soda apple) is a yellow fruited, thorny, a solanaceous weed common to Mexico, Honduras, the West Indies, South America, and India. This plant has high significance because of its high content of glycoalkaloid solasodine.

Foliar Diseases

Tropical soda apple also serves as a reservoir for various diseases and insect pests of solanaceous crop plants (Mc Govern et al. 1994). During the field surveys conducted in Florida, more than 45 pathogens from the foliage, stems, and roots, including fungal and bacterial isolates, were associated. Major reported are *Alternaria* sp., *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., *Helminthosporium* sp., *Phomopsis* sp., *Verticillium dahlia*, *R. solanacearum*, and *Pseudomonas syringae* pv. *tabaci* (Charudattan and DeValerio 1996).

Disease Management

Foliar spraying of *Pseudomonas* spp., or *Bacillus* spp. @ 0.5% is recommended as prophylactic measure. However, under severe infections, spraying with fungicides viz., carbendazim @ 0.2% or mancozeb @ 0.2% or chlorothalonil @ 0.2% can be applied either alone or in combination at initial stage of foliar diseases. Harvesting has to be followed 15 days after fungicidal application.

10.3.18 *Diseases of Stevia (Stevia rebaudiana Bertoni)*

Stevia rebaudiana Bertoni is commonly known as sweet honey leaf, candyleaf, sweetleaf, or sugar leaf, a member of the Asteraceae family. The leaves contain multiple diterpene glycosides used as nonnutritive sweeteners.

Foliar Diseases

In Japan during 1971, *Septoria* leaf spot disease of stevia was first identified from field plantings based on morphological characterization. Later, detailed symptomatology, morphological and multilocus sequence analyses were confirmed (Koehler et al. 2019). They described that the lesions often had a chlorotic halo that rapidly merged to form large necrotic areas resulting in defoliation.

Soil-Borne Diseases

The soil-borne pathogens, *S. rolfii*, *M. phaseolina* and species of *Ceratobasidium* AG-F, *F. oxysporum*, *F. proliferatum*, *Mortierella* sp., *Pythium irregulare*, *P. coloratum*, and *P. sylvaticum* are found to be associated in the highest frequency from stevia roots sampled from plants (Koehler and Shew 2017) which may become a threat in future when the crop is widely cultivated.

Disease Management

Avoid or reduce foliar diseases by spraying with biocontrol agents *viz.* *B.subtilis* @ 2% (2 g/L) twice at 15 days interval at onset of disease. Also, soil application of *T. harzianum* or *T. viride* and FYM minimizes the infection of root-associated diseases.

10.3.19 Diseases of Indian Ginseng (*Withania somnifera* (L.) Dunal)

Indian Ginseng (*Withania somnifera*, fam. Solanaceae) is commonly known as “Indian Winter cherry” or “Ashwagandha.” It is one of the most essential herb in the traditional system of medicine in India and other parts of the world, owing to its immense health benefits (Maiti et al. 2007a). The two most destructive fungal pathogens of this plant are *Alternaria* leaf spot and *Fusarium* wilt.

Foliar Diseases

A leaf spot caused by *A. alternata* was first reported in 1985 that causes on older and mature leaves during warm and humid climatic conditions leading to 50–60% yield loss causing considerable damage to the commercial fields of Indian Ginseng with severe defoliation. Substantial biodeterioration of its pharmaceutically important constituents was reported (Pati et al. 2008). Leaf blight caused by *Alternaria dianthicola* produces small, brown lesions that turn gradually dark brown with irregularly concentric rings, surrounded by yellow halo (Maiti et al. 2007a). *A. tenuis* causes dieback and necrosis of twigs (Mondal et al. 2018). A new leaf spot of Indian Ginseng caused by *Pithomyces charotarum* is reported (Verma et al. 2007). Another leaf spot disease caused by *Myrothecium roridum* was first reported in 1986 with initial symptoms appearing as small, dull, yellow to brown colored water-soaked spots on the leaves with brown to violet margin surrounded by chlorotic hallow. Leaf spot caused by *C. gloeosporioides* appears as small, yellowish to brown spots on the leaves that gradually become enlarged surrounded by a concentric ring (Sarkar and Dasgupta 2017).

Soil-Borne Diseases

Wilt of Indian Ginseng caused by *F. solani* is reported (Gupta et al. 2004) as a severe disease of the nurseries and the commercial fields; sometimes causes complete failure of the crop. *F. solani*, causes *Fusarium* wilt, whereas the yield loss is estimated to be 55–65%. Infection at the seedling stage leads to the complete death of seedlings. Under field conditions, no seed formation occurs or formed, the seeds are thin, tiny, and shriveled (Pati et al. 2008). Damping off of seedlings caused by *R. solani* is seen above the ground level during the rainy season (Jetawat et al. 2015). Root knot of Indian Ginseng caused by *M. incognita* infested on roots and formed several galls leading to root deformation (Pandey and Kalra 2003).

Disease Management

Foliar and soil-borne diseases can be controlled by adopting precautionary measures like seed treatment and sapling spray with mancozeb 0.25% or copper oxychloride 0.4% (Mondal et al. 2018). Plant growth promoting rhizobacteria also offers a vast scope of opportunities in managing diseases and promoting plant growth with quality produce. Conventionally, seed and planting material treatment with a mixture of *T. viride* + *P. fluorescens* at 4 g kg⁻¹, even followed by carben-dazim + mancozeb 2.5 g kg⁻¹ provided better prevention of foliar and soil-borne diseases. Treating the *Pythium* infected soil with a combination of *Azotobacter* sp. and *Trichoderma* sp. reduced the infection at nursery. *T. harzianum* is effective against *F. oxysporum* inciting wilt and leaf blight caused by *A. alternata* in Ashwagandha (Mondal et al. 2018; Rahman et al. 2020).

10.4 Recent Strategies in Management of Plant Diseases

Plant protection plays a prominent role in preventing or reducing infections by pathogen propagules. Practicing a single approach always has a drawback in disease management since a single crop may be affected by foliar and soil borne diseases, so always integrated approach delivers effective and economical control. Through combined physical, cultural, biological, biotechnological, and chemical practices at appropriate periods, Integrated Disease Management can ward off fungal, bacterial, and viral populations in a crop.

Effective disease-management under field conditions should include the precise diagnosis of diseases. These can be manifested in various types of symptoms, including anthracnose, cankers, damping-off, leaf spots, leaf blights, root rots, stem rot, scabs, powdery and downy mildew, mosaic, phyllody, vascular wilt, and rust. Timely diagnosis is crucial for deciding the proper use of management practices. Plant pathogenic fungi, bacteria, and parasitic nematodes spend part of their life on host plants and part in the soil or in plant debris. Therefore, soil disinfestation to eradicate soil-borne pathogens remains a big concern nowadays. Control of these can be achieved by adopting accurate cultural practices with soil solarization, and choosing resistant varieties, cultivars, and hybrids.

An environmentally-friendly alternative for the use of chemicals seems to come from medicinal plants themselves. Many plant-derived oils have been tested with noticeable results and could represent a promising more sustainable alternative for reducing the hazardous impacts. Several reports have proven that essential oils control soil-borne and foliar fungal diseases. The same solution can be adopted for other soil-borne pathogens, taking into account botanical agents for disease management. In recent years, nematodes and pathogenic microorganisms have been effectively controlled through biological methods and the use of plant-derived products.

No doubt, there is a need for chemical fungicides to combat severe infections with allowable dosages, especially in the initial growth period of crops, to ensure no residual effects. In that way, soil drenching with carbendazim (0.1%) or propiconazole (0.1%) tebuconazole (0.1%) or dipping stem cuttings with carbendazim (0.1%) is an effective treatment (Pandey 2017) for managing soil borne diseases of medicinal plants under unavoidable circumstances. Despite the continuous application of chemical fungicide results in the development of resistant strains of pathogen. Moreover, chemical treatments, especially in medicinal plants, could cause severe problems, reduce yields, and affect the quality of the essential oils and biologically active substances.

10.5 Concluding Remarks

Due to the increasing cultivation of medicinal plants, these crops are prone to be affected by pathogens and nematodes that affect crop yields and hinder production of quality phytochemicals of pharmaceutical importance. Knowledge, documentation and identification of emerging diseases must be given utmost priority also in order to conserve the medicinal flora of a country, like India. Understanding the physiology and behavior of major diseases of medicinal plants will direct us towards developing sustainable methods of protecting medicinal plants and preserving the quality of phytochemicals for pharma and nutraceuticals. In the current global trend of promoting sustainable agriculture and horticulture, promising organic disease management practices used with other integrated crop cultivation practices are crucial for increasing awareness in the outlook of environmental safety and the production of good quality drugs.

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Chapter 11

Harbouring the Potential of Medicinal and Aromatic Plants of India: Novel Biotechnological Approach and Extraction Technologies



Saahithya Rajamohan

Abstract The chemical diversity of medicinal and aromatic plants is used in multiple ways. The biochemicals and metabolites produced by plants are used in medicines, as flavouring agents, as agricultural chemicals and in cosmetic industry. Mostly plants harvested from their wild habitats are utilised. Recently, the usage and demand has doubled causing the over-exploitation of natural habitats. The extent of wild-crafting is also influenced by factors like cultivation, cost of production and the utilization rate of resources. In such cases, various biotechnological tools come handy in maintaining the potential of the so valuable medicinal and aromatic plants. This chapter deals, in brief, with the recent biotechnological approaches used and elucidates their core concepts and advantages. These techniques can be used either as a sole component or in combination with the other techniques to derive the maximum beneficial potential of the medicinal plants.

Keywords Medicinal plants · Aromatic plants · Biotechnological tools · Extraction techniques · Approaches

Abbreviations

AFLP	Amplified Fragment Length Polymorphism
ATPS	Aqueous Two-Phase System
CCC	Counter Current Chromatography
cDNA	Complementary Deoxyribonucleic acid
CPC	Centrifugal Partition Chromatography
CT	Critical Temperature

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DAF	DNA Amplification Fingerprinting
DES	Deep Eutectic Solvents
DNA	Deoxyribonucleic acid
GHz	Giga Hertz
HPLC-MS	High Performance Liquid Chromatography – Mass Spectrometry
IUCN	International Union for Conservation of Nature
MAE	Microwave Assisted Extraction
MAPs	Medicinal and Aromatic Plants
MHz	Mega Hertz
NADES	Natural Deep Eutectic Solvents
NGS	Next Generation Sequencing
PC	Critical Pressure
RFLP	Restriction Fragment Length Polymorphism
SFE	Supercritical Fluid Extraction
SNPs	Single Nucleotide Polymorphisms
SSR	Simple Sequence Repeats
UAE	Ultrasound Assisted Extraction
WHO	World Health Organization

11.1 Introduction

A medicinal plant by definition is any plant which, in one or more of its parts/organs, contain substances that can be used for therapeutic purposes or which is a precursor for the synthesis of useful drugs (Sofowora 2008; Evans 2008; Sofowora et al. 2013). One can differentiate between plants that are already established as medicinal plants with the requisite properties and those which need farther studies, although are already considered as medicinal plants (Sofowora et al. 2013), e.g. bark of Cascara; plants used for extraction of pure substances and metabolites either for immediate medicinal use or for the indirect production of medicinal compounds through intermediate metabolites; food, spice, and perfumery plants used medicinally, e.g. ginger.

Medicinal plants have been a resource for healing in local communities around the world since centuries. Still it remains of contemporary importance as a primary healthcare mode for approximately 85% of the world's population (Pešić 2015), and as a resource for drug discovery, with 80% of all synthetic drugs deriving from them (Bauer and Brönstrup 2014). Aromatic plants (also known as herbs and spices) come in Medicinal plants category (collectively they are known as Medicinal and Aromatic Plants) are the prime source of many therapeutics which are responsible for preservation of health and also used in multiple ways in many facets of our life (e.g., cosmetics, medicinal products, food and feed additives).

11.1.1 Medicinal and Aromatic Plants of India

In India, as much as 7500 species are utilised in ethnomedicines (Badola and Aitken 2003) which is half of country's Indian native plant species. Approximately 6000 species of plants that have medicinal properties are employed in China (Khan 2014) and over 5000 plant species are used for medicinal purpose in Africa (Iwu 1993).

Out of all the 250,000 higher plant species on earth, more than 80,000 species are reported to have at least some medicinal value and around 5000 species have specific therapeutic value (Marinelli 2004; IUCN 2007). Besides the usual botanical classification, they are also classified based on the plant organ used, their habit and habitat, therapeutic value etc. (Joy et al. 2001). There is a huge number of species to be reported out of which, based on therapeutic value, *Cinchona* sp., *Azadirachta* sp., *Acacia* sp., *Digitalis* sp., *Phyllanthus* sp., *Terminalia* sp., *Silybum* sp. etc., are few of the many notable medicinal plants used in day-to-day life by the world population.

The list of all the medicinal and aromatic plants are huge and the mere mentioning of the same is beyond the scope of this chapter.

11.1.2 Processing of Medicinal and Aromatic Plants: Approaches

The medicinal principles (biologically active substances) of plants are present in the different organs of the plant like root, stem, bark, heartwood, leaf, flower, fruit or plant exudates. These medicinal principles can be isolated by several processes, out of which extraction is the most frequently used. It implies the isolation of the required constituents from plant materials by using a solvent (Paroda 1993).

In many developing countries, there has been an increased awareness to promote and develop the production and use of medicinal and aromatic plants. Due to the general resurgence of interest towards plant based products, both researchers and producers are focussing on strategies to harness low cost and purpose oriented technologies which go hand in hand with the green consumerism, liberalised and free market economy, ultimately aiming at biodiversity conservation and sustainable use of natural resources (Joy et al. 2001).

For the majority of the world's population, Medicinal and Aromatic Plants (MAPs) tend to be an everlasting source of life saving drugs in primary healthcare. The issues put forward by growing human population, in conjunction with depletion in renewable resources, indirectly causes an increase in the global demand for medicinal plants. Under these circumstances, the ever-increasing demand for therapeutic molecules, triggered by the "green processes", and the depleting quantity of wastes have also come to the forefront of concepts for the viable production of phyto-pharmaceuticals from plants and plants waste. Industrial biotechnology seems to be a promising tool serving this purpose. In addition to this, the isolation and optimization of high value phytoconstituents through development of

alternative techniques have additional social and economic importance (WHO 2019; Fierascu et al. 2020).

11.2 Biotechnological Approaches

The following chapter summarizes the notable major biotechnological tools employed in plant industry to produce derive the secondary metabolites for both small scale and industrial purposes. Some of the conventional approaches, like extraction and chromatographic techniques are excluded. Focus is placed on the prime techniques, which are much more promising and “greener” in the near future. The various tools used in India are described in a brief manner without getting much deeper into the aspects of methods involved.

11.2.1 Micropropagation

Micropropagation also known as plant tissue culture is defined as aseptic asexual plant propagation on a defined culture medium, in culture vessels, under controlled conditions of light and temperature. Here, the isolated plant cells, tissues, organs or whole plants are grown on semi-solid or in liquid synthetic nutrient media, under aseptic conditions. The term micropropagation refers to the miniature size of shoots/plantlets initially produced in culture vessels (*in vitro*). Micropropagation allows to capture maximum genetic gains from the genetic variability of natural populations. Micropropagation of economically important plants can be feasible when relevant selection methods are adopted (Kane et al. 2008; Ganguli 2009; Kumar and Loh 2012; Máthé et al. 2015).

The conventional propagation methods are frequently time consuming, less efficient and occasionally even unsuccessful. Aseptic *in vitro* propagation techniques allow to achieve the multiplication of disease-free plants, on a large scale, in short period of time and throughout the year (Rout et al. 2000a, b; Rath and Puhan 2009; Lakshmi and Reddy 2009; Yaadwinder 2010). Plant regeneration from shoot meristems has yielded encouraging results in numerous medicinal plants: selected examples are: *Aegle marmelos* (Arumugam et al. 2003); *Aloe vera* syn *barbadensis* Mill. (Baksha et al. 2005; Ujjwala 2007), *Astragalus cicer* (Basalma et al. 2008), *Centella asiatica* L. (Tiwari et al. 2000), *Rhodiola rosea* (Tasheva and Kosturkova 2012) etc. Direct micropropagation without callus phase has been described in medicinal plants like *Leucojum aestivum*, *Eryngium foetidum* and *Lilium rhodopaeum* *Catharanthus roseus*, *Cinchona ledgeriana* *Digitalis* spp., *Rauwolfia serpentina* and *Isoplexis canariensis* and *Eryngium foetidum* (Arockiasamy et al. 2002). Mass multiplication by tissue culture mode was targeted and achieved in several threatened and endemic

medicinal plants; Mass clonal multiplication has been successfully achieved in several Himalayan medicinal plants including *Potentilla fulgens* using axillary buds (Thangavel et al. 2014; Sambyal et al. 2006). Another *in vitro* technique using somatic embryogenesis can rapidly produce uniform plants. It is a type of vegetative propagation based on plant cell totipotency. It has established itself as a powerful substitute to other vegetative propagation methods (Thangavel et al. 2014).

To date, micropropagation appears to be the most efficient and practical plant propagation technology used commercially. The success of *in vitro* culturing of plants depends on various factors viz., selection of the starting material, composition of the nutrient media, incorporation of the specific growth regulators and environmental factors. Direct organogenesis is generally considered the safer route for micropropagation of clonal, true-to-type plants (Sandhu et al. 2018); this involves synergistic interactions between physical and chemical factors (Chand et al. 1997) and is started within the shoot meristem of the explant (Altman and Loberant 1998). The advances made in the field of plant cell culture techniques could pave way even for manufacture of rare plants and their cells which would be capable of producing essential chemicals (Lemma et al. 2020).

11.2.2 Somaclonal Variation

Secondary metabolite production is often observed during the *in vitro* culturing of medicinal plants, especially in cell suspension and callus cultures compared to untreated plants (Ngezahayo 2018). An important feature of *in vitro* cultures is the occurrence of somaclonal variation as a result of gene mutation or epigenetic alterations (Larkin and Scowcroft 1981; Gould 1986; Kaepler et al. 2000).

Sources of variations detected in plant tissue culture include: the type of explant, its source, process of regeneration, extent of culture period, the number of subculture cycles, environment maintained for the culture, genotype, and ploidy, etc. Highly differentiated tissues (roots, leaves, and stems) generally produce more somaclonal variations than axillary buds and shoot tips (reviewed in Krishna et al. 2016).

It has been observed that callus, shoot tip cultures, and somatic embryogenesis are accompanied by microRNA gene expression in which microRNAs accomplish different roles such as target gene regulation, stress response, revitalization in micropropagated plants, formation of embryogenic callus and somatic embryogenesis, embryogenesis and postembryonic development, downregulation of target genes, response to light photoperiod (Qiao and Xiang 2013; Chávez-Hernández et al. 2015; Szyrajew et al. 2017; Ngezahayo 2018). MicroRNAs expression was observed in the callus culture of *Taxus* trees (Zhang et al. 2015). Other epigenetic variations, like histone modifications, are also bound to occur.

11.2.3 Synseed Technology

The process involves encapsulation of plant material by using explants, such as shoot tips, nodal segments, hairy roots, calli, protocorm like bodies along with encapsulating agent and matrix for the sustainability of the synthetic seed (Gantait et al. 2015). It is absolutely necessary that the artificial seed coat should be able to shield the explants, possess the efficiency to include nutrients as well as other growth and biological factors, protect the developed artificial seed through the entire process of storage and handling. It should be capable of elucidating mode of action for activating 'germination', should be edible, maintain a good affinity with the biological and chemical systems; and biodegradable (Khor and Loh 2005). The types of explants, the concentration of encapsulating agents used as well as matrix have prominent roles in the production of synthetic seeds in medicinal plants. The above three factors for the most part govern the success of synthetic seed production (Gantait et al. 2015).

In the recent past, increasing importance has been shown towards the usage of synthetic seeds produced via encapsulation technology. This is considered to be an exemplary route for safer conservation and exchange of the species. Synseed technology has shown promising results with unsteady genotypes, and in the preservation, as well as large scale micropropagation of hybrids of rare varieties. Similarly, the method has been described to yield positive results with genetically modified plants that require mycorrhizal-fungal association or do not produce viable seeds (Chaudhury and Malik 2003; Ara et al. 2000; Gantait et al. 2015).

In this method, seeds are, generally, produced as the outcome of a sexual procedure: as a result, in cross-pollinating species, the naturally produced seeds are genetically different from the individual parents (Senaratna 1992). Conservation of seeds can be useful in several tropical and subtropical plants with pharmaceutical values that are difficult to propagate owing to their its discreteness. A large number of medicinal plant species bear desiccation-sensitive or recalcitrant seeds that limit confine the storage duration only up to few weeks or months (Gantait et al. 2015).

11.2.4 Protoplast Culture

Protoplasts are living cells without their cell walls (Riazunnisa et al. 2007; Sinha and Caligari 2009; Yang et al. 2009). Usage of protoplasts for the manufacture of useful metabolites is that the metabolites are liberated readily into the culture medium. This has double benefits: they increase overall productivity and facilitate downstream processing in cases where the cell wall limits the secretion of useful products. Protoplast cultures represent a sustainable and relatively clean source of enzymes and useful secondary metabolites (Aoyagi 2011). However, the main constraint is that they cannot be used for long term production, as they are very fragile.

This can be overcome by usage of immobilization matrix and Alginate, as a common elicitor (Aoyagi 2011).

11.2.5 Development of Novel Transgenics

Transgenic crops are better defined as the genetically engineered crops. Those characters and traits which are not possible of introduction by conventional / usual streamlined approaches can be tailored via transgenics. It involves the introduction of agronomic, pathological, entomological, nutritional, therapeutic-, and vaccine-related characters/traits in plants (Khan and Malik 2018). There are multiple ways and means to transplant and introduce genes into the plant genome: these are based on the choice of explant to be used in transformation experiments, for example through *Agrobacterium*-mediated gene transfer (which tends to be the commonly used method), through gene gun, agro-infiltration method, sonication and treatment by polyethylene glycol. Of these, the *Agrobacterium* mediated and the so called “gene gun” methods are the most commonly used approaches (Saito et al. 1992). Nevertheless, this method involves tailoring of plants for desired traits, and also plays a role in the production of secondary metabolites, the transfer and expression of artificially manipulated foreign genes (Marchev et al. 2020).

11.2.6 Molecular Markers and Maps

Molecular markers provide information on diversity at the nucleotide level (SNPs) to gene and allele frequencies (genotype information), extent and distribution of genetic diversity, and population structure (Sarwat et al. 2012).

They can be used for germplasm cataloguing and are essential in formulating both *in-situ* and *ex-situ* germplasm conservation programs. They also aid in solving taxonomic problems and help in assigning plants to their correct taxonomic hierarchies which are critical in phylogenetic studies. Such information can be ultimately utilized for devising a proper conservation strategy, management of gene-bank, and germplasm collections (Sarwat et al. 2012).

11.2.6.1 Crop Profiling

Crop profiles are descriptions of crop production and pest management recommendations compiled by the state and commodity. They are considered as living documents. They provide agricultural statistics for the crop; here in this case, the entire information about the medicinal and aromatic plants on regions within the state; an inventory of pests and strategies used for management (e.g., cultural practices,

biological control, and pesticides); and lists of key contacts, references, and online resources are made available.

11.2.6.2 Genetic Fingerprinting

The concept of fingerprinting has been increasingly applied in the past few decades to determine the ancestry of plants. Genotypic characterization of plant species and strains is useful as most plants, though belonging to the same genus and species, may show considerable variation between strains (Henry 2001; Breithaupt 2003; Vasudevan 2009).

In the case of medicinal plants, their content of active principles may vary from plant to plant (Vasudevan 2009). This has been a problem in the production of standardized medicines. Climatic factors and adaptability dictate the viability of a particular species and subsequently the content of active principle. In such cases, variations can be observed in the genetic composition of the plants, in addition to varying amounts of the active principle (Henry 2001; Breithaupt 2003; Vasudevan 2009).

A recent offshoot of this method is the use of **biomarkers**. When using these, the chemical marker compound possesses an intrinsic biological activity. For this purpose, DNA fingerprinting is successfully employed for profiling. The various techniques used are microsatellites (Simple Sequence Repeats – SSR), Restriction Fragment Length Polymorphisms (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Random Amplified Polymorphic DNA (RAPD). Further techniques used are Single Nucleotide Polymorphisms (SNPs), DNA Amplification Fingerprinting (DAF) (Henry 2001; Vasudevan 2009).

11.2.6.3 Identification of Adulterations

Adulteration involves the deterioration, admixture, sophistication, substitution, inferiority and spoilage of the actual nutraceuticals (crude drug) obtained from the medicinal plants with variety of substances like heavy metals, organic pollutants, mycotoxins, endotoxins etc. (Al Lawati et al. 2017).

Preliminary identification of adulterants by exomorphic features like shape, size, colour, texture and odour of leaves, flowers, and fruits, leaf type, leaf margin, leaf tips, flowers and their characteristics, inflorescence etc. through the naked eye, should be done. This needs to be compared with the reference material in the form of herbarium or voucher specimens. Microscopic identification is using distinguished histological, cell morphological characters, cell type and cell contents. Advanced microscopic techniques, as the application of phase contrast microscopes, fluorescence and confocal microscopes, scanning electron microscope have greatly increased the accuracy and precision of identification (Sarvananda et al. 2019).

Identification can be implemented also through the study of organoleptic characters, the assessment of colour, odour, taste etc. The microscopic characters are

identified using research microscope. Analytical techniques like infrared, nuclear magnetic resonance spectroscopy, High Performance Liquid Chromatography coupled with mass spectrometry (HPLC–MS), tandem mass spectrometry is used and comparative studies are performed (Al Lawati et al. 2017; Sarvananda et al. 2019).

11.2.6.4 Marker Assisted Breeding

The problems faced in the conventional breeding of medicinal and aromatic plants have changed with the advancement of DNA based molecular markers and molecular breeding strategies. The uniqueness of the molecular markers' correlates to the plant's genotype. Molecular markers are unique and relate directly to the plant's genotype (Bhau 2012).

Except for *Artemisia annua*, only few reports exist for the improvement of medicinal plants through molecular marker based approaches (Graham et al. 2010).

Plant genome sequencing has progressed rapidly since the first genome (*Arabidopsis thaliana*) was completed in 2000 (Fraser 2000) followed by completion of 389- Mb rice genome in 2004 (Takuji 2005). DNA probes from one species can often be used to identify homologous sequences in another closely related species. There is a high degree of similarity in the DNA sequences of functional genes among the different plant species (Bhau 2012).

The aim of developing new breeding strategies lies with the usage of molecular markers wherein the objectives will be based on increasing the germplasm base increasing the number of traits which could be selected simultaneously. The above developments rely upon the technologies that offer cost effective screening of markers and high multiplexing capabilities (Bhau 2012).

11.2.7 Transcriptome Sequencing (Identification, Isolation and Cloning of Useful Genes)

Study of the entire pool of transcripts in an organism (or single cells), specifically the transcriptome, at certain physiological or pathological stage, is highly essential in unravelling the connection and regulation between DNA and protein. Our understanding of genomics in a faster, cost-effective, and tractable manner has been reformed by Next Generation Sequencing technology (NGS). Adopting NGS could lead to enhancement of elucidating genes responsible for the production of active compounds from the medicinal plants. This entire process involves stages right from the sampling of the plant material, preparation of cDNA library, deep sequencing and subsequently the processes involving bioinformatics to extract information (Han et al. 2016).

11.3 Novel Extraction Techniques of Medicinal and Aromatic Plants

The processing of bioactive compounds from medicinal and aromatic plants relies upon the important steps of pre-extraction and extraction procedures. Traditional methods such as maceration and Soxhlet extraction are commonly used at small scale. However, significant advances have been made in the processing of medicinal plants with the help of modern extraction methods like Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE) and Supercritical Fluid Extraction (SFE), wherein these advances are aimed to increase yield at lower cost. Moreover, the methods can be continuously improved and developed. With such wide choice of available methods, the selection of proper extraction method needs meticulous evaluation (Swami et al. 2008).

The pre-extraction method involves the preparation of plant samples to preserve the biomolecules prior to extraction. This requires proper and timely collection of the plant, authentication by an expert, adequate drying and grinding. Also, this comprises the determination of quantity and quality of bioactive compounds. Plants samples such as leaves, barks, roots, fruits and flowers can be extracted from fresh or dried plants material. Other methods like grinding and drying influences the preservation of phytochemicals in the final extracts (Swami et al. 2008; Azwanida 2015).

The purposes of extraction procedures for crude drugs are to obtain the therapeutically desired portion of active principles. In addition, they are expected to eliminate inert materials by selective solvent (*menstruum*) treatment. It involves the separation of medicinally active principles of the plant using selective solvents wherein it is intended to soften and break the plant's cell wall to release the phytochemicals. Commonly used extraction methods include maceration, infusion, percolation, decoction. The extract obtained could be ready for use as a medicinal agent, in the form of tinctures and fluid extracts, or further processed to be incorporated in any of the dosage forms: e.g., tablets, capsules. It can be fractionated to isolate individual chemical entities. The volume of solvents used in these processes play a critical role (Azwanida 2015). The following processes offer a brief introduction to some of the important extraction methods in use and their advantages.

11.3.1 Liquid Liquid Extraction

The Aqueous Two-Phase System (ATPS) one step liquid-liquid extraction is the most classical approach to the recovery of biomolecules. ATPS offers a faster separation of phases, low interfacial tension mass transfer and critical separation of compounds with or without little denaturation. An ATPS is formed by two water soluble polymers (e.g., polyethylene glycol/dextran) or a polymer and a salt (e.g., polyethylene glycol/phosphate) with the presence of more than 80% of water, in both phases. This technique is highly suitable for separation and purification of proteins,

active secondary metabolites, enzymes and cell organelles (Aguilar 2017; Fierascu et al. 2020).

11.3.2 *Natural Deep Eutectic Solvents*

The initially the use of petrochemical solvents and volatile organic compounds was mostly a mostly flammable, volatile and toxic process. In the past decade, the Deep Eutectic Solvents (DES) and their natural equivalents, the Natural Deep Eutectic Solvents (NADES) are being used which deliver promising results. They are produced from plant based primary metabolites (Ivanović et al. 2020). As a routine, DES are usually a combination of two or more solid organic or inorganic compounds which under optimal temperature and stirring time liquefies and forms a stable eutectic (Choi et al. 2011). DES/NADES might be the most widely used solvents in the near future owing to its adjustable physical-chemical properties and its “green” character. The use of DES/NADES in combination with other avant-garde extraction techniques can lead to enhancement in terms of extracted yields of selected bioactive compounds, as well as to the benefits in terms of economic and environmental safety. They are used to extract polar and non-polar natural compounds. Two medicinal plants *Sideritis scardica* and *Plantago major* were extracted using NADES; simultaneous extraction of hydrophobic and hydrophilic bioactive compounds from leaves of *Ginkgo biloba*; phenolic compounds from *Carthamus tinctorius* in DES; extraction of two major flavonoids myricetin and amentoflavone from *Chamaecyparis obtusa* with polyalcohol-based DES have been carried out successfully. According to Ivanović et al. (2020) the recovery of target compounds from the extracts obtained and the recycling of the DES/NADES solvents used are still great disadvantages of the method.

11.3.3 *Counter Current Chromatography and Centrifugal Partition Chromatography*

This technique is being currently used for the preparative isolation and purification of natural products are Counter Current Chromatography (CCC) and Centrifugal Partition Chromatography (CPC). In both of the above techniques, separation usually occurs between two divergent phases (stationary and mobile), which in turn generates droplets or film. In CCC, the stationary phase is maintained by the gyratory motion in the polytetrafluoroethylene coil, while in CPC, the stationary phase is maintained in a constant gravity field aided by single axis rotation through rotary seals (Fierascu et al. 2020). They are helpful in efficiently separating, isolating, purifying milligrams to multigram with retention of virtually all biological activity and molecular integrity (Arige et al. 2017). Some of the plants extracted using this

method include purification of oridonin and ponigidin from *Rabdusia rubescens*, diterpene compounds from *Pseudolarix kaempferi*, Saponins from *Codonopsis lanceolata* roots and ginsenosides from *Panax ginseng*.

11.3.4 *Ultrasound Assisted Extraction*

It is worth noting that, when in high frequency Ultrasound Assisted Extraction (UAE) ultrasound is employed, the extraction yield did not increase significantly, however, the degradation of the herb constituents was diminished. This becomes more important when alkaloids are extracted. This method could be employed as a tool to help in the extraction of medicinal compounds by using lower frequencies to assist in the degradation of toxic alkaloids during the process. This method can be used both on small- and large-scale processes (Vinatoru 2001). This technique has the major advantage of lesser energy consumption. In addition, it can be performed at a lower temperature in a short period of time. Moreover, the cells of plant material can be destroyed by hydrolytic enzymes. This method was proven to be useful in the extraction of rosmarinic acid from mycorrhizal hairy roots of *Ocimum basilicum* L., and in extraction of isoflavonoids from hairy root cultures of *Astragalus membranaceus*. Being a proper method, it can be used to extract polysaccharides from different materials (Guo et al. 2015), the release of the active compounds being obtained through the enzyme degraded cell walls (Jia et al. 2015; Fernando et al. 2017; Fierascu et al. 2020).

11.3.5 *Supercritical Fluid Extraction (SFE)*

The Supercritical Fluid Extraction (SFE) technique basically relies upon the fluid that is at supercritical condition: it is also referred to as a dense gas (a fluid above its Critical Temperature (TC) and Critical Pressure (PC)). The density of the supercritical fluids determines their properties when used as an extraction solvent. CO₂ is, till date, a high percentage of medicinal and aromatic plants have been scrutinized for possible extraction by supercritical CO₂, the only solvent used so far. Some of the plants extracted using SFE include *Taxus brevifolia*, *Taxus cuspidate*, *Hypericum perforatum*, *Echinacea purpurea*, *Serenoa repens* etc. Organic solvent-free products can be obtained and the low operating temperature makes it possible to preserve all their natural properties. The feasibility study on specific products can be performed rather easily at laboratory scale. However, accurate evaluation of production costs, including both capital and operating ones, must be done in order to exploit SFE at the industrial level (Chandrakant et al. 2011).

11.3.6 Microwave Assisted Extraction

Microwaves are part of electromagnetic spectrum of light with a range of 300 MHz to 300 GHz and wavelengths of these waves range from 1 cm to 1 m (Mandal et al. 2007; Akhtar et al. 2019). Microwave Assisted Extraction (MAE) method is a method of low energy-high efficiency. Extraction in use is feasible, as it has the positive aspects of processes with reduced energy consumption, decreased quantity of raw material with increased yield of final biologically active compounds (Fierascu et al. 2020). Practically, the hydrogen bonding destruction is achieved by microwaves which induce dipole rotation in organic molecules along with heating. The increased kinetic energies of the ions and their friction results in a heating effect (Akhtar et al. 2019). Destruction of hydrogen bonding also increases the penetrating efficiency of the solvents into the plant matrix (Hudaib et al. 2003; Datta et al. 2005; Akhtar et al. 2019). Some of the herbs extracted through this method are *Artemisia annua*, *Cortex fraxini*, *Curcuma longa*, *Azadirachta indica* etc. (Akhtar et al. 2019).

These techniques can be used to extract active metabolites on an industrial scale. They have the advantage of offering “green” characteristics (shorter extraction time, no use of toxic chemicals, higher extraction yields with low solvent and energy consumption). By this technique, small amounts of essential oil components – that often form a hydrosol with condensate water – can be separated and collected (Fierascu et al. 2020).

11.4 Conclusions

Efficient usage of medicinal and aromatic plants and their metabolites assisted by the above-mentioned biotechnological tools, singly or in combination, are expected to yield positive results, in the near future. Both in small- and large-scale applications, they are economical, environment friendly and efficient. At the same time, they are capable of maintaining the integrity and purity of the bioactive compounds obtained. *In vitro* micropropagation tools, usually in combination with *Agrobacterium* transformation, can be regarded as most promising methods adopted for genetic transformation, also in the case of important medicinal species. To date, micropropagation and the *in vitro* production of secondary metabolites appear to be the most efficient and practical technology used commercially. DNA microarray has proved itself as a potential tool in drug discovery and development. This chapter also highlights some biotechnological methods used in India, that are still in progress of development/improvement. Apparently, they are likely to assume an important role in the industrial scale utilization, processing, conservation, etc. of medicinal and aromatic plants, in the foreseeable future.

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Chapter 12

Medicinal and Aromatic Plants in the Cosmetics Industry



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Abstract Plants have an immense contribution to improving the lifestyle of human beings. They have been used for healing and curing purposes. Indian traditional medicine system, Ayurveda, documents over hundreds of plants for their medicinal properties. Advances in science and technology has helped to identify over thousands of these medicinal and aromatic plants (MAPs) that traditionally were also used as cosmetics, for preparing creams and formulations for skin and/or hair applications. Sophisticated chromatographic tools have led to the identification of unique bioactive compounds from individual plants. These bioactives offer a great potential for the pharmaceutical and cosmetic industry. This book chapter deals with the history of MAPs with a focus on their cosmetic applications, briefly highlighting important Indian plants with their past and present potential for the cosmetic industry.

Keywords Ayurveda · Cosmetics · CAM · MAPs · Pharmaceuticals · Siddha · Unani

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Abbreviations

BSE	Bovine spongiform encephalopathy
CAM	Complementary and alternative medicine
GAGs	Glycoaminoglycans
MAAs	Mycosporine-like amino acids
MAPs	Medicinal and aromatic plants
MMP	Matrix metalloproteinases
ROS	Reactive oxygen species
SAS	Supercritical anti-solvent
TEWL	Transepidermal water loss
TTO	Tea Tree oil

12.1 Introduction

In the past 70 years or so, humans have relied almost entirely on plants to treat all manners of illnesses, from minor problems such as cough and cold to life threatening diseases such as tuberculosis and malaria. The knowledge of medicinal and aromatic plants (MAPs) from ancient history until today has passed from generation to generation and has helped in improving health and life (Ali 2020). The oldest (5000–3000 BCE) written medicinal records suggested that humans understood diseases and the use of medicinal plants which could help in maintaining and restoring good health (Inoue et al. 2019).

Plants were the main source and foundation of cosmetics before methods of synthesizing substances with the same properties were discovered. Cosmetology is one of the major emerging branches which has globally gained the attention of many researchers, industries and general society. Nowadays, cosmetics have become an unavoidable part of life. The importance of herbal cosmetology is currently being highlighted because synthetic cosmetics may cause adverse reactions like skin irritation, allergies, photo-irritation, psoriasis inflammation etc. (Srikanth et al. 2010). The various uses of herbal products (like oils, powders and extracts) in cosmetics are illustrated in Fig. 12.1. These extracts are added to cosmetic preparations due to their properties, like antioxidant and anti-inflammatory characteristics. In addition, plant parts and plant extracts (e.g.: aloe-vera and coconut oil) are used instead of traditional synthetic products, as they consist of natural nutrients like fat soluble vitamin E that keeps skin healthy, glowing and beautiful. Secondary metabolites and marine biopolymers from marine macro and microorganisms have also been found to be helpful ingredients for making cosmetics as they have anti-inflammatory, antioxidant, anti-pigmentation properties and can also restore trans epidermal water loss (Alparslan et al. 2018). The positive attitude towards these products is reported by consumers, as they believe to prefer “natural rather than synthetic”.

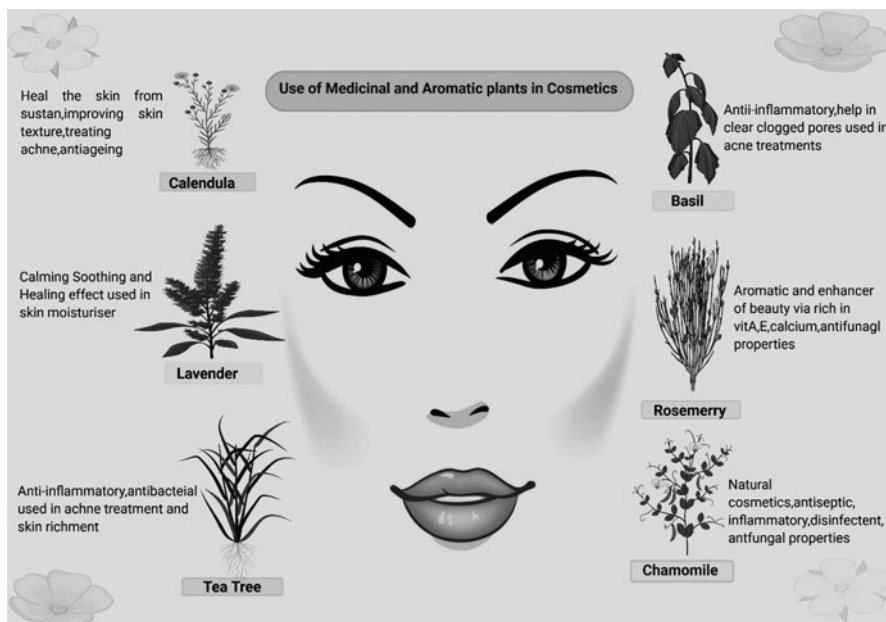


Fig. 12.1 Use of medicinal and aromatic plants in cosmetics

12.2 History of Cosmetic Uses of Medicinal and Aromatic Plants (MAPs)

12.2.1 Archive of MAPs: A Historical Background

Earliest evidences show that medicinal plants represent one of the oldest and most widespread forms of medication. Most of the medicines were derived from natural sources like plants and animals until last century. There is adequate archaeological prehistoric evidence which indicates that medicinal plants were regularly used or ingested by people for biomedically curative or psychotherapeutic purposes. The primates recognised and utilized the healing properties of plants by continuously observing how animals consume particular plant species for healing purposes such as analgesics, anti-microbial, anti-inflammatory, digestive aids and anti-diarrhoea etc. (Halberstein 2005). According to Huffman (1997) report the monkeys, gorillas, chimpanzees and humans selected the same medicinal plants for cure of similar illness.

In different cultures, like Indian, Chinese, European etc. the beliefs about medicinal plants were different. While in the fourth century the Greek philosopher Aristotle thought that plants were having a so called “psyche” form. During 1500 BC, as it is written in Hindu religious scriptures, people believed that many plants are sacred to specific divinities like Bael tree (*Aegle marmelos*) is known as

shelter Shiva. As mentioned in the Encyclopedia of Herbal medicines, around 3000 BC as civilization began to grow in Egypt, Middle east, Indian and Chinese herbs were started to be used more sophisticatedly. In developed cultures (500 BC) medicine began to be separated from the magical and spiritual world. The father of medicine, Hippocrates (Greek) said that “illness is a natural rather than a supernatural phenomenon” (Chevallier 2016). MAPs play a notable role in human society and help enhance their lives since ancient times. People have acknowledged their values and thereby are using the available historical data as a guide for the utilization of plant materials (Inoue et al. 2017). Around 2600 BC people had focused on plants for traditional medicines. The utilization of MAPs evolved in many cultures of countries like India, Greece, China and Egypt.

12.2.2 Archive of Indian Culture

Around 5000 years ago an Indian ancient culture developed along the bank of Indus river in Northern India. It is said that the old knowledge and wisdom was transmitted orally from “guru” (master) to “shishya”(followers) for several generations. This knowledge was written down in Sanskrit poetry called “Vedas”, in 1500 BC. Historical medicinal records recorded in ancient Indian literatures such as Rigveda (1700–1100 BC); in which therapeutic uses of 67 plants were mentioned, Yajurveda (1400–1000 BC); in this uses of 81 medicinal plants were enlisted and 290 plants of medicinal utilities were enlisted in Atharva Veda (1200 BC) (Adhikari and Paul 2018).

Punarvasu Atreya, the founder of the first Ayurvedic medical school in 400 BC, and his followers recorded medicinal knowledge. The scholar wrote “Charaka Samhita” within which uses of 341 plants were mentioned (Chevallier 2016). There are also some other manuscripts found such as Sushruta Samhita and Dhanwantari Samhita, in which uses of many plants and their polyherbal formulations are mentioned. India has a recognised system of medicinal practice which includes Ayurveda, Siddha, Unani, Yoga etc.

Ayurveda, “ayur” and “veda” these combinations of two Sanskrit words means the ‘science of life’. It is an oldest healing science originated by using herbs and considered by many scholars which is also well known as Mother of all healing. Approximately 90% of Ayurvedic preparations supported MAPs because it said that it may have stronger action on their body. Polyherbal combination, where around 3 to 30 plants utilized in which only one or two are active and others play supporting roles. This combination has proven that it possesses a long-lasting effect than a single herb.

12.3 Cosmetics Industry: Past and Present

The word cosmetics is procured from “*kosmetos*”, a Greek term meaning “ornament” or “adornment”. It was coined by Raymond Reed, founding member of the US Society of Cosmetic Chemists, in 1961 (Sharma et al. 2002). It is studied as a part of aesthetics, culture, social psychology and sociology. Each era has put up its standard of beauty as what is and what is not accepted. Beauty depends upon cultural as well as religious traditions and has evolved over the time. What may appeal to one community may leave another group of people cold or repulsed. Today cosmetics have become an integral part of our daily lives but, this habit traces back to the ancient Egyptians.

12.3.1 History of Usage of MAPs in Cosmetics

The Neanderthal man (100,000 B.C.) beautified their looks by painting patterns on the body using colourants manufactured from mud, dried plants and so on which fulfilled other purposes as well (Fenja 1973). This facilitated camouflaging into the surroundings while hunting animals and also because they believed that by mimicking the markings of an aggressive or powerful animal, one can acquire some of that animal’s power or characteristics. They considered sun a powerful force that brought daylight and secured them from all dangers of night and hence, colours similar to the solar spectrum were commonly used in paintings done on face and body. Till date, in Aborigine’s culture, red and yellow are used in daily rituals and ceremonies. It holds great significance and is a representation of peace.

The science of cosmetology is considered to be an ancient science. The very first use of cosmetics dates back to approximately 2500 and 1550 B.C during the Indus valley civilization (Lal 2002). Evidence found suggests that quite futuristic ideas of beautification and a wide range of products were in use by both females as well as males. All of these were carefully interwoven with the different “Rutus” (seasons) and with their “Dincharya” (daily life routine). This practice of using cosmetics was observed as a way of earning the “Punya” (virtues of life), “Anandam” (happiness) and “Aayush and Aarogyam” (longevity and good health), and not only as a process of maintaining good appearances. The very first reference of a beautician is in the greatest epic of “Mahabharata” when during the periods after Pandavas were out-casted from Hastinapur and Draupadi worked as a “Sairandhri” (a female attendant in the palace for the women’s section), for the queen of Virata (a northern district of India). There is a mention of a “Prasadhana petika” which is a case that contains all the items used for adornment like toiletries, accessories, cosmetics etc. (Lad 1978).

A variety of “Lepas” or masks were in use according to the different seasons for better enhancement. An ancient book of Ayurveda (around 1500 years) “Astanga Hridaya”, describes a wide range of formulas to be used for the six seasons throughout the year. Clarified butter (Ghritas) and oils (Tailams) were formulated for

application on face. There were depilatory products to remove excess hair as it was thought as disgrace. A whole array of hair care products for maladies like premature greying and hair fall also fumigants, dyes and hair rinses with fragrances were in use. There are also mentions about deodorants and bath powders full of aromas. Teeth care, colouring the lips and using mouth deodorants was a part of oral hygiene and a daily routine which was observed as a task to be religiously followed. All of these advanced ideas of cosmetics were brought into force with the help of natural resources available in plenty in the surroundings by the ancient Indians (Patkar 2008).

Raja Serfoji's reign lasted from 1788 A.D till 1832 A.D as the ruler of Thanjavur (Tanjore) which is located in Tamil Nadu, a state of South India. He is the founder of the "Serfoji's Saraswati Mahal" which is in Tanjore and is one of the greatest libraries. Research and medicinal formulations were his areas of interest and he tested many of them by actually administering them to patients and had British doctors to record their medical history. "Dhanvantri Mahal", an Institute of Medical Research, was built where research was carried out and then a few efficient preparations were selected by the king. The Tamil pandits prepared versus of these recipes and wrote on papers or palm leaves. These were then translated into "Bakhar Marathi" (colloquial Marathi or old spoken during eighteenth to nineteenth century A.D.) for the benefit of people who spoke Marathi. These formulations are referred to as "Anubhoga Vaidya Bhaga" which translates as preparations that are evaluated by observations. There was also an impressive herbarium in the palace which provided botanical material to the Dhanvantri Mahal. The king used artists to paint these plants in water colour in records for instant references (Rao 1952).

Cracked lips can quite dimmish a pretty face. The rind of *Aegle marmelos* Corr. (Bel fruit), is ground to powder and mixed with the milk of a woman. This paste is then used for curing cracked lips and within 10 days the cracks start healing (Rao 1952). A lighter skin tone has always been a desire for all girls. A mixture of roots of *Saussurea lappa* Clarke. (Kushtha or Costus) (Mukerji 1953), leaves of *Albizia lebbek* Benth. (Sirisa), seeds of *Sesamum indicum* Linn. (Til), wood of *Cedrus deodara* Roxb. (Devdar), leaves of *Pongamia pinnata* Pierr. (Chopda) and the wood of *Berberis tellate* DC. (Zadali Haled) is roasted together between dried buffalo dung cakes and ground to powder. This powder is made into paste and applied to the body as a scrub for almost 3 days for obtaining glowing skin (Rao 1952). Dandruff was cured by applying a mask made from crushing seeds of *Papaver somniferum* Linn. (Khas-khas) in milk (Rao 1952). "Kayakalpa" is an ancient well-known process of rejuvenation which means to make one look young, bring a change in skin texture, hair colour, improve vision and so on. Five ingredients namely, leaves of *Azadirachta indica* Russ. (Kadunimba), *Sphaeranthus indicus* Linn. (Mundi or Gorakhmundi) (Rao 1967), *Eclipta alba* Hassk. (Maka), *Vitex negundo* Linn. (Nirgundi, Nagoda or Nirgunda) (Watson 1866) and *Carum copticum* Benth. (Vova) are taken in equal amount and dried. It is then ground into fine powder and this is consumed two times a day, along with a diet of milk and rice. This will give a lustre to the skin and stop greying of hair (Rao 1952).

The process of depilation was practiced as the hair on face, arms, pubic area and legs was considered a taboo. A formulation made out of dried fruits of *Piper longum*

Linn. (Pimpali) and *Emblica officinalis* Gaertn. (Aavalakatti) by soaking them in the milk-coloured latex of *Euphoria nivulia* Ham. (Cactus or Nivadunga). This is applied to the necessary area and the hair from that area detach (Rao 1952). There is also mention of breast developer methods. A powdered mixture of root of *Withania somnifera* Dunal. (Ashwagandha), root of *Saussurea lappa* Clarke. (Kosta), fruit of *Scindapsus officinalis* Schott. (Gajapimpali) and rhizomes of *Acorus calamus* Linn. (Vekhanda) is mixed with butter (made out of buffalo milk) and this paste is massaged on the bust area. This treatment makes breast firm, gives them proper shape and enhances the bustline (Rao 1952). A face pack made out of *Lens culinaris* (Masura – a common lentil from India) by crushing it in honey (Madhu) into a paste. This paste when used on face at night for about a week, makes skin bright and gives a nice glow (Mishra 1897). A remedy for eliminating pimples is made by making a paste of *Coriandrum sativum* Linn. (Kustumburu, Coriander, dhana or dhaniya) (Nadkarni 1910), *Symplocos tellate* Roxb. (Lodhra or Lodhar) (Nadkarni 1910), *Acorus calamus* Linn. (Vekhanda) and *Saussurea lappa* Clarke. (Kushtha or kosta) is considered effective (Bhishagaratna 1962, 1963).

Mouth fresheners were made by making “Supari” (*Areca catechu* Linn. or betel nut) which was made by mixing together *Saussurea lappa* Clarke. (kushtha), *Myristica fragrans* Houtt. (Jatiphala), *Valeriana wallichii* DC. (Tagara), *Syzygium aromaticum* Merrill and Perry. (Lavanga), *Cinnamomum camphora* Nees and Eberm. (Karpooora) and *Ellettaria cardamomum* Maton. (Ela) (Sharma 1921). A piece of cloth is dipped in the juice prepared from the leaves of *Piper betel* Linn. (Phanivalli or paan leaves) which have been added with mercury (paratda), and are then tied to the head. This treatment eliminates nits and lice and clears the scalp (Sambashiva 1936). Oil prepared by cooking together Maka (*Eclipta alba* Hassk.) or Bhringaraja juice with iron (Lohakitta) with Triphala [*Terminalia chebula* Retz. – Harada (Watt 1908), *Terminalia bellerica* Retz. – Beheda (Mukerji 1953) and *Phyllanthus emblica* Gaertn. – Avala (Khory 1887)] or Phalatrikam is applied to the scalp. This treatment relieves dandruff, alopecia, itching and also enhances the hair colour (Gaud 1967). Deodorant powders are made from barks of *Punica granatum* Linn. (Pomegranate or dalimba) (Khory 1887) and *Mangifera indica* Linn. (Mango, aam or amba) which is mixed with fragrant shell (Shankha) powder and used on the body to remove bad odour. Also powder made from seeds of *Pongamia glabra* Vent. (Karanja) and *Tamarindus indica* Linn. (Chincha) is used for eliminating bad odour (Bhagirathaswami 1930; Upadhyaya 1965).

The Kautilya Artha Shastra, a text that mainly deals with Economics and Political science, also mentioned various Ayurvedic herbs. It has described many fragrant drugs like Agarar (*Aquilaria agallocha* Roxb.), Chandana (sandalwood) which were applied externally (Anulepan) for cosmetic purposes. Agarar (*Aquilaria agallocha* Roxb.) It is soft, heavy, greasy and has a uniform smell and is so adhesive to skin as it is not easily removed by rubbing. For the enrichment and protection of beauty of the body parts Vatsayana described Subhagamkarana yogas. Ointment prepared from kushtha (*Saussurea lappa* Clarke.), Tagara (*Valeriana wallichii* DC.) and Talisa patrakaar (*Abies webbiana* Lindl.) is used externally while powder of Utpala (*Nymphaea tellate* Willd.), Padma (*Nelumbo nucifera* Gaertn.), Nagakesara (*Mesua*

ferrea Linn.) along with ghee or honey used internally as Subhagamkarana. *Hibiscus rosa-sinensis* Linn., Japa flowers are ground using cow's (especially black) urine and applied externally to the scalp to get rid of Indralupta (alopecia). To get relief from Dharunaka (Dandruff) the paste of Aragvadhā pallava (leaves of *Cassia fistula* Linn), Dharati phala (fruit of *Emblīca officinalis*), prapunnata (seeds of *Cassia tora* Linn.) were applied externally. Badara's (*Zizyphus jujube* Lal.) seed powder was used to make a paste along with butter, honey and jaggery and when applied externally helps in Vyanga (hyperpigmentation) (Penchala et al. 2011).

12.3.2 Present Status of MAP Usage in Cosmetics

Ethnobotanical knowledge along with Ayurvedic references have been used as sources for overall healthcare. Nevertheless, with advances in technology, researchers have led to newer and improved formulations. Formulators are striving to prepare highly differentiated multifunctional products that also have the essence of aesthetics having active ingredients. The ubiquitous curiosity in medical treatment and beauty routine has now led to the requisition for certain fruitful botanical extracts (Alcalde 2008). Build-up of molecular damage due to ROS (Reactive Oxygen Species) like ions, peroxides and free radicals is termed ageing. The ageing of the skin has great social importance although the internal organs do not visibly age and hence, distinct from general ageing of the organism. Ageing is accelerated by various environmental factors like UV radiations, electromagnetic fields, chemicals and climatology which produces ROS damaging DNA telomeres, cell membranes and enzymes (González et al. 2008). All these factors are identified by the chemical and pharmaceutical industries and a new concept of "Cosmeceuticals" has come into being which is an intersection of both and involves cosmetics with therapeutic action (Pieroni et al. 2004). Over 60 different botanicals have been formulated into cosmeceuticals. But, as botanicals are more focused on the treatment of signs and symptoms of diseases, rather than improving total "body condition", dermatologists must have working knowledge of botanicals to provide optimal care.

The actions of ROS mechanisms at molecular levels on layers of skin also the chemical structures of a series of antioxidants obtained from plants. Have been studied (Jadoon et al. 2015). Green tea (*Camellia sinensis* L., Kuntze) leaves are rich sources of vitamin C although, lipid peroxidation is inhibited by polyphenols from grape seeds (*Vitis vinifera* L.) (Angerhofer et al. 2008). Seeds of green coffee (*Coffea arabica* L.) comprise certain compounds that encourage collagen and elastin production (Velázquez-Pereda et al. 2009). Cucumber (*Cucumis sativus* L.) has antioxidants which help in skin hyaluronidase and elastase inhibition (Nema et al. 2011). Rhizomes like ginger and turmeric have inhibitory effect on cutaneous tyrosinase and are hence used in anti-ageing formulations (Lee et al. 1997a). A central American tropical fern- *Polypodium leucotomos* Hook., is enriched with caffeic, ferulic and chlorogenic acid and its extracts are used in sunscreen (Nestor et al. 2014).

Previously chemicals like mercury, arsenic and lead were used for the process of bleaching and making the skin more definite till later, cases of toxicity came up leading to their ban. Hence, now hypoallergenic or “allergy tested” products are recommended by people especially with sensitive skin (Alcalde 2008). As the demand for cosmetics is now increasing globally, there is production of new raw materials and in concert new cosmetics. Also, there is research conducted on the positive effects as well as negative consequences of the new raw materials and products. Hence, laws have been passed to limit or ban the use of substances that can cause trouble but, this ends in a ceaseless cycle as new and new materials transpire daily. It was perceived during epizootic crisis like the “mad cow” bovine spongiform encephalopathy (BSE) or “bird flu” (H5N1 virus), that products from animal origin are particularly harmful (Soulioti et al. 2013).

As a result, the formulators started giving more emphasis on plant based natural, ecological, bio or green cosmetics (Alcalde 2008). The term “phytosome” was introduced in 2007, to describe a nanocomposite which is obtained from a phospholipid layer surrounding a phytoconstituent that the skin easily absorbs (Amit et al. 2007). Botox is a non-surgical cosmetic procedure wherein; tiny amounts of Botulinum A are injected directly into the muscle that lies below the wrinkle that the consumer wishes to erase. This results in the temporary relaxing of the muscle giving a smoother looking skin and lasts up to 3–4 months. However, 12 continuous months of treatment can last longer due to muscle atrophy and loss of the “habit of facial expression” (Hunt et al. 2011). The fungi *Fomes officinalis* (Will.) Bress., has been tested because of its botox effect (Santana et al. 2011). The pluripotent plant meristem cells of plant meristems from the common apple tree (*Malus pumila* Mill.) can be used to produce different types of plant tissues as well as seedlings, by nurturing in bioreactors that in result give secondary metabolites (Morüs et al. 2014). However, in some cases, the excess use of plant resources can lead to the deterioration of a species. Like the argan oil (*Argania spinosa* L., Skeels) case, which is an endemic species of Morocco, has very scant capacity to regenerate but, the market for its oil has led to a sudden decrease in their population (Faouzi 2015).

Marine ecosystems have also come into the picture to be a generator of unceasing exploitable resources complementary to the utility of terrestrial plants. They are thought to be less risky as they are phylogenetically far away from humans and other animals. Also, they are home to the most ancient life forms in the history of evolution (Nesse and Stearns 2008). Even though their environment is hostile making it difficult for carrying out research, it has been predicted that novel, healthy and promising particles will be discovered. Usually, macroalgae like *Chondrus crispus* (Stackh) and *Laminaria saccharina* (J.V. Lamour) have been used as a source of phycocolloids in thalassotherapy sessions as they are rich sources of minerals and amino acids (Bedoux et al. 2014).

12.4 Application of MAPs in Cosmetic Industry

Medicinal and aromatic plants have tremendous potential in the cosmetic industry. Several hundreds of compounds have been isolated and characterised from these MAPs. In recent times their application as moisturizers, in prevention of skin ageing, sunscreen lotions, skin whitening agents, hair care products, aromatherapy, treatments for dry skin, acne etc. has been greatly explored.

12.4.1 *Moisturizers*

It is necessary to maintain the hydration of the skin. Topical application of lipids is usually done to avoid dehydration. It is well known that omega-6 polyunsaturated fatty acids, especially C-18 fatty acids like linoleic acid and gamma-linolenic acid are capable to restore the trans-epidermal water loss (TEWL) to normal (Ziboh and Chapkin 1987). Thus, formulations comprising oil-in-water containing ingredients that can retain water in the skin see used for moisturizers.

Brown marine algae of genus *Laminaria* are widely used in cosmetics, especially moisturizers. *Laminaria ochroleuca* is claimed to moisturize skin while boosting the skin's barrier layer. Besides macroalgae, microalgae of genus *Nannochloropsis* are also found to have moisturizing properties as they have linolenic acid (Mourelle et al. 2017).

12.4.2 *Prevention of Skin Ageing*

Ageing of skin is caused due to degradation of extracellular matrix in epidermis and dermis layers of skin. It is caused by intrinsic factors like genetics, ethnicity, sex and extrinsic factors like exposure to UV radiations, harsh weather, pollution, smoking, stress and poor sleeping and also eating habits and exercise.

Carotenoids are considered major active ingredients with anti-ageing properties. Within its class of compounds, beta-carotene is the top pigment having an excellent capacity to prevent formation of Reactive oxygen species (ROS). The most important marine source of beta-carotene is halotolerant microalga *Dunaliella salina*. It produces more than 10% of beta-carotene than its dried weight and also synthesized 9-cis-beta-carotene which has high antioxidant activity, heals damages skin cells and also fights infections (Raja et al. 2007). Because of the environment in which it grows, it also has minerals like magnesium, potassium, calcium in addition to glyc-erine and iodine.

Fucoidan is a water soluble, sulphated polysaccharide found in the cell walls of brown algae. It is known to have anticoagulant, antiviral, anti-inflammatory and anticancer activities. Fucoidan extract from the phaeophyta *Undaria pinnatifida*

shows inhibitory action against bacterial collagenase and human neutrophil elastase. Extracts from *Fucus vesiculosus* and *Himanthalia elongata* also show significant inhibition of elastase.

Hyaluronic acid, also called hyaluronan, is an anionic, non-sulphated glycosaminoglycan distributed widely throughout epithelial, connective and neural tissues and is a major component of extracellular matrix of skin. Extract of giant brown seaweed *Macrocystis pyrifera*, found in Antarctica, stimulates the production of hyaluronic acid.

Jania rubens is recognised for its ultra-moisturizing and protective properties due to a high concentration of minerals and trace elements present in it. A combination of extracts from *Jania rubens* and *Meristotheca dakarensis* is found to stimulate keratin, glycoaminoglycans (GAGs) and collagen I and III synthesis.

The enzymes matrix metalloproteinases (MMPs) are involved in the skin ageing process. *Ecklonia stolonifera* extract has polyphenols like eckol and dieckol that inhibit the expression of MMP-1 in human dermal fibroblasts (Joe et al. 2006; Alparslan et al. 2018).

Turmeric (*Curcuma longa* L.) is a rhizomatous, herbaceous, perennial plant, belonging to the ginger family. The rhizomes have a distinct orange colour due to the presence of curcuminoid pigments like curcumin, demethoxycurcumin, bisdemethoxycurcumin and cyclocurcumin. Curcumin has low skin penetration but shows retention and hence is used in anti-ageing formulations. It has also shown antiparasitic, antispasmodic, anti-inflammatory and potential anticancer activity (Lin and Lin 2008; Goncalves et al. 2014). It also helps prevent premature ageing by inhibition of phosphorylase kinase activity (Heng 1999). Almost used for more than 2000 years, ginseng is known as a supreme traditional medicine. Although, *Panax ginseng* C.A. Meyer (Korean ginseng) is visibly and synthetically distinct from others and is discovered to decrease keratinization (Kim et al. 1989), provoke metabolism of the skin (Tanaka and Okada 1991), supply wetness and softness (Gezzi et al. 1986; Curri et al. 1986), brightens up and diminishes wrinkles. The 'anti-ageing' effect is the result of higher metabolism rate due to the blood circulation and cell build-up (Lee et al. 1997b). Literature also points out that the 'anti-ageing' activity might be because of the saponins that show free radical scavenging effect (ginsenosides) also lipoperoxidation restriction (Liu and Xiao 1992; Pan et al. 1993; Choi and Byun 1986; Choi and Oh 1985, 1984).

Camellia sinensis or tea leaves are harvested and steamed immediately followed by drying to yield green tea. They are a full package of various chemical compounds nearly more than 500 that includes tannins, flavonoids, caffeine, polysaccharides, vitamins, amino acids and so on. They are rich sources of vitamin C but, green tea has higher concentrations than black tea as major amount of vitamin C (90%) is demolished in the process of fermentation. It has nicotinic acid (vitamin B6), vitamin K and vitamin E (Lee et al. 1997b; Pietta et al. 1998). The polyphenol flavonoids are verified to be antioxidant, antibacterial, anti-inflammatory, antiallergic and antiviral whereas, tannins retain antioxidant and antiseptic properties (Schreiner et al. 1999; Katiyar and Elmets 2001). Catechins, flavonols, flavandiols and phenolic acids are prime polyphenols in green tea. Green tea has now gained popularity

due to its power to cure UV-photo damaged skin and also photo-toxicity (Elmets et al. 2001; Zhao et al. 1999a; Katiyar and Elmets 2001; Lee et al. 1997b) but mainly because of its antioxidant activity (Katiyar and Elmets 2001; Pietta et al. 1998; Miyazawa 2000; Fourneau et al. 1996; Mitscher et al. 1997). It enriches the lipid layer of skin by upregulating ceramides and sphingolipids formation and thus prevents/cures dry skin (Schreiner et al. 1999).

Vitis vinifera L., commonly known as grapes (have a wide variety), contain seeds rich in polyphenolic pro-anthocyanidins that form oligomers by binding to each other called procyanidins. Procyanidins are substantial antioxidants (Maffei 1994; Pietta et al. 1998), and resist lipid peroxidation to ease healing of wounds. They also guard deterioration of collagen and elastin. They are used in anti-ageing and skin lightening formulations as they showcase tyrosinase inhibiting activity (Maffei 1994).

12.4.3 Sunscreen Applications

Long-time exposure to sunlight can lead to skin damage by causing burns. The depletion of the ozone layer has increased our risk of sun damage from UV rays. The UV-A (400-320 nm) is linked to skin ageing and may lead to skin cancer while, UV-B (320-290 nm) can cause sunburn and skin cancer. Photosynthetic organisms rely on sunlight for energy, also need to protect themselves from the harmful UV radiations. Some plants have physical barriers like waxy cuticles, but marine organisms like algae have chemical compounds for photoprotection. Mycosporine-like amino acids (MAAs) are an interesting family of compounds having cyclohexanimine or cyclohexanone ring as core and various functional groups attached to the ring. They have low molecular weight, are water soluble and are stable when exposed to light and heat.

Palythine extracted from red algae *Chondrus yendoi* was found to protect lab cultured human cells from UV-A and UV-B damage under intense artificial sunlight (Lawrence et al. 2018; Alparslan et al. 2018). The leaves of *Camellia sinensis* undergo fermentation to give black tea which is as compared to green tea, black tea has lesser polyphenols and also shows lower antioxidant activity (Yokozawa et al. 1998; Halder and Bhaduri 1998). It reduces photochemical damage to skin when consumed orally and applied topically. Black tea obtained by oxidising green tea helps combating signs of UVB- induced phototoxic effects like skin thickness and sunburn (Zhao et al. 1999b).

Red clover (*Trifolium pratense* L.) has been used for psoriasis, acne, rashes and eczema in traditional medicine over the years. Its flowers have isoflavones that protect the skin from UV radiations, reduces caused by conditions like oedema and contact hypersensitivity. Equol is an isoflavone that is used in lotions that can be applied topically after sun exposure. It voluntarily keeps the immune system safe from the photo-suppression caused by sunburns (Widyarini et al. 2001).

12.4.4 Skin Whitening Properties

Pigmentation of skin is caused due to melanin, a pigment synthesized from amino acid L-tyrosine and converted to dopaquinone by enzyme tyrosinase. Melanin is produced and stored in the melanosomal compartment of the melanosome then transported to overlying keratinocytes. The most obvious target of depigmentation compounds is enzyme tyrosinase. Hydroquinone is generally used as an essential whitening agent in cosmetics but is now banned due to its ability to cause mutagenicity (Bhattar et al. 2015).

Arbutin is a glycosylated form of hydroquinone that is present in bearberry of genus *Arctostaphylos*. It prevents tyrosine activity and thus melanin is not formed. It is used in skin lightening treatments designed for long term and regular use.

The extract from green microalgae *Chlorella vulgaris* reduces pigmentation by more than 10%. It helps to reduce signs of dark circles from around the eyes. It is rich in amino acids, oligopeptides and more than 20 vitamins and minerals.

Ecklonia cava is a red alga which is used as food and medicine for cancer, arthritis, stress, obesity, heart disease, high cholesterol, diabetes etc. When chromatographic purification of its ethanolic extract and ethyl acetate soluble fraction is done we can isolate phloroglucinal, dioxinodehydroeckol and 7-phloroeckol of which 7-phloroeckol exhibits more tyrosinase inhibition activity than arbutin towards mushroom tyrosinase (Yoon et al. 2009). Zeaxanthin is a carotenoid generated from the supercritical anti-solvent (SAS) process of extracting microalga *Nannochloropsis oculata*, which showed antityrosinase activity in agar plate method against mushroom tyrosinase (Shen et al. 2011; Alparslan et al. 2018).

The roots of Manjistha (*Rubia cordifolia*) are traditionally used internally and externally to treat discoloration and gain lustre and glow of skin (Nadkarni 1998). It is considered as a magnificent aid in promoting complexion and treating pigmentation anomalies. Dried and crushed powders of orange peels, sandal, turmeric and Manjistha mixed together can be used as an effective face pack.

12.4.5 Acne, Spots and Pimple Treatment

Acne is one of the most common skin conditions observed mostly among teenagers. It is caused due to clogging of hair follicles with oil and dead skin cells and causes whiteheads, blackheads, pimples especially on the face, forehead, shoulders, back, neck, chest and upper arms. The blemishes sometimes also leave scars, marks or pitting which can be agonizing. Ayurveda describes this silk cotton tree thorn-like outburst on the face due to imbalance of *kapha*, *vata* and *rakta*.

Manjistha (*Rubia cordifolia* L.) is well-known as an excellent blood purifier and is used for blood, skin and urinary diseases (Shrotri et al. 2005; Gupta et al. 2005). It's stems and roots are rich sources of Anthraquinones which show anti-acne properties in gel (Meena and Chaudhary 2015). The root has a distinctive anti-acne

effect through anti-bacterial, anti-inflammatory, antioxidant and anti-androgen properties as these agents are chief pharmacotherapeutic agents to break the pathogenesis of acne. The root powder mixed with ghee, honey or rose water is also used for acne treatment (Joshani et al. 2010; Sharma et al. 2002). The pulp of the whole plant along with honey is rubbed for treating acne and dark spots by Vanraj tribes of Kumaun Himalaya (Bhatt et al. 2013).

Ocimum sanctum L. is determined the king of herbs as it is a very substantial medicinal plant. The oil obtained is fixed oil and has the potential to block pathways like cyclooxygenase and lipoxygenase of arachidonate metabolism which are known to be responsible for the anti-inflammatory activity as it contains linolenic acid (Singh and Majumdar 1997, 1999). As antibacterial activity is seen at quite low dilutions in most of the basil species (Sivropoulou et al. 1996; Lachowicz et al. 1998), *O. gratissimum* oil is used for acne (Orafidiya et al. 2002) and another species *O. basilicum* is known to have antimicrobial and antiseptic properties (Lachowicz et al. 1998).

Cucurbita pepo L. seeds yield oil rich in fatty acids like linoleic, oleic and stearic acid, that possess anti-inflammatory properties (Akhtar et al. 1980; Nesterova et al. 1990). This oil is used on acne vulgaris, herpes lesions, stubborn leg ulcers and venereal sores in India and Central America. The leaves are used on pulled ligament, sprains as poultice. The root inoculate is used for pimples, syphilitic sores, blackheads and herpes lesions (Morton 1981). *Allium cepa* (common red onion) has flavonoids like quercetin and kampferol that gives it anti-inflammatory and antiallergic properties (Griffiths et al. 2002; Dorsch et al. 1990; Dorsch 1996; Middleton 1984; Dorsch and Ring 1984). It also has antifungal (Conner and Beuchat 1984) as well as antimicrobial properties (Dorsch 1996; Arunachalam 1980). Conventionally, onion has been used externally to treat acne, blackheads, boils, abscesses as also for infections, to reduce inflammation and for speedy restoration. People in Africa use onion juice on burns, scalds and to avoid infection and blistering and in Eastern parts, the onion skin is used as bandage for body and facial sores (Dweck 1997b). The seeds of *Pisum sanctivum* L. (pea) carry carbohydrates, salts, proteins, fats, lecithin and are known to be antidermatosis. The crushed peas are used in face masks to treat wrinkles and acne (Dweck 1997b).

12.4.6 Dry Skin Treatment

Dry skin (Xerosis) is a condition caused due to loss of water and oil from skin causing cracking, roughness, scaling, itching or worse cause redness or deep cracks that bleed. It is caused by various factors like winter weather or hot, dry environments, severe deficiency of vitamin A, low moisture in air, soaking in hot or chlorinated water for long, inadequate hydration, harsh soaps and detergents, jobs that are rough on hands like mechanics or farming, age or other skin conditions like eczema or psoriasis.

Castor bean (*Ricinus communis*) contains 50% of fixed oil that is viscous, colourless with a slight odour. It is applied on skin as it acts as a barrier from harsh climate and comforts the skin. Ricinoleic acid along with its derivatives have potential to cure rough skin and acne as they possess polishing and moisturizing qualities (Miyahara and Sanbe 2002). Hydrogenated forms or esters of castor oil are used in toiletries, cosmetics, skin and hair care formulations also beneficial for skin conditioning and cleansing as they are useful carriers or emollients (Sato 2002). This oil also transforms into a pure, transparent, faint, odourless soap that dries and turns rigid thoroughly (Matsumura 2001). *Cocos nucifera* is also termed as “tree of life” or “Kalpa Vriksha” as it is one of the most useful trees in the world. The dry drupe yields an emollient oil that treats skin infections. It is modified and used as protectant to prevent drying of skin in pharmaceutical formulations and cosmetics as it contains polyunsaturated fatty acids as mono-, di- and triglycerides (Aburjai and Natsheh 2003). The seeds of *Helianthus annuus* accommodate polyunsaturated fats with high amounts of triglycerides of linoleic acid. As linoleic acid is one of the crucial fatty acids required to keep skin in good shape, sunflower oil boosts its level in the skin and also lowers trans-epidermal water loss. Hence, in patients with fatty acid deficiency it prevents scaly lesions and is also utilised to treat conditions like psoriasis and bruises (Dweck 1997a). A hybrid of sunflower oil that has high oleic acid content outstanding oxidative stability can serve as a natural raw material in cosmetics (Brown et al. 1993).

The fruit *Olea europaea* produces oil that is rich in fatty acids, triglycerides, pigments like chlorophylls and carotenoids, moisturizing agents like squalene, along with tocopherols, sterols with other volatile and flavour compounds. Since it has polyphenols, virgin olive oil has free radical scavenging effect and is used as a topical application to treat various skin damage conditions like eczema (severe hand and feet eczema), xerosis, contact dermatitis (in the diaper area), rosacea, psoriasis, atopic dermatitis and seborrhoea (Perricone 2001). As it has antioxidant properties, it is also operative on radiation burns like skin tumours induced by UVB (Budiyanto et al. 2000). Even though it is considered as a weak irritant, it can have detrimental cutaneous effects (Kranke et al. 1997). Cocoa butter is obtained from seeds of *Theobroma cacao* and consists majorly of oleic, palmitic and stearic acids also fats in the form of mono-unsaturates. It can be used for topical application on sunburn or windburn as it provides comfort (Aburjai and Natsheh 2003).

The rhizome of *Curcuma longa* L. is processed into fine powder that is commonly used as turmeric. Along with curcumin as one of the most active component, it has a whole lot of properties like anti-bacterial, antioxidant, anti-parasitic, anti-carcinogenic (Ozaki et al. 2000; Huang et al. 1988, 1991; Aruna and Sivaramakrishnan 1996), anti-HIV (Mazumber et al. 1995; Mesa et al. 2000), anti-inflammatory with toxic effects in a few cases (Rao et al. 1982) and also inhibition of lipid peroxidation thereby avoiding cell damage (Phan et al. 2001; Unnikrishnan and Rao 1995; Scartezzini and Speroni 2000; Pulla and Lokesh 1994). It is used for treating also preventing various skin conditions that include burns, acne, wounds, sun damage, eczema, psoriasis and also premature ageing. Samoans use turmeric on newly born infants to help them recover the cut of umbilical cord and also to avoid nappy rash

and keep skin pliable and soft. Its paste is also used for ulcers and considerable skin eruptions (Shah 1982; Uhe 1974).

Chamomile has flavonoids that give its most treasured anti-inflammatory activity. It also contains apigenin along with its glycosides which enhance healing of skin and abate itch as they are antipruritic, anti-erythema and anti-inflammatory. Along with them, chamazulene and levomenol terminate leukotriene production and enhance the anti-inflammatory and antioxidant properties (Wagner et al. 1986; Safahy et al. 1994). The plant extract is used through lotions, ointments and also essential oil is useful for inhalations and applications. It is used to treat eczema, skin inflammation and also in the mucous membranes (Hoermann and Korting 1994; Carle and Gomaa 1992). *Matricaria recutita* L. (German or true chamomile) and *Anthemis nobilis* L. (Roman chamomile) both are used as they possess almost similar compounds.

12.4.7 Hair Care Products

Alopecia (Hair loss) is a condition wherein there is sudden hair loss that causes bald patches which may further grow larger. There is itching and burning when the hair falls off. It is an autoimmune disease that attacks the hair follicles and is caused likely due to genetic inheritance or genetic disorders like down syndrome, and other conditions like asthma, pernicious anaemia, thyroid, vitiligo and other seasonal allergies.

In vitro studies of hair regrowth (Oyedeki et al. 2020) suggests that the flowers and leaves of *Datura metel* contain varying amounts of phytochemicals which are extracted using extracting solvents like n-hexane, methanol, ethanol etc. Both extracts show complementary effects in inducing hair regrowth and failed to cause any skin irritation. This extract contains hair restoration phytochemicals like saponins, sterols, glucosides, alkaloids, tannins, flavonoids, phenols, reducing sugar, and terpenoids. The flavonoids and triterpenoids strengthen the capillary wall of smaller blood vessels which supply to hair follicle to boost hair growth promoting activity and it also improves blood circulation to nourish hair follicle. The steroids function as a strengthening agent. For preserving hairs suppleness and shine, the mixture of triglycerides (sterols, fatty acids and ceramide) is produced in hair bulb, including waxes and squalene (metabolic precursor of sterols) which lubricate them by forming a film on the surface of the skin.

Amla (*Embllica officinalis*) or Indian gooseberry is known as the king of all medicinal plants. It is rich in quercetin, phyllemblic compounds, gallic acid, terpenoids, alkaloids, tannins, flavonoids, carbohydrates, pectin, vitamin C and various polyphenolic compounds. It is also rich in minerals like phosphorus, iron and calcium. It's most active ingredient with significant pharmacological motion is specified as "Phyllemblin" by Indian scientists. The dried fruit is boiled in coconut oil and this oil is used for prevention of premature greying of hair which is a basic sign of pitta dosha. Both coconut oil and amla penetrate into the roots and stimulate follicles. It is

rich in vitamin C which increases collagen that adds volume and length and replaces dead cells and regenerates new ones. Intake of amla through juice, powder and candy can avoid premature greying also it is a natural coolant. Powder can be used with henna as a hair pack. It's one fruit has 80% of moisture content hence, hydrates the scalp and avoids dandruff. It has anti-bacterial and anti-inflammatory properties which prevent itching and scaling. Amla hair oil serves as a good conditioner if used regularly as it absorbs extra oil from oily hair (Jain et al. 2016).

Grape seeds (*Vitis vinifera* L.) contain pro-anthocyanidins that accelerate the growth of hair follicles and transform hair-cycle from phase telogen to anagen (Takahashi et al. 1998). Maidenhair tree leaf decoction or *Ginko biloba* is used in hair tonic as it advocates regrowth and increase in hair follicles also reducing its death rate (Kobayashi et al. 1993; Kim et al. 1998).

Dandruff (pityriasis capitis) is the condition of the scalp that causes cracking of the scalp, flaking of skin and itching. It can also cause red, greasy patches of skin and conditions get severe in cold and dry seasons or when under stress. According to Ayurveda, it is caused due to the vitiation of *vata* and *kapha* dosha. Shikakai (*Acacia concinna* L.) or Soap pod, that grows in tropical jungles throughout India and is used as a laxative, emetic, expectorant and detergent. The fruit (pods) show presence of phytochemicals like saponin, alkaloid and sugar flavonoids (Khanpara et al. 2012). The fruit pods, leaves and bark powder are traditionally used in shampoo due to the saponin present in the pods and bark, which produces mild lather, has low pH and doesn't strip hair of its natural oils. Shikakai hair wash completely or partially relieves dandruff and it's symptoms like scaling, itching, dryness and greasiness of the scalp (Ediriweera et al. 2014). It also strengthens hair by promoting hair growth and avoiding hair splitting and keeps them in their original colour (Sharma et al. 2002).

12.4.8 Aromatherapy

Aromatherapy, also known as essential oil therapy, is a form of complementary and alternative medicine (CAM) that uses essential oils as a form of therapeutic agent on mood, behaviour and health (Herz 2009). Essential oils are complex mixtures of volatile compounds mainly consisting of terpenes, monoterpenes (hydrocarbon and oxygenated monoterpenes), sesquiterpenes (hydrocarbon and oxygenated sesquiterpenes) and also phenolic compounds (secondary metabolites of plants) (Dhifi et al. 2016). These volatile molecules are absorbed by the nasal mucosa which then are transformed into chemical signals traveling to the olfactory bulb which then further trigger the limbic system, cerebral cortex of brain and the olfactory sensory centre situated at the base of the brain. This results in the interaction with the neuropsychological framework producing characteristic physiological and psychological effects on target tissues (Lis-Balchin 1997). Essential oils have been used since ancient civilizations such as Indian, Chinese, Egyptian, Greek and Roman, where they were used as cosmetics, perfumes and drugs.

The oil extracted from *Melaleuca alternifolia*, a plant native to Australia, is popularly known as Tea Tree oil (TTO) or Melaleuca oil. This essential oil is considered precious as it has various properties like antibacterial, antifungal, antiviral and anti-inflammatory properties (Ali et al. 2015; Mertas et al. 2015). Hence, used in treatment of many infections as well as intimate or private care products like cosmetics, formulations of hair and cuticle creams (Priest 1999; Saller et al. 1998; Weseler et al. 2002; Carson and Riley 1994). It is constituted of monoterpenes, sesquiterpenes and allied alcohols but, 1,8-cineol, alpha-terpineol and terpinen-4-ol are the three most established compounds. Amongst these, terpinen-4-ol is determined to be the active component though, the interdependent influence of other terpenes cannot be ruled out (Altman 1988).

12.5 Conclusions

Natural ingredients are everywhere constantly gaining on popularity and the use of plant extracts in cosmetic formulations is also increasing. A cosmetic formulation with active ingredients of natural origin can protect the skin from external and internal harmful agents and can be a remedy for many skin conditions. In addition, natural products can be used in hair care products. Aromatic plants and oils have been used as incense, perfumes, etc. for thousands of years for their medicinal and culinary purposes. Today's generations are more aware of body and mental fitness, fashion and beauty and developments in the cosmetic industry. Cosmetics is now a multi-million-dollar industry that employs billions of people around the world. In the future it is possible that many new plant extracts, oils of commercial importance will be identified and many generic uses and claims of medicinal plants will be proven. New isolation and extraction technologies are anticipated to yield high quality products. Herbal medicine research is growing with hospital-based, pre-clinical studies. Many multinational pharmaceutical companies are willing to develop drugs based on MAP extracts that will eventually prove to be more effective than the various ingredients used in conventional drugs. Scientific and research practitioners of various disciplines can use ancient, heritage knowledge to ensure safe, cost-effective quality and to promote the sustainable use of indigenous medicines for the development of medically proven cosmetic agents and for the benefit of mankind.

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Chapter 13

Distribution, Chemical Composition and Ethnomedicinal Appraisal of *Acorus calamus* L. an Endangered Medicinal Plant Species of Kashmir Valley, India



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Abstract *Acorus calamus* L. often called as sweet flag, belongs to the family Acoraceae. It is also called as *Acorus odoratus*. There are 110 genera in the family Acoraceae and beyond 1800 species. The family comprises bulbous or rhizomatous herbs. In Kashmir, it is locally known by the name *Vai-gander*. Its rhizome is cut into small circular pieces, dried and made into a garland and is, as such, sold in the markets. Traditionally the rhizome portion is used for the treatment of cough, stomachache, swellings, fever, toothache diarrhea and joint pains etc. The rhizome is also used as abortifacient, carminative, diaphoretic, febrifuge, stimulant and vermifuge. This conventional scented medicinal herb, is also used to cure a large range of health disorders like neurological, metabolic, respiratory, kidney, and liver diseases etc. It is a popular conventional, critically endangered, curative and fragrant plant species, particularly from India and China. It grows in wetlands of Kashmir Valley, particularly in Hokhersar and Shalbug wetlands (Ganderbal), Manasbal Lake, Anchar Lake, Srinagar and other marshy tracts in huge quantities. *A. calamus* has a broad geographic distribution in India, from Kashmir to the North-East

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mounting to an elevation of 1500–2200 m in the Himalayas. Populations *A. calamus* from Kashmir valley are hexaploids ($2n = 6x = 72$) and this is the only variety in India that contains 5.2% of the β -asarone content in their oils. Presence of little quantity of β -asarone is an excellent feature, as β -asarone is highly carcinogenic agent which is present in large quantities in triploid and tetraploid varieties. Its populations in Kashmir are also genetically distinct from other states of India. Thus, the populations of *A. calamus* from Kashmir valley have a good potential for commercial cultivation. There is less or no flow of genes within these populations, therefore a favorable trait can be utilized for the preservation of the genetic uniqueness of the populations of *A. calamus*.

Keywords Rhizomatous · β -asarone · Antibacterial · Endangered · Glycosides · Flavonoids · Saponins · Hyperglycemia

13.1 Introduction

Acorus calamus Linn. – often called as sweet flag – belongs to the family Acoraceae. It is also called as *Acorus odoratus*. The name of genus *Acorus* is derived from Acoron (coreon means the pupil of the eye) and the species name *calamus* is derived from the Greek word *calamus* (a reed). There are 110 genera in the family Acoraceae and beyond 1800 species. The family comprises bulbous or rhizomatous herbs. In Kashmir, it is locally known by the name Vai-gander. Its rhizome is cut into small circular pieces, dried and made into a garland and is as such sold in market. Traditionally the rhizome portion is used for the treatment of cough, stomachache, swellings, fever, toothache diarrhea and Joint pain etc. (Rather and Baba 2015). The rhizome is also used as abortifacient, carminative, diaphoretic, febrifuge, stimulant and vermifuge (Devi et al. 2014). In commerce it occurs in both peeled and unpeeled forms. It produces flowers during summer season depending on the latitude, in the Himalayas, Manipur, and Naga Hills and in some parts of south India. The other species in this genus is *Acorus gramineus* native to Eastern Asia commonly called as Japanese sweet flag, Japanese rush, dwarf sweet flag is a wetland perennial with grass like foliage. However, *A. calamus* is a semi aquatic plant species, grows in a specific area only. The species is close to extinction because of excessive exploitation and loss of habitat (due to filling of wetlands) (Barbhutiya et al. 2009) and unfavored reproductive system.

13.2 Taxonomic Position

A. calamus belongs to a genus of monocot flowering plants. This genus was formerly set down in the Araceae family. However, the latest classifications have placed it in its own recognized family Acoraceae and order Acorales. This is the only genus

of the ancient live line of monocots. Presently *Acorus* is regarded as “colleague” to all other monocots. Liliopsida stands for one cotyledon and the cotyledon is having two prominent vascular bundles. Leaf venation is mostly parallel, and closed at the apex. Vascular bundles are usually without cambium. So these species are usually herbs. Their flowers are usually tri-merous, sometimes tetra-merous. Liliopsida most probably originated from some very ancient vesselless herbaceous member of Magnoliopsida that had atactostelic vascular system means a highly developed stele (Takhtajan 2009).

Class: Liliopsida; Order: Acorales; Family: Acoraceae; Genus: *Acorus*;
Species: *calamus*

English name: Sweet Flag, Local name: Vai-gander

13.3 Morphological Characteristics

It is a perennial wetland, *liliopsid* plant, with creeping and divided, scented rhizome, circular, with thickness of 2.5 cm, light brown to brown from external surface and white from interior side. The leaf of *A. calamus* has a single eminent mid-vein and moderately elevated secondary and tertiary veins (Bentley and Trimen 1983). This feature makes it different from *A. americanus*. The leaves are sword-shaped, 0.7 to 1.7 cm wide. The margin of the leaf is clearly edged. During the late spring numerous tiny, greenish yellow flowers are borne on 5–10 cm long spadex which grows from the sympodial leaf of *A. calamus* (Fig. 13.1). The sympodial leaf is somewhat shorter than the vegetative leaves. The fruits are small and berry-like, containing few seeds.



Fig. 13.1 Summer and autumn view of *Acorus calamus*

13.4 Geographic Distribution

A. calamus L. is a semi-aquatic plant found throughout the temperate to sub-temperate regions of Eurasia and America. The plant has an ethnobotanical history dating back possibly to the time of Moses in the Old Testament of the Bible and was used in early Greek and Roman medicine. *A. calamus*, which is supposed to be native to India and have roll out through trade routes to other countries Motley et al. (1994). It grows in wet lands of Kashmir valley particularly in Hokhersar and Shalbug wetlands (Ganderbal), Manasbal Lake, Anchar Lake Srinagar and other marshy tracts in huge quantities (Sharma et al. 1985). *A. calamus* has a great geographical distribution in India from Kashmir to the North-East with an altitudinal range of 1500–2200 m in the Himalayas. Therefore, presence of distinct *A. calamus* varieties in various habitats, provides the expectations or chances of elevated population disparity particularly with regard to ploidy level and active component β -asarone (Ogra et al. 2009). Various varieties of *A. calamus* have been recognized on the bases of poloidy level and geographical or topographical distribution. These varieties are (i) diploid ($2n = 2x = 24$; North America), (ii) triploid ($2n = 3x = 36$; Europe), (iii) the tetraploid ($2n = 4x = 48$; East Asia, India and Japan) and (iv) hexaploid ($2n = 6x = 72$; Kashmir area) (Ogra et al. 2009; Ginwal et al. 2011). Ginwal et al. (2011) studied a good number of populations of *A. calamus* from discrete areas of India and reported that populations from Kashmir valley were all hexaploids ($2n = 6x = 72$) and the one population A-44 from Khaziar lake (Himachal Pradesh) was diploid ($2n = 2x = 24$). All other populations were triploid and tetraploids.

The striking feature is that only diploid and hexaploids varieties are known to have low concentration of β -asarone, a carcinogenic agent. Some authors have reported contrasting chromosome number in this plant species and corresponded its ploidy status with the amount of active component β -asarone. For instance: *A. calamus* variety from Jammu region possesses a tetraploid $2n = 48 = 4x$ number of chromosomes and about 80% β – asarone, 13% α -asarone level in their oil. In contrast *A. calamus* variety from the Kashmir region is having the hexaploids $2n = 54 = 6x$ number of chromosomes and very little quantity i.e. 5.2% of the asarone level in their oils. Similarly, the cylindrical root as well as the leaves of the diploid variety $2n = 24 = 2x$ is known by the absenteeism of β -asarone and the triploid populations $2n = 36 = 3x$ identified by the appearance of 3–19% β -asarone in the rhizome oil and 31–44% in the leaf top oil; however, the tetraploids accommodate up to 96% of β -asarone in their rhizome oils and 60–70% in their leaf-top oils and 60.92–8.0%.

The tetraploids from Japan and far-east Russian are characterized by the presence of 10–40% of β –asarone in rhizomes and 20–50% β –asarone in their leaf top oils. The populations of *A. calamus* from Kashmir are genetically distinct as well as possess the low concentration of carcinogenic β -asarone in their essential oil. The germ plasm of *A. calamus* from Kashmir is having good potential for huge augmentation for commercial cultivation. There is very less flow of genes in between the

populations of *A. calamus* (diploid variety) of Kashmir region, which is a great tool in retaining of the genetic variations in the populations (Ginwal et al. 2011). It is a famous endangered aromatic and medicinal herbaceous plant species mostly found in Kashmir region of Jammu and Kashmir, India and also in China. (Avadhani et al. 2016). This plant species due to habit loss caused by drying up of wet lands have become rare in various pockets of the world (Dusek et al. 2007). It is a customary commodity in the world wide drug trade and the basic material is procured from the natural resource. In an International report conveyed by McAlpine Thorpe and Warriier Limited (1996), *A. calamus* is amidst the endangered therapeutic plant species whose population has been reported to decrease drastically.

13.5 Chemical Composition

So far nearly 145 compounds have been isolated from *A. calamus* rhizomes and leaves, viz. sterols, phenylpropanoids, glycosides, triterpene, triterpenoid sesquiterpenoids, saponins, alkaloids and monoterpenes. Amidst these, sesquiterpenoids and phenylpropanoids (chiefly, asarone and eugenol) have been reported as the primary efficient compounds (Sharma et al. 2020). Phenylpropanoids of *A. calamus* are with a skeletally divergent group of phenylalanine- derived secondary plant metabolites, like eugenol, α -asarone, β -asarone, isoeugenol, etc. (Soledade et al. 2010). A number of phenylpropanoids have been identified from *A. calamus* rhizome and leaves. α and β -asarone isolated from the rhizome are the prominent compounds present in this plant. A good number of aromatic oils with different structures have also been communicated from the rhizome part of the plant (Kumar et al. 2010; Kim et al. 2011; Haghighi et al. 2017).

The major chemical constituents of the essential oils of sweet flag are thermolabile squiterpenoids, phenylpropanes, mono-terpenes and phenylpropanes (Rost 1979; Bos 1979). Methyl eugenol, cis- methylisoeugenol, geranylacetate, 3-asarone, f-farnesene, epishyobunone, shyobunone, isoshyobunone are the most prominent chemical constituents comprising approximately 20% of the volatile oil (Rost 1979; Bos 1979). The rhizomes oil of *A. calamus* contain β -Asarone (83.2%) and α -asarone (9.7%) as the major constituents, while as the leaf oil contain β -asarone (85.6%) and linalool (4.7%) as the major constituents (Oprean et al. 2001; Raina et al. 2003).

The oil from rhizome was found to contain varying concentrations of camphor, a-asarone, b-asarone, c-asarone, calamene, calamenenol, calameone, a-pinene b-pinene, camphene, p-cymene, eugenyl acetate, eugenol, isoeugenol, methyl isoeugenol, calamol, azulene, eugenol methylether, 1,8-cineole, dipentene, terpinolene, methyleugenol, saronaldehyde, hydrocarbons and caryophyllene (Nigam et al. 1990; Srivastava et al. 1997; Mukherjee 2002). The oil also contains fatty acids such as heptylic acid, an ester of butyric acid, palmitic acid and its ester (Chaudhury et al. 1957). A lignin called as acoradin was isolated from the rhizomatous portion of the plant species (Raja et al. 2009). Imam et al. (2013) took studies of photochemistry of rhizome and revealed the existence of flavonoids, glycosides,

saponins, tannins, mucilage, volatile oil, polyphenolic compounds and pungent principle. The plant also contains glycoside, alkaloid and essential oil containing calamen, clamenol, calameon, asarone and sesquiterpenes and a bitter glycoside named acorine along with eugenol, pinene and camphene. Satyal et al. (2013) in Nepal reported (Z) asarone (78.1–86.9%), (E)-asarone (1.9–9.9%), (Z)-methyl isoeugenol (1.5–2.0%), linalool (0.2–4.3%) and small amounts of gamma-asarone (2.0–2.3%), in the essential oil of rhizome extract of the mentioned plant species.

13.6 Contribution of *A. calamus* L. to a Rural Livelihood-Based Cottage Industry in Kashmir

Of the all off-farm and on-farm income sources in District Ganderbal of Kashmir, in district Ganderbal of Kashmir, the total average collection/household/annum of *A. calamus* was 67.3 kg. From this a total average of 61.7 kgs, were processed and an average of 5.1 kgs were consumed by per family household providing a total average employment of 68.66 man days to the single sample household or an average income/household/annum as Rs. 2152.00 in the district. So the *Acorus* based cottage industry has been found as the fifth major source for generation income in the District Ganderbal of Kashmir. Usually of marginal landholders living in the vicinity of wetlands go for collection and sold it at the rate of Rs. 34 kg⁻¹ (Bhat et al. 2020).

13.6.1 Ethnomedicinal Uses

Locally all parts of the plant are used for treatment of one or other ailment. Mostly rhizome is dried and grounded into powder and the powder is utilized for treatment of skin diseases, wounds, urinary infections, deworming, gastrointestinal disorders, joints pains, stomachache, gout, burns and cough etc. In Ayurveda medicine, *A. calamus*, an excellent herbaceous plant species, is regarded as refresher or renovator for the brain and nervous system and also used for treatment of various digestive disorders. The cylindrical roots of *A. calamus* are utilized for a number of medicinal purposes particularly loss of appetite, stomachache, fever as well as toothache (Divya et al. 2011). This plant species is utilized generally in the Indian Ayurvedic tradition, as well as in the Chinese system of medicine for anodyne, febrifuge, tonic, anti-depressant, anti-obesity, and analeptic purposes; it is highly therapeutic for skin diseases. It is also utilized for treatment of neurological, respiratory, gastrointestinal, and various other health ailments. Rhizomes and leaves are found to be profusely practiced in the form of infusion, powder, paste, or decoction (Kingston et al. 2009; Napagoda et al. 2019). All these properties are due to the presence of active components like glycosides, saponins, resins and steroids.

Sharma et al. (2020) reported that *A. calamus* is a conventional Indian scented medicinal herb that is used to cure a large range of health disorders, like gastrointestinal, neurological, metabolic, respiratory, kidney, and liver diseases. They also reported that about 145 components are present the herb and have been isolated and identified. They include sesquiterpenoids, phenyl propanoids, and monoterpenes as well. A large number of evidence supports the bio-potential nature of its various extracts and active constituents in many neurological and metabolic diseases. The scented rhizomes of *A. calamus* are utilized considerably in conventional medicine internationally. These are regarded to hold anthelmintic and anti-bacterial properties and also utilized for cure of tumors, persistent diarrhea, stomach pain, dysentery acute rhinitis, chest diseases and fevers (Rani et al. 2003).

The phenylpropanoid β -Asarone, one of the power full components isolated from the roots and rhizomes of *A. calamus* was found to have antibacterial and anthelmintic activity (McGaw et al. 2002). The anticonvulsant property exhibited by volatile oil from rhizomes of *A. calamus* has also been attributed to the presence of β -Asarone in significant amounts (Mittal et al. 2009; Ponrasu et al. 2014). β -asarone easily passes through the blood-brain-barrier (BBB) and shows significant pharmacological effects on the cardiovascular and central nervous systems (Sadati et al. 2016).

13.6.2 *Anti-bacterial Activities*

The plant extract has antibacterial properties on all gram negative and gram positive bacteria and thus is convenient for the development of potent treatment for the eradication of contagious diseases. The ethanolic extract of rhizomes possesses very strong antibacterial activity against different strains of bacteria like *A. hydrophila*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Bhuvaneshwari and Balasundaram 2006).

13.6.3 *Hypolipidemic Properties*

The saponins found in the ethanolic extract of *A. calamus* demonstrate hypolipidemic properties. The concentrated aqueous extract of *A. calamus* has also demonstrated hypolipidemic activity (Sharma et al. 2014).

13.6.4 *Hyperglycemia*

According to Si et al. (2010), *A. calamus* improves post prandial hyperglycemia and cardiovascular complications.

13.6.5 Allelopathy

The aqueous extract of *A. calamus* has been observed to demonstrate allelopathic effects on the development of *Microcystis aeruginosa* and *Chlorella pyrenoidosa*, the two water bloom-forming algal species (Hur et al. 2004).

13.6.6 Anticancer Activity

Gaidhani et al. (2009), evaluated anticancer activity of *A. calamus* rhizomes by preparing concentrates of root of *Glycyrrhiza glabra*, rhizome of *A. calamus*, and *Terminalia chebula*. The β -asarone, has cancer causing property. It has shown anti carcinogenic activation of α -asarone on the human carcinoma cells as has been reported in some experimental findings (Das et al. 2019).

13.6.7 Antifungal Activity

A. calamus rhizome crude methanol extract shows strong antimicrobial action on numerous microorganisms including filamentous fungi, yeasts and bacteria. These crude methanolic extracts of rhizome of *A. calamus* exhibited high activity against filamentous fungi like *Trichophyton rubrum*, *Microsporum gypseum* and *Penicillium marneffeii*, average action against yeast particularly *Cryptococcus neoformans*, *Saccharomyces cerevisiae* and *Candida albicans*, and less action against bacteria (Phongpaichit et al. 2005). Kumar (2014) reported that the antimicrobial activity of *Acorus calamus* is due to the presence of flavonoids, glycosides, saponins, resins and steroids in its rhizome.

Rhizome and leaf ethyl acetate extracts exhibited pronounced antifungal activity as well as anti-yeast activity. Further, both α - and β -Asarones possesses a strong antimicrobial property against the yeasts as well as fungi than leaf and rhizome extracts (Devi and Ganjewala 2009). The ethanolic crude extract of rhizome of *A. calamus*, *Tinospora cordifolia* and *Celestrus paniculatus* showed anti-fungal activity against *Alternaria solani*, *Curvularia lunata*, *Fusarium* sp., *Bipolaris* sp. and *Helminthosporium* sp. at different concentrations. It also possesses antioxidant, antibacterial and anti-inflammatory property as well. (Singh et al. 2010). Dhiman et al. (2018) studied the effect of *A. calamus* extract on fungal growth on wooden samples of *Pinus roxburghii* and *Bombax ceiba* at different concentrations and found that wood samples treated with *A. calamus* L. extract were able to inhibit the fungal growth significantly. Dethoupa et al. (2019) studied the efficacy of *A. calamus* crude ethanol extract against eight plant pathogenic fungi: *Alternaria brassicicola*, *Colletotrichum capsici*, *Bipolaris oryzae*, *Lasiodiplodia theobromae*, *Phytophthora palmivora*, *Pyriculariaoryzae*, *Rhizoctonia solani* and *Sclerotium*

rolfsii and reported that that extract had significant antifungal activity both *in vitro* and under greenhouse conditions against *B. oryzae* in rice. β -Asarone and galangin were found to be the major antifungal compounds active against the tested plant pathogenic fungi. Thus *A. calamus* L. extract is a promising candidate as a botanical fungicide to control brown spot of rice.

13.6.8 Antioxidant and Radical-Scavenging Activity

Rhizome extract shows strong superoxide anion-scavenging activity and with the increase in the concentration of phenolic content of the rhizome, the antioxidant activity is also increased, however, the leaf extract possesses striking radical-scavenging activity and ferric reducing antioxidant power as well (Devi and Ganjewala 2011). The antioxidant effects may be because the rhizome concentrate has enough quantity of ascorbic acid and polyphenolic compounds in it. These compounds are having potential for enhancing antioxidant potential and function in the brain and that may be because of the presence of α -asarone, an anti-oxidant compound present in the rhizome (Rawat et al. 2016).

13.6.9 Phytoremediation Potential

Phytoremediation is a new cheap and eco-friendly technique that uses plants to clean the environmental pollution by heavy metals. The aquatic plant *A. calamus* was tested for its ability to accumulate Sb from contaminated water in laboratory experiments. The results showed that *A. calamus* serves as a good candidate for phytoremediation of water contaminated with Sb (Sytar et al. 2016).

13.6.10 Neurotransmission

A. calamus rhizome extract results in suppression of enzyme, acetylcholinesterase due to the presence of an active component β -asarone, hence aids in cholinergic neurotransmission (Feng et al. 2015).

13.6.11 Anti-depressant Activity

A. calamus methanolic extract, when was given to rats under experimental conditions for a period of 7 days manifested dose-dependent anti-depressant activity with a potential similar to 5 mg/kg imipramine (Chellian et al. 2018; Pawar et al. 2011).

However, aqueous extract of rhizomatous portion of the plant causes sedative effects on cardiovascular tissue under in vitro conditions that is 55–60% lowering in heart beat rate and atrium contractile force (Shah and Gilani 2012).

13.6.12 Increases Memory Power

Oral ingestion of few milligrams of active component, β -Asarone for approximately a month helps in preserving of perception in rats with a vigor in comparison to donepezil hydrochloride, which is associated with lowering of hippocampal cell death rate (Geng et al. 2010; Zhou et al. 2016).

13.6.13 Blood Pressure Regulation

Rhizome extracts helps in lowering the enhanced systolic and diastolic blood pressure. It also aids in decreasing the plasma renin as well as oxidative bio markers particularly glutathione in the kidneys (Patel et al. 2012).

13.6.14 Anti-helminthic Activity

Rhizomes of *A. calamus* also possesses anti-helminthic activity particularly against earthworms (Merekar et al. 2011). Bhakta et al. (2013) studied the anti-helminthic potential of crude methanolic, ethanolic and aqueous extracts of the leaves of *Acorus calamus* on Indian earth-worm (*Pheretima posthuma*). The activity involved the determination of time of paralysis (vermifuge) and time of death (vermicidal) of the worm and reported that all the extracts exhibited significant anti-helminthic activity at a concentration of 100 mg/ml, however, peak activity was exhibited by the methanolic extract at a concentration of 100 mg/ml. Calamus's essential oil possesses anticholinesterase effects (Mathew and Subramanian 2014) and antidiabetic potential (Rau et al. 2006) as well.

13.7 Conclusions

Acorus calamus L. is an endangered, rhizomatous or tuberous herb, found in marshy tracts of Kashmir, locally known as Vai-gander. *A. calamus* based cottage industry has been found to be the fifth major constituent of household income employment in one of the districts of Kashmir valley. It is locally used for the treatment of various kinds of ailments. Besides it is used as anti-depressant, increases memory

power, used in neurotransmission also regulates blood pressure, possesses anthelmintic, anti-bacterial, antifungal properties. There is higher degree of genetic divergence within the individuals of the population of *A. calamus* from Kashmir. Amplified RAPD and cpSSR fragments were found specific for them while these fragments were found completely absent in populations collected from other states of India. The populations of *A. calamus* from the Kashmir region of India need more focus in the conservation programs, as these populations are genetically distinct, and possess low concentration of carcinogenic β -asarone in their essential oil. *A. calamus* from the Kashmir region of Union territory Jammu and Kashmir, India has excellent potentials for the commercial cultivation. The moderate gene flow in the populations of *A. calamus* can be regarded as a useful feature for the preservation of the genetic distinctiveness of the populations from Kashmir (Ginwal et al. 2011).

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Chapter 14

A Multipurpose Medicinal Plant Species: *Tinospora cordifolia* (Willd.) Miers



Bharat Singh and Vinay Sharma

Abstract *Tinospora cordifolia* (Willd.) Miers (Fam. – Menispermaceae) is a perennial, dioecious, deciduous climber found in India. It is used in the treatment of jaundice, fever, urinary diseases, asthma, gout, diabetes, diarrhoea, skin disease, rheumatism, and snakebites. The crude extracts of *T. cordifolia* are used in the treatment of bone fracture, wound healing, jaundice, fever, urinary diseases, asthma, gout, diabetes, diarrhoea, and skin diseases. Various chemical constituents have been identified from *T. cordifolia* viz., tinoscorside A-D, berberine, jatrorrhizine, cordifoliside A-B, and amritoside A-D. The crude extracts and isolated compounds possess antimicrobial, anti-inflammatory, antioxidant, antirheumatic, and antidiabetic properties. In clinical trials, *T. cordifolia* extracts reduced the fasting blood glucose levels in the type-2 diabetic patients and also restored the changed liver functions of patients, although the drug itself, demonstrated mild to moderate adverse changes (in kidney, liver, intestine, and stomach) at a therapeutic equivalent dose ($\times 10$ dose level). The anti-inflammatory, antidiabetic, antimicrobial, anticancer, antistress, and antioxidant activities of *T. cordifolius* have been evaluated. Farther clinical applications should be preceded by *in vivo* model studies on the safety, potent doses, bioactive molecules, and the mechanisms of actions.

Keywords *Tinospora cordifolia* · Ethnomedicine · Cordifolisides · Pharmacology

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Tinospora cordifolia (Willd.) Miers. (Photo: Plantzoin)

14.1 Introduction

The plant-derived medicines are more economical, effective as well as easily available to the people of developing countries. *Tinospora cordifolia* is used in Indian traditional medicinal systems for the treatment of fever, asthma, gout, diabetes, diarrhoea, skin disease, and snakebites (Jakhar et al. 2004). *Tinospora cordifolia* (Willd.) Miers [syn. *Menispermum cordifolium* Willd., *Tinospora gibbericaulis* Handel-Mazzetti, *Tinospora mastersii* Diels, *Tinospora rumphii* Boerlage, *Tinospora thorelii* Gagnepain] is a both medicinally and commercially important plant species, commonly known as “Guduchi” or “Amrita”, and distributed in several states (Rajasthan, Uttar Pradesh, Bihar, West Bengal, Gujarat, Punjab, Tamil Nadu, Kerala and Karnataka) of India (Singh and Chaudhuri 2017). The methanolic, ethanolic, chloroform and aqueous extracts of whole plants are widely used in the treatment of jaundice, and rheumatism (Sharma et al. 2019), intermittent fever, eye and liver ailments (Choudhary et al. 2013). The alkaloids [tinoscorside A and B (Kiem et al. 2010), jatrorrhizine, palmatine, magnoflorine (Chintalwar et al. 2003; Sarma et al. 2009)], glycosides [cordifoliside D, cordifoliside D tetraacetate, cordifoliside E, cordifoliside E tetraacetate (Gangan et al. 1994, 1995)], diterpenoid lactones, daucane-type sesquiterpenes [tinocordifolin, tinocordifoliside and tinocordifoliside tetraacetate (Maurya et al. 1997)] and sesquiterpenoids have been isolated and identified from *T. cordifolia*. *T. cordifolia* has antioxidant (Polu et al.

2017), antiarthritic (Ramya and Maheswari 2016), antidiabetic (Khedekar et al. 2015), anti-inflammatory (Sannegowda et al. 2015), antiulcer (Antonisamy et al. 2014), and anticancer properties (Bhadane et al. 2018). This chapter provides the critical and comprehensive knowledge of the ethnomedicinal uses, phytochemistry, pharmacological activities, clinical trials and toxicological aspects of *Tinospora cordifolia*.

14.2 Ethnomedicinal Uses

T. cordifolia is a large deciduous climber, growing on wide range of hedges and trees (Anonymous 1976); its stem is green, and has succulent bark. The stem of *T. cordifolia* is used for stimulating gastric activity, bile secretions, enrichment of blood composition and to treat skin diseases, spleen enlargement, vaginal and urethral discharges (Sahu 2002). Stem decoction is used for washing of eyes and syphilitic sores, acts as an antidote to treat snakebites and scorpion stings (Trivedi 2006), and the treatment of pyorrhoea, malaria, chronic diarrhea, asthma, dysentery, urinary, skin diseases and respiratory problems (Ramadevi et al. 2018; Sharmila et al. 2018). The aqueous extract of roots is considered as an emetic and analgesic agent, and also useful in the treatment visceral pain (Stanely et al. 2000). The crushed leaves are mixed with honey and used to treat ulcers, gout and bacterial skin infections. Decoction of leaves is useful in the treatment of malaria as well as for enhancement of women's fertility (Singh and Chaudhuri 2017). The aqueous extract of dry stem bark showed anti-spasmodic, anti-pyretic, antiallergic and anti-leprotic effects (Spandana et al. 2013). The powder of fruits is mixed with ghee or honey and used as a tonic as well as in the treatment of jaundice and rheumatic complaints. The combination of ripened fruit juice and honey is recommended daily (for 3–5 days) for treatment of cold in children (Sahu 2002). The extract of whole plants is useful in the treatment of diarrhoea, stomach complaints, anemia, as well as healing of wounds (Alsuhaibani and Khan 2017). The *T. cordifolia* stem is a rich source of copper, calcium, phosphorus, iron, zinc, manganese hence, used to treat metabolic disorders of humans (Upadhyay et al. 2010; Dhama et al. 2017).

14.3 Phytochemical Characterization

The alkaloids, glycosides, diterpenoid lactones, flavonoids, steroids and sesquiterpenoids have been isolated and identified from *T. cordifolia*. The isolated and identified compounds and their structures have been mentioned in Table 14.1 and Fig. 14.1. The identified compounds of *T. cordifolia* is summarized below.

Table 14.1 Tree species heavily traded in the herbal industry

Species	Source	Parts used	Estimated consumption (MT)	Cat A & B	Cat C & D
<i>Abies spectabilis</i>	W	Leaf	571	1	99
<i>Acacia catechu</i>	W	Bark (stem), wood (heartwood)	411	32	68
<i>Acacia nilotica</i>	W/C	Gum, bark (stem)	454	40	60
<i>Aegle marmelos</i>	C/W	Leaf, fruit, bark (root, stem)	2939	33	67
<i>Albizia amara</i>	W	Leaf	270	100	0
<i>Alstonia scholaris</i>	W	Bark (stem)	181	1	99
<i>Aquilaria agallocha</i>	I/W	Bark (stem), wood (heartwood)	129	88	12
<i>Azadirachta indica</i>	C/W	Fruit (fruit, seed), flower, leaf, bark (stem)	2255	21	79
<i>Bombax ceiba</i>	W	Exudate of bark (stem), flower, root	166	17	83
<i>Boswellia serrata</i>	W	Oleo-gum resin	762	19	81
<i>Butea monosperma</i>	W	Bark (stem), flower, root, fruit (seed), wood, gum	463	39	61
<i>Caesalpinia sappan</i>	C	Wood (heartwood)	419	10	90
<i>Cassia fistula</i>	W/C	Flower, fruit (seed), bark (stem)	471	21	79
<i>Cedrus deodara</i>	W	Wood	930	16	84
<i>Emblica officinalis</i>	W/C	Fruit (seed)	16820	58	42
<i>Ficus benghalensis</i>	W/C	Bark (stem)	313	2	98
<i>Ficus religiosa</i>	W/C	Bark (stem), leaf	287	1	99
<i>Garcinia indica</i>	W/C	Fruit (fruit, peel)	493	100	0
<i>Gmelina arborea</i>	W/C	Bark (root)	1439	24	76
<i>Holoptelea integrifolia</i>	W	Bark (stem)	113	44	56
<i>Myristica fragrans</i>	C/W	Fruit	180	12	88
<i>Oroxylum indicum</i>	W	Bark (stem, root)	1201	29	71
<i>Pongamia pinnata</i>	C/W	Bark (stem), fruit (seed), leaf, root	277	21	79
<i>Premna latifolia</i>	W	Root, root bark	1003	22	78
<i>Pterocarpus marsupium</i>	W	Wood (heartwood), bark (stem), resin, fruit	522	9	91
<i>Pterocarpus santalinus</i>	W	Wood (heartwood)	442	14	86
<i>Santalum album</i>	W	Wood (heartwood)	291	13	87
<i>Sapindus emarginatus</i>	W/C	Bark (stem), fruit, leaf	182	36	64
<i>Saraca asoca</i>	W	Bark (stem)	2041	26	74
<i>Stereospermum chelonoides</i>	W	Root	1322	25	75

(continued)

Table 14.1 (continued)

Species	Source	Parts used	Estimated consumption (MT)	Cat A & B	Cat C & D
<i>Strychnos nux-vomica</i>	W	Fruit (seed), stem or bark	2891	97	3
<i>Symplocos racemosa</i>	W	Bark (stem)	629	30	70
<i>Terminalia arjuna</i>	W/C	Fruit (seed), bark (stem)	2355	10	90
<i>Terminalia bellirica</i>	W/C	Fruit	3424	31	69
<i>Terminalia chebula</i>	W	Fruit, galls	8158	23	77

C cultivated, I imported, W wild, C/W cultivated and wild, W/C wild and cultivated, I/W imported and wild, Cat A&B medium to large industries (~50 units), Cat C&D small to very small industries (~9000 units)

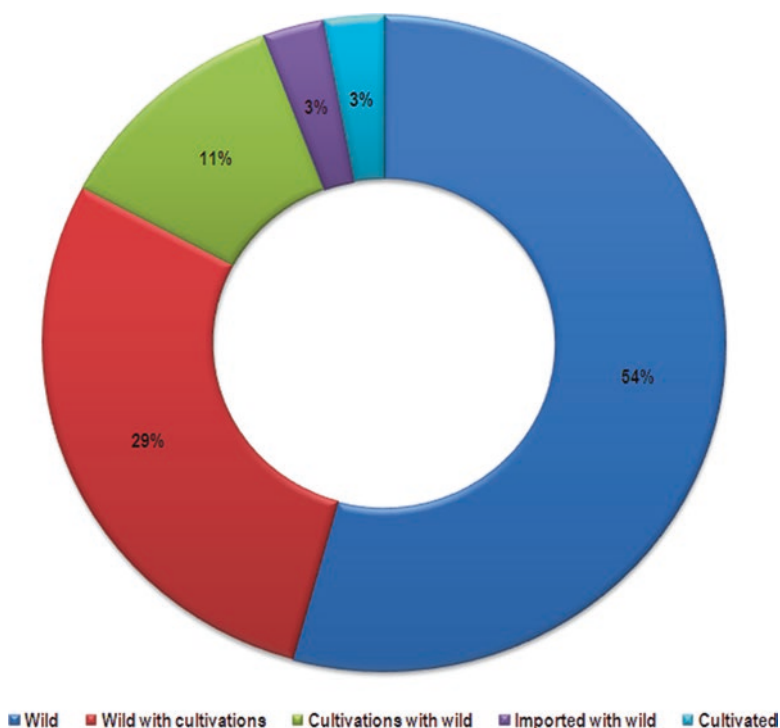


Fig. 14.1 Pie Chart showing the proportion of sources of collection of tree species used in Indian herbal industries

14.3.1 Alkaloids

Alkaloids are a large group of naturally occurring secondary products which contain nitrogen atom(s) in their structures. These nitrogen atoms are usually situated in cyclic ring system (Kurek 2019). Based on the cyclic ring system, alkaloids can

be grouped into several classes such as indoles, acridines, quinolines, isoquinolines, pyrrolidines, pyridines, pyrrolizidines, quinazolines and tropanes. Alkaloids are usually bitter, colorless, coloured (sanguinarine, berberine), odorless crystalline solids, but sometimes they can be yellowish liquids (nicotine). Nearly more than 3000 alkaloids have been investigated in different 4000 plant species (Ge et al. 2015; Gaziano et al. 2016) among them tinoscorside A and B (Kiem et al. 2010), jatrorrhizine, palmatine, magnoflorine (Sarma et al. 2009; Bala et al. 2015), berberine, isocolumbin, tembetarine (Srinivasan et al. 2008; Reddy and Reddy 2015) have been reported from aerial parts and stem of *T. cordifolia*.

14.3.2 *Clerodane-Furano-Diterpene-Glucosides and Norditerpene-Furan Glycosides*

The amritoside A, amritoside A pentaacetate, amritoside B, amritoside B pentaacetate, amritoside C, amritoside C pentaacetate, amritoside D, amritoside D tetraacetate (Maurya et al. 1995, 2004), tinosponone, tinosporaside, tinosporaside tetraacetate, tinocordioside, tinocordioside tetraacetate (Iqbal et al. 2005; Puratchimani and Jha 2007), tinoscorside C, borapetoside F, borapetoside B, cordifolide A (Kiem et al. 2010; Pan et al. 2012), tinosporafuranol, tinosporaclerodanol, tinosporafuradiol, tinosporaclerodanoid (Ahmad et al. 2010; Phan et al. 2010), cordioside (Wazir et al. 1995) from stem and stem bark (Kumar et al. 2019), syringin (Kiem et al. 2010), cordifoliside A, cordifoliside A tetraacetate, cordifoliside B, cordifoliside B tetraacetate, cordifoliside C, cordifoliside C tetraacetate, cordifoliside D, cordifoliside D tetraacetate, cordifoliside E, cordifoliside E tetraacetate; (Gangan et al. 1994, 1995; Sharma et al. 2018, 2019), 4,5,7-trimethoxy-2-naphthol-2-*O*- α -L-arabinofuranosyl-(2' \rightarrow 1'')-*O*- α -L-arabinopyranosyl-2''-*O*-pentane, β -D-arabinosyl-*O*-geranilan-10'-oate, 5,7-dimethoxy-2-naphthol-2-*O*- α -L-arabinopyranosyl-(2' \rightarrow 1'')- α -L-arabinopyranosyl-2''-*O*-decane (Sultana et al. 2017) have been isolated and characterized from the stem of *T. cordifolia* (Kattupalli et al. 2019).

14.3.3 *Daucane-Type Sesquiterpene*

Sesquiterpenes consist of three isoprene units (C₁₅H₂₄) with acyclic rings. Biochemical alterations such as oxidation or rearrangement form the related sesquiterpenoids (Davis and Croteau 2000). Cyclic sesquiterpenes are more common than cyclic monoterpenes because of the increased chain length and additional double bond in the sesquiterpene precursors (Chizzola 2013). The tinocordifolin, tinocordifolioside, and tinocordifolioside tetraacetate were isolated and identified from the stem of this plant species (Maurya et al. 1997; Maurya and Handa 1998).

14.3.4 Ecdysteroids and Steroids

Phytoecdysteroid-like compounds have been found in gymnosperms, angiosperms, fungi, and algae. These compounds accumulate in various plant organs, viz., fruits, seeds, flowers, anthers, leaves, and roots. Phytoecdysteroids (*Ajuga decumbens*) demonstrated significant inhibitory effects on early induction and potent antitumor-promoting activities of Epstein–Barr virus on a mouse skin. Besides anticancer properties, phytoecdysteroids are also being considered as nutraceutical additives to food products (Bajguz et al. 2015; Saleem and Nazir 2015). Plant steroids are derived from S-squalene-2,3-epoxide via acetate-mevalonate pathway and several phytosterols have been reported to possess hypocholesterolemic activity. Withanolides and brassinosteroids are a large group of steroidal compounds possess various pharmacological activities (Tarkowska 2019). The polypodine B 20, 22-acetonide, 20-*p*-hydroxyecdysone, β -sitosterol were isolated and identified from *T. cordifolia* aerial parts (Pathak et al. 1995; Kiem et al. 2010).

14.4 Pharmacological Properties

Ethanollic extract of *T. cordifolia* is extensively used in formulation of ‘Septilin’ syrup, recommended as remedy for the treatment of bronchitis and earache (Spelman 2001; Singh et al. 2003). The berberine and jatrorrhizine (from *T. cordifolia*) showed antimicrobial, anti-inflammatory (Patgiri et al. 2014), anthelmintic (Pawar et al. 2014), antineoplastic (Jagetia and Rao 2006), antidiarrheal, antiulcer (Kumar et al. 2014) and anti-diabetic activities (Sinha et al. 2004; Sangeetha et al. 2013). Similarly, the alcoholic and aqueous extract enhances cognition in normal rats (Agarwal et al. 2002). *Tinospora cordifolia* possesses several pharmacological properties and are mentioned below:

14.4.1 Antioxidant Activity

The oxidative stress is considered as a main cause for development of various diseases. The food materials promote antioxidative defences in human body to combat non-desirable effects of reactive oxygen species. Plants are able to biosynthesize a wide range of non-enzymatic antioxidative molecules capable of attenuating reactive oxygen species-induced oxidative destruction (Kasote et al. 2015). The ethanolic extract (300 mg/kg body weight) was administered (p.o.) to the cancer bearing animals for 30 days. It significantly ($P < 0.01$) reduced the levels of catalase and superoxide dismutase (57.05 ± 5.67 and 6.69 ± 0.19) in treated animals but, no significant changes observed in animals of control group (Jayaprakash et al. 2015; Polu et al. 2017). The superoxide dismutase converts superoxide radical into hydrogen

peroxide and molecular oxygen, while the catalase converts hydrogen peroxide into water. By this way, two toxic species (superoxide radical and hydrogen peroxide) are converted into the water in treated animals (Weydert and Cullen 2010).

14.4.2 Anti-diabetic Activity

The diabetes mellitus type 2 is an endocrine metabolic disorder that causes (microvascular and macrovascular complications) significant morbidity and mortality in humans. The human bodies perform enzymatic and non-enzymatic antioxidative activities which minimize the development of reactive oxygen species (significant cause for diabetes). Recently, the disease is rapidly increasing and affecting all parts of the world (Patel et al. 2011a, b). The methanolic extract was administered (p.o.) for 24 weeks to diabetic rats and levels of blood glucose were monitored in treated and non-treated groups. The methanolic extract lowered the blood glucose levels in treated animals significantly ($P < 0.001$) than normal rats (Agrawal et al. 2012). The ethanolic extract (100 and 200 mg/kg body weight) suppressed the levels of 6-phosphatase and fructose 1, 6-diphosphatase activities significantly ($P < 0.001$) but induced the restoration of glycogen contents in liver ($P < 0.005$). It is suggested that ethanolic extract induces activity of pancreatic cells for insulin secretion but suppresses the process of gluconeogenesis and glycogenolysis in treated animals (Sangeetha et al. 2011). The extract increased the body weight, total haemoglobin and hepatic hexokinase in rats but, decreased the levels of hepatic glucose-6-phosphatase and serum acid phosphatase, alkaline phosphatase, and lactate dehydrogenase activities in diabetic rats ($P < 0.001$; Stanely et al. 2000). The water extract+honey suspension reduced the levels of blood glucose and glycated haemoglobin in streptozotocin treated Wistar albino rats (Khedekar et al. 2015). Ethanolic extract of *T. sinensis* flowers (200 mg/kg body weight/day) altered the improper metabolic profile and it was led to hypolipidemia significantly ($P < 0.05$; Sandhyarani and Kumar 2014). Similarly, the ethyl acetate extract of leaves demonstrated significant ($P < 0.001$) antidiabetic activity (149 ± 0.66) at 200 mg/kg dose (Pimpriker et al. 2009).

14.4.3 Anti-arthritic Activity

Rheumatoid arthritis is a chronic systemic inflammatory disease that causes joint inflammation, synovial proliferation, and destruction of articular cartilage. The condition gets worse over the time unless the inflammation is stopped or slowed. It is the most common cause of physical disability in developed countries, and its prevalence is found between 0.3% and 1.50% in humans (Lin et al. 2006). The *in vitro* results were not found optimistic but oral administration of extract showed significant results (50 mg/kg body weight/day; given for 21 days followed by treatment for

12 weeks) to ovariectomy-induced bone loss (Abiramasundari et al. 2017). The minimum protein denaturation (23%, 36%, and 43%) was reported in animals treated with methanolic extract of stem bark (100 µg, 250 µg and 500 µg/ml). The methanolic extract of *T. cordifolia* demonstrated significant ability to inhibit the denaturation of proteins on comparison with diclofenac sodium in animals (Ramya and Maheswari 2016).

14.4.4 *Anti-stress Activity*

Stress is the nonspecific stimuli of the human body to any demand made upon it. Normally stress-induced alterations are self limiting events that override the 'threshold' limits become irreversible and pathological (Seyle 1973). Stress is involved in the development of various diseases such as hypertension, peptic ulcer, Alzheimer's disease, and depression (Piato et al. 2008). *T. cordifolia* stem is used in the management of depression, treatment of Alzheimer's disease and attention deficit hyperactivity problems. No evidence is available in the literature of serious toxicity of *T. cordifolia* in depression management, Alzheimer's disease and attention deficit hyperactivity disorder (Mutalik 2011). In chronic mental stress affected patients, the levels of serum glucose, triglyceride, cholesterol, anxiety, and depression were significantly higher than healthy humans. The patients were administered with *T. cordifolia* (3 g twice daily) and advised to do yoga daily. The experimental study was followed for 60 days and results revealed that *T. cordifolia* with continuous yoga practice in patients showed significant antistress activities as compared with adaptogenic agent diazepam ($P < 0.001$; Biswas et al. 2015).

14.4.5 *Anticancer/Antitumor Activity*

Cancer is one of the most dreaded diseases; occur in different forms in human population. It is the third leading cause of death worldwide following cardiovascular and infectious diseases (Kelloff 2008). Breast cancer is the most ordinary type of cancer in women. The maximum prevalence of breast cancer is found in South-Central Asian countries (Soliman et al. 2006). It has been established that several diseases occur in humans due to development of oxidative stress. The oxidative stress developed by free radicals, seek stabilization through electron pairing with proteins and DNA in healthy cells of human, cause DNA and protein damage. These alterations contribute to development of cancer, cardiovascular problems, and ageing disorders (Maxwell 1995; Braca et al. 2002). Due to lack of preventive measures, high cost of chemo- and radiotherapies, and the adverse effects of anticancer agents, cancer may be considered as a major cause of death. So, attempts are still being made to search for novel and effective plant-derived molecules that could suppress cancer development (Bhadane et al. 2018). Methylene chloride extract (100 µg/ml) induced the cell

killing effect (HeLa cells; 6.8-fold) as well as formation of micronuclei. The extract showed greater cell killing effect than doxorubicin (reference compound) in treated animals. On the basis of results, it is concluded that methylene chloride extract may be used for potent cytotoxic activity against HeLa cells (Jagetia et al. 1998). The methanolic extract (20 mg/kg) significantly suppressed tumour directed capillary formation (B16F10 melanoma cell-induced capillary) in rats. The results revealed that levels of IL-1 β , IL-6, TNF- α , granulocyte monocyte-colony stimulating factor were suppressed in treated animals while no change found in angiogenesis-induced control animals (Leyon and Kuttan 2004a, b). Hexane fraction of ethanolic extract induced the formation of apoptotic bodies, nuclear condensation, and activation of caspase-3, but inhibited increase in the cell number as well as volume of Ehrlich ascites tumor in mice (Thippeswamy and Salimath 2007).

14.4.6 Radioprotective Activity

The radiation exposure has been increased during last 100 years with the development and use of X-rays and radio-isotopes for therapeutic purposes, and also by testing of nuclear weapons (Donya et al. 2014). The radiation exposure can develop mutational changes, immune system alterations, and cancer development in humans (Mamedov et al. 2011). The administration of amifostine (aminothiols) to patients has been reduced the mortality rate but showed adverse effects. Unfortunately, no safe synthetic radioprotective compounds are available to date; therefore, the exploration of plants has been ongoing for several decades to treat radio exposed patients (Bhandari 2013). Aqueous-ethanolic extract (1:1, 200 mg/kg) improved the several parameters in treated animals e.g., the spleen weight (49% in irradiated control while 93% in treated), apoptosis (from 19% to 2.8%), DNA fragmentation (from 43% to 20.4%), macrophage adherence (75% in irradiated control while 120% in treated) and macrophage spread size (from 8 to 15 μ m). Extract also increased the levels of IL-1 β , GM-CSF [from 56 pg/ml and 53 pg/ml in irradiated group (control) to 59 pg/ml and 63 pg/ml (treated)] in experimental animals (Singh et al. 2007). Ethanol extract (200 mg/kg b.w.) displayed significant recovery of spleen weight (from 49% to 93%); apoptosis (from 19% to 2.8%); DNA fragmentation (from 43% to 20.4%). Extract also enhanced levels of IL-1 β and GM-CSF (from 56 pg/ml and 53 pg/ml to 59 pg/ml and 63 pg/ml; Singh et al. 2007). Aqueous extract of *T. cordifolia* showed radioprotective effects in Co-60 gamma irradiated Swiss albino mice. The control group of mice showed sickness, ruffled hair, and diarrhoea hence, died on 14th day. The oral administration of aqueous extract induced more survivability rate in irradiated rats (Pahadiya and Sharma 2003).

14.4.7 *Hepatoprotective Activity*

Liver is an important organ of human body and has roles in the maintenance, functioning and regulation of homeostasis in all the biochemical pathways of growth, defense mechanisms, digestion, energy supply and reproduction. Hence, it is an important to maintain healthy liver for healthy lifestyles. But sometimes healthy liver is exposed to various exogenous compounds like environmental pollutants, toxic drugs and alcohol which can eventually led to various liver diseases such as hepatocellular, cholestatic, or mixed type of liver disorders (Dange 2010). The effects of petroleum ether, ethanolic and aqueous extracts of leaf, stem and root of *T. cordifolia* were evaluated in carbon tetrachloride-induced liver damage in Wistar albino rats. Out of these tested extracts, the ethanolic extract exhibited significant ($P < 0.01$) hepatoprotective activity in the experimental animal models. Decrease in levels of serum bilirubin in animals after treatment with extract indicates the effectiveness of it in normal functional status of the liver. Reported findings suggest that the presence of flavonoids, alkaloids in ethanolic extract of *T. cordifolia* may be responsible for significant hepatoprotective activity (Kavitha et al. 2011). *T. cordifolia* ethanol extract (100 mg/kg/b. w. for 15 days) showed liver protected property in CCl₄ intoxicated rats. A significant decrease in serum levels of glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, bilirubin was observed in treated animals (Bishayi et al. 2002).

14.4.8 *Neuroprotective and Neuroregenerative Activity*

Neurodegenerative diseases are caused due to disruptions in functions of neurons of the central nervous system and often resulted in the deterioration of cognitive functions in individuals. The deterioration in cognitive functions includes gradual loss in memory, and motor coordination (Adewusi et al. 2010; Nieoullon 2011). Various plants and plant-derived products have been reported to be used in traditional medicine for neuroprotective, memory enhancement, and antiageing purposes (Iriti et al. 2010; Elufioye et al. 2017). Excitotoxicity is connected with the pathological processes of several neurodegenerative diseases viz., trauma, brain injury and memory loss. Butanol extract (20 µg/ml) of *T. cordifolia* showed significant ($P < 0.01$) neuroprotective activity against catastrophic glutamate-induced excitotoxicity (Sharma and Kaur 2018). Ethanolic extract (400 mg/kg) of aerial parts exhibited significant neuroprotective effects by enhancing the levels of dopamine against 6-hydroxy dopamine lesion rat model of Parkinson's disease (Kosaraju et al. 2014).

14.4.9 Anti-inflammatory Activity

Inflammation and pain are defense mechanism in the body of animals. The immune system perceives injured cells, allergens, and microbes, and it starts the process of healing. Non-steroidal drugs are normally used for the treatment of inflammations and pain; however, their prolonged use has adverse side effects (Bhadane et al. 2018). The anti-inflammatory effects of Guduchi Ghana (market product) and aqueous extract of *T. cordifolia* (50 mg/kg, p.o.) were compared in carrageenan-induced paw edema in albino rats. The Guduchi Ghana showed three-fold higher reduction in paw volume (33.06%) than aqueous extract (11.71%; Patgiri et al. 2014). Similarly, in other study, ethanolic extract exhibited maximum inhibition of edema (83.21%) at 500 mg/kg dose in male wistar rats. *T. cordifolia* demonstrated anti-inflammatory effects in both acute (Wesley et al. 2008) and sub-acute inflammations (Ghatpande et al. 2019). Methanolic extract (1 g/kg in a 2 ml volume) of aerial parts of *T. cordifolia* displayed anti-inflammatory activity against adjuvant arthritis model and bone damage in experimental animals. The antiarthritic activity difference was found significant ($P < 0.05$) in between extract-treated and control groups from day 17 to day 29. Methanolic extract also reduced the activity of IL-1 β , TNF- α , IL-6, and IL-17 producing T cells as well as production of chemokines significantly ($P < 0.05$). No major change was found in IFN- γ levels in animals of control group (Sannegowda et al. 2015). Aqueous extract of *T. cordifolia* protects dopaminergic neurons by inhibiting neuroinflammation in MPTP-induced Parkinsonian mouse model (Birla et al. 2019).

14.4.10 Immunomodulatory Activity

Immune system is the most complex defense system of body and it helps in protection of human body from various types of infections. Modulation in immune system of body by stimulation or suppression mechanisms, helps in maintaining disease-free lifestyles (Nfambi et al. 2015). Medicinal plants play significant roles in the prevention of humans from various pathogenic diseases hence, attempts are being made to search novel immunomodulator agents (Kumar et al. 2011). Guduchi (formulation of *T. cordifolia*; Himalaya Drug Company, India) showed maximum viability ($94 \pm 0.89\%$) in J774A macrophage cells at dose of 80 $\mu\text{g/ml}$ (More and Pai 2011). Guduchi ImP protein demonstrated significant mitogenic activity (~3-fold higher than control) against murine splenocytes (1–10 $\mu\text{g/ml}$). Due to presence of immunomodulatory protein (Guduchi ImP protein) in *T. cordifolia* stem, the guduchi may be used in Ayurvedic preparations for immunomodulatory actions (Aranha et al. 2012). Splenocytes were cultured in low concentration (1.56 $\mu\text{g/ml}$) of aqueous extract of *T. cordifolia* produced higher levels of IL-6 (as compared to unstimulated cells; Upadhyay et al. 2011). Ethanolic extract (8 mg/kg) significantly ($P < 0.001$) increased the neutrophil activity in *Oreochromis mossambicus* (Sudhakaran et al. 2006).

14.4.11 Wound Healing Activity

The healing of wounds is a complex process of repairing of injury of the skin and soft tissues. During healing, a process of inflammation occurs and the below layers of skin started to increase the formation of collagen layer. In later stage of development, the epithelial tissue is regenerated. Wound healing can be divided into four stages - inflammation, coagulation, proliferation, and remodelling at vicinity of the injury (Mutsaers et al. 1997; Garg and Paliwal 2011). It is often considered as a major problem in clinical practices (Kokane et al. 2009). The plant-derived extracts and pure compounds are gaining importance in wound healing (Ambika and Nair 2019). *T. cordifolia* cream (1 g powder added to 99 g of cold cream) was applied topically over the surface of wound of treated animals and healing was compared with untreated group (control). In treated animals, the process of epithelialisation of wound ($20.333 \pm 1.633\%$, percentage of original area in mm^2) was faster than control group of animals (24.500 ± 2.167 , percentage of original area in mm^2). The results were found significant ($P < 0.05$) in treated animals than control group (Girish and Kamdod 2012). The closure of wound excision in treated animals was not significantly different from that of control group on days 4, 8 but, on 16th (94.60 ± 0.40) and 18th day (99.40 ± 0.40), the significant ($P < 0.0001$) results were obtained. The breaking strength of 10 day old resutured incision in treated animals was significantly ($P < 0.0001$) higher (543.0 ± 34.63 g) than that of control (Hashilkar et al. 2016). Ethanolic extract significantly ($P < 0.05$) induced the healing process in resutured incision and dead space wound models also (Girish and Priyadarshini 2012; Singh et al. 2017).

14.4.12 Antimicrobial Activity

Plants and their products have been used as antimicrobial agents in traditional system of medicine. In developing countries, 70% population are treated with the plant-derived drugs (Mittal and Sharma 2014; Ojiako 2015). On first day, *Escherichia coli* (1×10 viable) cells were injected intraperitoneally for evaluation of antibacterial activity of *T. cordifolia* in adult Swiss albino mice. The aqueous extract (100 mg/kg/day by intragastric tube) of stem was administered to the albino mice. The mice mortality rate in control mice was 100% while in treated group (extract), authors reported mortality rate as 17.8% and 11.1% (gentamicin), respectively ($P < 0.001$; Thatte et al. 1994). Ethanolic extract of *T. cordifolia* leaf showed maximum inhibitory potency against *Klebsiella pneumoniae* (inhibition zone 12.0 mm) and *Pseudomonas aeruginosa* (inhibition zone 9.0 mm) at 400 $\mu\text{g/ml}$ concentration. Similarly, the ethanol extract of stem also exhibited maximum activity to *K. pneumoniae* (inhibition zone 15.0 mm) but displayed moderate effect against *P. aeruginosa* (inhibition zone 12.0 mm; 400 $\mu\text{g/ml}$; Jeyachandran et al. 2003; Agarwal et al. 2019). Ethanol extract (95%) of *T. cordifolia* showed significant antipyretic activity in Himalayan rabbits (50 mg/ml for 10 days; Vedavathy and Rao 1991; Prasad and Chauhan 2019).

14.4.13 Analgesic Activity

Pain is an unpleasant and sensory feeling associated with potential tissue damage and its control is the most important therapeutic priorities for human beings (Hassan et al. 2015). Many pharmaceutical drugs have been developed by pharmaceutical industries but they are associated with serious adverse effects such as ulceration, gastrointestinal bleeding, addictive potential, respiratory distress, drowsiness, nausea (Mate et al. 2008) hence, opportunities are available for researchers in searching of plant-derived molecules for the treatment of pain. Guduchi capsules (300 mg/kg; p.o.) showed statistically significant analgesia (60 min $P < 0.01$; 90 min $P < 0.01$; 120 min $P < 0.05$) in treated animals (Goel et al. 2014). Aqueous-methanol (30:70) extract of *T. cordifolia* stem (300 mg/kg) inhibited the number of writhes in dose-dependent manner (Hussain et al. 2015). Aqueous extract of *T. cordifolia* leaves (200 mg/kg) showed significant ($P < 0.001$) increase in the reaction time (pain threshold) in treated Swiss albino mice and Wistar rats (Siddalingappa et al. 2011).

14.4.14 Anti-psychotic/Antiepileptic Activity

Due to ambitious lifestyle, urbanization, and stressful environment, people are suffering by mental disorders. Psychosis is a one of the severe mental condition in which a sufferer experiences a distortion or loss of contact with reality and clouding of consciousness (Yadav et al. 2015). It is characterised by depression, delusion, hallucination, anxiety, sleep disturbance, thought disorder, social isolation and impaired role functioning (McNamara 1996). Methanol extract of *T. cordifolia* stem showed significant increase ($P < 0.05$) in serum prolactin while decrease in dopamine, superoxide dismutase and catalase levels in haloperidol and sulpiride treated animals ($P < 0.05$; Tiwari et al. 2019).

14.4.15 Antiulcer Activity

Ulcers are characterized by an open sore of skin or inflamed break in dead tissue of mucous membrane (Chan and Graham 2004). There are several types of ulcers found in human beings viz., mouth ulcer, esophagus ulcer, peptic ulcer, and genital ulcer (Paguigan et al. 2014). Out of these, peptic ulcer is commonly seen among many people, and characterized by erosion of lining of stomach or the duodenum (Vimala and Shoba 2014). Aqueous extract of *T. cordifolia* stem (400 mg/kg) showed significant ($P < 0.01$) decrease in gastric volume, total acidity and ulcer index in treated animals (Chandan et al. 2013). Ethanol extract of stem (600 mg/kg) demonstrated statistically significant ($P < 0.05$) better protection in aspirin and ethanol-induced ulcers in animals (Khan et al. 2015). Ethanol extract also showed significant protective activity against an 8 h restraint stress induced ulcerization (Sarma et al. 1995).

14.4.16 Antifertility Activity

Increasing human population throughout the world has detrimental effects on health care, food security, employment, education, housing, and environment hence, it suggests that fertility control is a problem but, in my opinion, it is an issue that needs to be tackled by public health (Mamatha et al. 2012; Devi et al. 2015). Ethanolic extract of *T. cordifolia* stem interrupted the process of spermatogenesis and formation of round spermatids (73.12%) in treated animals. It also reduced the serum testosterone levels and surface area of Sertoli cells when compared to controls ($P < 0.001$). Methanolic (70%) extract (100 mg/kg, given for 5 days) of its roots decreased the fertility index in fertile female albino rats (Fatima Rose et al. 2010).

14.5 Clinical Studies

The use of medicinal plants and their products has increased tremendously over the past few decades but incidents of adverse effects from usage of herbal medicines are being reported recently (WHO 2004, 2005). The adverse effects are attributable to several factors including wrong identification of plant species, adulteration of herbal products, and contamination with toxic compounds, improper use of herbal medicines by healthcare providers and use of herbal medicines concomitantly with other medicines therefore, rigorous clinical trials of medicinal plants and their products are to be conducted.

One tablet of *T. cordifolia* (500 mg) given to healthy volunteers with morning breakfast. As per clinical studies, no healthy volunteer complained of any negative effects on clinical chemistry and hematological measurements, blood pressure, heart rate, or body weight during and after the period of drug intake at the given dose and duration (Karkal and Bairy 2007). One tablet of *T. cordifolia* extract (500 mg) reduced the fasting blood glucose levels in the type-2 diabetic patients and also restored the changed liver functions of patients (Mishra et al. 2015a, b). Its pills demonstrated positive influence on surgical outcome (92% survival in treated patients and 40% in non-treated patients) of patients of obstructive jaundice (Rege et al. 1993). Capsules (formed from stem extract) improved the physical performance as well as suppressed over the activation of sympathetic nervous system which shows its adaptogenic property (Salve et al. 2015). Aqueous extract of *T. cordifolia* was found effective in relieving the clinical symptoms in patients of allergic rhinitis, cold and fever (Geeta et al. 2017). The test drug showed statistically significant increase in total leucocyte count ($P < 0.001$), absolute lymphocyte count ($P < 0.001$) and lymphocyte percentage ($P < 0.001$) as compared to placebo (Sharma and Sharma 2015).

14.6 Toxicological Effects

Most of the medicinal plants are non-toxic when used by humans but some are poisonous to both humans and animals causing damage to certain organs in the body (Okoye et al. 2014). As the global use of medicinal plants is growing and many more new plant-derived products are being introduced into the market so, proper attention is needed in safe use of medicinal plants for treatment of diseases (Ekor 2014). In alcoholics patients, the higher levels of γ -glutamyl transferase, aspartate transaminase, alanine transaminase, triglyceride, cholesterol, high density lipoproteins and low-density lipoprotein ($P < 0.05$) were reported but aqueous extract decreased their levels in treated patients (Sharma and Dabur 2016). Acute toxicity result showed that drug (*T. cordifolia* aqueous extract) did not produce any signs and symptoms of toxicity or mortality up to an oral dose of 2000 mg/kg in rats. Although the drug demonstrated mild to moderate adverse changes (in kidney, liver, intestine, and stomach) at therapeutic equivalent dose ($\times 10$ dose level) in a clinical trial (Gokarn et al. 2017). No acute toxicity was reported in male albino mice treated (p.o.) with aqueous extract (Sengupta et al. 2011). Embryo-toxic effect of aqueous extracts of leaf and bark was dependent on dose and time of exposure. Leaf extract (10%) showed maximum mortality (100%) while bark extract (10%) exhibited 33.33% mortality in treated animals (Zebrafish embryos; Romagosa et al. 2016).

14.7 Conclusions

Tinospora cordifolia is used in Indian traditional systems of medicine for the treatment of fever, urinary diseases, asthma, gout, diabetes, diarrhoea, and skin diseases. Its content of clerodane-furano-diterpene-glycoside showed promising anticancer properties. Its isoquinoline alkaloids exhibit antidiabetic activities against different models. It should be the task of further studies to fully characterize the crude extracts and transform to clinical efficacy the results obtained in the preclinical studies. It seems also important to conduct detailed chemical and pharmacological evaluation of *T. cordifolia*. More scientific studies are to be conducted on the standardization of Ayurvedic formulations based on the therapeutic and toxicological properties of ingredients. These results will help the development of new healthcare products in the future.

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Chapter 15

Recent Trends in Elicitation and Secondary Metabolic Pathway Analysis in Medicinal Plants: An Integrated Transcriptomics and Proteomics Approach



Archana Giri and Charu Chandra Giri

Abstract The medicinal plants are nature's treasured gift, which have immeasurable influence on human life and are the vital source of numerous structurally diverse pharmacologically active secondary metabolites. The secondary metabolites have immense pharmaceutical importance, where more than one-third of the drugs for human use contain these phytochemicals. *In vitro* plant tissue/organ culture is an emerging alternative to intact whole medicinal plant cultivation. A lot of research work has been done using various *in vitro* plant culture systems, such as cell, tissue, organ, and engineered cultures. "Elicitor is either a physical or chemical, biotic or abiotic agent or factor that enhances the synthesis of secondary metabolites in living cell system when introduced in small concentrations by imitating the natural stress situation". Elicitation not only increases secondary metabolite yield but also modulates the gene expression. Transcriptomics, deals with the study of entire pool of transcripts in an organism, at certain physiological or pathological stage. Frequently it is useful to unravel the correlation and regulation among DNA and proteins. A complete proteome analysis or the proteomics study furnishes the identification of different proteins involved in the biosynthetic pathways, at different developmental stages of plant. This is becoming a relevant strategy, as the secondary metabolites are developmental stage specific. Studies to increase the production of secondary metabolites in cell cultures using various approaches have been focused on the elucidation of biosynthetic pathways. This chapter deals with current trends in

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elicitation and secondary metabolic pathway scrutiny in medicinal plants, using an integrated transcriptomics and evidence-based proteomics approach.

Keywords Current trends · Elicitation · *In vitro* cultures · Medicinal plants · Metabolic pathways · Proteomics · Secondary metabolism · Transcriptomics

15.1 Introduction

The medicinal plants are the valuable gift from nature, which have immense influence on human life. Due to the increasing awareness on personal health, there has been a growing trend in the use of plant derived drugs and it has enhanced the dimensions of research on medicinal plants (Poddar et al. 2020). There is a rejuvenated interest in plant-based herbal drugs owing to fewer side effects in comparison to synthetic drugs. The medicinal plants synthesize enormous spectrum of bioactive chemical compounds used against array of human health problems and this has been well documented (Petrovska 2012). A detailed review has been published earlier on medicinal and aromatic plants depicting its role in health care (Máthé et al. 2015). In the developing countries, approximately 80% of people directly or indirectly depend on conventional medicine, which involves mostly the plant extracts and formulations. The WHO global report on traditional and complementary medicine 2019 indicates that currently, a project is underway to define and understand “integration as well as integrative medicine” to provide guidance to different countries on the criteria and best practices for integrating traditional and conventional medicine (T&CM) into national health systems in WHO Traditional Medicine Strategy (TMS) 2014–2023 (WHO global report on T&CM 2019).

15.1.1 Biosynthetic Pathway and Secondary Metabolites

The structurally diverse pharmacologically active metabolites of medicinal plants (MAPs) find extensive applications in pharmaceutical and cosmetic industries (Han et al. 2016). MAPs are a very huge resource of bioactive molecules, which in turn, can act as a template molecules for drug design and model development against many incurable diseases (Deng et al. 2016; Jain et al. 2016). Plant-based sources have a great potential for new drug discovery, therefore it is the need of the hour to have a system comprising the information on medicinal plant biodiversity, in an organized way.

The incredible adaptability of terrestrial plants to adverse environmental stress can be largely attributed to their broad metabolic fingerprint expressed in the form of their secondary metabolism. The secondary metabolic pathways are lineage specific. Secondary metabolites vastly outnumber the primary metabolites e. g. approximately about 5000 flavonoids, 21,000 alkaloids, and 22,000 terpenoids, however;

this is likely an underestimation of what is present in nature. The natural product secondary metabolites have immense pharmaceutical importance, where more than one-third of the drugs for human consumption (e.g., morphine, artemisinin, paclitaxel, and vincristine etc.) contain these phytochemicals.

The phytopharmaceuticals are synthesized via diverse complex and interconnected metabolic pathways. Inadequate information is available about these biosynthetic pathways, hence studies on the elucidation of these biosynthetic pathways related to the synthesis of these compounds is of great research interest (Tripathi et al. 2016).

The high number of sequenced genomes and the diversity of the biosynthetic pathways in bacteria, fungi, plants, and animals catalyzed the development of numerous bioinformatical methods to detect secondary metabolic pathways (Mutwil 2020). The studies related to biosynthetic pathways in the medicinal plants have been found to be very tiresome owing to their low concentrations and spatial and temporal regulation. Further, their highly complex multi-step nature makes its elucidation even more complicated. The mechanisms, even when equipped with the latest cutting-edge technologies are far from being capable of extracting all of the plant secondary metabolites.

The natural product secondary metabolites are produced by medicinal plants (in particular) in response to abiotic/biotic challenges, facing their very existence and survival. These metabolites have attracted the attention of pharmaceutical industries. The increased demand for these secondary metabolites has resulted in the destruction of natural resources. A case in point is that the chemical synthesis of these compounds is very laborious and difficult process, which is due to their complex chemical structures. Further, a little information is available about the biosynthetic pathways, the genes and gene regulation involved, and compartmentalization of these secondary metabolites in the plants (Erb and Kliebenstein 2020).

15.1.2 Plant Tissue Culture and Production of Secondary Metabolites In Vitro

The plant tissue/organ culture is an emerging alternative to whole medicinal plant cultivation as a source of secondary metabolites. A lot of research work has been done using various *in vitro* plant culture systems, such as cell, tissue, organ, and engineered cultures, as well as heterologous expression in microbial platforms to produce important plant secondary metabolites (Giri and Zaheer 2016; Chandran et al. 2020). The plant tissue culture and genetic engineering techniques are used as a powerful tool for the production of large amounts of secondary metabolites in bioreactors (Khanahmadi and Paek 2017; Werner et al. 2017; Yancheva et al. 2019; Valdiani et al. 2019). Moreover, the genomic, transcriptomic, proteomic and metabolomic tools, along with recombinant methods to overexpress, silence or disrupt one or more regulatory genes of the pathway of interest open up new exciting possibilities of metabolic engineering (Nielsen et al. 2019).

15.1.3 *Elicitor and Impact of Elicitation on Pathway and Secondary Metabolites*

“Elicitor is either a physical or chemical, biotic or abiotic agent or factor that enhances the synthesis of secondary metabolites in living cell systems when introduced in small concentrations, by imitating the natural stress situation”. Since the discovery of elicitors in 1968, the elicitors have been recognized as compounds stimulating any type of plant defense mechanisms indirectly helping overall growth and development. Elicitation not only increases secondary metabolite yields, but also modulates the gene expression. When plants undergo certain stress conditions, they produce secondary metabolites, in excess amounts. Once the elicitor is recognized by the receptor present on plant cells, a sequence of cytological and biochemical processes will occur inside the plant cells (Fig. 15.1).

These activated protein kinases intern activates mitogen activated protein kinases (MAPKs) cascade which upon a series of reactions transports to nucleus and activates transcription factors by phosphorylating specific sequence. Elicitation also activates G-protein which mediates Ca^{+2} release by producing secondary messengers (Ins P3, DAG) which trigger nitric oxide and act on adecanoid signaling pathway. Later on, a series of reactions occur such as acidification of cytosol by activated NADPH oxidase, cytoskeleton reorganization production of ROS followed by pathogen related protein accumulation.

All these reactions together activate transcription of defense response genes and causes the synthesis of JA and SA. These JA and SA acts as secondary messenger. There are some mechanisms hypothesized by many researchers to explain the elicitation process. The “Elicitors” bind to the receptor present on plasma membrane and allow Ca^{+2} to the cytoplasm from the extra cellular environment. Elicitors activate protein kinases (Mitogen Activated Protein Kinases (MAPKS)) and induce rapid changes in protein phosphorylation. The inactivation of $H^{+}ATPase$ results in acidification of cytoplasm. The increase in the extra cellular pH, decreases the membrane polarization and production of ROS and H_2O_2 which is involved in the production of bioactive fatty acid derivatives. This acts as secondary messengers which is involved in the transcriptional activation of genes (Giri and Zaheer 2016).

The different metabolic pathways, such as primary and secondary, manage the synthesis of diverse countless chemical compounds in plants. Primary pathways are dominant, directly involved in the growth, development of plants and participate in several vital metabolisms explicitly photosynthesis, respiration, transpiration etc. The combination of plant tissue culture and elicitation is one of the widely used approach for the enhancement of secondary metabolites (STMs) content using different culture systems (Choi et al. 2005; Giri and Zaheer 2016; Gao et al. 2018; Kar et al. 2019; Jeong et al. 2020; Koul and Sharda 2020).

Further, it has been established that chemical elicitor are having immense multi-tasking ability to drive a number of cellular functions at both biochemical and genetic level (Caarls et al. 2015; Giri and Zaheer 2016; Kar et al. 2019; Chandran et al. 2020). So far as our own experience is concerned the elicitation of *in vitro*

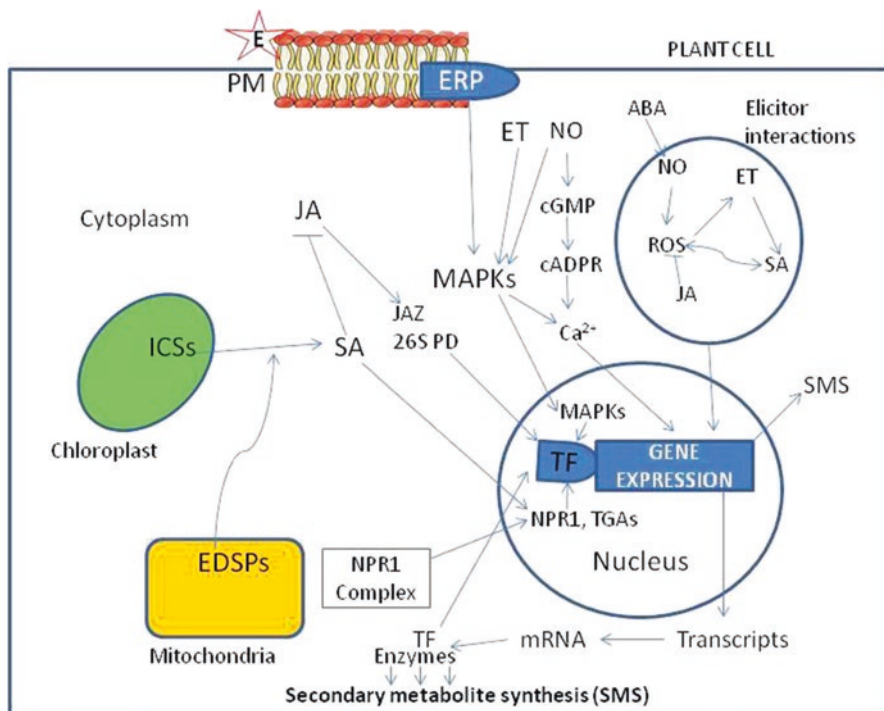


Fig. 15.1 Diagrammatic depiction of elicitors and their mode of action mimicking possible elicitation mechanism using elicited plant cell, tissue and organ cultures *in vitro*. ABA abscisic acid, Ca²⁺ Calcium ion, cADPR cyclic adenosine diphosphoribose, cGMP cyclic guanosine monophosphate, E elicitor, EDSPs enhanced disease susceptibility proteins, ERP elicitor receptor perception, ET ethylene, ICSs isochorismate synthases, JA jasmonic acid, JAZ jasmonate zim domain, MAPKs mitogen activated protein kinases, NO nitric oxide, NPR1 non-expressor of pathogenesis-related genes 1, PM plasma membrane, ROS reactive oxygen species, SA salicylic acid, TF transcription factors, TGAs leucine zipper transcription factors, 26S PD 26S proteasomal degradation Source: Giri and Zaheer (2016) *Plant Cell Tiss Organ Cult* 126: 1–18

tissue/organ cultures with signal molecules, a number of secondary metabolites have been improved to an accountable extent (Suryakala et al. 2012; Zaheer and Giri 2015, 2017; Zaheer et al. 2016; Giri and Zaheer, 2016; Bhuvneswari et al. 2012, 2013, 2014; Sandhya et al. 2021). The basic knowledge of elicitation mechanism at biochemical, genetic and molecular level will be helpful to manipulate secondary metabolic pathways to develop quality foods and pharmaceuticals in healthcare for practical applications (Teixeira et al. 2014; Giri and Zaheer 2016; Chandran et al. 2020; Nabi et al. 2021).

15.2 Transcriptomic-Pathways and Secondary Metabolites

The transcriptome, which deals with the study of entire pool of transcripts in an organism at certain physiological or pathological stage, is obligatory to unravel the correlation and interaction among DNA and protein. In the recent past, next-generation sequencing (NGS) has revolutionized our understanding of transcriptome in a fast, efficient mode. The adoption of NGS, has overwhelmingly enhanced the efficiency and outcomes towards elucidation of genes responsible for production of these bioactive secondary metabolites in medicinal plants. It involves sampling of plant material, cDNA library construction, deep sequencing followed by bioinformatics tools to pull together massive, incomplete data to characterise and generate information. The transcriptomics archive all the transcripts, that include mRNAs, small RNAs, and micro-RNAs, in target organisms deciphering the structural composition of genes and their related, posttranscriptional modifications, expression level study at different stages, or to understand the impact of various environmental factors. However, it is intrinsically challenging mission to completely attain the transcriptome of an individual plant (Han et al. 2016).

The knowledge of genomic and transcriptomic tools has witnessed a rise in the past decade. Next generation sequencing (NGS) technologies are being utilized for generation and analysis of enormous data of considerable significance. Among these, the de novo transcriptome sequencing has become one of the most sought-after tools for an easy resource to gain information (Oksman-Caldentey et al. 2004; Sangwan et al. 2013, 2014). With the advent of this technology, it is possible to go for a comprehensive analysis of various secondary metabolic pathways of medicinal plants. (Gupta et al. 2013; Sangwan et al. 2013; Tripathi et al. 2016; Luo and Chen 2019). The NGS has sparked a revolution in gene expression analysis, providing the capability to characterize transcriptomes from virtually any organism or cell type with unprecedented resolution on a massive scale for the identification of biosynthetic pathway genes.

15.2.1 *Transcriptomics in Relation to Secondary Metabolic Pathway*

The transcriptomics through RNA-seq technique has more advantage than whole genome sequencing owing to its rapid data output, less complexity, and cost effectiveness. RNA-seq technique is also advantageous for huge genome size. Approximately 20,000–60,000 genes can be sequenced out of which 15–25% contribute towards secondary metabolic pathway (Góngora-Castillo et al. 2012). Thousands of genes can be monitored simultaneously and data analysis tools enable us to decode the metabolic complexity of medicinal plant at genetic level. Secondary metabolites have diversity in their synthesis and in chemical structure, hence

transcriptomics can be utilized for pathway elucidation to understand their origin, biosynthesis and functional aspects (Tripathi et al. 2016).

15.2.2 Transcriptomics Techniques: A Brief Reference

The different advanced techniques and recent innovations has put transcriptomics study as emerging field to decipher unknown metabolic pathways. The mention of a few such as second-generation sequencing technologies following SGS platforms, produce large amounts (typically millions) of short DNA sequence reads have provided unparalleled opportunities for high throughput transcriptome sequencing. The 454 Sequencing by pyrosequencing approach, a sequencing-by-synthesis technique measuring the release of inorganic pyrophosphate (PPi) by chemiluminescence is another lead in this direction. The version of SOLiD system included SOLiD 3, SOLiD 4, and SOLiD5500xl, which was available and can produce 30–45Gb of sequence data per 1-day run. The first Solexa sequencer, the Genome Analyzer, was launched in 2006, followed by HiSeq2500, HiSeq2000, HiSeq1000, HiScanSQ, MiSeq, MiniSeq/Next Seq, and Nova Seq instruments. In third-generation sequencing technologies in comparison with the second-generation sequencer, the third-generation sequencer is completely revolutionary and is developing toward high-throughput, low-cost, and long read length direction. It has several advantages viz. single-molecule sequencing improves sample throughput and detection speed (Han et al. 2016).

The RNA direct sequencing reduces system error following in vitro reverse transcription and long-segment sequencing leads to continuity of DNA polymerase. In the HeliScope Single-molecule Sequencer HeliScope, the pioneer of the third-generation sequencer is the first single-molecule sequencer to be adopted. The PacBioRS combines a novel single-molecule sequencing technology, based on a novel Single Molecule Real Time (SMRT) technology (Han et al. 2016; Luo and Chen 2019).

15.2.3 Transcriptomics Case Studies: An Ephemeral Orientation

In *Arabidopsis* the signaling molecules alter gene expression depending on the plant organ (root or leaf) based on their application and signaling molecules and secretion of root-exuded phytochemicals by regulating transporters (ABC, MATE, MFS), transcription factors and metabolic biosynthesis for pathway elucidation. However, further studies are needed to dissect the specific genes involved in cross-talk of the signaling molecules, mediated defense responses, biosynthesis, transport and regulation of secondary metabolites (Badri et al. 2008). In a related study, Naoumkina

et al. (2008) analysed the transcription factors responding to yeast Extract elicitor (YE) or methyl jasmonate (MJ). Out of 502 differentially expressed TFs, WRKY and AP2/EREBP gene families were over-represented among YE-induced genes whereas Basic Helix-Loop-Helix (bHLH) family members were overexpressed among the MJ-induced genes. The Jasmonate ZIM-domain (JAZ) transcriptional regulators were highly induced by MJ treatment. To investigate potential involvement of WRKY TFs in signalling, four *Medicago* WRKY genes were expressed in tobacco. The levels of soluble and wall bound phenolic compounds and lignin were increased in all cases. Their results confirm that *Medicago* WRKY TFs play important role in orchestrating metabolic responses to biotic stress. The details on elicitation driven transcriptomics is summarized in Table 15.1.

The RNA-Seq was carried out to evaluate differential gene expression under different light treatments which effectively regulated flavonoid profiles, inducing a faster accumulation of phenolic compounds as a response to switching from white to blue light. The results demonstrated that HY5, MYB12, ANR and LAR were differentially regulated under light/dark conditions and could be targeted by overexpression aiming to improve catechin synthesis in cell cultures. The RNA-Seq analysis of cacao cells cultured under different light conditions provides a platform to dissect key aspects into the genetic regulatory network of flavonoids. These light responsive candidate genes can be used further to modulate the flavonoid production in in vitro systems with value-added characteristics (Gallego et al. 2018). In *Cyclocarya paliurus* jasmonic acid (JA) played important roles in the elicitation as signal molecules. Based on series of experiments of NO quenching by C-PTIO, H₂O₂ blocking by DMTU, and JA synthesis inhibition by IBU and NDGA, together with exogenous NO, H₂O₂ and JA addition experiments, it was deduced that ANE improved triterpenoids synthesis in the suspension cultured *C. paliurus* cells via a complex signal transduction network, in which three deduced and three hypothetical signal transduction pathways might be involved. JA was not only the junction of NO and H₂O₂ signal pathways, but also the critical point in the whole signal network. RNA-seq analysis showed that a total of three candidate JA synthesis pathway genes including one LOX and two OPR were found to be significantly up-regulated under the ANE stimulation, along with five down-regulated JAZs and one upregulated JAR1 regulating to JA signal transduction (Wang et al. 2020).

The transcriptome profiling of *Lycoris aurea* seedlings was carried out with the methyl jasmonate (MeJA) treatment to uncover the molecular mechanisms regulating plant secondary metabolite pathway by using short reads sequencing technology (Illumina). The DEGs encoding key enzymes involved in the secondary metabolite biosynthetic pathways, transcription factors, and transporter proteins were identified (Wang et al. 2017a, b). *Panax ginseng* adventitious roots were treated with MeJA to identify the genes responsible for ginsenoside biosynthesis. The transcriptome analysis identified genes related to ginsenoside biosynthesis (Um et al. 2017).

Table 15.1 Elicitation facilitated transcriptomics and gene regulation using *in vitro* cultures

Plant Name	Culture type	Elicitor	Gene regulation	References
<i>Psammosilene tunicoides</i>	HR	SA	Full length transcriptome data for saponins	Su et al. (2021)
<i>Cyclocarya paliurus</i>	SC	<i>Aspergillus Niger</i>	Signal molecules crosstalk in triterpenoid synthesis	Wang et al. (2020)
<i>Theobroma cacao</i>	SC	Light and dark	Transcriptomics for flavonoid production	Gallego et al. (2018)
<i>Panax ginseng</i>	AR	Me JA	Ginsenoside biosynthesis-related genes	Um et al. (2017)
<i>Lycoris aurea</i>	Seedlings	Me JA	TFs, and transporters	Wang et al. (2017a, b)
<i>Rehmannia glutinosa</i>	HR	SA	Identification of Acteoside biosynthesis genes	Wang et al. (2017a, b)
<i>Arabidopsis</i>	Seedling, ML, R	<i>Bacillus amyloliquefaciens</i>	Comparative digital gene expression	Hao et al. (2016)
<i>Sugarcane</i>	SC	<i>Colletotrichum falcatum</i>	Transcriptome analysis using pathogen elicitor.	Rahul et al. (2016)
<i>Panax ginseng</i>	AR	Me JA	Putative Ginsenoside biosynthesis and transport genes.	Hongzhe et al. (2015)
<i>Taxus _ media and Taxus globosa</i>	SC	CD and CORO	Gene transcription and taxane production	Ramirez-Estrada et al. (2015)
<i>Isatis indigotica</i>	HR	Me JA	Gene-to-metabolite network for biosynthesis of lignans	Chen et al. (2015)
<i>Taxus baccata</i>	SC	Me JA	Transcript profiling of b-phenylalanine-CoA ligase	Ramirez-Estrada et al. (2016)
<i>Salvia miltiorrhiza</i>	HR	Y E	Metabolomics and transcriptomics of tanshinone biosynthesis	Gao et al. (2014)
<i>Vitis vinifera</i>	SC	CD and me JA	Transcriptional response for phenylpropanoids and stilbene biosynthesis.	Almagro et al. (2014)
<i>Taxus x media</i>	SC	Me JA	Transcriptome re-programming	Sun et al. (2013)
<i>Taxus cuspidata</i>	SC	Me JA	Identification and expression analysis of MeJa responsive ESTs	Lenka et al. (2012)
<i>Taxus chinensis</i>	SC	Me JA	Transcriptional profiling	Li et al. (2012)
<i>Nicotiana sp.</i>	SC	Cr	Transcriptome analysis of new potential actors of calcium-dependent and calcium-independent plant defense pathways,	Amelot et al. (2012)

(continued)

Table 15.1 (continued)

Plant Name	Culture type	Elicitor	Gene regulation	References
<i>Medicago truncatula</i>	SC	Y elicitor	Integrated metabolite and transcript profiling	Farag et al. (2009)
<i>Arabidopsis sp.</i>	RC	Me JA, SA, NO	Transcriptome analysis for signal transduction, metabolic regulation and secretion.	Badri et al. (2008)
<i>Medicago truncatula</i>	SC	Y,me JA	Transcription factors for metabolic reprogramming	Naoumkina et al. (2008)

AR: Adventitious roots; CD: Cyclodextrins CORO: Coronatine Crgn: Cryptogein HR: Hairy roots MeJA: Methyl jasmonate ML: Mature leaf NO: Nitric oxide RC: Root culture; SA: Salicylic acid; SC: Suspension culture Y: Yeast; YE: Yeast extract.

15.3 Proteomic-Pathways and Secondary Metabolites

The mechanism behind the elicitation process at the functional level is incalculable. The solution for this can be envisaged by adopting proteomics approach. Proteins are large, complex nitrogenous molecules which play various essential roles in the functioning of a cell. They regulate most of the activities in cells and are required for the structure, function, and regulation of the several metabolic pathways in plants. All biological processes are regulated mostly at protein level. It can be at translational level or post translational level (Kosová et al. 2018; Perrar et al. 2019).

The proteins serve a basis of the tight homeostasis that characterizes any biological system. The insights into the protein localization and their interactions with other proteins at different stages of the plants will help in understanding the native biological activities within. The understanding of stress response is very important in plants which lead to the production of many secondary metabolites. These are useful in the synthesis of economically important compounds such as medicines, flavourings, and recreational drugs (Shakeel et al. 2016; Kallscheuer et al. 2019). It becomes inevitable to study the biosynthesis of secondary metabolites and their regulation and simultaneously, signaling mechanisms involved in stress can also be understood in non-model plants (Martínez-Esteso et al. 2015).

15.3.1 Proteomics in Relation to Secondary Metabolic Pathways

A complete proteome analysis furnishes the identification of different proteins involved in the biosynthetic pathways at different developmental stages of plant. It becomes a relevant strategy as the secondary metabolites are developmental stage specific. In a most recent study, Canola roots were treated with salt as abiotic stress revealed the involvement of proteins in glycolysis, stress, redox regulation, energy metabolism and transport majorly involved in production of glucosynolates

(Kholghi et al. 2019). The cellular regulatory processes are driven by the interactions between proteins and metabolites in organisms. The concept of the solitary role of substrates, products, or cofactors of enzymes, defense mechanism and growth-regulatory signal compounds has now been reformed by the recent findings which highlighted that plentiful plant metabolites hold many biological events.

15.3.2 Proteomic Techniques: A Brief Outline

The principle behind the technique is based on mass to charge ratio (m/z) value. This technique is sensitive, robust and inexpensive compared to high throughput techniques. Most of the proteomics data was produced in the literature through this approach. Though several technologies have been developed to identify and characterize metabolite–protein interactions, the systematic implementation of such methods in the plant field remains limited. Recent studies reported unexpected roles of metabolites, highlighted by the observations that several compounds with a supposed specific plant defense function also act as plant growth regulators. A systematic in-depth mapping of interactions between metabolites and their target proteins would lift our understanding of cellular homeostasis and plant physiology to unprecedented levels.

The proteomics term was thought up by Marc Wilkins in 1994. The proteomics is the mass-scale learning of proteins, particularly with reference to their structure and function. The methodical investigation of all of the proteins in totality in a cell, tissue, or organism is popularly known as proteomics. The proteome is the complete set of proteins expressed by a genome, cell, tissue or organism at a particular point and during the growth and development of the organism. The protein classification is envisaged by studying the expression, cell map or interaction pattern at functional and structural level.

The different steps in proteomic study include sample collection, handling and storage of proteins, protein separation, identification and characterization. An array of evolving techniques has been adopted in proteomics study which includes 2-D gel electrophoresis, ICAT (isotope coded affinity tags), Mass spectroscopy, MALDI, PMF (Peptide mass fingerprinting) and the ICAT is particularly useful for comparing relative protein abundance between two samples. Further, mass spectroscopy is used for protein identification and the mass spectrometer separates proteins according to their mass-to-charge (m/z) ratio used for determining masses of particles, for determining the elemental composition of a sample or molecule. The molecule is first ionized which results in the formation of charged particles. The ions are separated according to their (m/z) ratio in an analyzer by electromagnetic fields.

In addition to this the MALDI Matrix-assisted laser desorption/ionization is a soft ionization technique is used in mass spectrometry, allowing the analysis of biomolecules and large organic molecules. The PMF technique is used for rapid protein identification. The database for proteomics study includes the PRIDE-The PRIDE Proteomics which is a centralised public data repository for proteomics data availability to all. Recently, the PROTEICdb access-PROTEICdb is a tool developed

by INRA PROTIcDb provides means for data storage, enrichment, and dissemination of proteomics data. The PPDB stores experimental data from in-house proteome and mass spectrometry analysis, curated information about protein function, protein properties and subcellular localization.

15.3.3 Proteomic Studies: A Passing Angle

The proteomics is useful to study the candidate proteins/enzymes participating in different secondary metabolite synthesis in medicinal plants in general (Desgagné-Penix et al. 2010; Heazlewood 2011; Jia et al. 2019). In recent years, mass-spectrometry (MS) based proteomics helpful in protein identification, quantification and monitoring multiple reactions at the same level (Girolamo et al. 2013; Alonso-Gutierrez et al. 2015; Smith et al. 2019). The forward genetics is an approach used to identify genotype responsible for phenotype. At the outset, different techniques like mutagenesis followed by genetic mapping employed for study of plant secondary metabolites and elucidation of biochemical pathways. Extensive research has been done in *A. thaliana* using forward genetic approaches. Plants where genetic background is not very clear in non-model plants, it is difficult to map the genes without knowing the genotypes. In such situations, elicitation strategy can be exploited for differential gene expression studies particularly proteins. Differential expression of proteins upon elicitation is listed in Table 15.2.

The proteomic approaches have been used to identify the regulatory enzymes involved in secondary metabolism and their biochemical pathway elucidation (Jacobs et al. 2000). These unidentified genes can be studied at the functional level i.e. protein. Proteome analysis can be done by two- dimensional electrophoresis (2-DE) and Mass Spectrometry (MS) for quantification and identification of proteins. It is a combined technique to separate and visualize proteins and MS for protein identification (Gygi et al. 2000). These methods were used to identify and quantify proteins in non-model legume *Medicago truncatula* cell suspension culture (Lei et al. 2005; Gallardo et al. 2007). Recently, label free quantitative proteomics plays an important role as labelled proteomics limits to specific proteins. However, using label free quantitative proteomics, discovered a greater number of proteins and it expands the scope to understand the biosynthetic pathways in a better way (Abdallah et al. 2012). Proteome of different plants were studied using various explants, at different developmental stages, with variety of elicitors. In post genomic era, contemporary techniques in proteomics help in understanding morphological and physiological changes in plants (Ruiz-May et al. 2019).

In silico analysis of protein, using bioinformatics tools helps in identifying the preliminary physicochemical parameters which are needed for knowing the nature of protein and their properties. The primary, secondary and tertiary structure provides the structural information of a protein. In a most recent report, *Lens culinaris* L, revealed cloning of six defensin proteins, *in silico* analysis showed secondary structure, motifs and phylogenetic analysis which might be useful in manipulating

Table 15.2 Elicitation driven proteomics and differential expression of genes and proteins

Culture system	Plant species	Elicitor	Genes / Proteins	Mode of Manipulation	Compound of interest	References
SC	<i>Arabidopsis</i> sp.	Fumonisin B1	Peroxidase 52, subtilase-like, serine protease 1.7, and phospholipase c-like 1	UR	–	Smith et al. (2021)
Leaves	(<i>Lactuca sativa</i> var. capitata)	MJ	Hydroxybenzoic acids, Hydroxycinnamic acids, Galloyl hexose	UP & DR	Polyphenols and carotenoids	Jesus Omar et al. (2020)
Leaves	<i>Rubus fruticosus</i> cv. <i>Loch Ness</i>	Bacillus QV15	Flavonol-3-hydroxylase Quercetin-3-O-glucoside	UP & DR	Flavonols and anthocyanins	Gutiérrez-Albánchez et al. (2020)
Seedlings	<i>Glycine max</i> L. Merr.	Shade	Porphyryn and chlorophyll metabolism, photosynthesis-antenna proteins and photosynthesis	UPR & DR	–	Fan et al. (2019)
Seeds	<i>Ricinus communis</i> L.	Cold	Protein synthesis, stress-related proteins, fatty acid biosynthesis	UPR & DR	–	Wang et al. (2019)
Plants	<i>Lepidium draba</i> L.	Glucose	Unique proteins, photosynthesis, chaperones, energy related metabolism and signalling	UPR	Soulforaphane	Rezaee et al. (2018)
Leaves	<i>Persicaria minor</i>	MeJa	Stress response mechanism, lipid metabolism, secondary metabolite production, DNA degradation and cell wall degradation	UPR&DR	–	Aizat et al. (2019)
SC	<i>Sorghum bicolor</i>	Sorbitol	ECM related proteins	UPR	–	Ngara et al. (2018)
Canes	<i>Vitis vinifera</i> <i>Vitis rupestris</i>	<i>Neofusicoccum parvum</i> , <i>Diplodia seriata</i>	Different classes of proteins	UPR	Stillbene	Stempien et al. (2018)
Leaves	<i>Zostera muelleri</i>	Light	Protein folding, sorting and degradation functions	UPR	Light influencing pathways	Kumar et al. (2017)

(continued)

Table 15.2 (continued)

Culture system	Plant species	Elicitor	Genes / Proteins	Mode of Manipulation	Compound of interest	References
Plant	<i>Glycine max</i>	Male sterility	Energy supply, unbalance of protein synthesis and degradation, disruption of flavonoid synthesis, programmed cell death, abnormalities of substance metabolism	DR	-	Li et al. (2016)
Seedlings	<i>Glycine max</i>	Al ₂ O ₃ , ZNO, AG NP	Oxidation-reduction, stress signaling, hormonal pathways related to growth and development	UPR	-	Hossain et al. (2016)
SC	<i>Vitis vinifera</i> L. cv.	<i>Botrytis. cinerea</i>	Defense, oxidative stress, cell wall formation and protein folding	UPR	-	Dadakova et al. (2015)
SC	<i>Panax</i> sps	SA	Different classes of prtns	UPR	Ginsenosid	Sun et al. (2014)
SC	<i>Taxus cuspidata</i>	MeJa	Different classes of prtns	DR growth UPR cell cycle	Paclitaxel	Patil et al. (2014)
SC	<i>Ginkgo biloba</i>	NaCl, SA	GbPrx09, GbPrx10	DNR & UPR	Lignins	Novo-Uzal et al. (2014)
SC	<i>Catharanthus roseus</i>	Artemisinic acid	TDC, G-10H, T16H, D4H	UPR	VD VB	Liu et al. (2014)
SC	<i>Silybum marianum</i>	CDs, MeJa	ABC or MATE transporters	UPR	FLN	Prieto and Corchete (2014)
SC	<i>Scutellaria baicalensis</i> Georgi	GA3, ABA, and NaCl	Dioxygenases (SbCCD1, SbCCD4, and SbNCD)	UPR	Carotenoid	Kim et al. (2013)
SC	<i>Panax ginseng</i>	JA, DCCD	SQS, SEAND DS	UPR	GSN	Huang and Zhong (2013)
SC	<i>Taxus Media</i>	Coronatine MeJa	TXS, T13, OH, T7, OH, T2, OH, DBAT, BAPT, DB TNBT	UPR	Taxane	Omrubia et al. (2012)
SC	<i>Taxus cuspidata</i>	MeJa	Diff. Proteins		Taxol	Lenka et al. (2012)

Culture system	Plant species	Elicitor	Genes / Proteins	Mode of Manipulation	Compound of interest	References
SC	<i>Vitis vinifera</i>	-	Diff. Proteins	-	Resveratrol	Sharathchandra et al. (2011)
<p>DCCD- N,N -dicyclohexylcarbodiimide; T13,OH, taxadiene 13 -hydroxylase; T2,OH,- taxane 2 -hydroxylase; T7'OH, taxane 7 -hydroxylase; DBAT, 10-deacetylbaocatin III-10-O-acetyltransferase; BAPT - baccatin III-3-amino,13- phenylpropanoyltransferase; DBTNBT, debenzoyltaxol N-benzoyl transferase.; TXS- taxadiene synthase; CAOMT- caffeoyl-O-methyltransferase, 4CL- 4-coumarate: CoA ligase; CCOMT- caffeoyl CoA o-methyltransferase; TNMTTetrahydroprotoberberinecis-N-methyltransferase; TYDC- tyrosine/dopadecarboxylase; 4OMT 3-hydroxy-Nmethylcoclaurine 4-O-methyltransferase; 6OMT noroclaurine 6-O-methyltransferase; 4OMT- 3-hydroxy-(S)-Nmethylcoclaurine-4'-O-methyltransferase; CNMT- (S)-coclaurine N-methyltransferase; 6OMT- (S)-noroclaurine-6-O-methyltransferase; DHBO dilydrobenzophenanthridine oxidase; DBPA Dihydroform benzophenanthridinealkaloid;BPA - benzophenanthridine alkaloid; BBE - Berberine bridge enzyme; PAL- phenylalanine ammonia-lyase; CHS: chalconesynthase; CHI-chalcone isomerase; TDC- tryptophan decarboxylase; STR -stricotosidinesynthase; HMGR - HMG-CoA reductase; XEGxyloglucan endo-1,4-D glucanases; UPR- up regulated; DR- down regulated;CSD- citrus sudden death; GSN- Ginsenoside; PCL Pacilitaxel; FLN Flavonolignan; VB vinblastine; VD vindoline; GABA c-aminobutyric acid;</p>						

these genes (Drikvand et al. 2019). In *Eriobotrya japonica* Lindl., heterotrimeric G protein α subunit ($G\alpha$) gene (EjLGA1) was cloned, expressed and *in silico* analysis revealed $G\alpha$ subunit nucleotide and amino acid sequences are of highly conserved among 48 other seed plants, demonstrating that the loquat $G\alpha$ subunit likely has similar functions in other plants (Wu et al. 2018).

The functional interactions of a protein can be studied i.e. interactome studies which assist in crucial insights of plant growth, development, physiological and biochemical regulation. The major breakthrough using bioinformatics is drug designing for various diseases. Docking and ligand binding site predictions will further help in understanding the role of protein. Plants contain various pharmacologically important compounds and those are unique to each plant. There is a necessity in studying each plant with beneficial characters. In our literature survey from past to present, proteome analysis to understand the physiological, biochemical and molecular changes in plants such as suspension-cultures of ginseng cells were elicited with salicylic acid, fifteen different classes of proteins were induced by salicylic acid. They were involved in defense and stress response, energy metabolism, signal transduction/transcription, protein synthesis, metabolism and photosynthesis (Jiaman et al. 2014).

Using proteomic approaches, the biochemical pathway regulatory enzymes and their mechanisms were studied. This was reported in *Taxus X media* suspensions where cells treated with methyl jasmonate greatly promoted downstream gene regulation in the production of paclitaxel and baccatin III. Further, vanadyl sulfate had the same effect on the production of 10-deacetyl baccatin III simultaneously there was a down regulation in the HMGR (Bonfill et al. 2003). Likewise, in *Glycine max* seedlings treated with different elicitors such as Al_2O_3 , ZnO and silver nano particles enhanced the production of bioactive compounds. Proteome analysis revealed that type of elicitor plays a major role for elicitation (Hossain et al. 2016). In plant seedlings grown in shade or diffused light areas as elicitor, the photosynthesis related proteins were mostly influenced. However, in chemical elicitor treated seedlings the stress and secondary metabolite related proteins were regulated (Fan et al. 2019). Potato plants were infected with *Phytophthora infestans* to understand the developmental changes during leaf blight disease. Proteins up to 80% were up-regulated in the early stages of infection and 61% were down-regulated in late stages (Xiao et al. 2019). Similarly, *Ricinus communis L* a temperate plant the resistance to cold was analysed using proteome technology. It was observed that there was overall up-regulation of protein synthesis, cold stress related and fatty acid biosynthesis which conferred resistance to cold (Wang et al. 2019). Strawberry plants were treated with ozone to study the fruit senescence. The proteome analysis revealed 382 proteins were differentially expressed in four different treatments with delayed senescence upon ozone treatment and were linked to physiological traits of strawberry fruit senescence (Chen et al. 2019).

15.4 Proteomic Studies for Elucidation of Secondary Metabolite Pathways

15.4.1 Total Proteome Analysis of *Andrographis paniculata* Using Capillary LC-Q-TOF (MS/MS)

The average of the triplicate run was considered for individual abundant proteins. The criterion to identify a protein was validated where at least the presence of five biological replicates were selected and evaluated in duplicate run for a single peptide.

The one-dimensional (1D) analysis of crude proteome of jasmonic acid (JA) treated plants by silver nitrate staining showed that the proteins mostly present in the range of 20KDa to 40KDa, approximately. No significant visible difference was observed between control and treated samples in the protein profile and content in one-dimensional gel electrophoresis. PLGS software for peptide identification was considered with peptides having PLGS score greater than 100. The chromatograms based on MS/MS data were combined to search with a taxonomic restriction of Viridiplantae (green plants) against Universal Protein Resource (UniProt) with default parameters. Heat map was drawn for the triplicate run and shown in. In ratio 1, half of the proteome was showing 0.5-to-1-fold increased expression, the other half proteome occupied by 0.5-to-1-fold expression. In ratio 2, major proteome showed 0.5-to-1-fold decreased expression. In ratio 3, three parts proteomes were covered with 0.5-to-1-fold expression. The average of triplicate run for abundant proteins was selected and the fold expression was monitored.

Finally, 58,757 peptides were identified for control and 58,063 for treated sample using PLGS software. The analyzed data was submitted to PRIDE with ID PXD010682 (Bindu et al. 2020).

15.4.2 Peptide Assembly into Proteins from Proteome Data of *A. paniculata*

The contigs up to 3266 were assembled in control and 2494 for jasmonic acid (JA) treated proteome of *A. paniculata*. A total of 684 proteins were more in treated sample. A total of 2216 proteins were differentially expressed between control and treated samples. About 201 proteins were highly up regulated ranging from 2.0 to 81 fold. A total of 1393 were up regulated in a range between 0.5 and 1.0 fold. About 621 proteins were down regulated ranging from 0.02 to 05 fold. The analysis for integrity of the assembled contigs using Blast2GO revealed sequence distribution that the number of GO terms with sequences length mostly fall in the category between 60–100 amino acids. InterPro scan repeats analysis revealed that control sample contains 11 and treated 9 repeats with maximum length of 25 and 17

residues that are occupied by pentatricopeptide, respectively. The sites distribution in control and treated sample showed that control top 50 hits contain a greater number of sites than treated sample. The lengthy site in control was sulphate anion where as in treated it was phenylalanine/histidine site. The top 50 hits for families were identified among control and treated samples. InterProscan family analysis revealed that major part was occupied with energy related group but in control engaged by photosynthesis related proteins (Bindu et al. 2020).

15.4.3 Bioinformatics Analysis of Total Proteome in Jasmonic Acid Treated and Control In Vitro Grown *A. paniculata* Plants

The functional annotation of proteins by UniProt in both treated (50 μ M JA) and untreated control plant samples identified 40 metabolic processes. Amongst them, the highly affected pathway was protein modification with an increase of 5.7% in treated plants compared to control. Glycan biosynthesis was up-regulated up to 2.3% in treated plants. Metabolic intermediate biosynthesis such as in (R)-mevalonate biosynthesis (R)-mevalonate from acetyl-CoA was increased up to 1%.

In addition to primary metabolism, the secondary metabolite biosynthesis was also increased up to 1.4%. Likewise, other biosynthetic pathways such as amine and polyamine metabolism e.g. spermidine metabolism (0.9%), amino-acid biosynthesis (0.7%), aromatic compound metabolism e.g. phenylpropanoid biosynthesis (0.6%) were enhanced. The carotenoid and lycopene biosynthesis (0.5%), glycan metabolism (0.6%), isoprenoid biosynthesis (0.2%), nucleotide-sugar biosynthesis (0.3%), pigment and anthocyanin biosynthesis (0.5%), plant hormone biosynthesis (0.4%), plant hormone metabolism and auxin biosynthesis (0.1%), terpene metabolism, lanosterol biosynthesis and lanosterol from farnesyl diphosphate (0.2%) were increased in treated plants. On the other hand, lipid metabolism was decreased upto 2.8% in treated plants, followed by decrease in other metabolisms like amino acid degradation, carbohydrate biosynthesis, carbohydrate metabolism, co-factor biosynthesis and phenylpropanoid metabolism up to 1.3, 1.1, 1.1, 1.5 and 1.5%, respectively. In metabolisms like alkaloid biosynthesis 0.5%, carbohydrate degradation 0.8%, glycan and starch degradation 0.3%, nitrogen metabolism 0.3%, photosynthesis 0.9%, phytoalexin biosynthesis, 3,4',5-trihydroxystilbene biosynthesis and 3,4',5-trihydroxystilbene from trans-4-coumarate 0.6%, porphyrin-containing compound metabolism 0.8%, steroid biosynthesis 0.8%, sulphur metabolism 0.8%, and t-RNA modification 0.3% were down regulated (Bindu et al. 2020).

Overall, these metabolisms were categorized into three major processes such as molecular, cellular and biological function. At this level, we compared these three processes between controls and treated. It revealed that the biological process was 5.3% up regulated in treated sample. On the other hand, cellular function was 5.8% and the molecular function was 5.5% up regulated in treated sample. In each

sample, we analysed different enzyme classes and maximum number of enzymes found under the category transferases. The next highest category was hydrolases followed by oxidoreductases. The lyases, isomerase and ligases were observed in less number.

15.4.4 Differential Expression of Proteins in Control and Jasmonic Acid Treated *A. paniculata* Plants

The increased expression of different transcription factors was observed in treated sample compared to control. In control, MYC1 was expressed and involved in flavonoid biosynthesis and treated sample expressed MYC2, 3, 4. The expression of 26S proteasome subunits were up regulated up to 2.3-fold in treated sample than in control. The expression of other transcription factors was found to be involved in jasmonic acid signalling pathway, which included mybs, topless related proteins (TRP's), Skp1/Cullin/F-box (SCF), DREBs, and Glabra. Glabra was uniquely expressed in treated sample and absent in control. The expression of TRP's was up regulated up to 2.04-fold in treated plants. SCF was up-regulated to an extent of 0.6-fold in treated plants. The expression of dehydration responsive element binding proteins (DREB's) were more in treated sample compared to control. AP2/ERF expression was increased up to 1.63-fold in treated plants. The expression levels of MED was up regulated up to 2.9-fold in treated sample. The expression of WRKY transcription factors were up-regulated to 0.8-fold (Bindu et al. 2020).

15.4.5 Biosynthesis of Isoprenoids in Control and Jasmonic Acid Treated *A. paniculata* Plants

The HPLC analysis revealed that one-fold increase of andrographolide in treated sample compared to control. The KEGG analysis showed only limited number of terpenoid backbone biosynthesis proteins were expressed in control, but in treated sample most of the proteins were expressed particularly diterpene lactones.

The five terpenoid backbone related proteins were found to be expressed in control highlighted in green colour. These identified proteins included acetyl-CoA C-acetyltransferase (EC2.3.1.9), the second one is hydroxymethylglutaryl-CoA reductase (HMGR) inhibitor class protein (EC1.1.1.34). Likewise, the other proteins expressed in control are dimethylallyltranstransferase (EC2.3.1.1), farnesyl diphosphate synthase (EC2.5.1.10) and geranylgeranyl diphosphate synthase (EC2.5.1.29). It indicated that the plants in general and *A. paniculata* in particular much reduced number of secondary metabolites are accumulated in normal conditions without any stress.

In KEGG analysis, proteins expressed in treated sample are highlighted in purple colour. A total of more than 49 proteins were expressed in treated sample compared to untreated control. The enzyme involved in first step of MVA pathway i.e. hydroxymethylglutaryl-CoA synthase (HMGS) (EC2.3.3.10) was expressed. The next step and key regulator of MVA pathway, HMGR was up regulated up to 15.5-fold in treated compared to control sample. Other proteins found by KEGG analysis in MVA pathway were mevalonate kinase (EC2.7.1.185; EC2.7.1.36), mevalonate-3-phosphate 5-kinase (EC2.7.1.186), phosphomevalonate kinase (EC2.7.4.2), diphosphomevalonate decarboxylase (EC2.7.1.33) and phosphomevalonate decarboxylase (EC4.1.199).

The first committed step of MEP by 1-deoxy-D-xylulose-5-phosphate synthase (EC2.2.1.7) was expressed in treated sample. The other proteins/enzymes involved in MEP pathway, which were expressed sequentially. The detailed depiction of MEP pathway based on KEGG data is described in detail (Bindu et al. 2020). Isopentenyl-diphosphate delta-isomerase (EC 5.3.3.2) was expressed in treated sample compared to its absence in control, which interconvert IPP and DMAPP and are precursors for terpenes. The expression of squalene monooxygenase was down-regulated up to 0.5-fold in treated sample and squalene epoxidase to 0.2-fold. Downstream of both MVA and MEP pathways produce prenyl transferases which convert IPP and DMAPP to longer chain isoprenoid precursors such as geranyl diphosphate (GPP), farnesyl diphosphate (FPP) and geranylgeranyl diphosphate (GGPP). The key regulatory enzyme in the production of diterpenes is geranyl geranyl diphosphate synthase (GGPS) (EC 2.5.1.29). In the present study, a 0.5-fold up-regulation of GGPS was observed in jasmonic acid treated sample.

In diterpene production, only geranylgeranyl synthase was expressed in both control and treated samples compared to other remaining enzymes, which were expressed in treated sample. Those enzymes include ent-copalyl diphosphate synthase, ent-kaurene synthase, ent-kaurene oxidase, gibberellin 20-oxidase, gibberellin 3-beta-dioxygenase and trimethyltridecatetraene. Gibberellin 3-beta-dioxygenase expression was increased up to 1.8-fold (Bindu et al. 2020).

15.4.6 Biosynthesis of Other Groups of Secondary Metabolites in Control and Jasmonic Acid Treated A. paniculata Plants

In control sample, 3 enzymes were expressed compared to 10 enzymes in treated sample. Chalcone synthase was up regulated in treated sample up to 4.0-fold. In control, CHS and flavonoid 3'-monooxygenase were expressed. However, along with these two enzymes the remaining enzymes were expressed in treated sample. In the flavones and flavonoles only two enzymes were expressed in control sample and in treated four enzymes were expressed. The enzyme expressed in control was isoflavone/4'-methoxyisoflavone 2'-hydroxylase. Except this, remaining enzymes were expressed in treated sample.

Uniquely expressed proteins were identified in control as well as treated samples. In control sample, about 370 proteins were distinctively expressed and in treated samples 520 were solely expressed. In the control sample, the particularity is towards primary metabolism related proteins. However, in treated it includes different classes of secondary metabolism associated enzymes, transcription factors and phytoalexins (Bindu et al. 2020).

15.5 Conclusions and Future Perspectives

The basic knowledge of elicitation mechanism at biochemical, genetic and molecular level will be helpful to manipulate secondary metabolic pathways to develop quality foods and pharmaceuticals in health care for practical applications. Plant transcriptomics is widely used to study plant responses to various stresses. Transcriptomic studies have revealed many changes in expression levels of various genes during exposure to environmental extremes. NGS has revolutionized our view of how transcriptome can be sequenced and analyzed. On the other hand, the gradual maturation of single-cell whole transcriptome analysis allows researchers to tackle certain genes on a very micro scale.

The plant transcriptomics is an integral component of systems biology and is widely applied in plant sciences in both model as well as crop plants. Emerging area of multispecies transcriptomics holds promise to provide knowledge for the understanding of complex plant microbe interactions. Future development in plant molecular biology, computational biology and systems biology is based on plant transcriptomics for several aspects, thus, this field is expected to play a major role in future research on plant sciences. The synthesis and accumulation of specialized plant metabolites are strictly controlled in a spatial and temporal manner. This regulation can be affected by biotic and abiotic factors. The transcription factors (TFs) play a crucial role in the induction of specialized metabolite biosynthesis in response to stress (Meraj et al. 2020). Transcription factors are capable of interacting with specific DNA sequences (cis-like) in the promoter region of target genes. This interaction activates or represses the target gene expression in response to internal or external cues. The current state of knowledge surrounding TFs related to saponin. Biotechnological approaches using conventional and genetically modified biomass, such as cell, organ, and whole plant cultures are important alternatives to meet market demands of high quantity and quality metabolites. There are relatively few studies at the proteomic level with a focus on triterpene saponin metabolism and this should be a topic to consider in future research efforts, as it provides important information on general biochemical regulation. Overall, genetic engineering of triterpene saponin metabolic pathways is often focused on biosynthetic genes aiming at improved yields of target metabolites. However, an attractive set of transcription factors is being made available to reinforce and improve the metabolic engineering toolbox, potentially allowing simultaneous and fine control of enzyme encoding genes in a relatively less laborious fashion. The use of more recent technologies

such as genome editing is likely to increase in coming years and positively impact triterpene saponin production. Production systems of triterpene saponins in microbial hosts more amenable to industrial scale are still incipient. However, in the near future it is likely that large scale production of premium quality bioactive plant triterpene saponins will take place in stable, contained, and highly controlled industrial environments (Magedans et al. 2019).

The total proteome, differential expression of proteins and genes analysis revealed molecular insights involved in isoprenoids production. The isolation and characterization of genes for key enzymes will be beneficial in genetic manipulation for enhanced andrographolide production. These findings provide groundwork for future investigation on the signal mechanism involved in bioactive compounds production and in understanding the possibilities for the accumulation of secondary metabolites during stress response to unravel the camouflaged mechanisms in non-model plants like *A. paniculata*. Further research is necessary to validate these proteins for understanding elicitation mechanism through protein-protein interactions in detail. The integrative proteomic and bioinformatics approaches will help for the discovery of previously unknown metabolic pathways, enzymes and it is the first proteomic study of this plant species.

Along with the proteomic approaches, successful development of next-generation sequencing technologies will help in the identification, annotation of proteins and their isoforms in a particular plant species. Not only in the identification of proteins, but also it contributes to the understanding of the structural basis and their interactions between biological molecules. The next-generation metabolic engineering approaches towards the development of *in vitro* specialized culture systems, as biofactories, will be the future trend (Arya et al. 2020). In advanced “omics” technology, proteomics in combination with transcriptomics and bioinformatics will provide scope for the discovery and identification of new bioactive compounds.

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