P.E. Rajasekharan
Shabir Hussain Wani *Editors*

Conservation and Utilization of Threatened Medicinal Plants

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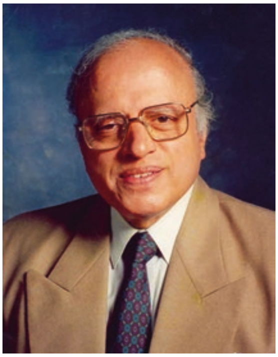
Foreword

Wild plant species form the foundation of healthcare practices throughout much of Asia, particularly traditional practices, such as traditional Chinese medicine, Ayurveda, Siddha, Unani and Tibetan medicines. Compounds such as reserpine from snakeroot and paclitaxel from Himalayan yew have important pharmaceutical uses in Europe, North America and elsewhere. Some species are in demand for their aromatic properties too. The use of Jatamansi oil dates back over 1000 years, whilst red sanders is in demand for its timber and as a source of red dye. In India, collection and processing of medicinal plants contributes at least 35 million workdays per year to the poor and underemployed, but rising demand is threatening this vital source of livelihood income in India and elsewhere.

Priority also needs to be given to wild land. There exists medium to high capability for research and use of improved methodologies for ex situ conservation. Nevertheless, strengthening of both technical and infrastructure capabilities is required in most cases. Crucial concerns associated with in situ and on-farm conservation through participatory approaches involving local communities to develop appropriate regeneration systems, maintenance and continuous cultivation in farmers' field, provision of adequate incentives to farmers with enough seed and planting material and promotion of village level nurseries/gardens to perpetuate local diversity need to be addressed. The static (ex situ) conservation strategy seeks to dramatically alter the original evolutionary trajectories of a plant species; a 'genetic snapshot' of sorts is conserved.

At the same time, the current status of technology does not allow many important species to be stored in genebanks, since they are all not propagated through orthodox seeds. In this context, this compilation is a welcome initiative as it discusses the state of the art related to conservation and use of threatened medicinal plants. This book provides a comprehensive overview using broad subject-based reviews about contemporary approaches to conservation and use in the framework of different technologies including biotechnological approaches as practised. The aim was to review the current status of threatened medicinal plants research in light of the surge in the demand for herbal medicine. The current volume brings together chapters on threatened medicinal plants of, and covers both wild (non-cultivated) and domestic (cultivated) crops with, therapeutic value. The work includes a brief chapter on the singular nature of threatened medicinal plant genetic resources giving rationale for it being distinct from field crop genetic resources. Other chapters give insight on protocols for conservation of selected threatened medicinal plants ex situ and focus on increased need to complement it with in situ conservation approach. Geospacial tools are also briefly described emphasizing on the gene pool in threatened medicinal plants. Legal and biotechnological aspects, namely morphological, genomics, chemical and molecular characterization, are also dealt with. The ways by which these resources are used with sustainable management and replenishment are described. The topics of interest include but are not restricted to research perspectives for sustainable development of various such plant species. The book will be a good reference tool, useful to horticulturists, botanists, policy makers, conservationists, NGOs and researchers in academia and industry.

I am happy to learn that Dr. P. E. Rajasekharan and Dr. Shabir Hussain Wani have edited this book titled *Conservation and Utilization of Threatened Medicinal Plants* to be published by Springer Nature. Both the editors have a rich and long experience in the area of plant genetic resources. I am impressed with their zeal and commitment for science, including research, teaching and dissemination of scientific knowledge. I congratulate both the editors for their timely initiative in bringing out this publication.



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M. S. Swaminathan

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 (MPs) 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 our knowledge regarding research and development, production, trade and utilization, and especially from the viewpoint of sustainability. This book on conservation and utilization of threatened medicinal plants of the world is aimed to look carefully at our present knowledge of this vast interdisciplinary domain. In the era of global climatic change, the series is expected to make an important contribution to the better knowledge and understanding of threatened MPs. The history of medicinal and utilization dates back to the beginnings of mankind. Our forefathers used natural substances, they could find in nature, to ease, cure their sufferings, illnesses, and to heal their wounds. This type of approach has survived in the traditional medicinal (TM) uses, until today, since nearly 80% of the world population still relies on MPs in their medications. The renaissance of MP-use in the high-income countries of the world has brought about a different type of use in the form of herbal medicines complementary and alternative medicines (CAM). MPs have become "industrial products" with new concepts like phytotherapy and veterinary medicinal uses, aromatherapy, nutraceuticals, cosmeceuticals, and animal welfare uses widening the scope of the utilization. New, innovative, value-added applications include their use in functional foods, animal husbandry, as well as plant protection in agriculture. In this regard, the versatile utilization of essential oils is promising. Modern approaches in production and uses have brought about an increased focus on the importance of quality, safety, and efficacy of both MPs and their produce. MPs will also maintain their importance in the search for new, valuable sources of drugs and lead compounds. In view of the steadily increasing demands on these important natural resources, attention should be paid to the sustainable forms of production and utilization.

Contributors of this volume were selected from a wide range of institutions for introducing a diversity of authors. At the same time, these authors were selected based on their vast expertise in specific areas of their choice to match the diversity of topics. These authors have a deep understanding of their subject to enable them not only to write critical reviews by integrating information from classical to modern literature but also to endure an unending series of editorial suggestions and revisions of their manuscripts. Needless to say, this is as much their book as ours. We hope that this volume will help our fellow researchers and a generation of students enter the fascinating world of threatened medicinal plants resources research and conservation with confidence, as perceived and planned by us. All these aspects are well covered in this volume.

The book is primarily designed for use by the undergraduates and postgraduates studying horticulture, sustainable crop production, agricultural sciences, and plant sciences. Horticulturists, plant and agricultural research scientists, and those in academia will find this book of great use. Libraries in all universities and research establishments where agricultural and horticultural sciences are studied and taught should have multiple copies of this valuable book on their shelves. Editors wish to thank all the contributors and staff of Springer for their cooperation in the completion of this book.

Bengaluru, Karnataka, India P. E. Rajasekharan
East Lansing, MI, USA Shabir H. Wani

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About the Editors

P. E. Rajasekharan is a Principal Scientist at the ICAR Indian Institute of Horticultural Research, Bengaluru. He completed his PhD in In Vitro Conservation of Threatened Medicinal Plants in the Department of Botany, Bangalore University. He is known for his contributions to the area of plant genetic resources, i.e. in vitro conservation and cryopreservation of horticultural crops. Also, he holds three postgraduate diplomas: Intellectual Property Rights from the National Law School of India University, Human Resources Management from Indira Gandhi National Open University (New Delhi) and Ecology and Environment from Indian Institute of Ecology, New Delhi. He supervised 20 MPharm students at Rajiv Gandhi University of Health Sciences. He also wrote many review articles and book chapters, participated in various national and international symposia and seminars and presented research results on cryopreservation and in vitro conservation. In addition, he has developed globally applicable cryopreservation protocols for the conservation of nuclear genetic diversity (NGD) in pollen of important vegetable, ornamental and endangered medicinal species. He also worked on conservation of threatened medicinal plants and established Field Gene Bank for the same at ICAR-IIHR, Bengaluru. He developed conservation protocols for several RET medicinal plant species including *Nothapodytes foetida*. Recently, he worked on *Madhuca insignis* which was rediscovered after 120 years and reintroduced in the natural habitats. He currently teaches courses on Plant Genetic Resources and Intellectual Property Rights in Agriculture. He has more than 200 articles and 2 books to his credit, one coedited with Dr. Ramanatha Rao published by Springer Nature, i.e. *Conservation and Utilization of Horticultural Genetic Resources***.** He is an expert reviewer for several international peer-reviewed journals, and sits on the editorial board of several journals. He is a Fellow of the Indian Society of Plant Genetic Resources and Indian Association for Angiosperm Taxonomy.

Shabir Hussain Wani is Senior Assistant Professor at Mountain Research Centre for Field Crops, Khudwani – 192101, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India. He received his PhD degree in Plant Breeding and Genetics on "transgenic rice for abiotic stress tolerance" from the Punjab Agricultural University Ludhiana, India. After obtaining his PhD, he worked as Research Associate in the Biotechnology Laboratory, Central Institute of Temperate Horticulture (ICAR), Srinagar, India. He then joined as Programme Coordinator in Krishi Vigyan Kendra (Farm Science Centre), Senapati, Manipur, India. He teaches courses related to plant breeding, seed science and technology and stress breeding and has published more than 100 papers/chapters in journals and books of international and national repute. He served as Guest Editor and Review Editor for the journal *Frontiers in Plant Science* (2015–2018). He has also edited several books on current topics in crop improvement for abiotic stress tolerance published by Springer Nature and CRC Press, USA. His PhD research won first prize in the North Zone Competition, national level, in India. He was awarded Young Scientist Award from the Society for Promotion of Plant Sciences, Jaipur, India, in 2009. He is a fellow of the Society for Plant Research, India. Recently he also received Young Scientist Award (Agriculture) 2015 from the Society for Plant Research, Meerut, India. He also served as Visiting Scientist in the Department of Plant Soil and Microbial Sciences, Michigan State University, USA, under the UGC Raman Postdoctoral Fellowship programme. Currently, he is in charge of wheat improvement programme at MRCFC Khudwani, SKUAST, Kashmir.

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Part I
Genetic Resources of Threatened Medicinal Plants at Crossroads

Chapter 1
Distribution, Diversity, Conservation and Utilization of Threatened Medicinal Plants

P. E. Rajasekharan and Shabir Hussain Wani

Abstract Rich biodiversity of India is under severe threat owing to habitat destruction, degradation, fragmentation, and overexploitation of resources. According to the Red List of threatened plants, 44 plant species are critically endangered, 113 endangered, and 87 vulnerable (IUCN, 2000). Widespread losses of plant species and varieties are eroding the foundation of agricultural productivity and threatening other plant-based products used by billions of people worldwide, as reported in a new study by the World Watch Institute, Washington, and worldwide some 3.5 billion people in developing countries rely on plant-based medicine for primary health care. Loss of habitat, pressure from nonactive species, and over harvesting have put one out of every eight plant species at risk of extinction, according to the world conservation union. Many medicinal plants are also in trouble from over harvesting and destruction of habitat. Since less than 1 percent of all species have been screened for bioactive compounds, every loss of a unique habitat and its species is potentially a loss of future drugs and medicines.

Keywords Threatened medicinal plants · Conservation · Red list · CAMP

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1.1 Introduction

In India, of the 17,000 species of higher plants, 7500 are known for medicinal uses (Shiva 1996). *Ayurveda*, the oldest medical system in Indian subcontinent, has alone reported approximately 2000 medicinal plant species, followed by Siddha and Unani. The Charaka Samhita, an age-old written document on herbal therapy, reports on the production of 340 herbal drugs and their indigenous uses. Approximately 25 percent of drugs are derived from plants, and many others are synthetic analogues built on prototype compounds isolated from plant species in modern pharmacopoeia (Rao et al. 2004). Further, the demand for medicinal plant-based raw materials is growing at the rate of 15 to 25 percent annually, and according to an estimate of WHO, the demand for medicinal plants is likely to increase more than US $5 trillion in 2050. In India, the medicinal plant-related trade is estimated to be approximately US $1 billion per year (Table 1.1).

1.2 Distribution

Macro analysis of the distribution of medicinal plants shows that they are distributed across diverse habitats and landscape elements. Around 70% of India's medicinal plants are found in tropical areas mostly in the various forest types spread across the Western and Eastern Ghats, Vindhyas, Chota Nagpur Plateau, Aravalis, and Himalayas. Although less than 30% of the medicinal plants are found in the temperate and alpine areas and higher altitudes, they include species of high medicinal value. Studies show that a larger percentage of the known medicinal plant occur in the dry and moist deciduous vegetation as compared to the evergreen or temperate with habitats.

Analysis of habitat of medicinal plants indicates that they are distributed across various habitats. One third are trees and an equal portion shrub and the remaining one third herbs, grasses, and climbers. A very small proportion of the medicinal

Table 1.1 List of medicinal plants traded in large volume internationally

Actaea racemosa	*Centella asiatica*	*Hydrastis perforatum*	*Silybum marianum*
Allium sativum	*Echinacea purpurea*	*Matricaria chamomilla*	*Silybum chirayita*
Aloe ferox	*Echinacea angustifolia*	*Melissa nettle*	*Tanacetum parthenium*
Aloe vera	*Echinacea sinica*	*Oenothera biennis*	*Taxus wallichiana*
Aloe Montana	*Ginkgo biloba*	*Papaver somniferum*	*Taxus brevifolia*
Atropa belladonna	*Glycyrrhiza glabra*	*Pelargonium sidoides*	*Taxus chinensis*
Carapichea ipecacuanha	*Hippophae rhamnoides*	*Sabal serrulata*	*Ulmus rubra*
Cassia senna	*Hydrastis Canadensis*	*Serenoa repens*	*Vaccinium macrocarpon*

plants are lower plants like lichens, ferns algae, etc. Majority of the medicinal plant are higher flowering plants.

1.3 Medicinal Plant Wealth of India

India is rich in medicinal plant diversity. All known types of agroclimatic, ecologic, and edaphis conditions are met within India. The biogeographic position of India is so unique that all known types of ecosystems range from coldest place like the Nubra Valley with 57 °C, 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. India is rich in all the three levels of biodiversity, such as species diversity, genetic diversity, and habitat diversity. There are about 426 biomes representing different habitat diversity that gave rise to one of the richest centers in the world for plant genetic resources. Although the total number of flowering plant species is only 17,000, the intraspecific variability found in them make it one of the highest in the world. Out of 17,000 plants, the classic systems of medicines like Ayurveda, Siddha, and Unani make use of only about 2000 plants in various formulations (Table 1.2). The classical traditions were prevalent in the past particularly in the urban elite society. The rural people who constitute 70–75% of the Indian populations live in about 576,000 villages located in different agroclimatic conditions. The village people have their own diverse systems of health management. While most of the common ailments were managed in the house by home remedies which included many species and condiments like pepper, ginger, turmeric, coriander, cumins, tamarind, fenugreek, and tulsi more complicated cases were attended by the traditional physicians who use a large number of plants from the ambient vegetations and some products of animal or mineral origin to deal with the local diseases and ailments. These are indeed community-managed systems independent of official or government system and are generally known as Local Health Tradition (LHT). The traditional village

Table 1.2 Distribution of medicinal plants by parts used (based on analysis of 1079 South Indian species)

Parts	Percentage (%)
Roots	26.6
Leaves	5.8
Flowers	5.2
Fruits	10.3
Seeds	6.6
Stem	5.5
Wood	2.8
Whole plant	16.3
Rhizome	4.4

physicians of India are using about 4500–5000 species of plants for medicinal purpose. However, there is no systematic inventory and documentation about the folk remedies of India. There is urgent need to document this fast disappearing precious knowledge system. The oral traditions of the villagers use about 5000 plant for medicinal purposes. India is also inhabited by a large number of tribal communities who also possess a precious and unique knowledge about the use of wild plants for treating human ailments. A survey conducted by the All India Coordinated Research Project on Ethnobiology (AICRPE) during the last decade recorded over 8000 species of wild plants used by the tribals and other traditional communities in India for treating various health problems. Some interesting observations made in the study are the use of the same species found in different regions for the same ailments, while some other species are used differentially.

Species Available in Phytoclimatic Zones in India
Our country is divided into tropical, subtropical, temperate, and alpine zones. The following medicinal plants are found in different phytoclimatic zones:

1. Tropical zone: *Acorus calamus*, *adhatoda vasica*, *aristolochia indica*, *azadirachta indica*, *cassia fistula*, *commiphora mukul*, *datura metel*, *evolvulus alsinoides*, *gloriosa superba*, *mucuna pruriens*, *psoralea corylifolia*, *pueraria tuberosa*, *tinospora cordifolia*, *tylophora indica*, *withania somnifera*, *chlorophytum arundinaceum*, *strychnos nux-vomica*.
2. Subtropical zone: *Acorus calamus*, *alpinia galanga*, *asparagus adscendens*, *curcuma zedoaria*, *holarrhena antidysenterica*, *urginea indica*.
3. Temperate zone: *Aconitum chasmanthum*, *artemisia maritima*, *berberis aristata*, *bergenia ciliata*, *colchicum luteum*, *daphne papyracea*, *datura stramonium*, *dioscorea deltoidea*, *fagopyrum esculentum*, *heracleum candicans*, *podophyllum hexandrum*, *rheum emodi*, *swertia chirata*, *urginea indica*, *viola odorata*, etc.
4. Alpine zone: *Nardostachys jatamansi*, *picrorhiza kurroa*, *dactylorhiza hatagirea*, *hyssopus officinalis*, *aconitum heterophyllum*, *a. balfourii*, *dictamnus albus*, *ephedra gerardiana*, *gentiana kurroo*, *jurinea dolomiaea*, etc.

1.4 Threatened and Endemic Plants of Indian Region

India with its varied climate, high mountains in the north, and sea on the other three sides supports a rich flora of tropical, subtropical, temperate, and alpine vegetation. It is estimated that over 17,000 species of higher plants occur in India, of which approximately one-third are woody species and another one-third endemic. It is also common knowledge that our forests with all this vegetation are gradually decreasing. Whereas we should have at least 33% of forest cover in order to have harmonious ecosystems, we are at present left with a mere less than 20%. Activities such as conservation of flora and afforestation should, therefore, go hand in hand and must be given top priority. The Indian efforts toward conservation of threatened biota through the Ministry of Environment and Forests are praiseworthy. India is one of

Table 1.3 National organizations working on medicinal plants

Sl. no	Organization	Headquarter	Area of work
1	CSIR-Central Institute of *Medicinal* and Aromatic *Plants*	Lucknow, Uttar Pradesh	Extending technologies and services to the farmers and entrepreneurs of medicinal and aromatic plants
2	ICAR-Directorate of Medicinal and Aromatic Plants Research	Anand, Gujarat	Quality production of medicinal and aromatic plants
3	JN-The Tropical Botanical Garden and Research Institute	Palode, Kerala	Conserving tropical plant genetic resource develops strategies for their sustainable utilization
4	CSIR-Indian Institute of Integrative Medicine	Jammu	Primary focus of research on drug discovery from medicinal plants
5	National Medicinal Plant Board	New Delhi	Development of medicinal plant sector through developing a strong coordination between various ministries/departments/organizations for implementation of policies/programs on medicinal plants

the signatories of the convention on the International Trade in Endangered Species of Wild Fauna and Flora. The National Committee on Environmental Planning and Coordination (NCEPC) and the National Committee on Man and Biosphere (MAB) have also been concerned with the protection of habitat having natural vegetation. Several natural areas have been identified for conservation as biosphere reserves throughout the country. Setting up of gene banks and gene sanctuaries are other major efforts of the government toward conservation (Table 1.3). The real challenge is to conserve the threatened endemic medicinal plants.

1.4.1 Distribution of Medicinal Plants by Habitats

Of the 386 families and 2200 genera in which medicinal plants are recorded, the families Asteraceae, Euphorbiaceae, Lamiaceae, Fabaceae, Rubiaceae, Poaceae, Acanthaceae, Rosaceae, and Apiaceae share the larger proportion of medicinal plant species with the highest number of species (419) falling under Asteraceae.

About 90% of medicinal plant used by the industries is collected from the wild. While over 800 species are used in production by industry, less than 20 species of plants are under commercial cultivation. Over 70% of the plant collections involve destructive harvesting because of the use of parts like roots, bark, wood, and stem and the whole plant in case of herbs. This poses a definite threat to the genetic stocks and to the diversity of medicinal plants if biodiversity is not sustainably used.

Medicinal plants have always been a basic resource for human health. Appreciation for the preventative and therapeutic value of herbal remedies and the additional benefits of their low cost, wide accessibility, and cultural relevance remain strong in many traditional cultures. Interest in and demand for traditional remedies and other plant-based health products (the so-called botanicals) are increasing worldwide, particularly in rapidly expanding urban societies. Increased consumption of medicinal plants, through expansion of local, regional, and global markets, has increased pressure on a resource that is largely harvested from depleted wild populations in shrinking wild habitats.

Research on the conservation and sustainable use of medicinal plants and their habitats has fallen far behind the demand for this globally important resource. More than 20,000 species of plants are used medicinally somewhere on earth. Nearly half of these species are potentially threatened by over-harvest or loss of habitat. Capacity to assess and monitor the conservation status of medicinal plants, to manage harvest within the limits of sustainability, and to devise cost-effective alternatives for the production of medicinal plants as a resource is extremely limited worldwide. The scale of consumption of this resource has overwhelmed knowledge and tools to effectively implement conservation activities.

The current and potential value of medicinal plants – their value to local community health, to regional markets, and to global health security and trade – is widely recognized as a reason to conserve tropical forest ecosystems. However, many other ecosystems worldwide support a medicinal flora that is important to local health and economy, as well as to regional and global supplies of plant-based medicines. The wide range of habitats, taxonomic groups, and the variety of cultural, social, and economic conditions affecting their use present substantial challenges to conservation and management efforts for these resources. At the same time, the capacity, experience, and expertise developed in meeting these challenges for medicinal plant resource management will contribute more broadly to biodiversity resource management capability in any natural and social environment where plants are used as medicines (Table 1.4).

1.4.2 Distribution of Threatened Medicinal Plants

Macro analysis of the distribution of medicinal plants shows that they are distributed across diverse habitats and landscape elements. Around 70% of India's medicinal plants are found in tropical areas mostly in the various forest types spread across the Western and Eastern Ghats, Vindhyas, Chota Nagpur Plateau, Aravalis, and Himalayas. Although less than 30% of the medicinal plants are found in the temperate and alpine areas and higher altitudes, they include species of high medicinal value. Macro studies show that a larger percentage of the known medicinal plant occurs in the dry and moist deciduous vegetation as compared to the evergreen or temperate habitats.

Analysis of habits of medicinal plants indicates that they are distributed across various habitats. One-third are tress and equal portion shrubs and the remaining one-third herbs, grasses, and climbers. A very small proportion of the medicinal

Table 1.4 Genetic diversity of some important medicinal plants

Sl. no	Species	Country	Genetic diversity	Reference
1	*Artemisia annua*	India	Eight individuals of a population showed chemotypic and genetic variation	Sangwan et al. (1999)
2	*Asparagus racemosus*	India	Accessions of A. racemosus and ornamental species showed 48.3%	Lal et al. (2012)
3	*Butea monosperma*	India	16 accessions from five provinces showed genetic divergence	Khan et al. (2008)
4	*Catharanthus roseus*	India	14 cultivars displayed 82% polymorphism	Shaw et al. (2009)
5	*Coleus forskohlii Coleus aromaticus*	India	Three species exhibited genetic diversity	Govarthanan et al. (2014)
6	*Dioscorea opposita*	China	28 cultivars exhibited 83% polymorphism	Zhou et al. (2008)
7	*Gymnema sylvestre*	India	Plants collected from 12 geographical regions recorded 85% polymorphism	Mouna et al. (2014)
8	*Ginkgo biloba*	China	Nine populations recorded 97.9% polymorphism	Fan et al. (2004)
9	*Hippophae* spp.	Different countries	Genetic diversity is high among populations, origins and subspecies	Cheng et al. (2007)
10	*Justicia adhatoda*	Pakistan	Genetic diversity was high (90%) within populations due to absence of genetic drift	Gilani et al. (2011)
11	*Morinda citrifolia M. tinctoria, M. pubescens*	India	22 accessions collected from four regions showed polymorphism	Singh et al. (2011)
12	*Ocimum basilicum*	India	All markers recorded 100% polymorphism	Lal et al. (2012)
13	*Oroxylum indicum*	India	Accessions collected from eight locations indicated high similarity with 49.6% polymorphism	Jayaram and Prasad (2008)
14	*Phyllanthus emblica*	India	Four populations exhibited genetic diversity	Shaanker and Ganeshaiah (1997)
15	*Rauvolfia tetraphylla*	India	Plants from five populations recorded 98% polymorphism	Saidi et al. (2013)
16	*Commiphora wightii*	India	Accessions collected from different locations recorded 83.5% polymorphism with 0.55–0.79 similarity coefficients	Suthar et al. (2008)
17	*Crocus sativus*	Iran	Observed and expected heterozygosities varied from 0.07 to 0.92 and 0.10 to 0.58, with 2.6 alleles/locus	Nemati et al. (2012)

(continued)

Table 1.4 (continued)

Sl. no	Species	Country	Genetic diversity	Reference
18	*Cephaelis ipecacuanha*	Brazil	50 wild clusters with 291 aerial stems showed no genetic differentiation at the cluster level	de Oliveira et al. (2010)
19	*Cassia occidentalis*	India	10 accessions from different districts had 71.2% polymorphism	Arya et al. (2011)
20	*Gardenia jasminoides*	China	Eight wild or cultivated populations registered 67.6% polymorphism	Han et al. (2007)
22	*Melissa officinalis*	Iran, Germany, Japan	Nine populations from Iran and each one from Germany and Japan revealed significant variation in morphoagronomic traits	Aharizad et al. (2012)
23	*Podophyllum hexandrum*	India	12 accessions displayed high degree of genetic diversity	Sultan et al. (2010)
24	*Mucuna monosperma*	India	25 accessions of five species collected from seven provinces displayed high polymorphism	Sathyanarayana et al. (2011)

plants are lower plants like lichens, ferns algae, etc. Majority of the medicinal plant are higher flowering plants.

Of the 386 families and 2200 genera in which medicinal plants are recorded, the families Asteraceae, Euphorbiaceae, Lamiaceae, Fabaceae, Rubiaceae, Poaceae, Acanthaceae, Rosaceae, and Apiaceae share the larger proportion of medicinal plant species with the highest number of species (419) falling under Asteraceae.

About 90% of medicinal plant used by the industries is collected from the wild. While over 800 species are used in production by industry, less than 20 species of plants are under commercial cultivation. Over 70% of the plant collections involve destructive harvesting because of the use of parts like roots, bark, wood, and stem and the whole plant in case of herbs. This poses a definite threat to the genetic stocks and to the diversity of medicinal plants, if biodiversity is not sustainably used (Table 1.5).

1.4.3 Threatened Medicinal Plant Resource Base

Medicinal plants are living resource, exhaustible if overused and sustainable if used with care and wisdom. At present 95% collection of medicinal plant is from wild. Current practices of harvesting are unsustainable, and many studies have highlighted depletion of resource base. Medicinal plant-based industries although old and vast are still being managed on traditional ethos and practices and lack a proactive and socially responsible image. Many studies have confirmed that pharmaceutical companies are also responsible for inefficient, imperfect, informal, and opportunistic marketing of medicinal plants. As a result, the raw-material supply

Table 1.5 International organizations working on medicinal plants

Sl. no	Organization	Headquarter	Area of work
1	Asia Pacific Information Network on Medicinal and Aromatic Plants	Philippines	To share databases of medicinal plants among its members
2	Botanic Gardens Conservation International	Kew, London	Forms the world's largest plant conservation network
3	Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES)	Washington, D.C.	Treaty to protect endangered plants
4	Fair-trade labeling organizations	Bonn, Germany	Product certification of medicinal plants
5	International organic trade association	North America	Focuses on the organic trade of medicinal plants
6	International Trade Center	Geneva, Switzerland	Providing trade technical assistance in countries all over the world
7	Medicinal Plants Specialist Group of the Species Survival Commission of IUCN	Gland, Switzerland	Conservation and sustainable use of medicinal plants
8	Trade Record Analysis of Fauna and Flora in Commerce (TRAFFIC)	Cambridge, United kingdom	Trade of wild plants and animal species
9	United Nations Environment Program (UNEP)	Nairobi, Kenya	Assists developing countries in implementing environmentally sound policies and practices
10	United Nations Industrial Development Organization (UNIDO)	Vienna, Austria	Promotion of international industrial cooperation
11	World Fair Trade organization	Culemborg, The Netherlands	Improving the livelihoods of economically marginalized producers
12	World Health Organization (WHO)	Geneva, Switzerland	Shaping the research agenda and stimulating the generation, translation, and dissemination of valuable knowledge
13	World Wide Fund for Nature (WWF)	Gland, Switzerland	Wilderness preservation

situation is shaky, unsustainable, and exploitative. There is a vast, secretive, and largely unregulated trade in medicinal plants, mainly form the wild that continues to grow dramatically in the absence of serious policy attention with environmental planning. Confusion also exists in the identification of plant materials where the original of a particular drug is assigned to more than one plant, sometimes having vastly different morphological and taxonomical characters. There are few others, where the identity of plant sources is doubtful or still unknown; therefore, adulteration is common in such cases.

The other main source of medicinal plant is form cultivation. Cultivated material is infinitely more appropriate for use in the production of drugs. Indeed, standardization whether for pure products, extracts, or crude drugs is critical, increasingly so, as quality requirements continue to become more stringent.

Given the higher cost of cultivated material, cultivation is often done under contract. In the majority of cases, companies would cultivate only those plant species which they use in large quantity or in the production of derivatives and isolates, for which standardization is essential and quality is critical. More recently, growers have set up cooperatives or collaborative ventures in an attempt to improve their negotiating power and achieve higher price.

Of the 270, 000 plant species in existence, 1 in 8 are considered endangered. One-quarter of plant species are at the risk of extinction within the next generation.

Special aspects of endangered species are as follows:

1. Limited amount of plant material available
2. Ability to test protocols severely limited
3. Plants located in remote areas
4. Resources available are also limited

Most biologists consider a species endangered if they expect it would die off completely in less than 20 years if no special efforts were made to protect it, or if the rate of decline far exceeds the rate of increase. Until the last few centuries, species became rare or died out as a result of natural causes. These causes included changes in climate, catastrophic movements in the earth's crust, and volcanic eruptions. Today, species become endangered primarily because of human activities. Species mainly become endangered because of (1) loss of habitat and (2) wildlife trade.

Many wild species are protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). This treaty, originally signed by 10 nations in July 1975, aims to control trade in wildlife, plants, and their products. By the late 1990s, over 140 countries had ratified it.

The International Union for the Conservation of Nature and Natural Resources (IUCN) and the World Conservation Union compile lists of endangered plants. Their lists include 34,000 species of plants that are threatened or endangered.

The IUCN Red Lists of Threatened Species are a compilation of plant or animal species categorized as critically endangered, endangered, or vulnerable according to the IUCN categories of threat. For the most part, the Species Survival Commission (SSC) Specialist Group covering the taxa in question makes categorizations with the newer 1994 IUCN criteria.

Many wild species are protected by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). This treaty, originally signed by 10 nations in July 1975, aims to control trade in wildlife, plants, and their products. By the late 1990s, over 140 countries had ratified it (Table 1.2).

The IUCN (International Union for the Conservation of Nature and Natural Resources) and the World Conservation Union compile lists of endangered plants and animals. Their lists include about 34,000 species of plants that are threatened or endangered. Protecting habitat is the key method of preserving endangered species. Many governments and organizations have set aside nature preserves.

Table 1.6 Wild collections used as medicinal plants in different countries

Country	Wild plants used as medicinal (%)
India	77
Europe	90
China	60–80
United States	90
Germany	70–90
Hungary	30–35
Spain	50
Ecuador	90
Albania	90–100
Romania	11,300

Since 1997, an extensive consultation has been carried out to review the IUCN Red List assessment system, and some changes to the categories and criteria have now been agreed on. Taking the data from the Red List analysis book, certain "hotspots" appear. The main areas where mammals, birds, and plants (trees) seem to require the most conservation effort are in the Neotropics (Brazil, Colombia, Ecuador, and Mexico), East Africa (Tanzania), and Southeast Asia (China, India, Indonesia, and Malaysia) (Table 1.6).

The goals of the IUCN Red List Program are to:

- Provide a global index of the state of degeneration of biodiversity.
- Identify and document those species most in need of conservation attention if global extinction rates are to be reduced.

The first of these goals refers to the traditional role of the *IUCN Red List*, which is to identify particular species at risk of extinction. The role of the *IUCN Red List* in underpinning priority setting processes for single species remains of critical importance. However, the second goal represents a radical new departure for the SSC and for the Red List Program, for it focuses on using the data in the Red List for multispecies analyses in order to understand what is happening to biodiversity more generally (Table 1.7).

To achieve these goals, the following objectives are proposed:

- To assess, in the long term, the status of a selected set of species.
- To establish a baseline from which to monitor the status of species.
- To provide a global context for the establishment of conservation priorities at the local level.
- To monitor, on a continuing basis, the status of a representative selection of species (as biodiversity indicators) that cover all the major ecosystems of the world.

Listing criteria are now as follows:

Listing in Appendix I (Table 1.8):

Table 1.7 Genetic erosion in medicinal plants

Country	Number of plants eroded	Plant species
China	3000	*Scutellaria baicalensis*, *Panax notoginseng*, *Acanthopanax senticosus*, *Asarum heterotropoides var. mandshuricum*, *A. lancea*, *Bupleurum chinense*, *Cistanche deserticola*, *Dioscorea zingiberensis*, *Ephedra sinica*, *Eucommia ulmoides*, *Magnolia officinalis*
Africa	59,000–90,000	*Warburgia ugandensis*
Chile		*Haplopappus taeda*
India	265	*Gnidia glauca* var. *sisparensi*, *Phyllanthus emblica*, *Calligonum polygonoides*, *Justicia adhatoda*
Brazil		*Carapichea ipecacuanha*
Nepal		*Swertia chirayita*
Europe	150	–
Croatia	17	–
Ukraine	202	–
Estonia	16	–
Finland	20	–

Table 1.8 Medicinal plant species listed in CITES (Appendix I)

	Source
Aloe barbadensis	(Korean Government) (Roberson 2008) (Hawkins 2008) (AHPAAmerican 2014)
Rauvolfia serpentina	(Schippmann 2001)
Saussurea costus	(BGCI) (R&D center of Flower Valley Agrotech 2005) (Hawkins 2008) (AHPAAmerican 2014)
Cyathea dregei	(Schippmann 2001)
Dioscorea deltoidea	(Schippmann 2001) (BGCI) (R&D center of Flower Valley Agrotech 2005)
Diospyros borneensis	(Department of Agriculture of Brunei Darussalam Government 2000)
Euphorbia spp.	(AHPAAmerican 2014)
Gnetum montanum	(Schippmann 2001)
Pterocarpus erinaceus	(Useful Tropical Plants 2017)
Swietenia humilis	(Useful Tropical Plants 2017)
Fraxinus mandshurica	(Korean Government)
Podocarpus neriifolius	(Schippmann 2001)
Picrorhiza kurrooa	(Schippmann 2001)
Nardostachys grandiflora	(Schippmann 2001)
Siphonochilus aethiopicus	(Schweinf and Burtt 2017)
Guaiacum officinale	(Schippmann 2001)

Source: Convention on international trade in endangered species of wild fauna and flora

Biological Criteria

A. Small population.
B. Restricted distribution.
C. Steep decline.
D. Risk of the above within 5 years.

Categories of Threatened Plants

The International Union for Conservation of Nature and Natural Resources (IUCN, 1995) has recognized the following updated categories of threatened plants on the basis of geographical range, populations, and fragmentation of population.

Extinct (EX): A taxon is *extinct* when there is no reasonable doubt that the last individual has died.

Extinct in the Wild (EW): A taxon is *extinct in the wild* when it is known only to survive in cultivation.

Critically Endangered (CR): A taxon is *critically endangered* when it is facing an extremely high risk of extinction in the wild in the immediate future (80% decline in the last 10 years).

Endangered (EN): A taxon is endangered when it is not critically *endangered* but is facing a very high risk to extinction in the wild in the near future (50% decline in the last 10 years).

Vulnerable (VU): A taxon is vulnerable when it is not critically endangered or endangered but is facing a high risk of extinction in the wild in the medium-term future (50% decline in the last 20 years).

Conservation Dependent (CD): Taxa which are the focus of a continuing taxon-specific or habitat-specific conservation program targeted toward the taxon in question, the cessation of which would result in the taxon qualifying for one of the threatened categories above within a period of 5 years.

Data Deficient (DD): A taxon is *data deficient* when there is inadequate information to make a direct or indirect assessment of its risk of extinction based on its distribution and/ or population status.

Low Risk (LR): A taxon is low risk when it has been evaluated and does not satisfy the criteria for any of the categories, critically endangered, endangered, vulnerable. Conservation dependent, or data deficient.

Not Evaluated (NE): A taxon is not evaluated when it has not yet been assessed against the criteria.

It is now realized all over the world that several species of plants are threatened; many are critically rare and a few already extinct. Studies in some parts of the world have shown that on an average about 10% of vascular plants fall into one or the other category of threatened species. Many countries in the world have taken stock of their rare plants and have developed provisional or fairly accurate lists of their threatened plants. Botanical Survey of India initiated work on rare and endangered species in India way back in 1980 and published several lists of rare and endangered species. "An Assessment of Threatened Plants of India" brought to light several hundred rare species from different parts of the country. This was followed by publication of 4 volumes of Red Data Book of India, which included nearly 620 threatened plants falling under various threat categories of ICUN further.

1.4.4 Current Status of Endangered Plants in India

In India following the general criteria laid down by IUCN (1978), the Botanical Survey of India has brought out several assessments of rare and threatened species in the country. These lists are fundamentally based on herbarium and partly field studies. It is estimated that about 3000 species of flowering plants out of 17,000 species fall in one or the other category of threatened plants, which also include several medicinal plants. These lists have also formed the basis for the publication of the 4 volumes of Red Data Books of India, wherein 620 species are included, of which 550 species are endemic including some valuable medicinal species.

These lists are however not without any drawbacks or limitations. Detailed field studies throughout the entire distribution range of these species to estimate the size and number of populations of particular species, their specific ecological niches, reproductive behavior, and demographic studies are lacking. Future studies should also highlight the correct status of threatened and endemic plants at state/regional level based on extensive field surveys.

1.4.5 Methodology for Assessment of Status of Plants: CAMP

The Conservation Assessment and Management Plan (CAMP) process is a methodology for rapid assessment of taxa in the wild. This methodology is a rational and objective method of assigning threat categories and deriving recommendations for conservation action plans through participatory group inputs from many stakeholders. A CAMP process is a platform for a congregation of 10–40 experts from related fields such as field biologists, ecologists, habitat experts, wildlife managers, forest officials, captive managers, university researchers, academicians, non-governmental organizations, policy makers, and other relevant stakeholders. The CAMP Workshop is organized and conducted by objective facilitators who do not have a professional or personal stake in the outcome of the assessments. The assessment is also followed by research and conservation recommendations for every taxon. CAMPs provide a rational and comprehensive means of assessing priorities for intensive management within the context of the broader conservation needs of threatened taxa.

The Conservation Breeding Specialist Group developed IUCN the CAMP process methodology first for identifying priorities in captive management planning for the global zoo community, which needed to know the in situ conservation status of species in their care. The methodology, however, has proved so effective for assessing status in the wild that IUCN SSC Specialist Groups, governmental and nongovernmental agencies, conservation action planners, and policy makers all over the world have recognized it. The CAMP methodology is emerging as an effective means of conducting biodiversity inventory, identification, and monitoring, thus satisfying Agenda Item 7 in the Conservation on Biological Diversity.

Medicinal plants as a group comprise approximately 8000 species and account for about 50% of all the higher flowing plant species of India. Millions of rural mass

Table 1.9 Global genetic resources of medicinal plants

Country	Total medicinal plants	Medicinal plants in use
India	7500–8000	960
Bulgaria	750	200–300
China	11,146	150
Ethiopia	1000	300
Finland	100	–
France	900	–
Hungary	270	–
Italy	1500	–
Jordan	363	–
Macedonia	700	150
Malaysia	1200	–
Malta	458	–
Nepal	1950	–
Pakistan	1500	–
Philippines	850	–
Republic of Korea	1000	–
Romania	283	–
Serbia	400	–
Slovenia	400	–
Sri Lanka	1414	208
Thailand	1800	–
Turkey	500	–
USA	2564	–
Vietnam	1800	–
Yugoslavia	>700	–
Myanmar	59	
Mongolia	92	
South pacific	102	
Papua New Guinea	126	
Republic of Korea	150	

use medicinal plants in self-help mode. One and a half million practitioners of ISM&H use medicinal plants in preventive/promotive and curative applications. There are about 460,000 registered practitioners of ISM&H using medicinal plants in the codified streams. Further, there are 7843 registered pharmacies of ISM and 851 of homoeopathy and a number of unlicensed small-scale units. Besides meeting national demands, they cater 12% of global herbal trade. In recent years, the growing demand for herbal products has led to a quantum jump in volume of plant material traded within and outside the country. An estimate of the EXIM Bank projects international market of medicinal plant-related trade at US $ 60 billion per year growing at a rate of 7% per year (Table 1.9).

India is blessed with two mega centers of biodiversity (the Hindustan Center of Origin and the Central Asia Center of Origin). This biodiversity is mainly distributed in Western Ghats, North-eastern India, and the Himalayan region. Floristically rich, India has about 141 endemic genera of 5150 species belonging to 47 families of higher plants. Among the different endemic species, 2532 species are distributed in Himalayas, 1788 spp. in the peninsular region, and 185 spp. in the Andaman and Nicobar Islands. About 43,000 plant species are said to exist in India of which 7500 plant species are referred in Indian folklore. However, only about 1700 plant species are mentioned in the documented form of old literature.

The vast degree of diversity present in this country is highly related to the highly divergent ecosystem and altitudinal variations. The agrobiodiversity in India is distributed in 8 very diverse phytogeographical and 15 agroecological regions. The range of distribution of these plants varies from the wet evergreen forests in the Western Ghats to the Alpine scrubs of the Himalayas, from the arid deserts of Rajastan to the mangroves along the east coast, from the vast deciduous forests of the Decan to the Sholas of the high ranges, and from the swamps of the Ganges to the moss laden tree trunks of the silent valley. The indigenous diversity of plant species of medicinal and aromatic value in the region is also unique. This is reflected from the Arogyapacha (*Trichopus zeylanicus*) of the Agastiar Hills to the Saalam Panja of the Himalayas, from the tiny *Drosera* of the Sholas to the huge dipterocarps of the Western Ghats, from the xerophytic aloes to the marshy land Brahmis, and from the wild turmerics to the cultivated peppers. Over 7000 species belonging mainly to the families Fabaceae, Euphorbiaceae, Asteraceae, Poaceae, Rubiaceae, Cucurbitaceae, Apiaceae, Convolvulaceae, Malvaceae, and Solanaceae are used from the ancient time by various health-care systems in the country. In other words, this number corresponds to more than one-fourth of the world's known medicinal plants, which are around 30,000 species. Analysis of these listed plants showed that they include all the major life forms, viz., trees, shrubs, climbers, and herbs. The proportion of ferns and lichens is much smaller as compared to the flowering plants.

Though India has rich biodiversity and is one among the 12 mega diversity centers, the growing demand is putting a heavy strain on the existing resources causing a number of species either threatened or endangered category. The IUCN report for the year 2000 revealed that India ranked fifth in the case of threatened plant species and birds. Recently, some rapid assessment of the threat status of medicinal plants using IUCN-designed CAMP methodology revealed that about 112 species in Southern India, 74 species in Northern and Central India, and 42 species in high altitude of Himalayas are threatened in the wild.

1.5 Collection and Conservation Efforts Undertaken

Collection of non-timber forest product (NTFP), which includes most of the medicinal plants, is associated with the livelihood of tribal and rural communities in and around the forest in India. Since the prices paid to the collectors are very low and most of the time exploitive in nature, they often overexploit the natural resources as their main objective is to generate substantive income.

Several medicinal plants have been assessed as endangered, vulnerable, and threatened due to over harvesting or unskillful harvesting in the wild (Table 1.10). Habitat destruction in the form of deforestation is an added danger. The Government of India has put 29 species in the negative list of export, which are believed to be threatened in the wild (Tables 1.11 and 1.12).

Table 1.10 Biological factors determining conservation methods

No	Biological factors	Preferred conservation methods	Remarks
1	Perennial species	In situ/field gene banks/seed and/or pollen storage	If tree species be required for utilization purpose
2	Annual species	Seed and/or pollen storage in vitro field gene bank	See also factors 3,4,6, and 7
3	Orthodox species	Seed storage	
4	Recalcitrant seeds	In vitro/in situ/field gene bank	
5	Synthetic seeds	As orthodox seeds	
6	Vegetatively propagated species with viable seeds	Field gene bank/pollen/in vitro/cryopreservation	
7	Vegetatively propagated species with nonviable seeds	Field gene bank/pollen/in vitro/cryopreservation	Field gene bank or genotype needs to be conserved
8	Long living pollen	Pollen storage	
9	Tissue culturing feasibility	If low, look for alternative method	
10	Cryopreservation feasibililty	If low, look for alternative method	
11	Genetic stability	If low for certain method, alternative method	

Table 1.11 Cultivation of medicinal plants globally

Country	Number of medicinal plants under cultivation	Area covered (ha)	Reported by
India	50	>95,000	Ved and Goraya (2008), Chaddha and Gupta (1995)
China	250	330,000–460,000	Akerele et al. (1991), Heywood (1999)
Europe	130–150	100,000	Lubbe and Verpoorte (2011)
Finland	30	<5000	–
Poland	60	20,000	–
Hungary	40	–	–
Romania	52	4000	–
Italy	100	–	–
Spain	16	6000	–
Latvia	20	300	–
Serbia	30	<5000	–
UK	26	4200	–

Table 1.12 Conservation of medicinal plants globally

Country	Conservation efforts
India	Botanical survey of India and CIMAP conserves 418 MPs in seed gene banks, 244 MPs in field gene banks, 44 MPs in in vitro gene banks, 53 MPs in DNA banks TBGRI conserves 30,000 plants
Croatia	900 accessions of 180 MPs are conserved
Czech republic	973 accessions of 78 MPs are conserved
Poland	159 accession of 13 MPs are conserved
Slovenia	650 accessions of MPs are conserved
Israel	197 in situ, 584 ex situ, and 576 seed accessions of 15 MPs are conserved

MPs medicinal plants

1.6 Conservation Strategy

The World Conservation Strategy defines conservations as "the management of human use of the biodiversity so that it may yield the greatest sustainable benefit to present generation while maintaining its potential to meet the needs and aspirations of future generations." The above definition invokes two complementary components "conservation" and "sustainability." The primary goals of biodiversity conservation as envisaged in the World Conservation Strategy can be summarized as follows:

1. Maintenance of essential ecological processes and life support systems on which human survival and economic activities depend.
2. Preservation of species and genetic diversity.
3. Sustainable use of species and ecosystems which support millions of rural communities as well as major industries.

Medicinal plants are potential renewable natural resources. Therefore, the conservation and sustainable utilization of medicinal plants must necessarily involve a long-term, integrated, scientifically oriented action program. This should involve the pertinent aspects of protection, preservation, maintenance, exploitation, conservation, and sustainable utilization. A holistic and systematic approach envisaging interaction between social, economic, and ecological systems will be a more desirable one. The most widely accepted scientific technologies of biodiversity conservation are the in situ and ex situ methods (Table 1.13).

1.6.1 In Situ Conservation

The aim of in situ conservation is to allow the population to perpetuate itself within a given ecosystem, to which it is adapted, thus ensuring its potential for continued evolution (5,6). For majority of situations, in situ conservation is the ideal method of conserving wild plant species.

Table 1.13 Most common methods used for germplasm conservation and the corresponding PGR categories

Sl. No	Methods	Predominantly conserved PGR categories by corresponding method
1	Biosphere reserve	Ecosystem/biodiversity by and large
2	Nature reserve	Specific habitat/wild and/or weedy species gene pool
3	Gene sanctuary	Ecosystem (specific)/wild species gene pool
4	On farm conservation (mass reservoirs, bulk hybrid populations)	Agro-ecosystems/land races
5	Botanical garden/arboretum	Wild species, obsolete cultivars, tree crop germplasm
6	Field gene bank	Wild species, vegetatively propagated crops, tree crop germplasm
7	Plant organ storage	Vegetatively propagated crops, mainly in the form of roots, tubers, and bulbs
8	Seed storage	All plant species which produce fertile and orthodox seeds
9	Pollen storage	In principle all species which produce long living pollen
10	In vitro storage	Wild and cultivated species which produce recalcitrant or no seeds, vegetatively propagated crops, disease free germplasm, as well as orthodox seeds
11	Cryopreservation	Germplasm mentioned above which permits cryopreservation
12	DNA and gene libraries	Special genetic stocks; in principle applicable for all germplasm

For and effective in situ conservation, it is important to identify the "hotspots" of genetic diversity. This can be done in two steps.

1. An extensive geographic distribution map of the species needs to be developed to identify sites (hotspots) with viable population sizes.
2. Among these sites, populations that are genetically rich need to be identified such sites can be considered for its in situ conservation of genetic resources. While there have been attempts to map the geographic distribution of the medicinal plants, that of identifying the hotspots of genetic action has been singularly lacking.

1.6.1.1 Advantages of In Situ Conservation

1. It usually allows increased probabilities of conserving a large range of potentially interesting alleles.
2. It is especially adapted to species, which cannot be established or regenerated outside the natural habitats. These species may be divided in three groups.

   (a) Species which are members of complex climax ecosystems
   (b) Species with seeds presenting fugacious germination or with seeds possessing dormancy, which cannot be broken by known artificial methods.

   (c) Species which have highly specialized breeding systems, depending on a single species of insect, bird, or bat for pollination which in turn is dependent on other components of the ecosystem.

3. It allows natural evolution to continue, a valuable option for conserving of disease and pest-resistant species, which can co-evolve with their parasites, providing breeders with a native source of resistance.
4. It can serve several sectors at once and gene pools of value to different sectors (e.g., crop breeding, forestry, forage production wild life).
5. It facilitates research on species in their natural habitats.
6. It assures protection of associated species biosphere reserve concept.

In July 1993, FRLHT, Bangalore, in close collaboration with state forest department of Karnataka, Kerala, and Tamil Nadu, research institutions, individuals, researchers, and environmental NGOs of southern India launched a pilot project for conservation and sustainable use of the region's medicinal plant diversity. This has been done through the establishment of a network of 30 medicinal plant conservation areas (MPCAs) in the three states. The MPCAs are each averaging 200 hectares. Degraded forest areas are taken up for production of medicinal plants. These are called medicinal plant development areas (MPDAs); six such MPDAs have been set up in the project states.

In has been well established that the best and cost-effective way of protecting the existing biological and genetic diversity is the in situ or on-the-site conservation wherein a wild species or stock of a biological community is protected and preserved in its natural habitat. The prospect of such an "ecocentric" rather than a species-centered approach is that it should prevent species from becoming endangered by human activities and reduce the need for human intervention to prevent premature extinctions. Establishment of biosphere reserves, national parks, wild life sanctuaries, sacred groves, and other protected areas forms examples of in situ methods of conservation. The idea of establishing protected area network has taken a central place in all policy decision process related to biodiversity conservation at national, international, and global level.

In India, 4.5% of its total geographical area constitute protected area network, comprising 8 designated biospheres, 87 national parks, and 447 wild life sanctuaries. This network encompasses various biogeographic zones and biomes rich in biotic diversity, including medicinal and aromatic plants. In addition to this, there are a number of sacred groves in different parts of the country particularly in South, West, and Eastern parts which are also active centers on in situ conservation of medicinal plants. Such conservation area network can attribute significantly toward the conservation and sustainable management of biological resources of our country.

However, experiences have amply demonstrated that in a densely populated developing country like India, where a sizeable population are living in close proximity to forests, declaring protected areas will not entirely be sufficient to ensure conservation on the fast eroding biological diversity. The success of any conservation program vests solely on the efficient management of protected areas. The involvement of local communities in conservation activities has now been increasingly realized. A people nature-oriented approach thus becomes highly imperative. This will help to generate a sense of responsibility among the local people about the values of biodiversity and the need to use it sustainably for their own prosperity and the maintenance of ecosystem resilience.

In situ conservation of medicinal plants in India can be accomplished through the active support and participation of people who dwell in or near and around the protected forest areas. Involving the local mass in all phases of conservation programs, such as planning, policy-decision process, and implementation will be a significant component in achieving efficient management and utilization of medicinal plant resources. A few such in situ conservation areas have been marked and declared as medicinal plant in situ conservation areas on the forests of three Southern States of Kerala, Tamilnadu, and Karnataka by the joint efforts of the forest departments of these States and FRLHT, Bangalore.

1.6.2 Ex Situ Conservation

Conservation of plant genetic resources outside their natural habitat is known as ex situ conservation, also known as “off-site conservation.” It can be achieved in the following five ways: (1) seed gene banks, (2) field gene banks, (3) botanical or herbal gardens, (4) medicinal plants conservation parks (MPCPs), and (5) in vitro repositories. Ex situ conservation facilitates conservation in controlled conditions and makes possible reintroduction of species into wild.

Conservation Medicinal plants conservtion can be accomplished by ex situ, i.e., outside, natural habitat by cultivating and maintaining plants in botanic gardens, parks, other suitable sites, and 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.

Botanical gardens can play a key role in ex situ conservation of plants, especially those facing imminent threat of extinction. Several gardens in the world are specialized in cultivation and study of medicinal plants, while some contain a special medicinal plant garden or harbor special collection of medicinal plants.

India has a network of about 140 botanical gardens which include 33 botanical gardens attached to 33 university botany departments. However, hardly 30 botanical gardens have any active program on conservation. Tropical Botanical Garden and Research Institute (TGBRI), located in a degraded forest region of Western Ghats mountains in Kerala, has an excellent example in ex situ conservation of plant diversity in India. The field gene bank program launched by TBGRI from 1992 to 1999 is now well acclaimed as a very effective method of conservation of medicinal and aromatic plant genetic resources. This field gene bank of medicinal and aromatic plants at TBGRI, Thiruvananthapuram, is essentially a blend of the ex situ and in situ situations.

Field gene bank of medicinal plants The concept of establishing field gene banks of plants provides ample options for long-term preservation of the genetic variability (interspecific) of species. Field gene banks are better established in a degraded forest where efforts could be made to reforest/restock the missing species complexes, trees, shrubs, herbs, climber, etc. It is indeed a recreation of a forest or rather simulation of a typical forest. Before attempting to establish such a field gene bank, it is essential to have a clear understanding of the natural ecosystem such as the spatial distribution and pattern of association, i.e., structure and functional dynamics of the species in question. After undertaking an in-depth study on the natural distribution pattern of the medicinal plants and the associated floristic elements – including their microecological niche – a well-planned action program of recreating the same in a degraded forest area or place close to the species found in nature can be attempted. TBGRI has accomplished this task of simulating the nature while establishing the field gene bank of medicinal and aromatic plants under the G-15-GBMAP sponsored by DBT, Government of India. TBGRI's experience now provides ample opportunity to repeat the same elsewhere in the country.

Identification of the keystone species and umbrella species is very important in these methods. After planting the keystone and umbrella species, other species complexes which include the medicinal aromatic plants in question have to be introduced. The sampling and selection of samples for introduction have to be highly knowledge and science intensive. To capture the maximum possible genetic diversity of the target species, it is extremely important to collect all valuable information such as morphological variants, chemical variants, or genetic variants or chemical screening of the population of the targeted species by using the latest methods and tools.

The field gene bank of TBGRI has covered 30,000 accessions of 250 medicinal and aromatic plant species which include 100 endemic, rare, and endangered medicinal and aromatic plants of the tropical region of India. A broad spectrum of the genetic diversity of these species were captured and introduced in this gene bank which covered morphotypes, cytotypes, and chemotype, and the number of samples from each species varied from 50 to 1000 plants.

1.7 Medicinal Plant Conservation Areas (MPCAs)

Since 1993, Foundation for Revitalization of Local Health Traditions (FRLHT), Bangalore, has pioneered the in situ conservation of India's medicinal plant diversity in conjunction with the state forest departments (SFDs) in the states of Karnataka, Tamil Nadu, Kerala, Andhra Pradesh, and Maharashtra, as well as with local communities, nongovernmental organizations (NGO), and research institutions. A medicinal plant conservation area (MPCA), a network of approximately 10 conservation sites, is officially designated for each state of 200–300 hectares. In Southern India, the sites are located in relatively undisturbed forests of varying vegetation types, lying in different altitude ranges, soil types, and rainfall patterns. This is an attempt to capture the wild populations of medicinal plant diversity of the state across the MPCA network. Forest areas with high biodiversity, sites traditionally valued for medicinal plant diversity, or sites with known red-listed medicinal plant species are identified for creating an MPCA. The MPCA boundaries may correspond to the natural boundary features of the selected site, and ideally an MPCA should be located in a discrete micro watershed (Somashekar 2011). The MP-CAs are categorized as no-harvest sites. Their protection and management involve the participation of the local communities. In order to meet the community requirements, the forest department is required to establish medicinal plant nurseries in the MPCA and to supply local households with (1) plant species of high economic value to grow and sell and (2) medicinal plant seedlings for their primary healthcare needs. These act as live field gene banks for medicinal plants. In situ conservation will not be restricted to medicinal plants and other plant species, and the fauna of the area will also be protected. This is to conserve medicinal plants within their ecosystems. In Karnataka, 13 such MPCAs were established representing all major forest types and different altitude zones of the state (Somashekar 2011).

1.8 Medicinal Plant Development Areas (MPDAs)

MPDAs are small areas in non-timber forest product (NTFP) circles and on degraded forests which are used for production of medicinal plants by planting the locally available indigenous species of medicinal plants and trees through people participation. The local communities and the Forest Department share the returns through sustainable harvesting of plants. A total of 12 MPDAs were established in three Southern India states Kerala, Karnataka, and Tamil Nadu under the Danish International Development Assistance (Singh et al. 2008). MPDA facilitates conservation and sustainable use of medicinal plants because it ensures people's participation in conservation and development of medicinal plants and its contribution to the welfare of the participating community (Singh et al. 2008). Twenty-one medicinal plant development areas declared in Arunachal Pradesh, Chhattisgarh, and Uttarakhand have concentrated efforts in MAP species diverse locations under the UNDP project on Mainstreaming Conservation and Sustainable Use of Medicinal Plants Diversity in three Indian States by the Government of India (www.in.undp.org).

1.9 Sacred Groves

These are the forest fragments of varying sizes, which are communally protected and which usually have a significant religious connotation for the protecting community. They are also known as "sacred natural sites" as per the ICUN (Oviedo et al. 2005). There exist more than 10,000 sacred groves in the tribal inhabited belt in India (http://www.ecoheritage.cpreec.org). Hariyali sacred grove, near Ganchar in Chamoli District of Uttarakhand, and the deodar grove in Shipin near Simla in Himachal Pradesh are the largest sacred grove in India. A good number of studies have shown the presence of many endemic and rare species in the groves (Bhakat and Pandit 2003).

1.10 Seed Gene Banks

In seed banks, germplasm is stored as seeds of various accessions. It is the easy, most effective, and efficient way of conservation of species that produce orthodox seeds. Orthodox seeds are seeds which will survive drying and/or freezing. Most of crop seeds belong to this category. Seed banks facilitate conservation of wide genetic variability in less space. However, success depends on careful monitoring of controlled conditions and testing of seed viability. Seed viability can be 5–25 years in medium-term storage (0–50C and 35% RH), whereas it can be up to hundred years in long-term storage (−10 °C to −20 °C) (Srivastava and Kumar 2010). There are four national seed gene banks for medicinal and aromatic plants at Tropical Botanical Garden and Research Institute (TBGRI), Thiruvananthapuram; Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow; National Bureau of Plant Genetic Resources (NBPGR), New Delhi; and Indian Institute of Integrative Medicine (IIIM), Jammu, in India where base collections are conserved. A total of about 6123 accessions of prioritized species are conserved as base collections at NBPGR, New Delhi (Annonymous 2012). About, 48 trait-specific germplasm accessions of 39 MAP species were also registered with NBPGR (Annonymous 2012).

1.11 Botanical or Herbal Gardens

Botanic gardens contain collections of plants for education, scientific purposes, and display, now means they play a key role in plant conservation particularly of rare and threatened plants. According to the IUCN Red List of threatened plants, 34,000 taxa are considered globally threatened with extinction. As per the estimate of the Botanic Gardens Conservation International (BGCI), currently, over 10,000 threatened species, approximately a third, are in botanic garden cultivation. These plants contribute to species recovery programs and provide long-term backup collections. These gardens are maintained by ancient doctors, healers, sages, royal families, and others supporting conservation. National biodiversity authority (NBA), an autonomous and statutory body of the Ministry of Environment and Forests, Government of India, listed existence of 109 botanical gardens across 18 states in India (http://nbaindia.org/link/241/34/1/SBBs.html). Ministry of agriculture under horticultural division has established 16 herbal gardens all over the India which are maintaining about 150 medicinal plants (Srivastava and Kumar 2010). The major botanic gardens in India include Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram (Kerala); Medicinal and Aromatic Plant Garden and Herbarium, Pune; Lal bagh Botanical garden, Bangalore; Royal Botanical garden, Kolkata; and Lloyd Botanical Gardens, Darjeeling.

1.12 Ex Situ Conservation

The work of a medicinal plant gene pool should employ a combination of methods; the appropriate strategy depends on factors such as geographic sites, biological characteristics of plants (Table 1.10), available infrastructure and network having an access to different geographical areas, human resources, and number of accessions in a given collection.

1.12.1 Conservation of Plants

Most of the medicinal plants can be conserved in field gene banks (FGB). In the different forms of ex situ conservation, field gene banks are probably the most costly in terms of establishing and maintaining of requirements, especially labor and supports. Due to economic as well as practical problems in management of field gene banks and simultaneous urgent needs for crop improvement, it has become evident that firm linkages between conservation and use needed for the sustainable long-term management of field gene bank. Field gene banks are established in the form of medicinal plants conservation parks (MPCPs) where plants of medicinal value are planted in near in situ locations and maintained as live specimens. Other strategies include ethnomedical forests (EMFs), herbarium material, and raw drug containing plant parts. In India, about 3200 home herbal gardens (HHGs) have been established in over 300 villages of the southern region. Besides this, the existing botanical gardens, arboreta, etc. are useful in conservation ex situ.

1.12.2 Conservation of Seeds

Seeds are best suited for storage in gene banks, but for species that do not set seeds or produce them sterile or recalcitrant, it is difficult to conserve. Seeds of most of the medicinal plants belong to this category. Seeds also should be able to survive when subjected to drying, i.e., desiccation up to 10%. Hence, only those species whose seed could be survive this conserved.

1.12.3 Conservation of Tissues

Six major steps defined in the conservation use cycle (40) are collection, quarantine, propagation, characterization, evaluation, monitoring, storage, and distribution. The role of in vitro conservation techniques in the overall conservation strategies should be indicative of the fact that it should complement other conservation strategies within the total program of a given species or population. The methods chosen should be carefully considered taking into account the feasibility, practicality, economy, and security.

Generally, field conservation of medicinal plants requires more space and is labor intensive and expensive. They also run the risk of being damaged by natural calamities and biotic stress factors. Techniques to conserve such species in vitro have recently been developed. For some species, while in situ conservation is the only option available, tissue culture systems offer advantages, which are as follows:

1. Very high multiplication rates.
2. Aseptic system
   - Free from fungi, bacteria, viruses, and insect pests.
   - Production of pathogen free stocks
3. Reduction of space requirements
4. Genetic erosion reduced to zero under optimal storage conditions.
5. Reduction of the expenses in labor costs.

In vitro collections of species could be maintained at the same or separate site, but should have clear linkages with field gene banks. The properties required for a successful in vitro conservation system as defined by Grout are as follows:

1.13 Way Forward

The increase in human population is one of the main causes for concern in meeting the daily requirements of medicine as the economy and livelihoods of human societies living in developing countries primarily depend on bioresources. This phenomenon is leading to continuous erosion of medicinal plant populations in the wild, thus making challenge to meet the requirements as well as to conserve them. In situ conservation along with ex-situ or off-site conservation is especially desirable in case of species where wild populations have dwindled to critical levels and viable populations for some of these species are not available. Biotechnological approaches are imperative for rapid multiplication and conservation of the threatened medicinal plants. Thus, strategies and vision for conservation of threatened medicinal plant diversity and sustainable use of the same in the 21st century is of far reaching significance for sustainable development

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Chapter 2
Threatened Medicinal Plants of Eastern Ghats and Their Conservation

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Abstract Traditional medicine has a long history of cultural heritage and ethnic practices in India and in recent years has gained much recognition worldwide. The Eastern Ghats, inhabited by nearly 54 tribal communities, constituting nearly 30% of total population, are a diverse and rich source of threatened medicinal and aromatic plants used in drug, pharmaceutical, and perfumery industries. Out of 2500 species of flowering plants belonging to angiosperms, gymnosperms, and pteridophytes known to occur in Eastern Ghats, about 77 species (67 dicots, 9 monocots, and 1 gymnosperm) are endemic. The variations in altitude and climatic conditions, especially in rainfall, have immensely contributed to the evolution of rich ethnic floristic diversity in the Eastern Ghats. At least 788 medicinal plant taxa and 40 aromatic plants are concentrated in this area which are used in various medicinal systems including codified and folklore which belong to 132 families and 384 genera. The dominant medicinal plant families in the Eastern Ghats are Leguminosae (67 spp.), Apocynaceae (29 spp.), Malvaceae (26 spp.), Euphorbiaceae (25 spp.), Orchidaceae (22 spp.), Solanaceae and Rubiaceae (16 spp. each), Asteraceae (15 spp.), Acanthaceae, Asteraceae and Lamiaceae (14 spp. each), Cucurbitaceae and Zingiberaceae (13 spp. each), Rutaceae (12 spp.), and Araceae (10 spp.). These medicinal plant genetic resources are distributed in various vegetation types in the Eastern Ghats region. Ethnobotanical knowledge from the Eastern Ghats region has

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been recorded by several workers. Indian systems of medicine are reported to utilize around 2500 plant species of which about 800 species are used by the industry and approximately 25% of species are under cultivation. India ranks sixth in essential oil production and export of products derived from medicinal plants. It is estimated that India has a potential to export plant base crude drugs to the tune of Rs. 400 billion but manages to export produce worth only about Rs. 12.6 billion. India with its rich biodiversity and tradition of use of herbal drugs in healthcare holds tremendous opportunity for growth in a multibillion global trade, particularly in the herbal area, which has vast potential for developing multiple products for nutrition, cosmetics, and prevention and cure of diseases. This article provides an overview of the threatened medicinal plants of the Eastern Ghats, their distribution, and reported uses in local health traditions. Blending traditional knowledge with modern science including genomics is a priority area to meet the forthcoming challenges in the light of climate change, and thus conservation strategies, both ex situ and in situ, for these diverse species are also discussed.

Keywords Eastern Ghats · Conservation · Threatened medicinal plants

2.1 Introduction

Plants are being utilized as medicines for thousands of years all over the globe and are a source of many potent and powerful drugs. Traditional medicine has become more popular in the treatment of many diseases due to belief that these are safe, easily available, and with fewer side effects. At least 80% of the population of developing countries depend on plant drugs for their primary healthcare needs (Farnsworth et al., 1985). Medicinal plants are vital components and play a significant role in the healthcare of rural people all over the world. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed (Reddy et al., 2019). There are many traditional systems of medicine associated with their own different philosophies and cultural origins. The herbal medicines/ traditional medicaments have been derived from rich traditions of ancient civilizations and scientific heritage. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman, and Syrian texts dates back to about 5000 years. The classical Indian texts including *Rig-Veda*, *Charaka Samhita*, and *Sushruta Samhita* are the evidences for these age-old traditions (Kamboj, 2000).

The Eastern Ghats have a diverse and rich source of threatened medicinal and aromatic group of plants used in drugs, pharmaceutical, and perfumery industries. In the modern era, though synthetic chemicals are contributing appreciably in the pharmaceutical application, the plant-based drugs remain vital source of modern medicine. The spurt in demand of the raw material in world trade has caused large-scale collection of the naturally occurring populations, thus threatening the very existence of these irreplaceable gene pools. The quantity gathered from natural habitats is so large that even protected areas are no longer safe, despite notification from the Government time to time. Even the ban on ruthless collection of medicinal plants/species has not improved the frequency of their distribution. For example, Eastern Ghats region has lost large populations of several medicinal plants in the past, viz., *Rauvolfia serpentina*, *Commiphora wightii*, *Chlorophytum tuberosum*, *Dioscorea deltoidea*, etc. Threatened medicinal plants are used in various indigenous systems of medicine such as *Siddha*, *Ayurveda*, *Amchi*, *Unani*, and even in allopathy, with pharmaceutical industries depending on plants for preparation of the medicines. Herbal drugs or medicinal plants, their extracts, and their isolated compounds have demonstrated a wide spectrum of biological activities. Such natural medicines have been used and continue to be used as medicine or as food supplements for various disorders as described in various texts and folklore. Safe, effective, and inexpensive indigenous remedies are currently gaining popularity among the people of both the urban and rural areas in India. Plant-based ethnic and traditional knowledge systems have become a recognized tool in search of drugs and pharmaceuticals (Reddy et al., 2019). An attempt has been made to review the conservation strategies, traditional knowledge systems of threatened medicinal plants prevailing in Eastern Ghats of India.

2.2 Eastern Ghats

The Eastern Ghats, one of the major hill ranges of India, located between $77^0 22'$ and $85^0 20'$ E and $11^0 30'$ and $21^0 00'$ N, form an assembly of discontinuous ranges, hills, plateaus, escarpments, and narrow basins and spread in an area of about 75,000 km^2. The Eastern Ghats stretching from Odisha, Chhattisgarh, through Andhra Pradesh to Tamil Nadu and parts of Karnataka are endowed with a large number of biological species, geological formations, and indigenous tribal groups (Fig. 2.1). For Eastern Ghats, the Mahanadi basin marks the northern boundary, while the southern boundary lies in the Nilgiri hills. The tips of Bastar, Telangana, Karnataka plateaus, and Tamil Nadu uplands form the boundary in the West, while the coastal belt forms the boundary in the East.

The Eastern Ghats region is inhabited by nearly 54 tribal communities, which constitute nearly 30% of total population (Chauhan, 1998). The major tribes in the Eastern Ghats are *Arondhan*, *Irular*, *Kota*, *Kotanayakam*, *Kurmar*, *Puniyan*, *Pulayan*, *Sholaga* and *Tuda*, and *Malayali* in the southern region; *Bagata*, *Chenchu*, *Gadaba*, *Jatapu*, *Kammara*, *Kondadora*, *Konda Kapu*, *Konda Reddy*, *Kandha*, *Kotiobenthu Oriya*, *Koya/Goud*, *Kulia*, *Mali*, *Mukadora*, *Mannedora*, *Nayaka*, *Paraja*, *Reddidora*, *Savara*, *Valmiki*, *Yenadi*, and *Yerukala* in central region; and *Bathudi*, *Birjhal*, *Bhuiyan*, *Dhuma*, *Bhumis*, *Bhuttada*, *Gond*, *Khana*, *Kisan*, *Kolba*, *Munda*, *Oraon*, *Soarha*, and *Sounti* in the northern region. The variations in altitude and climatic conditions, especially in rainfall, have immensely contributed to the evolution of rich ethnic floristic diversity in the Eastern Ghats. This region is very rich in terms of natural wealth, which is manifested, in its greatest biological diversity. Out of 2500 species of flowering plants belonging to angiosperms, gymnosperms, and pteridophytes known to occur in Eastern Ghats, about 77 species (67 dicots, 9 monocots and 1 gymnosperms) are endemic.

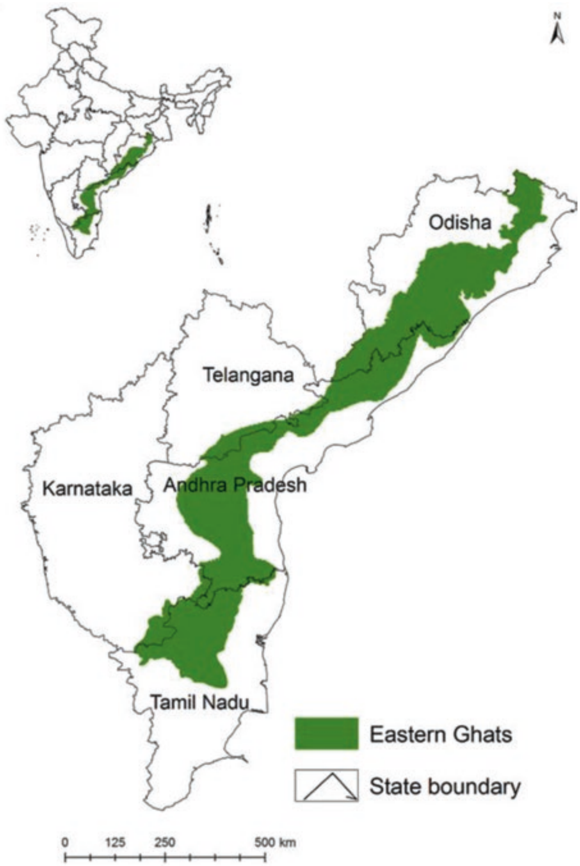

Fig. 2.1 Location map of Eastern Ghats, India. (Courtesy: Dr Sudhakar Reddy, NRSC, Hyderabad)

2.3 Status of Medicinal Plant Genetic Resources in Eastern Ghats

The rich and diverse heritage of traditional indigenous medicinal and aromatic plants in Eastern Ghats is threatened due to various abiotic and biotic stresses coupled with the technological advancement. With increasing interest in herbal medicines worldwide, conservation of medicinal plants in Eastern Ghats has assumed considerable importance. The Eastern Ghats are endowed with rich floristic diversity consisting of more than 2000 species of plants including medicinal plant species (1800) belonging to angiosperms, gymnosperms, and pteridophytes. Eastern Ghats vegetation includes 454 endemic species belonging to 243 genera and 78 families (Reddy et al., 2002a, b). At least 788 medicinal plant taxa and 40 aromatic plants are concentrated in this area which are used in various medicinal systems including codified and folklore which belong to 132 families and 384 genera. The dominant medicinal plant families in the Eastern Ghats are Leguminosae (67 spp.), Apocynaceae (29 spp.), Malvaceae (26 spp.), Euphorbiaceae (25 spp.), Orchidaceae

(22 spp.), Solanaceae and Rubiaceae (16 spp. each), Asteraceae (15 spp.), Acanthaceae, Asteraceae and Lamiaceae (14 spp. each), Cucurbitaceae and Zingiberaceae (13 spp. each), Rutaceae (12 spp.), and Araceae (10 spp.). These medicinal plant genetic resources are distributed in various vegetation types in the Eastern Ghats region (Table 2.1). A total of 560 tree taxa fewer than 262 genera belonging to 80 families are reported to occur in the Eastern Ghats (Rani and Pullaiah, 2002). Dye yielding plants occurring in the southern most point of Eastern Ghats is recorded (Krishnamurthy et al., 2002). The Eastern Ghats region is being exploited in an unregulated manner for this natural wealth. Several published floras by eminent botanists from the region are available on Eastern Ghats such as H.H. Haines, J.S. Gamble, C.E.C. Fischer, C.A.Barber, C.H. Beddome, T. Spring, J.L.Ellis, K.M. Matthew, R.S.Rao, G. Rao, B. Suryanarayana, T. Pullaiah, H.F. Mooney, etc., and changes in plant biodiversity pattern of the region were also a subject of review recently (Pandravada et al., 2004). Genera such as *Anaphalis*,

Table 2.1 Predominant medicinal taxa including threatened species occurring in different vegetation types of Eastern Ghats

Scrub	Deciduous	Evergreen/semi-evergreen
Acacia chundra	*Adina cordifolia*	*Arisaema* sp.
Albizia amara	*Andrographis paniculata*	*Bridelia tomentosa*
Anogeissus latifolia	*Bauhinia vahlii*	*Callicarpa tomentosa*
Apluda mutica	*Boswellia ovalifoliolata*	*Calycopteris floribunda*
Atalantia monophylla	*Bridelia retusa*	*Celtis cinnamomea*
Capparis sepiaria	*Careya arborea*	*Centella asiatica*
Carissa spinarum	*Cassia fistula*	*Cinnamomum zeylanicum*
Cassia auriculata	*Cipadessa baccifera*	*Coelogyne nervosa*
Cissus quadrangularis	*Dendrocalamus strictus*	*Couroupita guianensis*
Curculigo orchioides	*Garuga pinnata*	*Dillenia pentagyna*
Cymbopogon flexuosum	*Grewia tiliifolia*	*Elaeocarpus serratus*
Decalepis hamiltonii	*Helicteres isora*	*Entada pursaetha*
Dichrostachys cinerea	*Kydia calycina*	*Ichnocarpus frutescens*
Dodonaea viscosa	*Madhuca longifolia*	*Ixora montana*
Eclipta alba	*Memecylon umbellatum*	*Macaranga peltata*
Emblica officinalis	*Mucuna pruriens*	*Mallotus philippensis*
Euphorbia antiquorum	*Pterocarpus marsupium*	*Mangifera indica*
Euphorbia tirucalli	*Schleichera trijuga*	*Mesua nagassarium*
Hemidesmus indicus	*Sterculia urens*	*Michelia champaca*
Holarrhena antidysenterica	*Terminalia arjuna*	*Naravelia zeylanica*
Hugonia mystax	*Terminalia chebula*	*Ochna gamblei*
Pergularia daemia	*Terminalia tomentosa*	*Pimpinella tirupatiensis*
Phyllanthus amarus	*Tinospora cordifolia*	*Plumbago zeylanica*
Santalum album	*Toddalia asiatica*	*Rauvolfia serpentina*
Strychnos nux-vomica	*Woodfordia fruticosa*	*Toona ciliata*
Tridax procumbens	*Wrightia tinctoria*	*Xylia xylocarpa*

Source: Sivaraj et al. 2006

Bulbophyllum, *Callicarpa*, *Clematis*, *Debregeasia*, *Delphinium*, *Dillenia*, *Ensete*, *Eulophia*, *Exacum*, *Lobelia*, *Mallotus*, *Meliosma*, *Mucuna*, *Pimpinella*, *Prunus*, *Raphidophora*, *Sapium*, *Saussurea*, *Syzygium*, *Tinospora*, *Vanilla*, and *Viburnum* present in the Eastern Ghats are common to the Himalayas, Khasi, and Jaintia hills of Meghalaya and the Western Ghats (Reddy et al., 2002a, b; Sahu and Dhal, 2012; Sivaraj et al., 2015). *Cycas beddomei*, *Cycas circinalis*, and *Gnetum scandens* and about 30 species of ferns including *Cyathea gigantea*, a tree fern, are also distributed in this region. The following are the red list categories which are applicable to threatened medicinal plant taxa of Eastern Ghats region:

- Critically endangered (CR) – in a particularly and extremely critical state (e.g., *Rauvolfia serpentina*, *Litsea glutinosa*, *Cycas beddomei*, etc.)
- Endangered (EN) – very high risk of extinction in the wild, meets IUCN criteria for endangered (A-E) (e.g., *Homalium zeylanicum*, *Butea monosperma*, *Rhynchosia heynei*, *Tephrosia calophylla*, *Saraca asoca*, *Entada rheedii*, *Plumbago indica*, *Strychnos colubrina*, *Ceropegia spiralis*, *Decalepis hamiltonii*, *Plectranthus barbatus*, *Piper nigrum*, *Santalum album*, *Vanilla wightiana*, *Acorus calamus*, etc.)
- Vulnerable (Vu) – meets one of the five red list criteria and thus considered to be at high risk of unnatural (human-caused) extinction without further human intervention (e.g., *Hildegardia populifolia*, *Sterculia urens*, *Aegle marmelos*, *Rubia cordifolia*, *Gymnema sylvestre*, *Oroxylum indicum*, *Euphorbia fusiformis*, *Phyllanthus indofischeri*, *Stemona tuberosa*, *Gloriosa* superba, etc.).

Detailed threatened category of medicinal plants of Eastern Ghats, species, and family wise are provided in Table 2.2.

2.4 Traditional Knowledge on Threatened Medicinal Plant Systems

Ethnobotanical knowledge from the Eastern Ghats region has been recorded by several workers (Saxena and Dutta, 1975; Banerjee, 1977; Reddy, 1980; Rao and Harasreeramulu, 1985; Thammanna and Rao, 1998; Ravisankar and Henry 1992; Goud and Pullaiah, 1996; Rao and Henry, 1996; Vedavathy et al., 1997; Pandravada and Sivaraj, 1999; Pandravada et al., 2000, 2006; Pullaiah, 2002; Rao and Reddi 2002; Reddy et al., 2002a, b; Basha and Sudarsanam, 2010, Dikshit and Sivaraj, 2014). The tribes living in the Eastern Ghats depend mostly on various forest products, but their careless collection resulted in much damage to the forest wealth particularly rare and endangered medicinal plant species. Many tribal communities are practicing their local health traditional methods using medicinal herbs to cure various ailments. Their understanding of the medicinal flora around them and related indigenous knowledge systems are transmitted through successive generations and practiced as a part of their tradition and culture. Medical practices of local and indigenous people have remained unchanged over long periods of time. In the face

Table 2.2 Threatened medicinal plant families of Eastern Ghats

Family	Threatened status	Botanical name	Distribution
Acanthaceae	Endangered	*Phlebophyllum jeyporense* (Bedd.) Bremekamp	Chhatisgarh, Odisha, Andhra Pradesh
		Santapaua madurensis Balakr. & Subram.	Tamil Nadu
	Extinct or possibly extinct	*Neuracanthus neesianus* (Wight ex T. Anders.) Clarke	Tamil Nadu
	Indeterminate	*Lepidagahis difusa* Clarke	Karnataka, Tamil Nadu
		Strobilanthes dupenii Bedd. ex Clarke	Peninsular India (Anamalais)
	Rare	*Lepidagathis barberi* Gamble	Tamil Nadu
		Mackenziea caudata (T. And.) Ramam.	Karnataka, Tamil Nadu
		Nilgirianthus circarensis (Gamble) Bremek.	Andhra Pradesh, Odisha
Amaranthaceae	NA	*Aerva wightii* Hook. f.	Tamil Nadu
Anacardiaceae	Endangered	*Nothopegia aureo-fulva Bedd. ex Hook. f.*	Tamil Nadu
Annonaceae	Endangered	*Desmos viridiflorus (Bedd.) Safford*	Tamil Nadu
		Uvaria eucincta Bedd. ex Dunn	Odisha
	Rare	*Goniothalamus rhynchantherus Dunn*	Tamil Nadu
		Orophea uniflora Hook. f. & Thoms.	Tamil Nadu, Karnataka
		Polyalthia rufescens Hook. f. &Thoms.	Tamil Nadu
		Popowia beddomeana Hook. f. &Thoms.	Tamil Nadu
	Vulnerable	*Miliusa nilagirica Bedd.*	Tamil Nadu
Apiaceae	Endangered (globally)	*Pimpinella tirupatiensis* Balakr. *et* Subram.	Andhra Pradesh
	Rare	*Peucedanum anamallayense* Clarke	Tamil Nadu
		Vanasushava pedata (Wight) Mukh. *et* Const.	S. India (Shervaroy, Pala ni, and Anamalis hills)
Apocynaceae	Critically endangered	*Rauvolfia serpentina*	Andhra Pradesh
	Endangered	*Anodendron paniculatum*	Andhra Pradesh
	Near threatened	*Holostemma ada-kodien*	Andhra Pradesh
Aponogetonaceae	Indeterminate	*Aponogeton appendiculatus* van Bruggen	Tamil Nadu

(continued)

Table 2.2 (continued)

Family	Threatened status	Botanical name	Distribution
Araceae	Endangered	*Acorus calamus*	Andhra Pradesh
		Lasia spinosa	Andhra Pradesh
		Rhaphidophora decursiva	Andhra Pradesh
	Vulnerable	*Amorphophallus sylvaticus*	Andhra Pradesh
		Cryptocoryne cognatoides Blatt. &McC.	Karnataka, Maharashtra
Asclepiadaceae	Endangered	*Ceropegia barnesii* Bruce *et* Chatterjee	S. India
		Ceropegia omissa Huber [C. intermedia Wight var. wightii Hook. f.]	Tamil Nadu
		Toxocarpus longistigma (Roxb.) Wight & Arn. Ex Steud.	Andhra Pradesh
	Endangered (globally)	*Decalepis hamiltonii*	Andhra Pradesh
	Extinct or possibly extinct	*Ceropegia fantastica* Sedgw.	Karnataka, Goa
		Ceropegia maculata Bedd. [C. parviflora Trimen]	Tamil Nadu
	Near threatened	*Holostemma ada-kodien*	Andhra Pradesh
	Rare	*Ceropegia decaisneana* Wight	Kerala, Tamil Nadu
		Ceropegia metziana Miq.	Karnataka, Tamil Nadu
		Ceropegia pusilla Wight *et* Arn.	Karnataka, Tamil Nadu
		Marsdenia raziana Yog. *et* Subr.	Karnataka
		Toxocarpus beddomei Gamble	Tamil Nadu
	Vulnerable	*Ceropegia fimbriifera* Bedd.	Karnataka, Tamil Nadu
		Ceropegia spiralis Wight	Andhra Pradesh, Karnataka, Tamil Nadu
		Ceropegia thwaitesii Hook.	Tamil Nadu
		Gymnema sylvestre	Andhra Pradesh
Asparagaceae	Indeterminate/ insufficiently known	*Asparagus rottleri* Baker	Deccan Peninsula
	Least concerned	*Chlorophytum arundinaceum*	Andhra Pradesh
Asteraceae	Endangered	*Senecio kundaicus* Fischer	Tamil Nadu
	Extinct or possibly extinct	*Vernonia recurva* Bedd. ex S. Moore	Tamil Nadu
	Rare	*Helichrysum perlanigerum* Gamble	Tamil Nadu

(continued)

Table 2.2 (continued)

Family	Threatened status	Botanical name	Distribution
		Senecio mayurii Fischer	Karnataka
Athyriaceae	Rare	*Diplazium travancoricum* Bedd.	South . India
Balsaminaceae	Endangered	*Impatiens neo-barnesii* Fischer	Tamil Nadu
		Impatiens nilagirica Fischer	Tamil Nadu
	Rare	*Impatiens talbortii* Hook. f.	Karnataka
Bignoniaceae	Vulnerable	*Oroxylum indicum*	Andhra Pradesh
Burseraceae	Endangered (globally)	*Boswellia ovalifoliolata*	Andhra Pradesh
Callophyllaceae	Not evaluated	*Mesua ferrea*	Andhra Pradesh
Capparaceae	Indeterminate	*Cleome burmanni* Wight *et* Arn.	Tamil Nadu
	Rare	*Capparis fusifera* Dunn	Kerala, Tamil Nadu
		Capparis rheedii DC.	Tamil Nadu, North Kanara
	Vulnerable	*Capparis diversifolia* Wight & Arn.	Tamil Nadu
		Capparis shevaroyensis Sundararaghavan	Tamil Nadu
Caryophyllaceae	Vulnerable	*Polycarpaea diffusa* Wight & Arn.	Tamil Nadu
Celastraceae	Endangered	*Euonymus angulatus* Wight	Karnataka, Tamil Nadu
	Extinct or possibly extinct	*Euonymus serratifolius* Bedd.	Tamil Nadu
	Extinct or possibly extinct	*Salacia malabarica* Gamble	Karnataka
	Near threatened	*Celastrus paniculatus*	Andhra Pradesh
	Rare	*Salacia beddomei* Gamble	Tamil Nadu
Caesalpiniaceae	Endangered	*Saraca asoca*	Andhra Pradesh
	Extinct or possibly extinct	*Euonymus serratifolius* Bedd.	Tamil Nadu
		Salacia malabarica Gamble	Karnataka
	Near threatened	*Celastrus paniculatus*	Andhra Pradesh
	Rare	*Salacia beddomei* Gamble	Tamil Nadu
Combretaceae	Endangered	*Terminalia pallida*	Andhra Pradesh
Commelinaceae	Endangered	*Belosynapsis kewensis* Hassk.	Tamil Nadu
	Indeterminate	*Cyanotis cerifolia* Rolla Rao *et* Kammathy	Tamil Nadu
	Rare	*Commelina indehiscens* Barnes	Karnataka, Tamil Nadu

(continued)

Table 2.2 (continued)

Family	Threatened status	Botanical name	Distribution
		Murdannia lanuginose (Wall. ex Clarke) Bruckn.	Deccan Plateau, Sahyadri hills
	Vulnerable	*Commelina tricolor* Barnes	Tamil Nadu
		Commelina wightii Rolla Rao	Tamil Nadu
		Murdannia lanceolata (Wight) Kammathy	Tamil Nadu
Convolvulaceae	Least concerned	*Merremia turpethum*	Andhra Pradesh
Crassulaceae	Rare	*Kalanchoe olivacea* Dalz.	Tamil Nadu
Cucurbitaceae	Near threatened	*Trichosanthes cucumerina*	Andhra Pradesh
Cyatheaceae	Endangered	*Sphaeropteris crinita (Hook.) Tryon [Cyathea crinita (Hook.) Copel.]*	Tamil Nadu
Cycadaceae	Critically endangered	*Cycas beddomei*	Andhra Pradesh
	Vulnerable	*Cycas beddomei* Dyer	Andhra Pradesh
Cyperaceae	Indeterminate	*Carex pseudo-aperta* Kuekenth.	Tamil Nadu
		Carex vicinalis Boott	Tamil Nadu
	Indeterminate or possibly extinct	*Carex christii* Boeck.	Tamil Nadu
Dicranopteridaceae	Vulnerable	*Dicranopteris linearis* (Burm. f.) Underw. var. *sebastiana* Panigr. & Dixit	Tamil Nadu
Dioscoreaceae	Near threatened	*Tacca leontopetaloides*	Andhra Pradesh
Dipterocarpaceae	Rare	*Hopea jacobi* Fischer	Karnataka
	Endangered	*Shorea tumbaggaia*	Andhra Pradesh
	Near threatened	*Shorea robusta*	Andhra Pradesh
Elaeocarpaceae	Rare	*Elaeocarpus blascoi* Weibel	Tamil Nadu
		Elaeocarpus recurvatus Corner	Tamil Nadu
Elaphoglossaceae	Endangered	*Elaphoglossum nilgiricum* Krajina ex Sledge	Tamil Nadu
Euphorbiaceae	Endangered	*Phyllanthus narayanaswamii* Gamble	Andhra Pradesh
	Indeterminate	*Pseudoglochidion anamalayanum* Gamble	Tamil Nadu
	Rare	*Dalechampia stenoloba* Sundararaghavan *et* Kulkarni	Karnataka
		Phyllanthus talbotii Sedgw.	Karnataka
	Vulnerable	*Euphorbia fusiformis*	Andhra Pradesh
		Phyllanthus indofischeri	Andhra Pradesh

(continued)

Table 2.2 (continued)

Family	Threatened status	Botanical name	Distribution
Fabaceae	Endangered	*Crotalaria clavata* Wight *et* Arn.	Tamil Nadu
		Crotalaria fysonii Dunn var. *glabra* Gamble	Tamil Nadu
		Crotalaria kodaiensis Debberm. *et* Biswas	Tamil Nadu
		Crotalaria longipes Wight *et* Arn.	Tamil Nadu
		Crotalaria sandoorensis Bedd. ex Gamble	Karnataka
		Entada pursaetha	Andhra Pradesh
		Humboldtia bourdilloni Prain	Tamil Nadu
		Humboldtia unijuga var. *unijuga* Bedd.	Tamil Nadu
		Butea monosperma var. lutea	Andhra Pradesh
	Near threatened	*Pueraria tuberosa*	Andhra Pradesh
	Rare	*Acacia campbellii* Arn.	Andhra Pradesh
		Albizia thompsonii Brandis	Andhra Pradesh, Tamil Nadu, Odisha
		Crotalaria digitata Hook.	Tamil Nadu
		Crotalaria globosa Wight *et* Arn.	Tamil Nadu, Karnataka
		Crotalaria lutescens Dalz.	Karnataka, Maharashtra
		Crotalaria peduncularis Grah. ex Wight *et* Arn.	Tamil Nadu
		Crotalaria priesleyoides Benth. ex Baker	Tamil Nadu
		Crotalaria rigida Heyne ex Roth	Tamil Nadu, Karnataka
		Crotalaria scabra Gamble	Tamil Nadu
		Cynometra travancorica Bedd.	Karnataka
		Eleiotis trifoliolata Cooke	Karnataka
		Humboldtia decurrens Bedd. ex Oliver	Tamil Nadu
		Indigofera barberi Gamble	Andhra Pradesh, Tamil Nadu
		Indigofera constricta (Thw.) Trimen	Goa, Karnataka
		Kingiodendron pinnatum (Roxb. ex DC.) Harms	Karnataka, Tamil Nadu
		Rhynchosia beddomei Baker	Karnataka

(continued)

Table 2.2 (continued)

Family	Threatened status	Botanical name	Distribution
		Tephrosia barberi Drumm.	Tamil Nadu
		Tephrosia calophylla Bedd.	Tamil Nadu, Karnataka
	Vulnerable	*Cynometra bourdillonii* Gamble	Karnataka
		Rhynchosia velutina Wight *et* Arn.	Tamil Nadu
	Endangered (globally)	*Pterocarpus santalinus*	Andhra Pradesh
Flacourtiaceae	Endangered	*Hydnocarpus macrocarpa* (Bedd.) Warb. ssp. *macrocarpa*	Tamil Nadu
Gesneriaceae	Rare	*Didymocarpus missionis* Wall. ex R. Br.	Tamil Nadu
Lamiaceae	Endangered	*Leucas mukerjiana* Subba Rao *et* Kumari	Andhra Pradesh
		Plectranthus barbatus	Andhra Pradesh
		Pogostemon paludosus Benth.	Tamil Nadu
	Indeterminate	*Acrocephalus palniensis* Mukherjee	Tamil Nadu
		Plectranthus bourneae Gamble	Tamil Nadu
	Possibly extinct	*Plectranthus bishopianus* Gamble	Tamil Nadu
	Rare	*Anisochilus wightii* Hook. f.	Tamil Nadu
		Leucas angustissima Sedgw.	Karnataka
		Pogostemon atropurpureus Benth.	Tamil Nadu
	Vulnerable	*Anisochilus argenteus* Gamble	S. India
Lauraceae	Critically endangered	*Litsea glutinosa*	Andhra Pradesh
	Endangered	*Actinodaphne bourneae* Gamble	Tamil Nadu
		Actinodaphne lanata Meisner	Tamil Nadu
	Rare	*Actinodaphne lawsonii* Gamble	Tamil Nadu
Liliaceae	Endangered	*Iphigenia sahyadrica* Ansari *et* Rolla Rao	Karnataka
		Urginea congesta Wight	S. India
	Indeterminate	*Dipcadi minor* Hook. f.	Deccan Plateau
	Possibly extinct	*Dipcadi concanense* (Dalz.) Baker	S. India

(continued)

Table 2.2 (continued)

Family	Threatened status	Botanical name	Distribution
	Presumed extinct	*Urginea polyphylla* Hook. f.	Deccan Peninsula
	Vulnerable	*Gloriosa superba*	Andhra Pradesh
Linaceae	Rare	*Hugonia belli* Sedgw.	Karnataka
Loganiaceae	Endangered	*Strychnos colubrina*	Andhra Pradesh
Loranthaceae	Indeterminate	*Viscum mysorense* Gamble	Karnataka
Malpighiaceae	Rare	*Aspidopteris canarensis* Dalz.	Karnataka, Maharashtra.
		Aspidopterys tomentosa var. hutchinsonii (Haines) Srivastava	Odisha
Malvaceae	Endangered	*Decaschistia rufa* Craib	Peninsular India
	Rare	*Decaschistia trilobata* Wight	Peninsular India
Marattiaceae	Endangered	*Angiopteris evecta*	Andhra Pradesh
Melastomataceae	Endangered	*Kendrickia walker* (Wight) Hook. f. ex Triana	Tamil Nadu
		Memecylon flavescens Gamble	Tamil Nadu
	Indeterminate	*Memecylon sisparense* Gamble	Tamil Nadu
Meliaceae	Vulnerable	*Aglaia talbotii* Sundararaghavan	Karnataka
Myrsinaceae	Rare	*Antistrophe serratifolia* (Bedd.) Hook. f.	Tamil Nadu
Myrtaceae	Endangered	*Eugenia discifera* Gamble	Tamil Nadu
		Meteoromyrtus wynaadensis (Bedd.) Gamble	Tamil Nadu
		Syzygium courtallense (Gamble) Alston	Tamil Nadu
		Syzygium gambleanum Rathakr. *et* Chitra	Tamil Nadu
		Syzygium alternifolium	Andhra Pradesh
	Extinct or possibly extinct	*Eugenia singampattiana* Bedd.	Tamil Nadu
Orchidaceae	Endangered	*Nervilia aragoana*	Andhra Pradesh
	Extinct or possibly extinct	*Anoectochilus rotundifolius* (Blatt.) Balakr.	Tamil Nadu
	Indeterminate or insufficiently known	*Chrysoglossum hallbergii* Blatt.	Peninsular India (Tamil Nadu)
	Possibly extinct	*Vanda wightii* Reichb. f.	Tamil Nadu
	Rare	*Bulbophyllum acutiflorum* A. Rich.	Tamil Nadu

(continued)

Table 2.2 (continued)

Family	Threatened status	Botanical name	Distribution
		Bulbophyllum albidum Hook. f.	Tamil Nadu
		Corymborkis veratifolia (Reinw.) Bl.	Tamil Nadu
		Eria albiflora Rolfe	Tamil Nadu, Karnataka
		Habenaria barnesii Summerh.	Tamil Nadu
		Oberonia brachyphylla Blatt. & McCann	Karnataka
		Vanilla wightiana Lindl.	Tamil Nadu
	Vulnerable	*Bulbophyllum elegantulum* (Rolfe) J.J. Sm.	Karnataka
		Coelogyne mossiae Rolfe	Peninsular India
		Liparis biloba Wight	Tamil Nadu
Periplocaceae	Endangered	*Utleria salicifolia* Bedd.	Tamil Nadu
Piperaceae	Endangered	*Piper nigrum*	Andhra Pradesh
Plumbaginaceae	Endangered	*Plumbago indica*	Andhra Pradesh
Poaceae	Presumed extinct	*Eragrostis rottleri* Stapf	S. India
		Eriochrysis rangacharii Fischer	Tamil Nadu
		Hubbardia heptaneuron Bor	Karnataka
	Rare	*Glyphochloa divergens* (Hook.) Clayton	Karnataka
		Isachne mysorensis Raghavan	Karnataka
Podostemonaceae	Rare or vulnerable	*Indotristicha tirunelveliana* Sharma, Karthi. & Shetty	Tamil Nadu
Ranunculaceae	Indeterminate	*Thalictrum dalzellii* Hook.	Karnataka, Maharashtra
	Rare	*Clematis theobromina* Dunn	Tamil Nadu
Rosaceae	Vulnerable	*Cotoneaster buxifolius* Wall. ex Lindley	Tamil Nadu
Rubiaceae	Endangered	*Acranthera grandiflora* Bedd.	Tamil Nadu
		Hedyotis albonervia Bedd.	Tamil Nadu
		Psychotria globicephala Gamble	Tamil Nadu
	Indeterminate	*Neanotis carnosa* (Dalz.) Lewis	Karnataka
	Near threatened	*Paederia foetida*	Andhra Pradesh
	Possibly extinct	*Hedyotis hirsutissima* Bedd.	Tamil Nadu
		Pavetta wightii Hook. f.	Tamil Nadu

(continued)

Table 2.2 (continued)

Family	Threatened status	Botanical name	Distribution
	Presumed extinct	*Ophiorrhiza brunonis* Wight *et* Arn.	Tamil Nadu, Karnataka
		Wendlandia angustifolia Wight ex Hook. f.	Tamil Nadu
	Rare	*Hedyotis buxifolia* Bedd.	Tamil Nadu
		Hedyotis cyanantha Kurz	Tamil Nadu, Maharashtra, Karnataka
		Hedyotis eualata (Bedd. ex Gamble) Henry et Subramanyam	Tamil Nadu
		Hedyotis swersioides Hook. f.	Tamil Nadu
	Vulnerable	*Hedyotis barberi* (Gamble) Henry *et* Subramanyam	Tamil Nadu
		Hedyotis ramarowii (Gamble) Rolla Rao *et* Hemadri	Tamil Nadu
		Neanotis prainiana (Talbot) Lewis	Karnataka
		Ochreinauclea missionis (Wall. ex G. Don) Ridsd.	Tamil Nadu, Karnataka
		Pavetta hohenackeri Brem.	Tamil Nadu
		Rubia cordifolia	Andhra Pradesh
		Tarenna agumbensis Sundararaghavan	Karnataka
Rutaceae	Endangered	*Zanthoxylum rhetsa*	Andhra Pradesh
	Rare	*Glycosmis macrocarpa* Wight	Tamil Nadu
	Vulnerable	*Aegle marmelos*	Andhra Pradesh
		Melicope indica Wight	Tamil Nadu
Santalaceae	Endangered	*Santalum album*	Andhra Pradesh
Sapotaceae	Indeterminate	*Isonandra villosa* Wight	Tamil Nadu, Andhra Pradesh
	Insufficiently known	*Madhuca diplostemon* (Clarke) van Royen	Peninsular India
	Possibly extinct	*Madhuca insignis* (Radlk.) H.J. Lam	Karnataka
Smilacaceae	Rare	*Smilax wightii* A. DC.	Tamil Nadu
Stemonaceae	Vulnerable	*Stemona tuberosa*	Andhra Pradesh
Sterculiaceae	Endangered	*Hildegardia populifolia* (Roxb.) Schott & Endl.	Andhra Pradesh, Tamil Nadu
	Rare	*Pterospermum reticulatum* Wight & Arn.	Karnataka, Tamil Nadu
	Vulnerable	*Eriolaena lushingtonii* Dunn	Andhra Pradesh, Tamil Nadu

(continued)

Table 2.2 (continued)

Family	Threatened status	Botanical name	Distribution
		Sterculia urens	Andhra Pradesh
		Hildegardia populifolia	Andhra Pradesh
Thelypteridaceae	Endangered	*Pseudocyclosorous griseus* (Baker) Holtt. & Grimes [*Neprodium griseum* Bake]	Tamil Nadu
Vitaceae	Vulnerable	*Cayratia roxburghii* (Wight *et* Arn.) Gagnepain	Tamil Nadu
Zingiberaceae	Endangered	*Zingiber roseum*	Andhra Pradesh
	Near threatened	*Costus speciosus*	Andhra Pradesh
	Rare	*Amomum microstephanum* Baker	Tamil Nadu
	Vulnerable	*Paracautieya bhatii* Smith	Karnataka

of increasing industrialization and modernization, the knowledge base of local health traditions has begun to erode. *Acacia catechu*, *Acacia concinna*, *Cassia auriculata*, *Cassia fistula*, *Cassia javanica*, *Cassia senna*, *Ceratonia siliqua*, *Glycyrrhiza glabra*, *Mucuna pruriens*, *Psoralea corylifolia*, and *Pueraria tuberosa* are some of the medicinal legumes, and *Caesalpinia* and *Indigofera* are some of the dye yielding plants from Eastern Ghats in India. Legumes used for treating various ailments of the body, i.e., ear, nose, throat, and eyes (ophthalmic, odontalgic, sternutatory); chest and lungs (antiasthmatic, demulcent, expectorant); heart and blood (cardiac, blood purifier, vasodilator); liver and kidneys (hepatic, antbilious); stomach (emetic, stomachic, digestive); bowels and bladder (purgative, laxative, carminative); nerves and muscles (antispasmodic, nervine); bones (anti-inflammatory, antirheumatic); skin, hands, and feet (acrid, skin applications); sex and reproduction (abortifacient, aphrodisiac, galactogogue); wounds and bruises (antiseptic, poultice, vulnerary); fever (febrifuge); infectious diseases (antiperiodic, VD); bites and stings (antidote, stings); cancer (cancer); and fungi and bacteria (antibacterial, antifungal) are reviewed and reported earlier (Pandravada et al., 2006; Varaprasad et al., 2006).

The Malayali tribes of the Southern Eastern Ghats region are using 189 plant species belonging to 86 families for the treatment of 85 diseases (Suresh, 2010). Tribals of Rayalaseema region of Eastern Ghats are using about 54 plant species belonging to 50 genera and 34 families for treating asthma alone (Anjaneyulu and Sudarsanam, 2013). The tribal areas of Rayalaseema have reported about 70 medicinal plant species for gynecological and abortive properties (Nagalakshmi, 2001). Eastern Ghats of Odisha has a potential ethnomedicinal resource for treating various human diseases particularly rheumatism for about 62 genera with 78 plant species including *Acanthus ilicifolius*, *Thunbergia fragrans*, *Cerbera odollam*, *Guizotia abyssinica*, *Derris scandens*, *Flacourtia indica*, *Pandanus fascicularis*, *Sesamum indicum*, and *Stachytarpheta jamaicensis* (Panda et al., 2014). Some of the major Eastern Ghats ethnic groups and their traditional healthcare knowledge systems on threatened medicinal plant taxa are presented in Table 2.3.

Table 2.3 Ethnic groups and traditional healthcare knowledge systems in Eastern Ghats

Tribal group	Number of plant families/genera/ species used	Major species and ailments	Reference(s)
Southern-Eastern Ghats			
Malayalis	86 plant families/147 genera/250 species	*Achyranthes aspera* (piles) *Aegle marmelos* (fever) *Andrographis paniculata* (poisonous bite) *Clematis gouriana* (eye diseases) *Macaranga peltata* (kidney stones) *Michelia champaca* (scorpion sting), *Naravelia zeylanica* (skin disease), *Nymphaea nouchali* (urinary problem) *Randia dumetorum* (lice and dandruff) *Tinospora sinensis* (rheumatism), *Wattakaka volubilis* (diabetes)	Alagesaboopathi et al. (1999) Dwakaran et al. (1994) Francis Xavier et al. (2011) Karthik et al. (2011) Murugesan et al. (2011) Prabu and Kumuthakalavalli (2012) Senthilkumar et al. (2013) Suresh (2010) Suresh et al. (2011) Vaidyanathan et al. (2013)
Irulas	57 species	*Achyranthes bidentata* (antifertility) *Blepharis maderaspatensis* (mother care) *Caralluma attenuata* (urinary troubles) *Cymbopogon citratus* (repellent) *Datura innoxia* (mental illness) *Ocimum americanum* (lice treatment) *Solanum virginianum* (cough)	Tariq et al. (2012) Kadavul and Dixit (2009) Karthick (2013)
Nakkala, *Sugalis* or *Lambadas*, *Yerukalas*	120 families/179 genera/204 species	*Abrus precatorius* (gonorrhea, night blindness) *Cassia auriculata* (bone fracture) *Nerium oleander* (cuts and wounds)	Anjaneyulu and Sudarsanam (2013) Naidu et al. (2012) Thammana and Rao (1998) Vedavathy and Rao (1994) Vedavathy et al. (1997)

(continued)

Table 2.3 (continued)

Tribal group	Number of plant families/genera/ species used	Major species and ailments	Reference(s)
Middle Eastern Ghats			
Chenchus	69 plant species	*Syzygium cumini* (earache, dysentery) *Andrographis paniculata* (fever, jaundice) *Euphorbia hirta* (ulcers and fissures, warts) *Andrographis echioides*, *Boerhavia diffusa*, *Canavalia ensiformis*, *Phyllanthus amarus*, *Physalis minima*, *Tephrosia purpurea* (liver ailments)	Rao and Sunita (2011) Sabjan et al. (2014)
Gonds	59 plant species	*Acacia arabica*, *Albizia odoratissima* (antidote) *Atalantia monophylla* (rheumatism) *Cayratia pedata* (uterine disorder, *Convolvulus sepiaria* (fertility) *Cyanotis tuberosa* (cough) *Litsea glutinosa* (wound healing) *Putranjiva roxburghii* (impotency) *Sterculia urens* (male sterility) *Xylia xylocarpa* (skin)	Murthy (2012) Kumar et al. (2013)
Bagatas, *Konda doras*, *Kotias*, and *Konds*	98 species	*Annona squamosa* (wounds) *Polyalthia longifolia* (rheumatism) *Cissampelos pareira* (stomachic) *Nelumbo nucifera* (dysentery) *Brassica juncea* (diarrhea) *Ziziphus xylopyrus* (asthma) *Pterocarpus marsupium* (eczema)	Padal et al. (2010) Padal et al. (2013)
Northern Eastern Ghats			
Paroja, *Saora*, *Bhumia*, *Godaba*, *Dogaria*, and *Kondha*	77 plant species	*Caryota urens*, *Curcuma montana*, *Sansiveria roxburghiana*, *Sesbania grandiflora*, *Elephantopus scaber* (liver disorders)	Smita et al. (2012) Panda and Misra (2011) Panda et al. (2014)
Bonda, *Didayi*, *Koya*, *Bhatoda*, and *Kondh*	34 plant species	*Barleria prionitis* (cough) *Bauhinia vahlii* (dysentery) *Cassia fistula* (leprosy) *Plumbago zeylanica* (abortifacient) *Ricinus communis* (headache) *Semecarpus anacardium* (wound healing) *Pterocarpus marsupium* (diabetes)	Pattanaik et al. (2009)

(continued)

Table 2.3 (continued)

Tribal group	Number of plant families/genera/ species used	Major species and ailments	Reference(s)
Santhals, *Kols*, and *Kharias*	34 plant families/58 species	*Aristolochia indica* (snake bite) *Morinda citrifolia* (body pain) *Pueraria tuberosa* (joint pains) *Soymida febrifuga* (malarial fever) *Syzygium cerasoides* (leucorrhoea)	Rout et al. (2009)
Juang, *Kondha*, *Kol*, *Bhomij*, *Bhuiya*, *Bathudi*, *Kharia*, *Gond*, *Makid*, *Pauri-Bhuyan*, *Mahalis*, *Sounti*, and *Saharas*	551 plant species	*Oroxylum indicum* (dysentery) *Paederia scandens* (diarrhea) *Piper cubeba* (carminative) *Pterocarpus marsupium* (diabetes) *Santalum album* (gonorrhea, syphilis) *Scindapsus officinalis* (asthma) *Semecarpus anacardium* (ovarian cancer) *Smilax zeylanica* (gynatone) *Solanum khasianum* (cough, asthma)	Pandey et al. (2002) Dikshit and Sivaraj (2014) Rout and Pandey (2007) Mohanta et al. (2006)

2.5 Medicinal Plant Wealth in Traditional Health Practices

Eastern Ghats tribal communities use threatened medicinal plants for treating various ailments. The medicinal plant taxa used in local health traditions are enlisted further (disease wise).

2.5.1 Abortifacients

Abrus precatorius, *Acacia leucophloea*, *Lawsonia inermis*, *Gloriosa superb*, *Sterculia urens*, *Madhuca longifolia var. latifolia*, *Ricinus communis*, *Aristolochia bracteolate*, *Plumbago zeylanica*, *Plumbago indica*, *Holoptelea integrifolia*, *Dolichos biflorus*, *Plumbago rosea*, *Rhynchosia beddomei*

2.5.2 Antidote for Poisonous Bites (Snakes, Scorpion)

Boswellia ovalifoliolata, *Pimpinella tirupatiensis*, *Habenaria roxburghii*, *Gymnema sylvestre*, *Rauvolfia serpentine*, *Vernonia cinerea*, *Aristolochia indica*, *Cassia glauca*, *Asparagus racemosus*, *Hemidesmus indicus*, *Cissampelos pareira*, *Corallocarpus epigaeus*, *Strychnos nux-vomica*, *Holarrhena antidysenterica*, *Acalypha indica*, *Leucas aspera*, *L. cephalotes*, *Uraria picta*, *Symphorema polyandrum*, *Celastrus paniculatus*, *Tinospora cordifolia*, *Soymida febrifuga*, *Dalbergia paniculata*, *Sapindus emarginatus*, *Cleistanthus collinus*, *Butea monosperma*, *Ziziphus xylopyrus*, etc. are for poisonous snake bites. *Santalum album*, *Canavalia virosa*, *Strychnos potatorum*, *Ziziphus mauritiana*, *Cassia auriculata*, *Tridax procumbens*, *Martynia annua*, *Andrographis paniculata*, *Leucas cephalotes*, *Aegle marmelos*, *Leonotis nepetifolia*, *Geodorum candidum*, *Rauvolfia serpentine*, *Soymida febrifuga*, *Clerodendrum serratum*, *Calotropis gigantea*, *Boswellia serrata*, etc. are used for scorpion sting.

2.5.3 Antifertility (Contraceptives)

Achyranthes aspera, *Aristolochia bracteolata*, *Mitragyna parvifolia*, *Allium sativum*, *Embelia tsjeriam-cottam*, *Cuminium cyminum*, *Schleichera oleosa*, *Plumbago zeylanica*, *Piper nigrum*, *Zingiber officinale*, *Capsicum annuum*, *Argyreia nervosa*, *Abrus precatorius*, *Aristolochia indica*, *Tamarindus indica*, *Salvadora persica*, *Ricinus communis*, *Crotalaria juncea*, *Phyllanthus amarus*, *Momordica dioica*, *Saccharum officinarum*, *Hibiscus rosa-sinensis*, *Dodonaea viscosa*, *Nymphaea nouchali*, *Strychnos nux-vomica*, *Butea monosperma*, *Balanites aegyptiaca*

2.5.4 Aphrodisiacs and Nervine

Curculigo orchioides, *Hybanthus suffruticosus*, *Clitoria ternatea*, *Decaschistia cuddapahensis*, *Maerua oblongifolia*, *Ipomoea mauritiana*, *Bombax ceiba*, *Hemidesmus indicus*, *Cuminum cyminum*, *Mucuna pruriens*

2.5.5 Arthritis, Body Pains, and Fits

Dichrostachys cinerea, *Azima tetracantha*, *Barleria prionitis*, *Lawsonia inermis*, *Limonia acidissima*, *Derris indica*, *Moringa concanensis*, *Sterculia urens*, *Cassia tora*, *Capparis sepiaria*, *Dregea volubilis*, *Ailanthus excels*, *Celosia argentea*, *Terminalia arjuna*, *Delonix alata*, *Ficus religiosa*, *Erythrina indica*, *Vitex negundo*, *Plecospermum spinosa*, *Diplocyclos palmate*, *Albizia lebbeck*, *Semecarpus anacardium*, *Dodonaea viscosa*, *Cassytha filiformis*, *Atalantia monophylla*, *Atylosia scarabaeoides*, *Alstonia scholaris*, *Leonotis nepetifolia*, *Hemidesmus indicus*, *Aristolochia indica*, *Derris indica*, *Butea monosperma*, *Trianthema portulacastrum*, *Boerhavia diffusa*, *Acalypha indica*, *Elytraria acaulis*, *Cryptolepis buchanani*, *Decalepis hamiltonii*, *Erythrina suberosa*, *Holarrhena antidysenterica*, *Mimosa rubicaulis*, *Zingiber roseum*, *Bacopa monnieri*, *Gossypium herbaceum*, *Bridelia retusa*, *Garuga pinnata*, *Phyllanthus emblica*, *Gardenia turgid*, *Holoptelea integrifolia*, *Cassia occidentalis*, *Morinda tomentosa*, *Clerodendrum phlomidis*.

2.5.6 Child Care

Acorus calamus, *Cryptolepis buchanani*, *Pterocarpus marsupium*, *Holostemma ada-kodien*, *Emilia sonchifolia*, *Oxalis corniculata*, *Helicteres isora*, *Sida acuta*, *Dichrostachys cinerea*, *Phyla nodiflora*, *Mukia maderaspatana*, *Casearia elliptica*, *Aegle marmelos*, *Cucurbita maxima*, *Citrus aurantifolia*, *Curcuma longa*, *Chloroxylon swietenia*, *Terminalia bellerica*, *Aristolochia indica*, *Aristolochia bracteolata*, *Ximenia americana*, *Blepharispermum subsessile*, *Gymnema sylvestre*, *Argemone mexicana*, *Tridax procumbens*, *Cynodon dactylon*, *Ailanthus excelsa*, *Pavonia odorata*, *Ziziphus xylopyrus*, *Blumea eriantha*, *Ziziphus rugosa*, *Lepidagathis hamiltoniana*, *Lepidagathis cristata*, *Hygrophila auriculata*, *Tamarix ericoides*, *Borassus flabellifer*

2.5.7 Cough and Cold

Leucas aspera, *Hemionitis arifolia*, *Abrus precatorius*, *Euphorbia tirucalli*, *Pergularia daemia*, *Trachyspermum ammi*, *Solanum surattense*, *Azanza lampas*, *Acacia torta*, *Acacia caesia*, *Leucas linifolia*, *Leucas aspera*, *Leucas cephalotes*, *Phyla nodiflora*, *Ficus racemosa*, *Ficus benghalensis*, *Cardiospermum halicacabum*, *Cadaba fruticosa*, *Coccinia grandis*, *Pergularia daemia*, *Solanum nigrum*, *Leucas aspera*, *Barleria prionitis*, *Elephantopus scaber*, *Vanda tessellate*, *Rhynchostylis retusa*, *Sesamum indicum*, *Strychnos nux-vomica*, *Tinospora cordifolia*

2.5.8 Diabetes

Rauvolfia serpentina, *Aegle marmelos*, *Gymnema sylvestre*, *Strychnos potatorum*, *Acacia chundra*, *Syzygium cumini*, *Azadirachta indica*, *Flacourtia indica*, *Coccinia grandis*, *Barleria prionitis*, *Leucas linifolia*, *Pterocarpus santalinus.*

2.5.9 Diarrhea and Dysentery

Lantana camara var. aculeata, *Holoptelea integrifolia*, *Desmodium gangeticum*, *Grewia hirusta*, *Diospyros exsculpta*, *Brassica juncea*, *Abrus precatorius*, *Anogeissus acuminata*, *Cassia auriculata*, *Cassia holosericea*, *Justicia glauca*, *Lannea coromandelica*, *Euphorbia prostrata*, *Helicteres isora*, *Psidium guajava*, *Carmona retusa*, *Terminalia pallida*, *Terminalia chebula*, *Anisomeles indica*, *Cassia auriculata*, *Solanum erianthum*, *Maytenus emarginata*, *Tectona grandis*, *Triumfetta rhomboidea*, *Cyanotis tuberos*, *Zanthoxylum rhetsa*

2.5.10 Dysmenorrhea

Andrographis paniculata, *Coccinia grandis*, *Soymida febrifuga*, *Momordica charantia*, *Holarrhena antidysenterica*, *Citrullus colocynthis*, *Cardiospermum canescens*, *Capparis sepiaria*, *Musa paradisiaca*, *Citrus aurantifolia*, *Pergularia daemia*, *Semecarpus anacardium*, *Butea monosperma*, *Sphaeranthus indicus*, *Arachis hypogaea*, *Haldina cordifolia*, *Sesamum indicum*, *Maytenus emarginata*, *Cassia auriculata*, *Cuminum cyminum*, *Sorghum vulgare*, *Eclipta alba*, *Elettaria cardamomum*, *Curcuma longa*, *Momordica dioica*, *Madhuca longifolia var. latifolia*, *Phaseolus radiates*, *Erythrina suberosa*, *Ougeinia oojeinensis*, *Butea monosperma*, *Atylosia scarabaeoides*, *Sida cordifolia*, *Soymida febrifuga*, *Eriolaena hookeriana*, *Securinega leucopyrus*, *Cassia auriculata*

2.5.11 Epilepsy

Solanum indicum, *Helianthus annuus*, *Gardenia turgida*, *Maytenus emarginata*, *Hemidesmus indicus*, *Brassica nigra*, *Chloroxylon swietenia*, *Holoptelea integrifolia*, *Vitex negundo*, *Cassia occidentalis*, *Acalypha indica*, etc.

2.5.12 Eye Diseases

Curculigo orchioides, *Ocimum americanum*, *Carmona retusa*, *Chloroxylon swietenia*, *Phyllanthus amarus*, *Cassia occidentalis*, *Soymida febrifuga*, *Achyranthes aspera*, *Ocimum tenuiflorum*, *Careya arborea*, *Strychnos potatorum*, *Tinospora sinensis*, *Cassia absus*, *Ziziphus mauritiana*, *Achyranthes aspera*, *Argemone mexicana*, *Eclipta alba*, *Aloe barbadensis*, *Gymnema sylvestre*, etc.

2.5.13 Facial Paralysis

Flacourtia indica, *Capparis sepiaria*, *Dichrostachys cinerea*, *Gmelina arborea*, *Capsicum annuum*, *Holoptelea integrifolia*, etc.

2.5.14 Fertility-Promoting Plants

Maerua oblongifolia, *Ferula asafoetida*, *Grewia tenax*, *Ficus religiosa*, *Terminalia bellirica*, *Smilax zeylanica*, *Tectona grandis*

2.5.15 Heart Disorders

Pterocarpus santalinus, *Atalantia monophylla*, *Sida acuta*, *Terminalia arjuna*, *Terminalia alata*, *Cardiospermum halicacabum*, *Mitragyna parvifolia*, etc.

2.5.16 Hepatic Disorders

Phyllanthus amarus, *Lagenaria siceraria*, *Ficus hispida*, *Luffa acutangula* var. *amara*, *Trachyspermum ammi*, *Andrographis paniculata*, *Azadirachta indica*, *Holarrhena antidysenterica*, *Cordia dichotoma*, *Benincasa hispida*, *Cassia tora*, *Curcuma angustifolia*, *Diospyros montana*, *Lawsonia inermis*, *Oroxylum indicum*, *Curcuma longa*, *Phyllanthus amarus*, *Solanum nigrum*, *Ricinus communis*, *Boerhavia diffusa*, *Leucas linifolia*, *Leucas aspera*, *Leucas cephalotes*, *Cassia occidentalis*, *Papaver somniferum*, *Eclipta alba*, *Acalypha indica*, *Balanites aegyptiaca*, *Butea monosperma*

2.6 Immunity Modulators

Aegle marmelos, *Ailanthus excelsa*, *Albizia lebbeck*, *Andrographis paniculata*, *Asparagus racemosus*, *Atalantia monophylla*, *Azima tetracantha*, *Capparis sepiaria*, *Clerodendrum phlomidis*, *Dichrostachys cinerea*, *Gmelina arborea*, *Hemidesmus indicus*, *Hesperethusa crenulata*, *Holarrhena antidysenterica*, *Moringa oleifera*, *Oroxylum indicum*, *Plumbago zeylanica*, *Pterocarpus marsupium*, *Solanum surattense*, *Soymida febrifuga*, *Stereospermum suaveolens*, *Terminalia chebula*, *Tinospora cordifolia*, etc.

2.6.1 Leucorrhea

Hibiscus micranthus, *Cassytha filiformis*, *Ficus racemosa*, *Mangifera indica*, *Syzygium cumini*, *Cassia occidentalis*, *Curcuma longa*, *Argemone mexicana*, *Aerva lanata*, *Cuminum cyminum*, *Bombax ceiba*, *Vernonia anthelmintica*, *Terminalia* *bellirica*, *Tephrosia purpurea*, *Sida acuta*, *Abrus precatorius*, *Derris indica*, *Mimosa pudica*, *Erythrina indica*, *Cuminum cyminum*

2.6.2 Malaria and Other Fevers

Andrographis paniculata, *Cissampelos pareira*, *Nyctanthes arbor-tristis*, *Soymida febrifuga*, *Vitex peduncularis*, *Terminalia alata*, *Ailanthus excels*, *Mimosa pudica*, *Paederia foetida*, *Cleome pentaphylla*, *Flacourtia indica*, *Aristolochia indica*, *Rauvolfia serpentina*, *Evolvulus alsinoides*, *Aganosma caryophyllata*, *Aerva lanata*, *Malaxis rheedii*

2.6.3 Miscarriage of Pregnancy

Vernonia cinerea, *mimosa pudica*, *Achyranthes aspera*, *Eclipta alba*, *Ocimum sanctum*, *Caesalpinia bonduc*, and other species

2.6.4 Menorrhagia

Bauhinia racemosa, *Prosopis cineraria*, *Canavalia virosa*, *Hemidesmus indicus*, *Hemidesmus indicus*, *Lepidagathis hamiltoniana*, *Abelmoschus ficulneus*, *Terminalia alata*, *Argemone mexicana*

2.6.5 Mother Care

Acacia catechu, *Butea monosperma*, *Allium sativum*, *Zingiber officinale*, *Capsicum annuum*, *Cuminum cyminum*, *Cinnamomum zeylanicum*, *Acacia catechu*, *Acacia chundra*, *Hesperethusa crenulata*, *Holoptelea integrifolia*, *Chloroxylon swietenia*, *Alangium salviifolium*, *Oroxylum indicum*, *Cassia occidentalis*, *Asparagus racemosus*, *Dillenia pentagyna*, *Piper longum*, *Salvadora persica*, *Dichrostachys cinerea*, *Brassica nigra*, *Symphorema involucratum*, *Canthium parviflorum*, *Trachyspermum ammi*, *Derris indica*, *Holoptelea integrifolia*, *Mundulea sericea*, *Mollugo pentaphylla*, *Ixora arborea*, *Tectona grandis*, *Oryza sativa*, *Raagi java*, *Eleusine coracana*, *Sorghum vulgare*, *Achyranthes aspera*, etc.

2.6.6 Paralysis

Smilax zeylanica, *Azima tetracantha*, *Symphorema involucratum*, *Derris indica*, etc.

2.6.7 Respiratory Disorders

Boswellia serrata, *Dolichandrone falcata*, *Strychnos potatorum*, *Tridax procumbens*, *Strychnos nux-vomica*, *Ocimum sanctum*, *Trachyspermum ammi*, *Achyranthes aspera*, *Capparis zeylanica*, *Andrographis paniculata*, *Anisochilus carnosus*, *Vernonia anthelmintica*, *Semecarpus anacardium*, *Euphorbia thymifolia*, *Barringtonia acutangula*, *Aegle marmelos*, *Anogeissus latifolia*, *Pergularia daemia*, *Leucas aspera*, *Ocimum sanctum*, *Borassus flabellifer*, *Evolvulus alsinoides*, *Dendrocalamus strictus*, *Echinops echinatus*, *Solanum surattense*, *Acalypha indica*, *Plumbago zeylanica*, *Solanum trilobatum*, *Cissus quadrangularis*, *Ziziphus oenoplia*, *Euphorbia hirta*, *Calotropis gigantea*, *Echinops echinatus*, *Albizia lebbeck*, *Alangium salviifolium*, *Leucas cephalotes*, *Helicteres isora*, *Mitragyna parvifolia*, *Derris indica*, *Terminalia arjuna*, *Pterocarpus marsupium*, *Cassia occidentalis*, *Aristida adscensionis*

2.6.8 Skin Diseases

Albizia thompsonii, *Nervilia aragoana*, *Paederia foetida*, *Grewia rhamnifolia*, *Urginea indica*, *Urginea raogibikei*, *Urginea nagarjunae*, *Elytraria acaulis*, *Opuntia dilleni*, *Holoptelea integrifolia*, *Cissus pallida*, *Ventilago calyculata*, *Ximenia americana*, *Boswellia seretta*, *Premna tomentosa*, *Ochna squarrosa*, *Ziziphus mauritiana*, *Eleusine coracana*, *Ailanthus excelsa*, *Tamarindus indica*, *Alangium salviifolium*, *Phyllanthus emblica*, *Argemone mexicana*, *Moringa oleifera*, *Albizia amara*, *Hyptis suaveolens*, *Annona squamosa*, *Terminalia chebula*, *Anisochilus carnosus*, *Coldenia procumbens*, *Commiphora caudata*, *Colocasia esculenta*, *Piper longum*, *Ficus hispida*, *Urginea nagarjunae*, *Solanum melongena*, *Holoptelea integrifolia*, *Ocimum americanum*, *Dendrocalamus strictus*, *Madhuca longifolia*, *Barleria prionitis*, *Rubia cordifolia*, *Trichosanthes tricuspidata*, *Terminalia arjuna*, *Pterocarpus santalinus*, *Mundulea sericea*, *Nerium indicum*, *Hesperethusa crenulata*, *Santalum album*.

2.6.9 Viral, Bacterial, and Fungal Attacks

Lygodium flexuosum, *Curcuma longa*, *Abrus precatorius*, *Mimosa pudica*, *Solanum surattense*, *Xanthium strumarium*, *Adiantum lunulatum*, *Chenopodium anthelminticum*, *Aristolochia indica*, *Barringtonia acutangula*, *Schleichera oleosa*, *Hemidesmus* *indicus*, *Solanum indicum*, *Azadirachta indica*, *Commiphora mukul*, *Brassica juncea*, *Acorus calamus*, *Achyranthes aspera*, *Brassica juncea*, *Acorus calamus*, *Calotropis gigantea*, *Leucas aspera*, *Albizia lebbeck*, *Morinda tomentosa*, *Gardenia gummifera*, *Gardenia resinifera*, *Clerodendrum viscosum*, *Solanum giganteum*, *Polycarpaea corymbosa*, *Selaginella rupestris*, *Adiantum incisum*

The detailed treatise on Eastern Ghats tribal medicine for various ailments and vast recipes is provided by Hemadri (2011).

2.7 Conservation Strategies for Threatened Medicinal Plants

The conservation of threatened medicinal plant genetic resources involves two basic strategies: (i) in situ and (ii) ex situ (Fig. 2.2). In situ conservation of medicinal plant taxa has to be carried out in original forest habitats where threatened medicinal plants occur naturally. Ex situ conservation requires collection and systematic storage of seeds/propagules outside the natural habitats of species for short, medium, and long term after proper characterization and evaluation. Threatened medicinal plant taxa under ex situ conservation in the country are to be characterized and evaluated in a phased manner. Storage of medicinal plant parts at an ultralow temperature, such as that of liquid nitrogen (−196 °C) termed cryopreservation, is one of the promising approaches being pursued to achieve prolonged preservation of medicinal plant genetic resources.

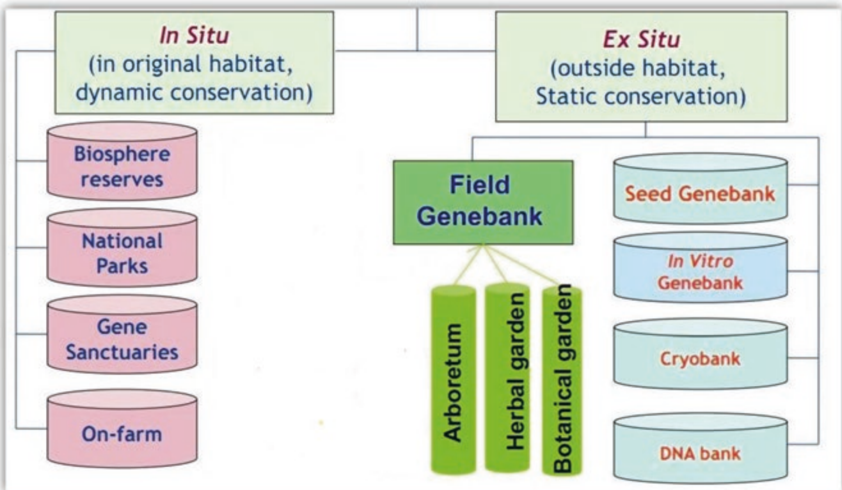

Fig. 2.2 Conservation strategies for threatened medicinal plants

Conservation programs are being implemented in Eastern Ghats region at three levels, viz., genotypes, species, and ecosystems. In situ conservation of wild flora through protection of habitats and ecosystems is being implemented by the Ministry of Environment and Forests and Climate Change. Fourteen biosphere reserves have been identified on the basis of survey data and have already been made operational in India. Concerted efforts were made for conservation of threatened medicinal plant taxa in India and particularly in Eastern Ghats region. Endemicity and usefulness leading to overexploitation are essentially two reasons for a medicinal plant species to come under threat. Apart from this, rapid change in land use pattern has resulted in degradation of specialized natural habitats and along with it rapid depletion of medicinal plants restricted to these habitats. Specialized habitats and threatened plants undeniably deserve exceptional concern for conservation and sustained monitoring in Eastern Ghats region of the country. Collaboration, coordination, and harmonization among institutions and also among naturalists, plant taxonomists, forest and protected area managers, and volunteers possibly give the essential support to realize this objective. Ex situ conservation of genetic variability of medicinal plants is the sole responsibility of the CSIR-CIMAP and ICAR-National Bureau of Plant Genetic Resources (NBPGR) that operates under the Indian Council of Agricultural Research (ICAR). The Indian National Genebank (NGB) was established at ICAR-NBPGR to conserve the PGR including medicinal plant taxa for posterity in the form of seeds, vegetative propagules, in vitro cultures, budwoods, embryos/embryonic axes, genomic resources, and pollen. The NGB has four kinds of facilities, namely, seed genebank (−18 °C), cryogenebank (−170 °C to −196 °C), in vitro genebank (25 °C), and field genebank, to cater to long-term as well as medium-term conservation. Numerous botanical gardens managed by the Botanical Survey of India and several other organizations help in ex situ conservation of endangered, threatened, and rare plant species.

The seed material of different seed-bearing orthodox medicinal plant species collected is stored at −20 °C with seed moisture brought down to 5–8% and RH being maintained at 25–32% in the National Genebank at NBPGR. In some difficult species, which are recalcitrant, pollen and seed material is stored at -180 °C in liquid nitrogen in the cryotanks at ICAR-NBPGR.

For medium-term conservation, the seed material is stored at 7 °C with the seed moisture brought down to 5–8% and RH being maintained at 30–35% in the cold storage modules at NBPGR Regional Station, Hyderabad. The medicinal plant species, which are non-seed bearing, and those that are multiplied by vegetative means (stem cuttings/root cuttings/whole plant) are being maintained in the glass house/field genebank at NBPGR Regional Station, Hyderabad, in live condition.

Regional Stations of the ICAR-National Bureau of Plant Genetic Resources (NBPGR) located at Cuttack and Hyderabad have made extensive exploration surveys and collected about 1800 accessions of medicinal and aromatic plant species from Eastern Ghats region, and the same has been documented. Some of the endangered/endemic medicinal plants collected include *Acorus calamus*, *Aegle marmelos*, *Costus speciosus*, *Cycas bedomei*, *Gloriosa superba*, *Gymnema sylvestre*, *Mucuna pruriens*, *Plumbago indica*, *Rauvolfia serpentina*, and *Withania somnifera*.

Collections of dye-yielding plants include *Bixa orellana* and *Mallotus philippensis*, while collections of aromatic plants include *Artemisia* spp., *Cymbopogon* spp., *Ocimum* spp., *Vetiveria zizanioides*, etc.

Genomic resources of threatened medicinal plant diversity such as cloning vectors, expression vectors, binary vectors, RFLP probes, cloned genes, promoters fused to reporter genes, subgenomic, cDNA, EST, repeat enriched libraries, BAC, YAC, PAC clone set from sequencing projects, genomic, mitochondrial or chloroplast DNA, and cloned DNA from wild medicinal plant species produced exclusively for the repository can be stored in the repository by the following storage methodologies:

- 1–2 years at 4 °C, 4–7 years at −20 °C, and greater than 5 years when stored at −70 °C
- ESTs, full-length cDNAs, BACs, PACs, and YACs, maintained in 96-well or 384-well micro plates at −80 °C
- cDNA clones as plasmid DNA at −20 °C
- Lyophilized DNA for long-term storage
- Ambient temperature storage

To effectively plan a conservation program especially for in situ approaches, the occurrence/ passport data enlisted will be useful in delineating species-rich areas, in general, and diversity-rich pockets, in particular, in the surveyed region. The Medicinal Plants Conservation Center (MPCC), Hyderabad, created eight medicinal plant conservation areas in the Eastern Ghats region of Andhra Pradesh, and a total of 715 medicinal plant species have been identified and conserved in these areas (Jadhav and Reddy, 2002). Based on the deliberations during the Conservation Assessment Management Plan (CAMP) workshop organized by the MPCC in 2001, the threat status for some of the medicinal plant species of this region has been assessed. Concerted and collaborative efforts are highly warranted for sustainable management of threatened medicinal plant wealth in the Eastern Ghats.

2.8 Conclusion and Way Forward

Eastern Ghats are endowed with a rich diversity of medicinal plant species. The ever-increasing growth of global and national herbal-based healthcare and wellness sector is putting enormous pressure on the available medicinal plant resources of this region. It has given rise to concerns about the conservation and sustainable utilization of threatened medicinal plants. Some of the threatened medicinal plant species of Eastern Ghats are in high commercial demand. It calls for active management plans so as to ensure proper conservation strategies of medicinal plant genetic resources and sustained supply of authentic and quality herbal products. Local healthcare traditions, evolved since ancient times, draw heavily from the available plant genetic resources of Eastern Ghats region which are thus increasingly becoming threatened. Conservation of threatened medicinal plant resources is significantly assuming a very high priority. The following are some of the action points for effective conservation of threatened medicinal plant genetic resources:

- Management of Eastern Ghats genetic resources: Priority management interventions are required on threatened species with a long-term national program on in situ conservation, development, and sustainable utilization of threatened medicinal plant resources of Eastern Ghats.
- Urgent need to strengthen ex situ collections in genebanks, botanical gardens, arboretum, and herbal gardens through systematic germplasm surveys for threatened medicinal plant germplasm collection, characterization, and documentation.
- Networking and coordination of efforts of stakeholders/organizations engaged in medicinal plant conservation are warranted toward focused output for conservation of valuable genetic resources of Eastern Ghats.
- Standardization of protocols for cryopreservation and in vitro conservation of threatened medicinal plant resources of Eastern Ghats.
- Consolidated inventory of threatened medicinal plant genetic resources of Eastern Ghats needs to be prepared, and their commercial demand needs to be worked out.
- Complex state-wise regulatory regimes are to be made uniform for sustainable utilization (collection, cultivation, transport, and trade) of medicinal plant genetic resources of Eastern Ghats.
- Capacity building of local communities/stakeholders on awareness of threatened medicinal resource conservation needs to be encouraged.

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Chapter 3
Indian Medicinal Plants Database (IMPLAD) and Threatened Medicinal Plants of India

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Abstract Medicinal plants have become of great relevance to the health care of the people, with a vast global population still relying on them. While developing the nation's most comprehensive, multidisciplinary database on flora, fauna, metals, and minerals of traditional Materia Medica from primary texts over the period 1500 BC to 1900 AD, IMPLAD (Indian medicinal plants database) has grown into a multifaceted platform for research, education, and outreach. This has referenced searchable botanical information (botanical names and its synonyms, 200 thousands vernacular names in 32 Indian languages, distribution, threat status study, state inventories, GIS maps, plant images) and traditional knowledge (Sanskrit names, bibliography from 20 major classical texts of Indian systems of medicine Ayurveda, Ayurvedic pharmacology and pharmacopoeia data, original Sanskrit Shloka references, glossary of technical terms) of around 6500 medicinal plants of India.

This facility has been empowering people with knowledge on traditional health care and natural resources as its one of the major objectives. It focuses on innovation, designing, and developing informatics facilities for the purpose of understanding, conserving, and propagating Indian systems of medicine, bearing in mind the

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requirements of education, research, and application in the sector. IMPLAD has improved our current understanding of medicinal plants and traditional knowledge in India and detailed field level information on many endangered species of conservation concern.

Keywords Database · Threatened Indian medicinal plants · Indian system of medicine · IMPLAD

3.1 Introduction

There are various estimates and guesstimates of the total number of plant species known to be in medicinal use in the country. It is understood that the most appropriate way to resolve the issue relating to these figures is to create a "referenced" database from the "published" sources. These published sources have to include materia medica publications of Ayurveda, Siddha, Unani, Tibetan, and Homeopathy as well as various ethnobotanical works like floras, published peer reviewed papers, books, and doctoral thesis. A species is tagged as medicinal only if it has cited use in any of the abovementioned references.

During 1993–2012, TDU-FRLHT team has endeavored to catalog the plant entities recorded in medicinal use in the codified systems of Indian medicine, namely, Ayurveda, Siddha, Unani, Swa-rigpa (Tibetan), and Homoeopathy as well as the ones documented in medicinal use in the folk practices, from the publications covering different regions of the country. Such an enlistment of Indian medicinal plants has been the central pillar of computerized database on Indian medicinal plants. This has also supported flagship project of FRLHT during 1993–2012 to locate, identify, and conserve medicinal plants both in in situ and ex situ conservation areas in different states of India.

Multidimensional data relating to medicinal plants mentioned in Ayurveda, Unani, Siddha, and homeopathy systems of medicine (AYUSH) and its diverse aspects (agro-technology, distribution, trade, etc.) and features of each of the listed medicinal plant entity can serve the needs of a range of stake holders including students and researchers of the Indian systems of medicine (ISM) as well as the policy makers, foresters, and farmers, and the herbal industries.

Several gaps exist today in the availability of comprehensive and authentic information. For example, data on Sanskrit *sloka* references, hundreds of bibliography data of Sanskrit names on plant entities mentioned in classical texts are not available for in-depth study and research. There is no geographical distribution data on plant entities of different zones, agro technology is not adequately available, there are confusions regarding the correlation of nomenclature of vernacular names to botanical entities, and there is limited data on the threat and trade status of species.

Thus, TDU-FRLHT database of Indian medicinal plants has great scope in addressing these gaps and helping to take informed decision making in conservation of medicinal plants and providing authentic information on health care through medicinal plants. The database was named as Indian medicinal plants database (IMPLAD Team 2016).

The purpose of the IMPLAD is to provide compiled, categorized intelligently analyzed data to different user groups on Indian medicinal plants. To achieve this goal, it was decided to classify this domain based on two major divisions such as botanical and traditional knowledge on medicinal plants.

It is also required to visualize this in two different prospective from structural and user-friendly way and organizing in linking data (in the way of looking design). This has uniqueness in providing content and linkages (through different system medicine) to botanical and traditional knowledge.

The database is a main decision making tool for conservation of medicinal plants and use of natural resources used as per traditional knowledge in India. The database is also serving as a backend of various educational tools like mobile apps, educational packages on compact disks (CDs), and websites or portals on medicinal plants and Indian systems of medicine.

3.2 Objectives of the Medicinal Plant Database

The objectives of this database are knowledge generation, data analysis, and dissemination to different user groups for revitalizing the Indian medical heritage. The purpose of the IMPLAD is achieved through various means.The database is also envisaged to support and act as decision making tool for conservation of medicinal plants and sustainable use of natural resources used as per Indian systems of medicine. The database is also serving as a backend of various educational tools like compact disks (CDs), websites or portals, and apps on medicinal plants and traditional knowledge.

3.2.1 Target Users of the IMPLAD

The IMPLAD is aimed to provide authentic information on Indian medicinal plants at two major levels: (1) botanical information and (2) traditional knowledge related to medicinal plants.

The main user group is researchers in botany, those who are focusing of medicinal plants and students, teachers, and practitioners of Indian systems of medicine (ISM).

Students of Indian system of medicine can make use of this database to authentically identify their medicinal plants by searching by millions of vernacular names and can correlate botanical sources with images.

Researchers will be benefited with quick reference, research data, and search facilities, which will help them to filter various data components. Practitioners would use this as a tool for searching appropriate information on formulations.

Students of Indian systems of medicine (ISM) especially at postgraduate and doctoral levels can get benefited with complete plant profile information from various sources through the query system.

A common man make use of IMPLAD in searching for medicinal plants that can be used for primary health care (PHC). The database is expected to serve development of websites, portals, and mobile apps on medicinal plants with customized data in a user-oriented manner.

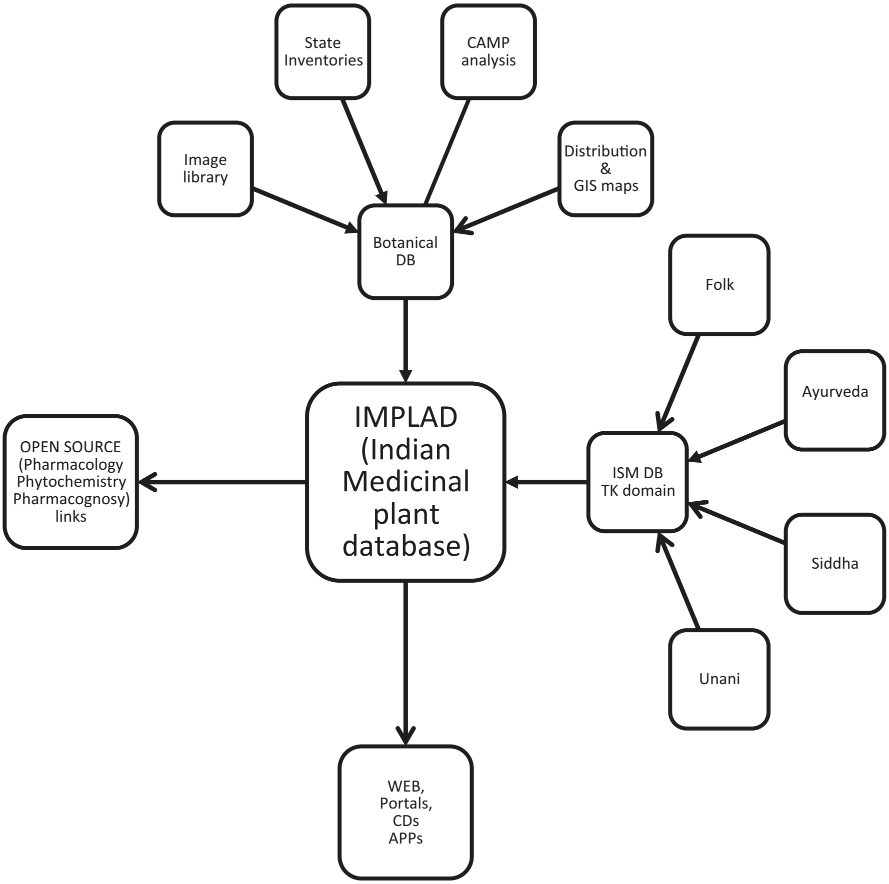
3.3 Methodology

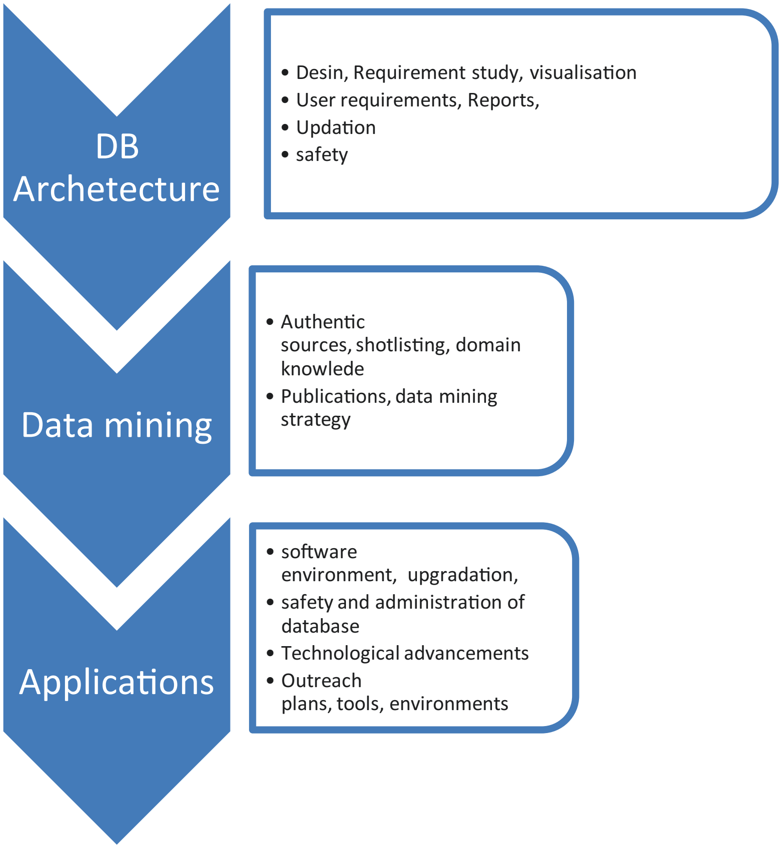
A *database* consists of an organized collection of data for one or more uses, typically in digital form. Digital databases are managed using database management systems, which store database contents, allowing data creation and maintenance, search, and other access.

3.3.1 The Database Architecture and Access

Database management systems are created based on specific design principles, including process models, parallel architecture, storage system design, transaction system implementation, query processor and optimizer architectures, and typical shared components and utilities. The architecture design based on the requirement analysis is flexible enough to accommodate any changes. IMPLAD has followed a network style architecture in an open-source environment to support in-house requirement of various departments of the organization on free access. However, certain modules of the IMPLAD were made available to public platform on specified terms of use.

3.3.1.1 Components of the IMPLAD Database





DB (database), CAMP (Conservation Assessment and Management Prioritization), GIS (geographical information system), ISM (Indian systems of medicine), TK (traditional knowledge)

3.3.2 Determination of the Workflow of the Database

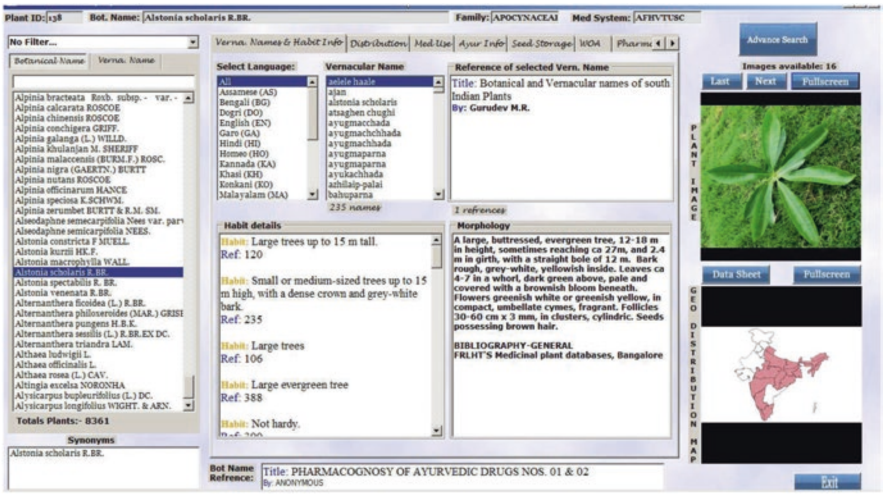
The purpose of the IMPLAD is to provide botanical and traditional information on medicinal plants for different user groups. The workflow for developing the database has been discussed with contributors and managers and knowledge providers in this task. A prior confirmation on allocation of resources and manpower is a prerequisite for this venture. To better control, streamlining the activities is a must for completing the prescribed tasks in a time bound manner.

3.3.3 Organization of Information

Searching for the source of data is the prerequisite and first phase of any database development activity. A detailed literature review is required to study the nature data and its role in increasing the authenticity of the database. The criteria for selecting the source books also determine the value of the database in a long run.

3.3.4 Botanicals and Nomenclature Correlation Exercise

As part of the medicinal plant conservation project that was initiated in Southern India in 1993, a database building activity was undertaken to understand what are the medicinal plants mentioned in the codified medical traditions such as Ayurveda, Siddha, and Unani. In the Ayurvedic database, the first effort was to build a database on correlation of Sanskrit names with botanical names mentioned in the secondary literature (nonclassical). The bibliographic sources for this activity included 21 books belonging to last 100 years of works by Ayurvedic experts, botanists, and pharmacognosists attempting to correlate Sanskrit names with botanical names. This work ended in a correlation of around 20,000 Sanskrit names to 1750 species belonging to 830 genera. These reference represent botanical correlations of Sanskrit name, and other botanical information of plants, accepted as authentic sources of information.



3.3.5 ISM Database Section of Traditional Knowledge (TK) Domain on Medicinal Plants

To develop TK database on medicinal plants, the texts were selected that are major milestones in the area of Ayurveda and are in contemporary use and covered various types of texts like treatises (*samhita*), compendiums (*samgraha*), and lexicons (*nighantus*).

This database has sourced appropriate information prescribed in 20 textbooks of Ayurveda on medicinal plants in its first phase. These texts cover a majority of the information on formulations and single drug remedies. Please refer to Appendix 3.2.

These references selected are considered as the major milestones in the history of development (pharmacognosy and pharmacology) of Ayurveda. Among this *Brihatrayi*, the major trio (*Charaka Samhita*, *Susruta Samhita*, and *Astanga Hrdayam*) are the most important classical works in Ayurveda.

Another focal area of this domain is lexicons (*Nighantus*), which are major works of Ayurvedic materia medica. This is taken into consideration for incorporating plants, nomenclature, classification systems, and properties, etc. These lexicons belong to different time periods (10th–18th Common Era (CE) when substantial contributions have been made to Ayurvedic materia medica).

3.3.6 Determine the Structure of the Database

The architecture of IMPLAD database has not followed common typical RDBMS (relational database management system) components like process control, client communication, pelational query processor, transactional storage, shared component utilities in its full potential. This was designed for user groups dedicated for research studies and education.

Two major divisions of this database, i.e., botanical and TK, have various modules which are linked together. This was possible because of a common standard followed and agreed mechanism to exchange data. These are rigorously designed and tested by set of experts and users from different knowledge domains it represents over a period of time.

Data entry and categorization process was followed based on the structure of the database (DB). In this case, a tentative structure of the DB was designed first, and component wise data entry was carried out based on prioritized books or publications. Data items were spread across the table with a common ID which is plant ID. This plant ID links with different tables of the IMPLAD database. Detailed data tables were populated with data upon approved fields and user requirements.

Refinement of the DB design is achieved over a period of time. This is a result of testing the functionality of the database, data integrity, scope of redundancy, and possible errors which may happen due to data deficient situations and links that are not logical. Testing procedures after populating the data table with data, query development, and user requirements are part of refining the design of the database.

3.3.7 Determination of Search and Reporting Facilities

Search facilities are the cardinal part of the database utility. DB can be searched based on various parameters, most often predetermined tags. In the case of IMPLAD, this should incorporate tags in each categories or global search facilities available in different data sets, for example, search by botanical name, vernacular names, disease entities in Sanskrit or English, or any terms which can appear in the database. The report should be available for use in the form of soft or hard copy preferably flexible enough to use them for customized requirements. Data export to tables or excel sheets or in a word format or web format will increase the user friendliness of the DB at large.

3.3.8 Data Management and Security

Special precaution has been taken to document different steps involved in the development of various modules of the database table and coding associated with application development. User and admin access to the database and application is controlled using username and password security features at different levels of its operation.

An administration module to facilitate data entry and editing is controlled by the administrator. Print facility and data export facility have been provided at user end as well as in admin module.

A time schedule for adding info from new source books into the database is permitted in the network on approval of the admin. Periodic backup of the source code and backup facility has been implemented. A login facility with username password has added more safety to the control facility and able to help track the record of the database users.

3.4 Nomenclature Specialties of the IMPLAD

3.4.1 Botanicals and Its Nomenclature Correlation Sources

The central part or the IMPLAD is the taxonomy details of plant species. The international code of botanical nomenclature standards is one protocol followed in this aspect. However, it is not strictly followed at this point of time due to fear of repeating the same exercises followed elsewhere. The number of levels represented in one taxa is limited to family, genus, species, varieties, etc. The information from other international databases also referred to confirm the authenticity of existing taxonomical nomenclature of plant species recorded in the IMPLAD.

Plants are known by their names and credited with their uses. Polynomial nomenclature system of Indian knowledge systems and different cultures has contributed a new dimension in plant nomenclature. Even though there are few problems with the polynomial system due to its specificity, it was not practically possible to a country like India to communicate with single language and single name for a plant. The knowledge and culture are still diverse, and it holds absolutely logical to follow the documentation of hundreds of names for a plant species.

The names are ascribed to a plant for describing its form or habit (*svarupabodhaka),* revealing its quality (*gunabodhaka),* action (*karma*) of the plant. The IMPLAD has documented 2 lakhs vernacular names in 32 Indian languages from published sources. It comprises around 25,000 names in Sanskrit alone. The utility of this data bank is found very useful when one is searching for identity of plant species using a vernacular name. This will also help to identify and trace older manuscripts written in many languages using the plant names of the region.

3.4.2 System-Wise Inventorization: Tagging

IMPLAD has endeavored to catalog the plant entities recorded in medicinal use in the codified systems of Indian medicine, namely, Ayurveda, Siddha, Unani, Swarigpa (Tibetan), Homoeopathy, traditional Chinese medicine and western medicine, as well as the ones documented in medicinal use in the folk practices, from the publications covering different regions of the country. Such an enlistment of Indian medicinal plants can constitute the central pillar of a computerized database on Indian medicinal plants. Multidimensional data relating to diverse aspects (nomenclature correlation, distribution, trade, etc.) and features of each of the listed medicinal plant entity can then be built around this central pillar.

A comprehensive tabulation of botanical names (genus, species, author citation) of plant entities recorded in each medicinal use in one or more of the Indian systems of medicine along with tags of related specific medical systems is one of the unique aspects of the database.

3.4.3 Toward Solving Problems in Plant Identification

This effort was to develop a bibliographic/reference database based on primary sources, i.e., the classical texts of Ayurveda. For this purpose, 20 classical texts covering a chronological period of around 2200 years were prepared. The texts were selected that are major milestones in the area of materia medica (Ayurvedic pharmacology) and are in contemporary use and covered various types of texts like treatises, compendiums, and lexicons. This also tried to cover different geographical locations to get maximum variations in the usage of plants.

The field Sanskrit names pertain to resource name, which was classified into plant, animal, and minerals, and metals. Gender in which the Sanskrit name was used was important as some names in female gender pertain to tender climbers and the same name in male gender meant trees. For example, *amrita* (Terminalia chebula) is different from amritaa (*Tinosporacordifolia*). To differentiate, this gender was taken as a separate field. Plant part, products, and groups were differentiated by tags.

This component of the IMPLAD has primary information from classical texts on the number of medicinal plants described in Ayurveda. At present, this consists of around 23,000 Sanskrit names relating to 12,2000 references across 20 texts. Tentative botanical correlation with pictures based on the earlier database and interface facility of searching references from individual texts, across texts, synonym, and basonym search have been created.

This database now helps in analyzing and searching the data for research purposes. It was found that around 70% of the materials used in Ayurveda are plants, 20% animals, and 10% minerals. This was an analysis of Nighantus of medieval period. The following charts show this (Unnikrishnan 1997).

3.4.3.1 Challenges Faced

A number of challenges were faced during development of this database. One of the challenges was selection of texts. The question was what can be defined a classical text. It was decided that Sanskrit literature that have not incorporated modern views, ideas, or botanical correlations could be selected for this purpose. Another criterion was that the text has to be in mainstream and used in different parts of the country. The criterion had to also cover major chronological milestones so as to incorporate various temporal ideas and different geographical locations to cover spatial variation ideas.

Different publications of these texts carried different *sloka* numbers or even differences in the verses. Thus, critical editions were needed. Collecting critical editions of classical literature and ascertaining time periods of these classical texts were difficult. For standardizing this, those texts with original Sanskrit slokas with commentaries and widely accepted publishers' books were selected (Unnikrishnan 2007), for example, Charaka Samhita text with English translation and critical exposition based on Cakrapani Dutta's Ayurveda Dipika, by R.K. Sharma and Bhagwan, Das, and published by Chowkhamba Sanskrit series from Varanasi.

This exercise has highlighted various problems like grammatical complexities of plant names in its gender variations used in Sanskrit language, differences of names across different time periods, different views by commentators on identity, etc.

According to the text *Dhanvantari Nighantu*, all these features in the classical texts are owing to the nomenclature system, which is designed based on reproductive characters, physical characters, color, potency, taste, and specific action. It is mentioned that common synonyms have to be decided according to the meaning, context, tradition, and reasoning so that each contextual reference becomes important.

Another limitation was that the bibliography selected for this work did not represent the regional literature in which a plethora of information is available.

Another major challenge was correction of data after compilation. Intricacies in classifying references into qualities and actions, different therapeutic and nontherapeutic groups, pharmaceutical preparation, and clinical application groups were yet another issue. Similarly, classifying into revealing form, *guna*-revealing quality, revealing action names, was also a challenge. This was essential to understand the specific context. All these were identified as key issues to be looked into for preparing an inventory of medicinal plants in classical texts of Ayurveda.

These challenges were identified during the effort to make the classical text database. Even after completion of this database, the major issue of critical nomenclature correlation remained unsolved. It was learned that this issue of nomenclature correlation could be solved only by a combination of approaches such as in-depth studies of classical literature, documentation of understanding of living traditional practitioners, and pharmacognostic and pharmacological studies. To solve some of these issues, a mere reference database was not sufficient. It had to have all the contextual details explained in each of these references. Only then studies on individual drugs could be taken up. Thus, building of detailed individual text databases was initiated. This included databases on three major texts *Brihatrayi* – Charaka Samhita, (Yadavji Trikamji Acharya 1992), Susruta Samhita, Astanga Hrdayam – and database of major lexicons.

3.4.4 Addressing the Issue of Correlating Sanskrit Synonyms of Plant Drugs

A number of nomenclature correlation-related issues came out of building up this database. Ayurveda follows polynomial system of nomenclature. In this system, a plant is identified through multiple names. Each of these names pertain to a specific character or feature of the plant. When all the names are grouped together, one gets a picture of the plant. For example, *Guduci* which is correlated to *Tinospora cordifolia* has around 70 names described in the classical literature. In this system of polynomial nomenclature, same names are used for different plants as well (Unnikrishnan 1997).

As there are multiple names and common synonyms, it was difficult to decide which has to be taken as the basic name for a plant. This issue was not addressed by any of the authors in the bibliographic sources that we used for the database. Thus, we found that many of the correlations were casual and noncritical of Sanskrit names with botanical names by these authors without having sufficient referencing or voucher specimens of the plants. Confirming the identity through descriptions was also not done in majority of these works.

3.4.4.1 Grouping of Synonyms and Fixing a Basonym

Synonyms appear in every places in a text representing a common drug or plant. The same plant names were used in different variant forms. For example, *yasti*, *yastika*, *yasteeka*, *yastiahvika*, *yastimadhuka*, *yasteemadhuka*, *madhuka*, *madhuyastika*, *madhuyasti*, etc. pertain to the same name *yastimadhu* that is *Glycyrrhiza glabra*. These variations had to be considered while grouping the synonyms. There were a number of synonyms used in the same text in different context. Since the effort of this database was to prepare a unique list of plant names from each of these texts, it was necessary to group the variants and synonyms of each plant and link it with a basic name. This had to be done by grouping the number of references.

Since the plants had a number of names in the same text, grouping of synonyms had to be done. This was found difficult without having complete descriptions and commentators' view on each plant. While grouping the references, contextual differences had to be considered. For example, in some context, *kustha* means a skin disease, whereas elsewhere it means a plant. Similarly, the name *tikta* means bitter as well as the plants *katuka* and *kiratatikta* which is *Andrographis paniculata.* Thus, these references had to be screened carefully.

In the name grouping, another difficulty was that of classification on part used. At times, the part used has a different name altogether which is considered as an entity. For example, *moca-rasa* is the exudate of Salmali (gum of *Bombax malabarica*), and it is mentioned as a different entity.

After building individual text databases, the following steps were done to find out the unique plants in each of these texts:

- Collect plant references.
- Collect commentators' views on references.
- Fix tentative basonym – based on commentary, frequently used names.
- Mark grammatical variants linked to basonyms.
- Mark synonyms based on suggestions of commentators.
- Mark gender variations.
- Mark plant names which pertain to groups, e.g., *triphala*.
- Mark plant names as basonyms if the plant name correlated by the commentator is not found marked under synonym or variant name.
- Mark tentative basonyms as basonyms if they are not linked to synonyms or variant names.
- Give exceptions in case of popularly used synonyms – e.g., Guduci, amrita.
- Compare botanical correlations done by selected subject experts.
- Fix status of identification by giving flags like noncontroversial, controversial, or unidentified based on these studies.

This analysis has now culminated in the following data in Charaka Samhita (1500 BCE–200 CE). There are 12,870 references related to plants in Charaka Samhita. After grouping the synonyms, there are 617 unique plant names. Out of this, 508 are identified, and they are correlated to 630 botanical species. There were around 500 synonyms, 817 variant names, and 56 group names in Charaka Samhita (Venugopalan 2001).

There are around 1630 formulations recorded in Charaka Samhita. Now this has become a unique inventory of plants of Charaka Samhita.

3.4.4.2 Threatened Plants as Found in Charaka Samhita (1500 BCE–200 CE)

The recent study on plants in Charaka Samhita (conducted by the authors) has revealed 668 distinct plant species in Charaka Samhita. Out of this, there are 482 species with different levels of controversy in its identification, and around 100 are unidentified or probably extinct. It is understood that around 60 plants in Charaka Samhita are having some threat status and falling under *Red List* as per Indian flora.

3.4.5 Authenticated Botanical Names Against Their Correlated Sanskrit Names

The foremost reason is the increased demand for correct identity of botanicals used in ISM and cosmetics. The global use of Ayurvedic products is rapidly increasing all over the world. The majority of drugs harvested in India are of plant origin, and plants provide the predominant ingredients of medicinal products. To make traditional plant products be acceptable to modern society, it is necessary to have reliable identification tools at the base level for identification of medicinal plant species.

A severe problem of the global Ayurvedic products is that many inaccurate substitutes and adulterants are traded due to their lower costs and due to misidentification of species with similar morphological features. For example, the plant *Shankhpushpi (usually correlated to Evolvulus alsinoides or Clitorea ternatea)* has more than 10 botanical correlations based on its usage in various parts of the country (IMPLAD 2016). For the protection of consumers and developments of relevant industry, authentication of plants is a critical issue.

Through this task, we tried to give a clear understanding regarding the correlations of the plants with its equivalent species by authenticating them. Only reputed publications were short listed to accomplish the above work. Please refer to Appendix 3.2 for the list of reputed works referred for authentication of botanical names against their Sanskrit counterparts.

IMPLAD has followed an authentication method based on the credibility of the texts/publication, expertize in field by cross-checking with the particular texts.

Steps involved the following:

- Segregate the records relevant to the textbooks they belonged.
- Verify and provide tags as:
  - Accepted source (AS) – which denotes there is no doubt about its botanical identity
  - Most probable (MP) – which denotes the nearest possible candidate which can be correlated to it
  - Controversial (C) – which indicates the different species of different genera being used for a single plant, e.g., *Pasanabheda* and *Murva*, wherein different species are being used for a single plant
  - Substituted source (SubS) – which denotes substitutes being used for the given plant
  - Suggested source (SS) – wherein the real correlation is unknown and the author tries to suggest a correlation based on its etymology, properties, etc.
  - Market source (MS) – which denotes the given species is used in the market for the particular plant
  - Not identified (Ni) – which denotes plants that are not known like *Soma*

Authentication process which is being followed in this process will help us to know the right candidate of the plant which will further go to the right collection of raw material to the finished product. In addition, these can be helpful to eliminate adulterants. Depending on the correct identification of species, it will ensure safety, herbal drug quality, and therapeutic efficacy of the product.

3.5 Materia Medica in IMPLAD

The materia medica of Ayurveda or *Dravyaguna* is essentially a compilation of ancient Indian medical knowledge on plants, metals and minerals, and animal products. IMPLAD has a dynamic section on Indian materia medica focusing on plants used in Indian systems of medicine.

3.5.1 *Classification of Plants in* Brihatrayi *(Three Main Treatises of* Ayurveda*)*

The classification of medicinal plants in Ayurveda is not binomial as the modern classification. The plants are named as per habitat, shape, size, therapeutic utility, etc. One plant can have up to 50 names, and one name can be given to various plants.

1. Charaka Samhita (1500 BCE–200 CE) – Explains 50 groups of medicinal plants classified as per their therapeutic indication. Each group has 10 plants in it and is called "group of decoctions (*Mahakashay*)" which are highly beneficial. Example of some of the "group of decoctions" is enlivening, anti-aging group, nourishing, increasing weight, and stamina. The basic principle of the treatment was to use medicines with virtues opposed to the system of the disease.

   Acharya Charaka also mentions many other types of classification including as per their taste and basic elements (*panchamahabuta*) and according to their plant part used (roots, fruits), etc.
2. Susruta Samhita (1500 BCE–400 CE) – It classifies the medicinal plants in group of drugs as per their therapeutic use. The groups are named as per the herbs included in them and not as per their therapeutic indication. These groups represent the collection of herbs with similar indications, e.g., *Aragvadadigana* constitutes *Cassia fistula*, *Azadiracta indica*, *Tinospora cordifolia*, etc., for wound cleansing (Sharma 1976).
3. Acharya Vagbhata, the author of Astanga Sangraha (600 CE), has classified herbs as per their therapeutic application and indication (Garde 1996). This makes it difficult to understand and interpret the classification as varied names of herbs have been used. Yet, this classification is widely used by traditional practitioners successfully, indicating the evidence of their practical applicability.

3.5.1.1 Classification of Plants in Lexicons (Nighantus) of Ayurveda

Lexicons (*Nighantus*) are considered to be the nucleus of Ayurvedic philosophy. Scholars in the medieval time felt the need to assemble the work on Ayurveda at one stage, and several lexicons were composed. Majority of the work was done between eight and fifteenth century C.E. It was the period of *Nighantus*; the botanical description of the medicinal herbs came into description. It differs with respect to the modern taxonomy which is totally on basis of anatomical morphology of the plant.

Pertaining to materia medica (*Dravyaguna*), the lexicons (*Nighantus*) give a vivid drug-to-drug description along with its pharmaco-vigilant aspects. Mentioned further are two of the most important lexicons among others.

Dhanvantari Nighantus (tenth century CE) is one of the oldest texts with a distinctive categorization of drugs in the form of seven different groups (*vargas*) based upon their morphology and therapeutic value.

Raja Nighantu, a lexicon written between the fourteenth and fifteenth centuries CE, is considered to be one of the important lexicons of Ayurveda. It describes various drugs, being classified under 23 groups along with certain properties and actions of individual drugs or medicines (*dravyas*). The nine groups are predominantly medicinal plant based, one is dealt with vegetables known as *Moolakadivarga*, and one group *Suvarnadivarga* deals with minerals and metals having medicinal value. Groups like *Paniyaaadi*, *Ksheeradi*, and *Shaliyadi* typically focus on food items.

3.5.1.2 Nomenclature of Medicinal Plants in Ayurveda

Medicinal plants in Ayurveda have several Sanskrit names and synonyms ranging from two to many. The scholars classified medicinal plants mostly on basis of morphological and organoleptic characters. The name *Ashwagandha* (*Withania somnifera*) has been derived from the smell of the plant resembling that of horse stool. *Sarpagandha* (*Rauvolfia serpentina*) has been derived from serpentine shape of roots. Although classification mentioned in Ayurvedic texts is of little significance in today's scientific world, its importance cannot be ruled out. Some drugs used in Ayurveda are of controversial origin, and the ancient knowledge can be of great help in naming the plants according to taxonomic standards.

3.5.1.3 Ayurvedic Pharmacology

In Ayurvedic pharmacology, the drug action is attributed to certain principles, namely, taste (*Rasa*), properties (*Guna*), potency (*Verya*), post-digestion and metabolism effect (*Vipaka*), and specific or synergetic actions (*Prabhava*) (Sharma 2006). IMPLAD has incorporated this details into the database with appropriate tagging and translation of technical terms.

3.5.2 Ayurveda Pharmaceutics

Around 2.5 lakh (0.250 million) herbal formulations in the traditional formulae of Ayurveda (www.tkdl.res.in) have been estimated. These formulae get modified to suit local conditions with an equivalent either listed in the formula/basic principles

(*Sutras*) or selected on the basis of the principles of Ayurvedic pharmacology mentioned in the materia medica.

The Ayurvedic drug formulation is based on what is known as five basic methods of preparation of medicine (*Panchavidha Kashaaya*) concept. According to this concept, there are five basic forms of formulation known as fresh expressed juice (*Swarasa*), a fine paste obtained by grinding fresh or wet dried plant material (*Kalka*), the decoction (*Kwaatha*), the cold water infusion (*Sheeta/Hima*), and the hot water infusion (*Faanta*) (Sharma 2011). Hence, almost every substance has to undergo a specific processing to acquire a form of palatable drug. The first two forms are prepared from freshly collected plant material and are directly put to patient use, whereas the last three forms, decoction, cold water infusion, and hot water infusion, are aqueous extracts prepared from the dried plant material.

Ayurvedic dosage forms are classified into four main groups depending upon their physical forms:

1. Solid dosage forms: tablet form (*Gutika*, *Vatika)*
2. Semisolid dosage forms: confections/linctus/(*Avaleha*, *Paka*), *Ointments* (*Lepa*), *Ghee Ghrta*
3. Liquid dosage forms: fermented preparations (*Asava*, *Arista*), *distilled extracts* (*Arka*) *oils* (*Taila*, *Dravaka*)
4. Powder dosage forms: different types of calcinated powders (*Bhasma*, *Satva*, *Mandura*, *Pisti*, *Curna*)

3.5.3 Glossary of Technical Terms

The Ayurvedic texts are in Sanskrit, and Ayurveda contains many terms that will be new to most people. All these terms are listed in the exhaustive glossary. The Ayurvedic terminology carries different connotations of the same words which mean something else in general Sanskrit. Therefore, to convey the proper meaning into any other language is extremely tricky. The commonest meanings have been included and have been defined in the simplest way possible. It may be noted that some of these words may have more than one meaning as is very common with Sanskrit words.

Attempt has been made to convey the inherent sense of the original Sanskrit technical term based upon the original textual reference to the context as also the available authentic commentaries. All the English terms included in this document are those that are present in universally recognized English dictionaries.

Most terms in English correspond well to the primary translation of the Sanskrit original, but there may well be exceptions. These exceptions are both expected and accepted because of the following reasons:

Homonyms Sanskrit boasts myriad shades of meanings for a single term. For example, the term “rasa” carries no less than 41 meanings. Such homonymous terms with practical significance were included with reference to the context.

Grammatical Nuances Sanskrit is a wonderfully flexible language and has complex nuances in word formation which often change the meanings diametrically. Some literal translations may fail to carry the full purport of the inherent sense (Unnikrishnan 2001). The glossary attempts to be as faithful as possible to the original meaning and is not merely a simplified translation. IMPLAD has a component of Ayurvedic pharmaceutics incorporating the above modules with various search options designed for different user groups.

3.5.4 Search Facility in IMPLAD

- Terms to be searched may be entered or selected from corresponding help menus or any technical term appearing in the database.
- Plant names can be searched through botanical or vernacular names.
- Search by clinical terms containing in-depth information on Ayurveda pharmacology, viz., *rasa* (taste), *guna* (quality), *virya* (potency), *vipaka* (post-digestion and metabolism effect), *karma* (actions), *dosha karma* (effect on humors – *vata, pitta, kapha*), *dhatu karma* (action on body tissues), *rogaharatva* (action on diseases).
- Advanced search facility provides combination of technical terms for an intelligent search.
- A common help file and glossary of technical terms and Sanskrit sloka references from classical texts are linked to the IMPLAD.

A large part of the materia medica features of the IMPLAD is made available for public use and can be accessed at www.medicinalplants.in (Ved et al. 2014).

3.6 State Inventories of Medicinal Plants of India in Determining the Threatened Plants

3.6.1 State Inventories of Medicinal Plants of India

Inventory is developed based on a thorough review of published floras and plant species recorded in each state, along with appropriate tagging of species which have been recorded in medicinal use in one or more of the codified Indian systems of medicine, namely, Ayurveda, Siddha, Unani, Homoeopathy as well as in the Folk traditions. This has detailed references of more than 200 published sources ranging from scholarly commentaries on classical texts relating to codified systems as well as published ethno-medico-botanical studies pertaining to each state. The inventory of medicinal plant database for different states has incorporated exhaustive correlation between the botanical names of medicinal plant entities and their vernacular names. These vernacular names belonging to several Indian languages have been

Table 3.1 Medicinal plant list enlisted in each state

State level inventory	Number of medicinal plant species
Chhattisgarh	1524
Madhya Pradesh	1736
Rajasthan	1017
Orissa	1643
West Bengal	1901
Uttarakhand	1608
North-Eastern States	
Sikkim	1618
Nagaland	1226
Tripura	858
Arunachal Pradesh	1654
Mizoram	679
South Indian States	
Kerala	2052
Karnataka	1838
Tamil Nadu	1840

included in the database. The purpose of this inventory database has served as a multifaceted, high potential tool for conservation of medicinal plants, providing deeper understanding of medicinal plants of the state (Table 3.1).

The total number of botanical names enlisted in the inventory of different states is given in the table; the list of medicinal plants given above excludes synonyms.

3.7 Nature and Kind of Threats to Medicinal Plants as the Variables for a Database

Nature of threats Destructive method of harvesting is not the sole cause of threat to wild populations of medicinal plants, as it is widely thought. In fact, on the contrary, a wide range of both extrinsic and intrinsic factors, many of which operate in close connection to each other, constitute the complex bundle of threats that affect the survival of medicinal plants in wild (Somashekhar 2015). A better understanding of such factors will provide a better and comprehensive backdrop against which the threats to a focal medicinal plant species are examined, threat status assigned, and appropriate conservation measure indicated. Such threat categories as assigned to prioritized plants become focal variables in the database of threatened medicinal plants.

Range of threats Different natural and anthropogenic factors are known to jeopardize wild populations of medicinal plants. They range from inherent ecological compulsions that affect the natural distribution of a species (such as natural rarity, endemic nature, restricted distribution, disjunct occurrence) to inherent biological, genetic, and physiological complexities of a species (such as long gestation period, dioecious nature of flowering, incompatibility of flowering flushes, poor flowering, hurdles for natural regeneration such as seed dormancy, lack of seed germination, and seed predation) and from anthropogenic pressures (shifting cultivation, forest clearing, forest fragmentation, habitat degradation, population decline, shrinking population size etc.) to market forces (fluctuations in the demand and its intensity, pricing, and supply of medicinal plants), and several such factors are known to affect the survival of wild populations of medicinal plants (Somashekhar 2015). An understanding of such critical variables and their role in increasing the severity of threats will allow help identify the causes of threats. Inclusion of such causes as a set of critical variables will further enhance the usability of the database of threatened medicinal plants.

An understanding of the threats and causes of such threats will offer a better picture of the complexity of the forces affecting the survival of wild populations of medicinal plants, an understanding necessary for assessing the threats and assigning a threat category, to a prioritized medicinal plant in a CAMP exercise based on IUCN criteria. The IUCN criteria of threat assessment and the threat categories make use of the data and finer details with respect to the causes of threats to the survival of medicinal plants, as detailed above, while assessing the threats.

The variables related to threats and causes of threats and the data pertaining to these variables which could be included in a database of threatened medicinal plants will serve two focal purposes: firstly, it serves as a reliable data source for developing taxon datasheets, which are the comprehensive profiles of a prioritized plants, in disguise. These taxon datasheets serve as the referral guidelines during the CAMP exercise. Secondly, these variables, included in a database, will provide a "framework" for building the consolidated picture of the threatened status of a prioritized species of medicinal plants.

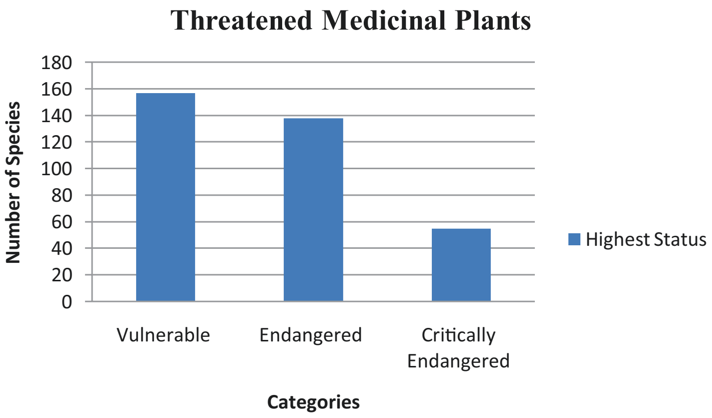
3.7.1 Threatened Medicinal Plants Across 19 Indian States

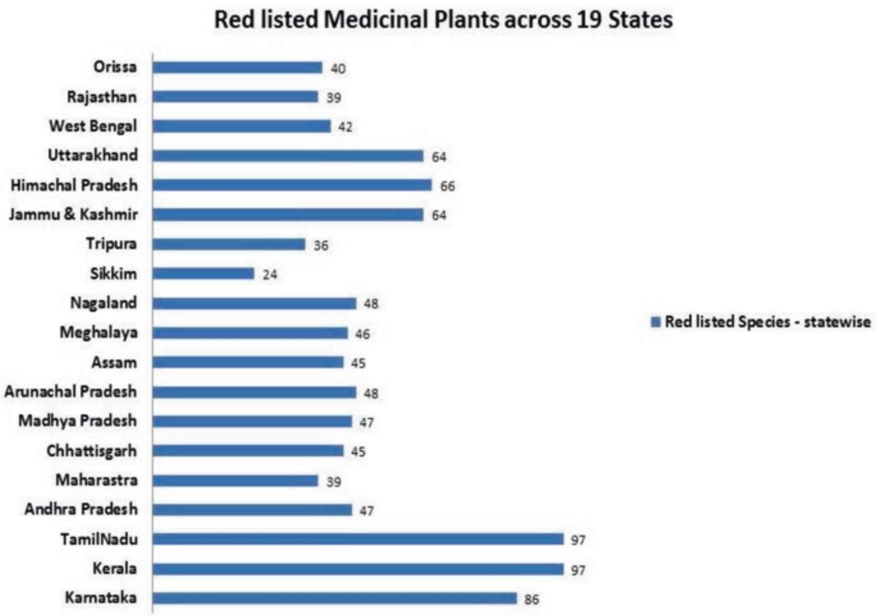
The Red-listed status has been assigned through the Conservation Assessment and Management Prioritization (CAMP) process, coordinated by FRLHT since 1995, for 19 states of India using Red List categories and criteria. A total of 388 species were assessed resulting in assignment of Red List status ranging from near threat (NT) to critically endangered (CR) (Ved et al. 2007).

The breakup of these assessment is as follows:

350 medicinal plant species assessed as threatened across 19 states of India
Vulnerable (VU) status: 157
Endangered (EN) status: 138

Critically endangered (CR): 55 as per assessment based on IUCN Red List category and criteria





Much more field work to assess the status of wild population is required. Obviously, there is a need to undertake more such assessment with respect to several other medicinal species of conservation concern (Ved et al. 2016) (Table 3.2).

Table 3.2 Table showing overlap of medicinal plants across different knowledge systems

Indian_System	*Ayurveda*	*Folk*	*Homeopathy*	*Siddha*	*TCM**	*Tibetan*	*Unani*	*Western*
Ayurveda	*205*	*120*	*27*	*107*	*47*	*59*	*65*	*9*
Folk	*120*	*271*	*22*	*99*	*43*	*47*	*54*	*9*
Homeopathy	*27*	*22*	*29*	*23*	*13*	*16*	*19*	*8*
Siddha	*107*	*99*	*23*	*125*	*31*	*48*	*52*	*7*
TCM	*47*	*43*	*13*	*31*	*63*	*20*	*25*	*9*
Tibetan	*59*	*47*	*16*	*48*	*20*	*59*	*44*	*4*
Unani	*65*	*54*	*19*	*52*	*25*	*44*	*66*	*6*
Western	*9*	*9*	*8*	*7*	*9*	*4*	*6*	*13*

TCM Traditional Chinese medicine

3.8 The Image Library

The image library has been developed over a period of time by the field botanists. This task involves proper labelling and digitization of plant images duly identified and photographed by TDU-FRLHT's botanical team members during their field work. Each species has more than one image for highlighting different features relating to the botanical features like flowers, fruits, habitat, parts used, etc., as the purpose of these authentic plant images is to facilitate identification of the plant entities.

Each image carries a file name establishing its linkage with the appropriate botanical entity recorded in the data table cataloging the entire list of botanical names of Indian medicinal plants.

The image library incorporated into the IMPLAD has a collection of around 25,000 images to facilitate plant identity. The image library has been updated with an average of 500 new images per year; superfluous ones have been deleted. Images were edited for providing watermark so that each image is established with due credits and shows a responsible proof for its authenticity.

3.9 Distribution and GIS Mapping of Threatened Plants

3.9.1 Distribution Database and Mapping of Wild Medicinal Plants of Conservation Concern in India

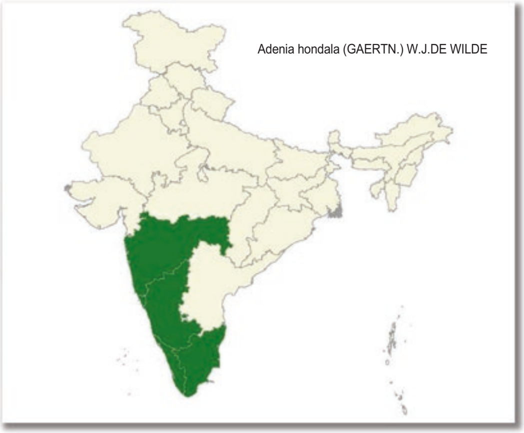
Information on the natural distribution of wild medicinal plants, across the geographical regions of the country, was not readily available. As per the database (IMPLAD Team 2016), there are 6500 medicinal plant species for which the distribution pattern was not well documented. Hence, the efforts were focused on building a distribution database (Ved et al. 1998). The distribution database is in a constant process of updating and curation referring available published authentic sources comprising more than 200 Floras of covering States and Districts of India. The gaps in distribution patterns and understanding were a roadblock for conservation action and hampered the assessment of the conservation status of wild medicinal plant species and identification of specific locations for informed conservation action. In order to address this gap, the activity has focused on building a database of recorded geographical distribution of the wild medicinal plant species at two levels: (1) building a distribution database through a data management module and (2) generating geographical distribution maps through a map module (Ved et al. 2014).

Building a Distribution Database Through a Data Management Module This module facilitates entry and management of distribution data which is an ongoing process. So far, data is compiled from more than 200 published floras; the information on distribution is being digitized at three levels (Global, National, and Regional), with related information like ecology, altitude, and associated species. So far, more than 75,000 distribution data records have been curated and incorporated into the IMPLAD.

Based on a thorough review of the relevant publication and a baseline information from the distribution database supplemented with field-based botanical surveys by our in-house botanical team, the distribution datasheets have been prepared for more than 2000 wild medicinal plants.

Generating Distribution Maps Through a Map Module The geo maps were prepared by using a map module based on Microsoft .NET framework, where the states, districts, and talukas can be selected for each species which will generate a map. The administrative boundaries of India for all state, districts, and talukas were downloaded from http://www.gadm.org/home website. The International Taxonomic Database Working Group's world geographical scheme for recording plant distributions was downloaded from https://www.kew.org/gis/tdwg/ website as ArcView shape files for use in the map module. This system provides researchers with codes for recording plant distributions at four scales or levels, from "botanical continents" to parts of large countries. Maps generated using the map module were made available into the IMPLAD database to provide the users with a quick pictorial map representation of the distribution of the species, which will be a ready reckoner from a user's perspective. In a particular genus like *Adenia*, five species are considered as medicinal according to IMPLAD database; among this, *A. hondala* and *A. wightiana* are reported from Southern Indian states. The maps that explain the distribution pattern of the species are shown below.

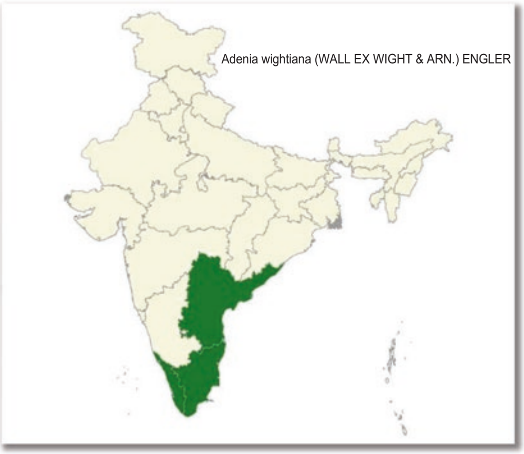
3.9.2 Genus: Adenia



Distribution: In India reported to be in Western Ghats - Maharashtra, Karnataka, Kerala & Tamil Nadu.

Synonym: *Adeniapalmata Engl. Modecca palmate Lam.*

Adeniahondala (Gaertn.) W.J.De Wilde



Distribution: In India reported to be present in Kerala, Tamil Nadu, Andhra Pradesh.

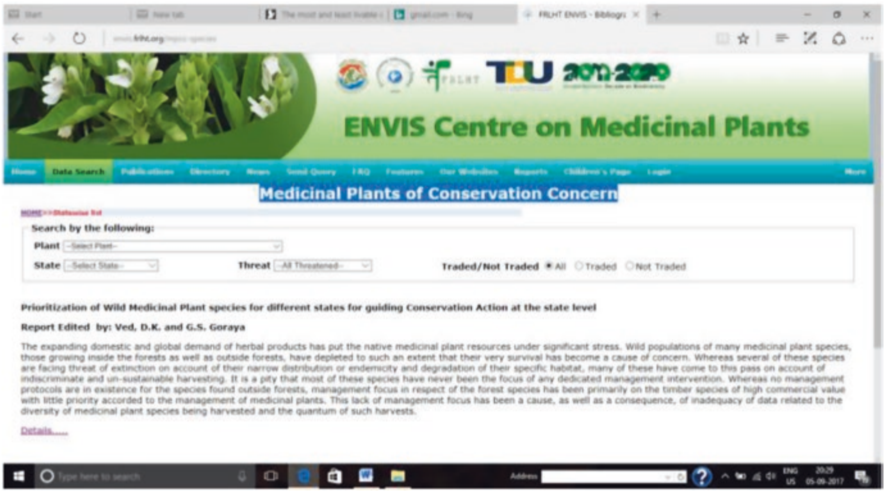
Synonym: *Modecca wightiana Wall.ExWt & Arn.*

***Adeniawightiana** (Wall.Ex Wight & Arn.)Engler.*

This activity aims at providing reliable data on the natural distribution of wild medicinal plants within India, for the use of forest managers, conservationists, and researchers. Geographical distribution maps for 2000 medicinal plant species with state presence were prepared with datasheets and incorporated into the IMPLAD. The outputs are also being disseminated on FRLHT-ENVIS portal (Ved et al. 2017) and BHUVAN (http://bhuvan-staging.nrsc.gov.in/events2/forest/frlht) portal to facilitate access to stake holders.

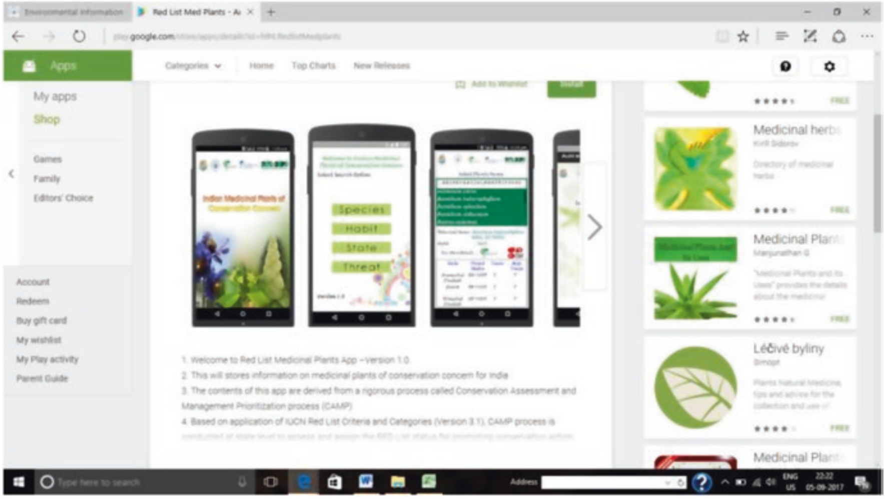
3.10 Websites and Mobile Apps on Red-Listed Medicinal Plants of India from IMPLAD

In the envis.frlht.org and frlhtenvis.nic.in websites (Ved et al. 2017), Red List medicinal plant species of India App, information related to 359 species that are of conservation concern is shared at state level. User-friendly graphical interface is designed for wide range of audience. Search can be done by three ways: scientific names, state wise, and trade wise. Contents are sourced from the various reports (Ved et al. 2016), generated through a rigorous process called Conservation Assessment and Management Prioritization (CAMP) for management action programs conducted by FRLHT/TDU, Bangalore, in collaboration with (the Ministry of Environment and Forest and Climate Change) MOEF and CC, State Forest Department and Biodiversity Boards, NMPB (National medicinal plant board), and UNDP (United Nations Development Projects) over two decades. Even CAMP reports from premier research institutions are shared on the websites. The threat categories for the species are assigned based on application of IUCN Red List Criteria and Categories (Version 3.1). Results of CAMP process are used to promoting conservation action and management programs for specific species. This website and app draws attention of managers, researchers, academicians, policy makers, and politicians to guide conservation action research programs.



The present version of the website and mobile app comprises information for 17 states with 359 medicinal plants species with their assigned Red List Status. Habit-wise analysis of 359 medicinal plant species reveals 125 herbs, 107 trees, 64 climbers, and 36 shrubs. Among the 359 species, 44 are endemic to India and are listed in IUCN Red List species. Each of the plant profile gives complete details of a species from botanical names, threat status across different states, and IUCN details. Table 3.1 shows total number of species with various threat categories across different states.

http://envis.frlht.org/mpcc-species, frlhtenvis.nic.in& Red List Medicinal Plants App –Version 1.0. https://play.google.com/store/apps/details?id=frlht.RedlistMedplants&hl=en



ENVIS App on Indian Medicinal Plants https://play.google.com/store/apps/details?id=com.envis_frlht

This particular app (Desale et al. 2017) is a ready reckoner on Indian medicinal plants recorded from various published sources, specifically designed for lay person, researchers, academicians, resource managers, ISM physician, etc. This app provides information on 6500 species with correlation of scientific and local names and associated information.

Neighborhood Medicinal Plant App: Version 0.5.0 (Bangalore city) https://play.google.com/store/apps/details?id=frlht.neighbmpblrcity

This app is exclusively designed for students and nature lovers, who are interested to learn about their neighborhood plants that are medicinally important. This app provides information on 300 common medicinal plant species of Bangalore city which is categorized by habit (Herb/shrub/tree/climbers) and flower colors. Each plant profile will give you colorful images; scientific name; local names such as English, Hindi, Sanskrit, Kannada, Tamil, and Telugu; and appearance, with medical system tag and indication of uses.

The development of these e-resources is exclusively supported by the Ministry of Environment, Forest, and Climate Change, Government of India, under ENVIS scheme. Technical inputs and data and design are supported by FRLHT-TDU team at Bangalore. These apps were officially launched during the evaluation meetings held during 2015–2017. More information can be obtained from envis@frlht.org, frlhtenvis.nic.in.

3.11 Conclusion

The IMPLAD (Indian medicinal plants database) and its various components on traditional knowledge on medicinal plants, pharmacopoeia, distribution mapping, and threatened plants and its various tools for knowledge dissemination are successful model in meeting the objectives of this unique venture and continue to move forward with more new activities for the future. This has served as a decision making tool for the policy makers and government agencies involved in the conservation of medicinal plants and traditional knowledge and contemporary research in the field of medicinal plants conservation especially of threatened plants. IMPLAD plays an integral part of many education, research, and outreach activities of transdisciplinary science.

Acknowledgments ENVIS (Environmental Information system), Ministry of Environment Forest Climate Change, Govt. of India (MoEF & CC)

- UNDP-GEF (United Nations Environment Fud, Global Environmental Facility)
- National Medicinal Plant Board (NMPB) AYUSH Ministry, Govt. of India
- FRLHT (Foundation for Revitalisation of Local Health Traditions). Bangalore, India

Appendix 3.1: Reference Text Selected for Ayurvedic Information on Plants

No	Text name	Chronology	Author	Plant name references
1	Charaka Samhita	1500 BCE–200 CE	Agnivesa, CharakaDrdhabala	12,850
2	Susruta Samhita	1500 BC–500 AD	Susruta, Nagarjuna	9650
3	Astanga Sangraha	500 AD	Vagbhata	20,500
4	Astanga Hrdayam	600 AD	Vagbhata	9900
5	Astanga Nighantu	800 AD	Vagbhata	2100
6	Paryayaratnamala	900 AD	Madhava	1900
7	Dhanvantari Nighantu	200 AD–1000 AD	Unknown	3250
8	Chakradatta	1075 AD	Chakrapanidatta	12,300
9	Dravyaguna Sangraha	1075 AD	Chakrapanidatta	320
10	Madhava dravyaguna	1250 AD	Madhava	750
11	Sarngadhara Samhita	1300 AD	Sarngadhara	4200
12	Nighantu Sesa	1200 AD	Hemachandra	2950
13	Siddhamantra	1210 AD–1247 AD	Kesava	950
14	Hrdayadipaka Nighantu	1260 AD–1271 AD	Bopadeva	820
15	Madanapala Nighantu	1374 AD	Madanapala	3000
16	Bhavaprakasha	1550 AD	Bhavamisra	11,200

No	Text name	Chronology	Author	Plant name references
17	Bhavaprakasha Nighantu	1550 AD	Bhavamisra	2600
18	Raja Nighantu	1700 AD	Naraharipanda	7300
19	Saligrama Nighantu	1896 AD	Saligramavaisya	4200
20	Siddhabhesajamanimala	1896 AD	Krshnaramabhatta	620

Appendix 3.2: Reference Texts for Botanical Information and Correlations

The bibliographic sources for this activity included 21 books belonging to last 100 years of works by Ayurvedic experts, botanists, and pharmacognosists attempting to correlate Sanskrit names with botanical names.

No	Name of the work	Author	Year
1	Pharmacognosy of Ayurvedic drugs Vol. 1, 2, 3, 10	K.N.Iyer, A. N. Namboodiri and M.Kolammal	19511957,1979
2	La-Harita Samhita	AlixRaisom	1974
3	Astanga Hrdaya Kosha	Anonymous	1936
4	Ayurvedic Pharmacopoeia of India Vol. 1	Ministry of Health and Family Welfare	
5	Ayurvedic Formulary of India Part 1	Controller of publications	1978
6	A Dictionary of Economic Products of India	George Watt	1889
7	Indian Medicinal Plants Vol. 4	Kirtikar and Basu	1935
8	Handbook of Medicinal Plants	P.N.V.Kurup	1968
9	A Catalog of Indian Synonyms	Moodeen Sheriff	1988
10	Single Drug Remedies	N.S.Moos	1976
11	Ganas of Vahata	N.S.Moos	1980
12	Indian Pharmaceutical Codex Vol. 1	B.Mukherji	1953
13	Indian Materia Medica Vol. 2	K.M.Nadkarni	1954
14	Indian Medicinal Plants Vols. 1–5	S.RaghunathIyer	1993–96
15	Dravyagunavijnana Vols. 2 and 5	P.V.Sharma	1994
16	Ayurvedic Drugs and Their Plant Sources	Sivarajan and I.Balachandran	1994
17	Glossary of Vegetable Drugs in Brhattrayi	Thakur Balwant Singh &Chunekar	1972
18	Nighantu Adarsha Vols. 1 and 2	VaidyaBapalal	1968
19	Some Controversial Drugs of India	VaidyaBapalal	1982
20	Studies on Medicinal Plants in Dhanvantariya Nighantu Vol. 1	VaidyaD.K.Kamat	1972
21	Materia Medica	Whitelaw Ainslie	1984

These references represent botanical correlations of Sanskrit name, and other botanical information of plants, accepted as authentic sources of information in the IMPLAD.

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Chapter 4
Harnessing the Potential of Medicinal, Aromatic and Non-timber Forest Products for Improving the Livelihoods of Pastoralists and Farmers in Himalayan Mountains

Madhav B. Karki

Abstract Medicinal, aromatic, wild food and other health and wellness-related natural plant resources found in Himalayan highlands include rare, endangered and threatened plant species and non-timber wild products. These are commonly described as NTFPs and MAPs. Sustainable wild harvesting and primary processing of these herbs for addressing poverty of poor pastoralists, farmers and local traders is a major challenge. Medicinal plants not only play a pivotal role in providing primary healthcare for poor people in mountain areas; increasingly, these niche products are being gathered, processed and sold in national and international markets for higher cash income. Prominent examples of high-value but threatened medicinal plants that are commonly used in the Ayurvedic and Tibetan systems of traditional medicine (Sowa Rigpa) are as follows: *Ophiocordyceps sinensis*, *Neopicrorhiza scrophulariiflora*, *Picrorhiza kurroa*, *Nardostachys grandiflora*, *Dactylorhiza hatagirea*, *Podophyllum hexandrum*, *Aconitum* spp., etc. Experience gathered to date suggests that technical, socioeconomic, institutional and policy inputs and instruments are required to develop niche and high-volume production in pastoral systems. This chapter analyses and recommends the following actions in enhancing future scope: (a) raising awareness through different formal and informal education means, (b) skill development in sustainable harvesting as well as grazing management, (c) production of organic and sustainably managed products, (d) integration of agricultural and pastoral livelihoods with off-farm activities through value chain development of major niche products that have high-value capturing

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potential, (e) improvement of degraded pasture and farmlands to enhance productivity of niche products and services, (f) conservation through sustainable use-oriented policy and legal reforms to implement integrated strategies of linking conservation of wild fauna and flora with sustainable pastoral production systems and (g) expansion of ecologically sensitive low-input high-return tourism, using pastoralists to provide services, particularly through their indigenous knowledge and improved local production practices. These measures are expected to help Himalayan countries to achieve several SDGs especially goal nos.1 and 2.

Keywords Globalization and economic liberalization · Medicinal and aromatic plants · Ayurveda · Sowa Rigpa · Natural ecosystems · Organic niche products · Value chain · Pastoralism · Non-timber forest products · Sustainability · Biodiversity · Poverty reduction · Green mountain economy

4.1 Introduction and Background

Mountains occupy 24% of the global surface area and are home to 12% of the world's population. They have ecological, socioeconomic, spiritual and cultural significance, not only for those living in mountainous areas but also for people living beyond (SDC/ICIMOD/MP 2012). The international community recognized the importance of mountains at the United Nations Conference on Environment and Development (UNCED) in Rio de Janeiro, Brazil, both in 1992 and 2012 with the adoption of Chap. 13 in Agenda 21 and Para 210–212 in the Rio+20 outcome document: *Future We Want*. This underscores the role of mountains in implementing the global sustainable development agenda. Mountain ecosystems are among the most varied and rich in terms of endemic and high-value species (e.g. Vare et al. 2003; Moser et al. 2005; Spehn and Korner 2005). Mountains support about one-quarter of the planet's biodiversity and have nearly half of the world's biodiversity hotspots (Singh 2011). Mountain systems provide niche habitats for many rare and/or endangered endemic species (ICIMOD 2011).

Mountain communities are mainly traditional farmers and pastoralist societies. They have developed and maintained vast knowledge and experience on the use of natural resources including plant resources. Much of the mountain's rural economic activities, however, are based on unsustainable use of natural resources, resulting in deforestation, loss of biodiversity and degradation and destruction of natural habitats. Cultural and traditional knowledge systems and high values for nature are also fast vanishing along with natural resources.

Efforts to sustainably manage the region's medicinal, aromatic, natural and other medicinal, aromatic and non-timber forest product (MAPs and NTFPs) resources, especially in the least developed mountain countries, have not achieved the desired goals. Balancing the four pillars of sustainable development—social, environmental, economic and institutional—has been one of the key challenges for these countries. Although the traditional drivers—population growth, agriculture intensification and unsustainable harvesting—continue to have an influence, new drivers of change such as climate change, globalization and outmigration of youth to overseas labour markets have added new problems and but also provided some opportunities such as increased remittance flow (ICIMOD 2019).

The markets for natural products, especially pharmaceuticals, food and nutrition products, are growing. Medicinal and aromatic plant products alone are estimated to command a market of more than USD $80 billion. However, with the rise in demand for natural products, there is also a rise in biodiversity loss and an increase in the number of poor people dependent on forest products such as NTFPs/MAPs for livelihoods. Therefore, it is necessary to develop a sustainable use as well as economic growth strategy that can secure an equitable living standard for forest-dependent people while conserving ecosystem resources. For such a development model, which is now called green mountain economy, the importance of natural capital or ecosystem services will be high. Here the role of NTFPs and MAPs cannot be overstressed. Many local economies, especially in mountain ecosystems, are highly dependent on NTFPs and associated natural resources. Their role can be enhanced through green technologies, green growth strategies and by generating green jobs. Many countries—both developing and developed—already have institutions and governance systems that are implementing sustainable management of natural resources ensuring an equitable flow of benefits to the people involved. Many of these traditional institutions that have evolved over generations have led to a number of good practices that have been helping indigenous and local communities to cope with financial, ecological and social changes and challenges, protecting against the consequences of unavoidable changes in the external environment. In the Hindu Kush Himalayan (HKH) region, many pro-poor value chain development pilots conducted by research and development organizations have been successful (MoA/SNV 2011; Karki 2017).

Sustainable use and management of biodiversity resources such as NTFPs and MAPs are a high-priority topic in sustainable mountain development agenda. In recent years, ecological, social and economic roles of NTFPs are becoming increasingly significant owing to better understanding and appreciation of their contribution in promoting green economic growth. Growing market preference for green and natural products and consumers' emphasis on efficient and sustainable use of natural resources have also highlighted the added importance of sustainable commercialization of NTFPs and MAPs (Karki 2017). In recent years, NTFPs have gained much needed recognition along with the realization of the need to conserve forests and protect the biodiversity and ecosystem goods and services they provide. In many countries, especially in Nepal and other HKH countries enhanced access to NTFP/MAP resources has been providing a powerful incentive to local communities to protect forest tree cover while harvesting forest undergrowth only. In fact sustainable management of medicinal plants has been helping to achieve sustainable management of forest resources in many countries (IUFRO 2012).

4.2 Current Understanding of MAP and NTFP Subsectors

There is no universally accepted definition of the term "non-timber forest products". FAO uses the term "nonwood forest products" and defines them as "products of biological origin other than wood derived from forests, other wooded land, and trees outside forests; they may be gathered from the wild, or produced in forest plantations, agro-forestry schemes and from trees outside forests" (FAO 1999). Ahenkan and Boon (2011) have done an excellent compilation and analysis of the semantics and the difficulties in defining NTFPs. In some countries, NTFPs are also referred to as minor or special forest products (Hammett 1999). In some definitions, NTFPs include non-consumptive ecosystem services enjoyed by humanity such as ecological/environmental, cultural and religious and tourism and recreation values (Walter 1998). MAPs are not well defined in the literature but in general any plant or parts thereof used in any medical system such as *Ayurveda*, *Siddha*, *Unani*, *Sowa Rigpa* or in the ethnic healing system are generally categorized as medicinal plants. Aromatic plants are those that have aroma in their parts that are extractable in the form of essential oils (Sharma 2007). Together these groups of plants are called NTFPs and MAPs.

In this chapter, the NTFPs found in mountain and hilly ecosystems are considered to comprise non-timber floral, faunal and recreational products, including fuel wood, wood crafts, animal fodder and compost materials; medicinal, aromatic and dye plants; wild mushrooms, floral greens, decorative greenery and wild foods (nuts and seeds, berries, oil seeds, etc.); craft species; and products of ecotourism value derived from forests, rangelands and protected areas (Ghimire et al. 2008a). They also include game animals, furbearers, etc. NTFPs are increasingly considered high-value ecosystem goods and services that can transform the economies of forest-rich developing countries into low-carbon or green-growth-based economies. The common factor that cuts across all forest and biodiversity-dependent communities in the mountainous regions is the existence of high poverty and deprivation amidst rich biodiversity. Hence, there is a need to provide forest and biodiversity-based employment and sustainable livelihoods to the poor and marginalized communities while ensuring conservation of forests and natural habitats, which are becoming increasingly threatened. In this context, the role of non-timber forest products (NTFPs) becomes extremely important, because cutting and using timber products increases carbon intensity. With an expected increased investment in forestry and green sector, there is a real need for more systematic research and knowledge generation on the role and potential of NTFPs in assisting the attainment of sustainable development goals. This is the main argument of this chapter.

4.3 Livelihood Importance of MAPs and NTFPs in Mountains

Persistent poverty in developing mountainous countries in South Asia is generally linked with small, fragmented or no landholdings, accompanied by low productivity. Dependence on collection and gathering of NTFPs from forests to ensure food security goes largely unnoticed and is not accounted in the calculations of gross national product (GNP). Some of the products meet a global demand (e.g. raw material for pharmaceutical industries, edible nuts, honey, bamboo and cane products); others reach specific markets (e.g. crude herbs, aromatic and chemical products), while some NTFPs and MAPs are collected and consumed locally.

Forest-dependent communities across the mountainous regions derive their sustenance from NTFPs in periods of financial stress and have used them as raw materials for producing items of daily use in normal times. In least-developed mountainous countries such as Afghanistan, Nepal, Bhutan and Myanmar, NTFPs provide food, medicine, nutrition and cash income to poor and vulnerable households. NTFPs are extracted primarily from the wild for meeting the food, medicine and supplementary cash needs for the subsistence of poor households in these countries (Karki and Bhattarai 2012).

The role of the medicinal and aromatic plant resources in the economy of developing countries becomes even greater when high-value service sectors such as health, nutraceutical, organic and certified products and ecotourism and health tourism are taken into account and linked to overall sectoral development of forest conservation and development (Karki 2003, 2004, 2015; Karki et al. 2004).

4.4 Market Potentials and Constraints

It is estimated that more than 150 NTFPs are traded in international markets (FAO 1997). Among these, medicinal and aromatic plant products alone are estimated to command a market of more than USD $80 billion (Karki and Nagpal 2004). The World Health Organization (WHO 2002) estimates that 80% of the global population relies on plant-based medicines for primary healthcare needs. Agrawal (2007) estimates the global market potential for NTFPs to reach as high as USD $225 trillion by 2050. It is clear that NTFPs, besides providing multiple intangible benefits, also have huge economic potential and generate cash incomes, particularly for women and families that do not have access to agricultural lands and major markets, particularly in developing countries.

However, the inadequacy of market-related information and negotiation skills with the upstream producers in dealing with market forces, as well as unequal power relationships or lack of a level playing field between buyers and sellers, disadvantages the growers, collectors and local traders of NTFPs in mountainous regions. The supply chain of NTFP products is unnecessarily long, with a large number of commission agents eating into the returns that could go to the farmers. These are the major obstacles to the small-scale producers and growers of NTFPs that prevent them from benefitting from higher values. Forest users, landowners, harvesters and processors and policymakers can influence how NTFP resources are managed through the knowledge, practices and policies they suggest, design and implement, if they can all work within one single framework linking producers to markets and consumers.

The annual revenue from the sale of more than 33,000 tonnes of NTFPs is estimated to be between 13 and 26 million USD (GoN 2010). Most of the products are exported to India in crude or semi-processed form. But in the last few years, semi-processed or processed NTFPs are being exported to both Himalayan and other countries. Essential oils are the major exported commodities among processed herbs that are extracted from more than 18 aromatic plants (Prakrit 2007; Ghimire et al. 2008b). The oils are mostly exported to Japan, the USA, Germany, Belgium and many other countries. The NTFPs other than MAPs exported by Nepal are handicraft items whose value was about Rs 300 million in 2004/2005 (Acharya 2006). The NTFPs thus are the major exports of Nepal. Nepal however also is one of the biggest consumers of processed medicinal products, most of which are imported from India, which is growing at an annual rate of 20%, (Ghimire et al. 2008a, b). Therefore, there is a tremendous possibility of improved management, processing and value addition of herbal products and other NTFPs in Nepal that can help alleviate poverty by meeting domestic as well as foreign markets and creating income generating opportunities locally (Tewari 2004; Sekar et al. 1996).

4.5 Employment, Health and Income Potential of MAPs and NTFPs

The NTFP sector is a very important source of rural employment (Ghimire et al. 2008a). According to FAO (1997, 1999), NTFPs contribute about 50% of forest revenue and 70% of income through export of different food, medicine and aroma products (Sekar et al. 1996). In India, the NTFP sector, including bamboo and rattan, medicinal plants and other subsectors, is estimated to employ poor people for more than 100 million person days (Tewari 2004) mainly in rural areas; about 200–300 million villagers depend on NTFPs to varying degrees. NTFPs also contribute 10–40% of income to the 50 million tribal households in India (FAO 1997). In Nepal, rural mountain communities derive up to 50% of their total family income from NTFPs including MAPs (Pyakurel and Baniya 2011). Thus, NTFPs can significantly help in livelihood diversification of vulnerable mountain communities affected by downturns in other resource sectors as a result of land and forest degradation, which is often aggravated by growing climate variability. Ayurveda, the oldest medical system in the Indian subcontinent, and traditional Chinese medicine (TCM) have alone reported using approximately 2000–3000 medicinal plant species (Prajapati ND et al. 2003). The *Charaka Samhita*, an ancient handwritten document on herbal therapy in India, reports on the production of 340 herbal drugs and their indigenous uses based on wild collection of NTFPs (Bhattacharya, Rajasri et al. 2006). Worldwide, it is estimated that approximately 25% of all pharmaceutical drugs are derived from plants, and many others are synthetic analogues built on prototype compounds isolated from plant species (Rao et al. 2004).

4.6 Key Issues in Sustainable Use of NTFPs and MAPs

Mountainous countries face numerous challenges in instituting sustainable use policies. Different countries are interpreting sustainable use regime differently and are embarking on different approaches to promote NTFP-based economic growth concepts and practices for sustainable development. NTFP-based green economic development can be a means to achieve sustainable use of MAPs and NTFPs in mountains. However, the common challenges mountain countries are confronting or will face in future are as follows: (a) How to document sustainable NTFP/MAP management cases on which the future sustainable development pathways can be charted? (b) How effective are the current approaches, and what lessons can be learned from the experiences, particularly in terms of management systems, and their successes and failures? Although NTFPs can be viewed from the perspective of economic development, they must also be considered in terms of biodiversity conservation and sustainable use (Karki 2017). The supply of wild plant NTFPs/MAPs is dwindling given the threats of increasing demand, a rapidly increasing human population and rampant destruction of plant-rich habitats. Medicinal and aromatic plants provide a good example. At the current rate of consumption and use, the status of many of these plants along with the future supply of raw materials and benefits generated by them is likely to be severely threatened. Although cultivation is playing an increasing role in the supply of MAPs, most will be obtained from wild collection in the foreseeable future; thus, their sustainable management is essential. There is no "golden rule" that can be applied universally to ensure conservation and sustainable medicinal plant management, because what is defined as conservation and sustainability will vary with type of plant, part used, locality and other factors. Bhutan banned the export of medicinal plants and other NTFPs in 1988 as a measure to conserve biodiversity and to prevent uncontrolled exploitation of these resources (FAO 1996). The "Framework for Collection and Management of Non-Wood Forest Products" (RGoB 2009) has permitted communities to collect medicinal plants and other NTFPs for non-commercial uses, considering conservation and sustainability of the resources. The government has identified seven species as "extremely rare" and 26 species as "rare" and has launched conservation and management initiatives for protecting them.

In China, the state has protected 116 species of medicinal plants used in TCM (CCTHM 1995). The government has proposed six large important plant areas (IPAs) for medicinal plants and other NTFPs in the Chinese Himalayan region, covering an area of 434,200 km^2 (Hamilton and Radford 2007). There are 2400 nature reserves covering 14.8% of the total land and 60% of the country's plant species that are designated for in situ conservation and management for sustainably harvesting medicinal plants benefiting the local population. Regarding ex situ conservation, there are ten state-managed medicinal plant gardens and germplasm banks, 220 botanical gardens (2006), about 5000 species of medicinal plants and other NTFPs cultivated in these botanical gardens (Pei and Sajise 1993). In India, Conservation Assessment and Management Plan (CAMP) workshops, following the IUCN criteria, have been organized in major parts of the country, including all the Himalayan states.

The National Medicinal Plants Board (NMPB) of India, chaired by the Union Health Minister, was established in 2000 and has prioritized 31 species of medicinal plants for conservation, management and cultivation. State-level Medicinal Plants Boards have been established in 26 states of the country. Considering the state-level activities for conservation and management of MAPs/NTFPs, in 2004, Uttarakhand declared itself as an Herbal State with a plan of action for the conservation, management and development of the NTFP sector. The Uttarakhand state government has prioritized 26 species of medicinal and aromatic plants for conservation in the wild and for cultivation. The state is also supporting farmers for cultivating the 26 prioritized species with 50% assistance on cultivation cost up to a maximum of 1,000,000 Indian rupees (USD 2000). By 2010, about 8000 private organic herbal farms had been registered. The state government has established large number of medicinal plant nurseries and provides free planting materials for registered farmers and *Van Panchayat* (Forest Council) members as a strategy to enrich plantations in the forests. In 1998, the Government of Sikkim imposed a ban on grazing in reserved forests, on plantation areas and around water source areas, and in 2000, it imposed a total ban on lopping of selected trees and collection of selected medicinal herbs. Sikkim has brought 34,000 farmers cultivating 18,000 ha in the organic farming regime.

The Government of Nepal has imposed different levels of restrictions in the collection, trade and export of some of the highly traded medicinal plants to safeguard them in the wild and to promote cultivation practices. The CAMP workshop (Tandon et al. 2001) evaluated 51 commercial MAPs and NTFPs for their status in the wild. In 2000, Nepal established the high-level Herbs and NTFP Coordination Committee (HNCC), chaired by the Minister of Forests and Soil Conservation, to formulate and implement MAP/NTFP-related policies and to streamline the NTFP sector in the country. The Herbs and NTFP Development Policy 2004 is a milestone in the country's strategy to conserve and sustainably manage the MAPs and NTFP sectors. It includes six policy objectives, five policy groups and 28 development strategies. In general, the policy identifies national challenges, opportunities and priorities and provides an outline for moving forward. The HNCC prioritized 30 species of MAPs/NTFPs for conservation, research, development and management, including 12 species recommended for cultivation (GoN 2010).

Pakistan, in 2001, assessed the threat of 52 species of commercial medicinal plants following the IUCN criteria. Later in 2010, the government prioritized 24 commercial medicinal plant species (including 12 endangered and 12 vulnerable species) and has made provisions to conserve and manage them through different administrative and management units (Hamilton and Radford 2007).

4.7 Area for Improvement: Local Value Additions and Value Chain Development

The world market for natural products and organically derived NTFPs, including medicinal plant products, has been increasing, and consumers have become more conscious of the source and quality of the products they purchase. According to FAO, organic trade is expanding at the rate of 15–20% per year, and more than 100 countries currently export certified organic products (Choudhary and Bhattarai

2008). However, the global trade in organic products is hindered by a multitude of standards, regulations and conformity assessment systems. There are currently two international standards for organic agriculture: the FAO/World Health Organization (WHO) Codex Alimentarius Commission Guideline-based standards and the International Federation of Organic Agriculture Movements (IFOAM) basic standards. This means that products certified as organic in one system may not be easily recognized as organic under another, causing problems and increased costs for organic producers and exporters who want to sell in different markets.

The potential for small holders and other marginal community groups to diversify and enhance their livelihoods is particularly significant when harvesters become involved in "value addition" activities associated with the packaging of goods or the manufacture of secondary products and when they engage in responsible trade of medicinal plants and other NTFPs. Investigating the market and the means to access it can enable NTFP cooperatives and other farm organizations to understand opportunities and develop strategies to meet the needs of its members and buyers. The objective is to create economic enterprises in which the livelihood base and activities of entire communities are upgraded and not just a few micro-entrepreneurs. Clearly, providing a delicate balance between the two depends on socioeconomic and cultural factors as well as the more obvious technological and biological support systems.

At the local level, improved marketing requires capable organizations such as cooperatives or other farm associations. These organizations can help take decisions of common interest and undertake collective actions. By working together, members of an organization can gain bargaining power with traders and middlemen and maximize their incomes. An organizational marketing strategy can also help reduce risks for producers.

A number of factors influence the ability of producers to respond to customer needs and wants. Some can be influenced by farmers and producers, while others are beyond their control. Although small-scale farmers have some marketing skills, they could benefit from the specialized expertise and more efficient marketing made possible through marketing associations. This means that capacity building is needed at village, regional and national levels to identify promising NTFPs and to manage their harvesting, production and marketing. Extension workers, nongovernmental organizations and community leaders can be important agents for introducing marketing to small farmers.

Indigenous and local knowledge on plants and the innovation practices of traditional communities can be useful tools in developing new ways of conserving and using NTFPs for the benefit of mountain communities. As well, integrating the indigenous knowledge based good practices with scientific knowhow will provide robust knowledge and good practices for achieving the UN sustainable development goals. The approach has to document this knowledge and apply it to bridge the gap between the understanding and needs of government agencies, the public sector, local communities and the private sector based on systematic NTFP/MAP-based knowledge management. One aim is to provide local NTFP users with viable incentives to refrain from unsustainable harvesting and of NTFPs while providing local and national economic benefits.

ICIMOD has pioneered development of commodity-wise value chains for selected NTFPs in the Hindu Kush Himalayan region (ICIMOD 2011). ICIMOD has developed a mountain-specific value chain approach and framework for more participatory and equitable engagement of collectors, producers, local traders and processors in NTFP value chain development and livelihood improvement. One project, for example, analysed the prevailing supply chains of *Cinnamomum tamala* (Indian bay leaf) in Nepal and India (Choudhary et al. 2011). Through awareness raising, training and capacity building of both producers and buyers, it helped establish a business partnership between poor producers and markets trading in essential oils and spices. This has doubled the income of producers in the Chamoli district of Uttarakhand, India, and the Udayapur district of Nepal. A detailed analysis showed that around 900 tonnes of raw bay leaves were harvested in Udaipur district, Nepal, and 20–40 tonnes in the Indian project sites were produced and exported annually. In the Nepal case, a local company, with a buy-back relationship with local producers, was using nearly 25% of the total bay leaf, producing essential oil. An estimated 2150 tonnes of bay leaves were sent from Nepal to India every year. Farmers in Nepal earned a gross margin of 11% and traders 34%; collectors in India had a margin of 10% and traders 17% (Choudhary and Bhattarai 2011). The bay leaf value chain has shown that by addressing underlying inequality and power differences between the upstream producers and downstream actors, we can achieve equitable benefit sharing (ICIMOD 2011).

4.8 NTFP-Based Mountain Green Economy: Challenges and Opportunities for Mountainous Regions

The green economy as we understand today has been around – at least conceptually – for a very long time. Communities and societies in forest- and biodiversity-rich mountain countries that were forced by technological and other resource constraints and by the inaccessibility, marginality and fragility of their environment to live at subsistence level have developed cultural norms, social contracts and management systems to ensure their livelihoods and the sustainability of the resource base. The original idea of the green economy as developed by ecologists and environmentalists was largely based on sustainable extraction and utilization of natural products while meeting high social standards. This approach, however, was limiting the kind of economic growth that the current green economy approach expounds (IUFRO 2012). Medicinal and aromatic plant (MAP) conservation and development and organic agriculture efforts practiced in Bhutan, India and Nepal provide examples of growth models based on this kind of economic development approach. Karki (2011) recently conducted a comprehensive assessment of successful case studies in the Asia Pacific mountain regions in the context of sustainable mountain development in which forest and NTFP management figure prominently. The case studies suggest that NTFPs are the most important biological resources for socioeconomically uplifting poor and marginal communities. NTFP sector development has impact on all three pillars of sustainable development—ecological, economic and social—in a balanced manner. NTFPs meet the criteria for green economy and green growth in that the resources are plentiful, management technologies are simple and accessible to poor and enterprising communities and markets (especially for herbal medicines, nutraceuticals and organic food) are growing worldwide.

Some of the key issues identified were lack of organizing skills among the producers, lack of market information and access to producers, absence of technologies for value addition, lack of sustainable harvesting and management skills, lack of capacity to conform to market requirements, policy hurdles to access to NTFP resources on government land and bureaucratic hurdles. Interventions were identified based on the issues identified, using a multistakeholder approach integrating poverty and gender dimensions. Market information, especially product prices, was gathered systematically. Partnerships between concerned government line agencies and the research team focused on building the capacity of local institutions in skills such as collection, grading, sorting and packaging of bay leaves. Training programmes also focused on group formation, bay leaf cultivation and management, sustainable harvesting and community-based enterprise development. Networks of buyers, local traders and exporters and producers were formed and strengthened. An effort to improve access to markets by bringing them closer to the production sites was piloted in India.

The value chain interventions led to immediate benefits for the poor producers in terms of increased income, increased knowledge and skills and gender equality. The outcomes could also be seen in improved education and health of the children of the producer families. Improved harvesting practices lead to improved quality of raw materials and finished products. With the market for NTFPs, especially medicinal plants, growing in South Asia and particularly in India and China, ICIMOD is scaling up and scaling out these experiences and promoting cross-border learning and sharing of good practices.

4.9 Conclusions and Recommendations

An NTFP/MAP-based green mountain economy not only should aim to increase production and income but also provides a basis for integrated and sustainable management of mountain natural resources. Taking the concept of green economy forward would call for a balanced and holistic approach to NTFP and MAP resources, as well as fundamental institutional changes and governance reforms. Technical inputs combined with traditional knowledge produce an adaptive technology that is based on the cultural, social, environmental and economic factors that are relevant to the local population; if adopted systematically, it can improve livelihoods.

Local knowledge about plants and the innovation systems of individuals and communities are useful in the search for new ways to conserve and use plants for the benefit of the communities as well as for achieving wider development goals. Given the overlapping benefits of enhancing access to affordable healthcare to poor through traditional system of medicine, providing livelihoods to local communities and enabling them to practice sustainable use of medicinal plants, specifically rare, endangered and threatened species is a viable policy. It is clear that work to promote the sustainable conservation and management of NTFPs and to build on indigenous and local knowledge and traditional practices can make valuable contributions to achieving the general socioeconomic advances spelled out in the Unsustainable Development Goals (SDG) including Paris Agreement.

Much has been said about the impact of globalization and economic liberalization on the lives of the poor. No doubt poor and disadvantaged mountain communities have been mostly losers. Therefore, there is an urgent need to undertake liberalization from the point of view of the poor. Specifically in promoting NTFP/MAP-based green economy, there is a need to use adaptive technologies and improved collection, processing and trade channels on a rational and efficient manner. Also, appropriate processing and value addition facilities need to be developed as close to the production and collection areas in mountain regions. It will be possible to bring about positive change in the livelihoods of NTFP/MAP-dependent poor people by developing transparent value chain development and fair and equitable sharing of benefits in marketing practices. It is also necessary to develop new products and new uses for popular products, with a reliable market destinations. In addition, the new attitude of green consumerism resulting from the concern for environmental conservation and the consequent preference for natural products is providing new opportunities for NTFPs.

A systematic approach to enhancing the contribution of NTFPs should involve the usual planning cycle: formulation of objectives, preparation of strategy, action planning, implementing, monitoring and appraisal of conservation and development projects and programmes. There is also increased requirement for NTFP managers to understand the resource status, potential and trend to develop sustainable use regime. All major stakeholders need to participate in decision-making and cost and benefit sharing and that effective procedures are implemented to resolve conflicts. Finally, policymakers and development agencies need to better understand the changing role of NTFP and MAP resources, especially those harvested from wild sources, for improving local livelihoods.

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Part II
Conservation of Threatened Medicinal Plants: Concepts and Practices

Chapter 5
Conservation of Threatened Medicinal Plants in India: Concepts and Practices

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Abstract This chapter starts with illustration of the Indian floristics and their current scenario, endemic plants, and factors causing the decline in plant populations. It covers about the medicinal plants used in the Indian Systems of Medicine and number of threatened medicinal plants. Outline is provided on the Conservation Assessment and Management Prioritization (CAMP) held in 19 states and their outcome. Conservation approach in conserving the medicinal plants is provided through in situ and ex situ method. This chapter lists out the threatened and traded medicinal plants. It summarizes the efforts to be taken to conserve the threatened medicinal plants.

Keywords Medicinal plants · MPCA · Conservation · In situ · Ex situ · Threatened · IUCN · CAMP · IUCN

5.1 Introduction

Conservation of medicinal plants (MPs) is receiving increased attention in view of resurgence of interest in herbal medicines for health care all across the globe (Franz 1993; Gupta et al. 1998). The goal of conservation is to support sustainable development by protecting and using biological resources in ways that do not diminish the world's variety of genes and species or destroy important habitats and ecosystems. In

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general, it involves activities such as collection, propagation, characterization, evaluation, disease indexing and elimination, storage, and distribution. The conservation of plant genetic resources has long been realized as an integral part of biodiversity conservation. There are two methods for the conservation of plant genetic resources, namely, in situ and ex situ conservation. On the other hand, ex situ conservation involves conservation outside the native habitat and is generally used to safeguard populations in danger of destruction, replacement, or deterioration. Approaches to ex situ conservation include methods like seed storage, DNA storage, pollen storage, in vitro conservation, field gene banks, and botanical gardens (Sarma 2003).

5.2 Indian Context

India has the distinction of being one of the seventeen megadiverse countries of the world, possessing four out of thirty six of the world's biodiversity hot spots, viz., Eastern Himalaya, Indo-Burma, Western Ghats and Sri Lanka, and Sundaland (Nicobar Islands) (Myers et al. 2000; Arisdason and Lakshminarasimhan 2017). Among the Himalayan region, the north-east Indian region harbors several floristically rich forest patches and a high number of endemics. It has been estimated that the north-eastern region comprises of approximately 7500 species of flowering plants that constitute nearly 40% of the total floristic wealth of the country which is about 19,400 taxa (Karthikeyan 2000). A total of 4381 species and infraspecific taxa of vascular plants belonging to 1007 genera and 176 families are recorded as strict endemics to the Indian political boundary, of which 4303 species and infraspecific taxa are angiosperms, 12 species are gymnosperms, and 66 are pteridophytes (Singh et al. 2015). Endemic species across phytogeographic region is provided in Fig. 5.1.

Unfortunately, these endemics along with the native medicinal plants are facing varying degrees of risk of extinction due to various factors like loss of habitat, overexploitation, and urbanization.

The pioneering works to enumerate and prioritize the threatened species in India were undertaken during 1980s and 1990s (Jain and Rao 1983; Nayar and Sastry 1987–1990). Nayar and Sastry (1987–1990), in their seminal work titled, Red Data Book of Indian Plants (RDB), listed 602 threatened vascular plants. Subsequently, the number of threatened plants increased to 1255 (Rao et al. 2003). In India, around 1700 of the 18,043 listed plant species have been reported to be threatened (Arisdason and Lakshminarasimhan 2017 & http://www.bsienvis.nic.in/Database/Status of Plant Diversity in India 17566.asps).

CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) is an international treaty and one of the oldest agreements between governments, aimed at regulating and monitoring the worldwide trade of selected species of plants and animals to ensure that it does not endanger the survival of such populations in the wild. CITES accords varying degrees of protection to *ca* 5000 species of animals and 29,000 species of plants which are traded as live specimens or as dried or preserved materials. Of them, 13 are Indian medicinal plants, namely,

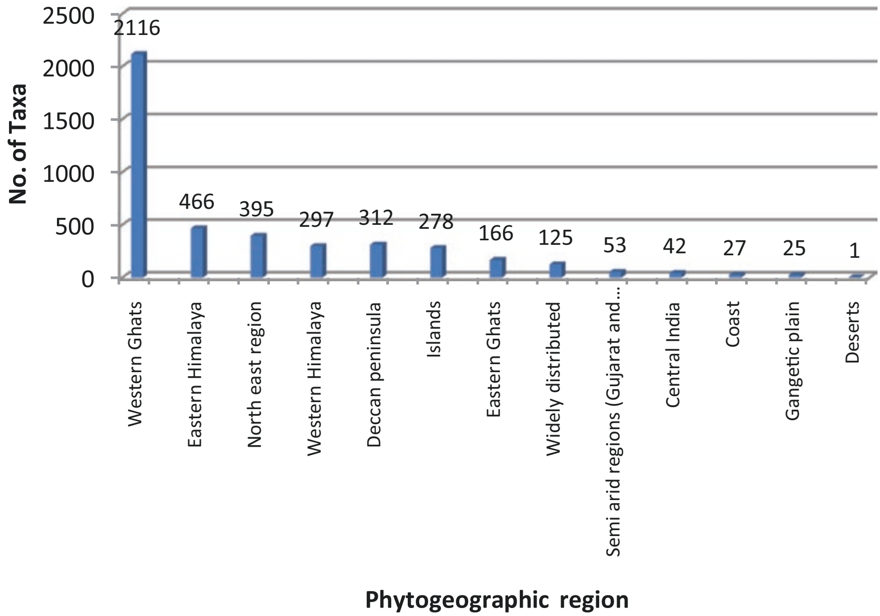

Fig. 5.1 Distribution of endemic angiosperms in different phytogeographical regions. (Source: Singh et al. 2015)

Saussurea costus (Falc.) Lipsch., enlisted under Appendix I. Twelve of the remaining species, namely, *Aquilaria malaccensis* Lam, *Cycas beddomei* Dyer, *Dioscorea deltoidea* Wall. *ex* Griseb., *Rauvolfia serpentina* (L.) Benth. *ex* Kurz., *Cibotium barometz* (L.) J.Sm., *Sinopodophyllum hexandrum* (Royle) T.S.Ying, *Pterocarpus santalinus* L. f., *Nardostachys jatamansi* (D.Don) DC., *Nepenthes khasiana* Hook. f., *Picrorhiza kurrooa* Royle, and *Taxus wallichiana* Zucc. are included under Appendix II (http://www.bsienvis.nic.in/Database/bsi_3949.aspx).

The threat of extinction of tree species is looming large due to their removal and rapid reduction/fragmentation of habitats. For such species and taxa, more intensive management becomes necessary for their survival and recovery. Increasingly, this rigorous management will have to include habitat management and restoration, extensive information, and possible conservation breeding.

5.3 Medicinal Plants in India

According to the Annual Report of All India Coordinated Research Project on Ethnobiology, 8000 plant species have been recorded for medicinal use by different communities across our entire country (Anonymous 1995). This constitutes nearly 45% of the known flowering plant species of India. It has also been estimated that nearly one-third of these plant species are endemics or near endemics and are exclusive to India or that they have only marginal presence elsewhere (Ravikumar and Ved 2000). In 1997, the International Union for Conservation of Nation (IUCN) published a compilation of threatened plants of the world (Walter and Gillett 1998) which enlists more than 34,000 vascular plant species in the threatened category suggesting that, across the globe, nearly 12.5% of known flowering plants are threatened with extinction. In the absence of any systematic assessment of such threatened medicinal plant species in India, it may be reasonable to extend the same proportion (12.5%) to the 8000 medicinal plant species enlisted in India. This suggests that over 1000 medicinal plant species of India may be threatened with extinction and more than 300 of these are likely to be endemics or near endemics. Since approximately 90% of India's flowering plant diversity is estimated to exist in the forests, the action for conservation of medicinal plant resources has to be targeted in these forest areas (Ravikumar and Ved 2000).

Over the last few years, an attempt has been being made at Foundation for Revitalization of Local Health Traditions (FRLHT) to catalogue (in a computerized form) the botanical names of the plant species that are recorded for medicinal use by the various systems of medicines that has been practiced in India. The information has been culled out from a variety of published literature, ranging from scholarly commentaries on classical texts relating to codified systems like Ayurveda, Siddha, Unani, etc., to ethnomedical and botanical studies. This database currently has 7334 botanical names, and each botanical name bears one or more tags of medical systems ranging from Folk (F) to Ayurveda (A), Siddha (S), etc. By identifying and enlisting the botanical synonyms, among these names, the number of plant species has been worked out to 6550 (FRLHT 2017).

Using a Conservation Assessment and Management Prioritization (CAMP) technique, an initiative to assess conservation status of wild medicinal plants species has been in operation since 1995 which is being led by FRLHT. Thus far, this initiative covers a total of 19 states in India. A compilation of the results of these exercises has resulted in enlisting of 388 wild medicinal plant species that have been assigned Red List status ranging from near threatened (NT) to critically endangered (CR) in one or more states (Table 5.1).

Conservation Assessment and Management Prioritization (CAMP) is a technique that allows rapid assessment of the conservation status of wild medicinal plants. Essentially involving 30–40 experts consisting of well-known field taxonomists, forest managers, traders, as well as knowledgeable local practitioners of Indian Systems of Medicine (ISM), this exercise is carried out in the form of 3 days workshop. These workshops, usually organized regionally with states as a unit, assess conservation status of prioritized medicinal plant species of the State using IUCN Red List Criteria and Categories and draw upon the collective knowledge of the participants in the workshop.

In 2012, 312 plant species were assessed threatened as per ver. 2011.2 (IUCN 2012). As per the version 2017-3, 390 plant species are threatened in India (Fig. 5.2). After 6 years, only 78 plant species were assessed and added to the IUCN database. These 78 plant species include 46 endemic medicinal plants that have been added from FRLHT database based on the CAMP assessments held from 1995 to 2017 in 19 states of the country (Table 5.2).

Table 5.1 Summary of the CAMPs held in 19 states of the country anchored by FRLHT

S.N.	State	No. of red list species with assessed conservation status	Year and location of CAMP workshop (1995 to 2017)
1	Andhra Pradesh	47	2001 at Hyderabad
2	Arunachal Pradesh	44	2003 at Guwahati
3	Assam	16	2003 at Guwahati
4	Chhattisgarh	47	2003 at Bhopal
5	Himachal Pradesh	62	1998 at Kullu, 2003 at Shimla
6	Jammu and Kashmir	62	1998 at Kullu, 2003 at Shimla
7	Karnataka	81	1995,1996,1997,1999 all at Bangalore
8	Kerala	85	1995,1996,1997,1999 all at Bangalore
9	Madhya Pradesh	50	2003,2006 both at Bhopal
10	Maharashtra	35	2001 at Pune
11	Meghalaya	25	2003 at Guwahati
12	Nagaland	28	2015 at Dimapur
13	Orissa	40	2007 at Bhubaneshwar
14	Rajasthan	38	2007 at Jaipur
15	Sikkim	24	2003 at Guwahati
16	Tamil Nadu	80	1995,1996,1997,1999 all at Bangalore
17	Tripura	21	2016 at Agartala
18	Uttaranchal	60	2003 at Shimla
19	West Bengal	43	2007 at Kolkatta

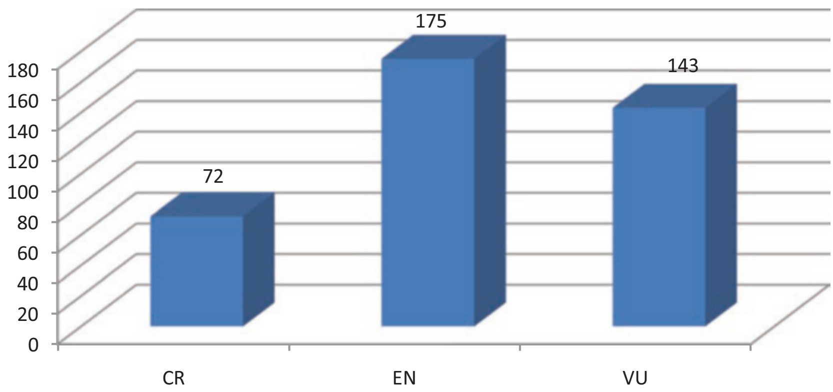

Fig. 5.2 Summary: Red List category of plant species (ver. 2017.3)

Table 5.2 List of 47 endemic medicinal plants added from FRLHT database based on the CAMP to IUCN Database

S.N.	Botanical name	Family	Habit	IUCN status
1	*Aconitum chasmanthum* Stapf *ex* Holmes	Ranunculaceae	Herb	CR
2	*Aconitum heterophyllum* Wall. *ex* Royle	Ranunculaceae	Herb	EN
3	*Aconitum violaceum* Jacquem. *ex* Stapf	Ranunculaceae	Herb	VU
4	*Angelica glauca* Edgew.	Apiaceae	Herb	EN
5	*Boswellia ovalifoliolata* N.P.Balakr. & A.N.Henry	Burseraceae	Tree	VU
6	*Calophyllum apetalum* Willd.	Clusiaceae	Tree	VU
7	*Cayratia pedata* (Lam.) Juss.*ex* Gagnep. var. *pedata*	Vitaceae	C	VU
8	*Cayratia pedata* (Lam.) Juss.*ex* Gagnep. var. *glabra* Gamble	Vitaceae	C	CR
9	*Chlorophytum borivilianum* Santapau & R.R.Fern.	Liliaceae	Herb	CR
10	*Cinnamomum macrocarpum* Hook.f.	Lauraceae	Tree	VU
11	*Cinnamomum sulphuratum* Nees	Lauraceae	Tree	VU
12	*Cinnamomum wightii* Meisn.	Lauraceae	Tree	EN
13	*Commiphora wightii* (Arn.) Bhandari	Burseraceae	Shrub	CR
14	*Coptis teeta* Wall.	Ranunculaceae	Herb	EN
15	*Coscinium fenestratum* (Goetgh.) Colebr.	Menispermaceae	Liana	DD
16	*Decalepis hamiltonii* Wight & Arn.	Periplocaceae	Liana	EN
17	*Diospyros candolleana* Wight	Ebenaceae	Tree	VU
18	*Diospyros paniculata* Dalzell	Ebenaceae	Tree	VU
19	*Dysoxylum malabaricum* Bedd. *ex* C.DC.	Meliaceae	Tree	EN
20	*Garcinia indica* (Thouars) Choisy	Clusiaceae	Tree	VU
21	*Gentiana kurroo* Royle	Gentianaceae	Herb	CR
22	*Gymnema khandalense* Santapau	Asclepiadaceae	Climber	EN
23	*Gymnocladus assamicus* P.C. Kanjilal	Caesalpiniaceae	Tree	CR
24	*Humboldtia vahliana* Wight	Caesalpiniaceae	Tree	EN
25	*Hydnocarpus pentandrus* (Buch.-Ham.) Oken	Flacourtiaceae	Tree	VU
26	*Illicium griffithii* Hook.f. & Thomson	Illiciaceae	Tree	EN
27	*Iphigenia stellata* Blatt.	Liliaceae	Herb	EN
28	*Lamprachaenium microcephalum* (Dalz.) Benth	Asteraceae	Herb	EN
29	*Lilium polyphyllum* D. Don *ex* Royle	Liliaceae	Herb	CR
30	*Malaxis muscifera* (Lindl.) Kuntze	Orchidaceae	Herb	VU
31	*Michelia nilagirica* Zenker	Magnoliaceae	Tree	VU
32	*Myristica dactyloides* Gaertn.	Myristicaceae	Tree	VU
33	*Nardostachys jatamansi* (D. Don) DC.	Valerianaceae	Herb	CR
34	*Nepenthes khasiana* Hook.f.	Nepenthaceae	Climber	EN
35	*Nilgirianthus ciliatus* (Nees) Bremek.	Acanthaceae	Shrub	VU
36	*Phyllanthus indofischeri* Bennet	Euphorbiaceae	Tree	VU
37	*Pimpinella tirupatiensis* N.P.Balakr. & Subram.	Apiaceae	Herb	EN

(continued)

Table 5.2 (continued)

S.N.	Botanical name	Family	Habit	IUCN status
38	*Piper barberi* Gamble	Piperaceae	Climber	CR
39	*Piper pedicellatum* C.DC.	Piperaceae	Shrub	VU
40	*Salacia oblonga* Wall. *ex* Wight & Arn.	Hippocrateaceae	Climber	VU
41	*Saussurea costus* (Falc.) Lipsch.	Asteraceae	Herb	CR
42	*Shorea tumbaggaia* Roxb.	Dipterocarpaceae	Tree	EN
43	*Syzygium alternifolium* (Wight) Walp.	Myrtaceae	Tree	EN
44	*Terminalia pallida* Brandis	Combretaceae	Tree	VU
45	*Tribulus rajasthanensis* Bhandari & V.S. Sharma	Zygophyllaceae	Herb	CR
46	*Utleria salicifolia* Bedd. *ex* Hook.f.	Periplocaceae	Shrub	CR
47	*Valeriana leschenaultia* DC.	Valerianaceae	Herb	CR

Source: IUCN (2017)

5.4 Conservation Approach

5.4.1 In Situ Conservation of Wild Medicinal Plants: Medicinal Plant Conservation Area (MPCA) Network an Innovative Approach – FRLHT Experience

MPCAs are managed as "hands off" areas with only the following interventions, wherever required – fire management, soil and moisture conservation, and weed management/encouraging native vegetation. In addition, the following activities are also to be allowed: on-field research, collection of germplasm for research and multiplication and right of way, and water to the local communities. All harvesting operations, thus, stand suspended in the MPCAs.

5.4.1.1 Medicinal Plant Conservation Area (MPCA)

MPCAs were sites with known medicinal plant richness (literature/local interaction), less disturbed but easily accessible, and relatively free from local rights/livelihood issues, form compact manageable units, and covered different forest/vegetation types and altitude ranges. The MPCAs were established to conserve the medicinal plants in the wild, to conduct studies on the status and conservation approaches of wild medicinal plants, and to design and develop mechanisms for medicinal plant conservation.

Depending on the status of data and assessment relating to the medicinal plant resources of a state or region, two types of MPCA were established:

MPCAs that capture the diversity of native medicinal plants are referred to as "Diversity-Focus MPCAs." These MPCAs were established before the

Plate 5.1 Some of the Medicinal Plant Conservation Area (MPCA) in India

prioritization of species, and related assessment about the potential sites of their populations in the state was studied (Plate 5.1).

Under DANIDA Project initiated in 1993, a network of 34 MPCA in 3 Southern Indian States of Karnataka, Kerala, and Tamil Nadu was established. Under CCF-I project during 1999–2003, 8 MPCAs were established in Andhra Pradesh and 13

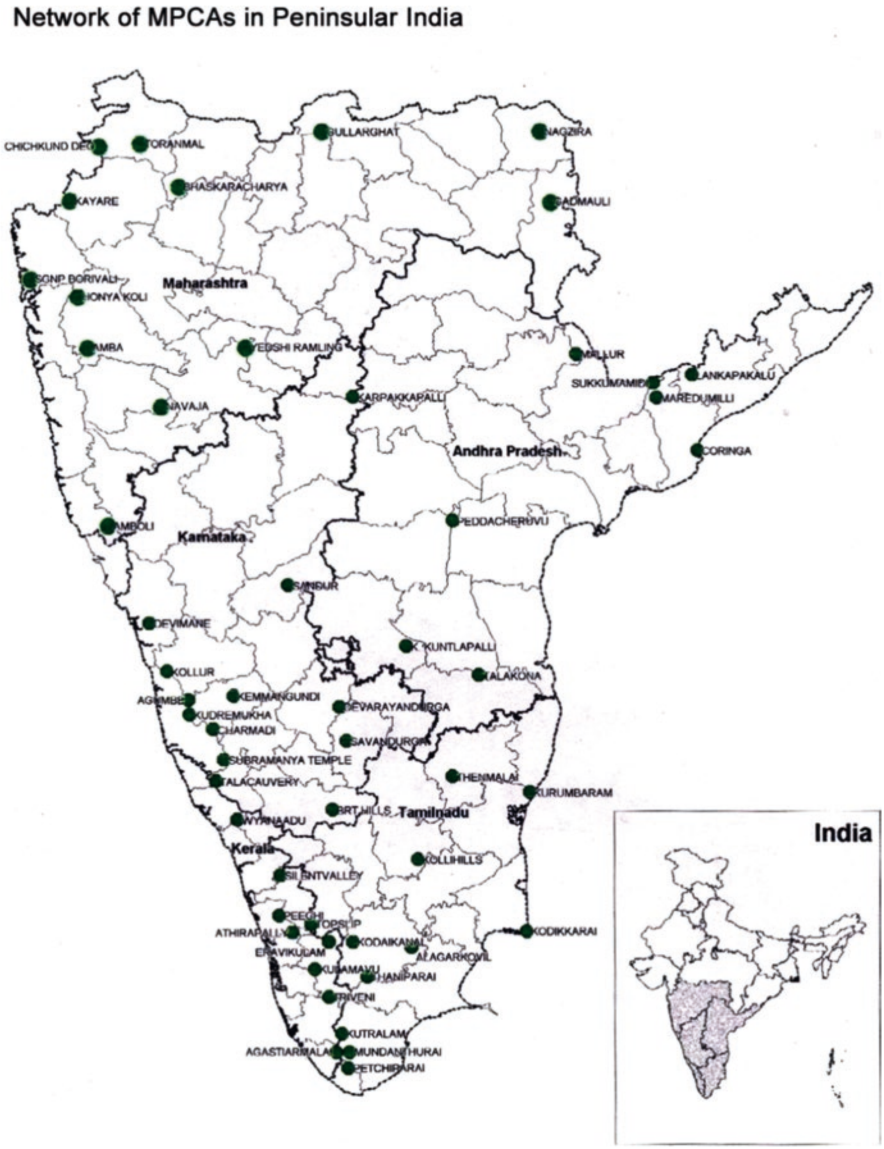

Fig. 5.3 A network of 55 Medicinal Plant Conservation Areas (MPCAs) established across different vegetation types in 5 states of Peninsular India. Each approx. 200 ha

MPCAs were established in Maharashtra. Thus, a total of 55 MPCAs were established in peninsular India (Fig. 5.3).

Further, MPCAs were established under CCF-II Project (2006–2010) and GEF project (2008–2014). Thus, till date 110 Medicinal Plant Conservation Areas have been established in the country (Fig. 5.4). The National Medicinal Plant Board,

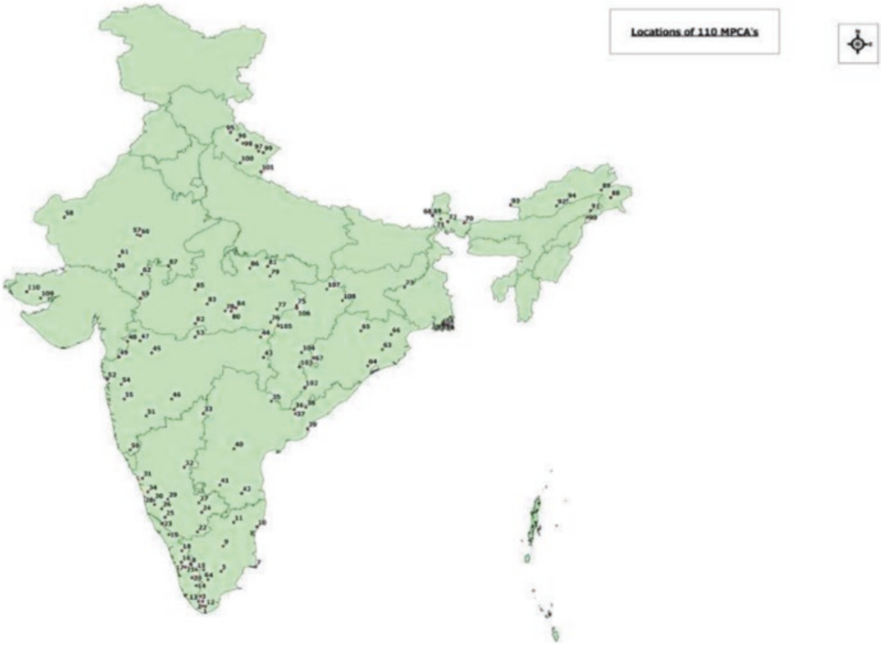

Fig. 5.4 A total of 110 Medicinal Plant Conservation Areas established across the country

Government of India, has established the MPCAs (https://www.nmpb.nic.in/content/achievements-nmpb-0). MPCAs were used for conservation education to sensitize local communities and others to the conservation value of medicinal plants resources. Capacity building was key activity, wherein State Forest Department (SFD), staff and village communities were sensitized in matters relating to medicinal plant conservation.

Species-focus MPCAs were established to conserve prioritized medicinal plants of high conservation concern. For example, *Saraca asoca* (Roxb.) Willd. occurs naturally in the states of Western Ghats like Maharashtra, Goa, Karnataka, and Kerala; in the states of Eastern Ghats like Odisha; and the north-east states, namely, Meghalaya and Mizoram. However, their occurrence in wild is sparse. A rapid assessment of its conservation status assigned it the Red List status of *Endangered*. Analysis of its global distribution revealed that this species is near endemic to India along with limited occurrence in Sri Lanka. The wild populations of this tree species, being threatened with extinction in the wild, call for urgent action for its conservation. This prompted FRLHT to undertake detailed field surveys for locating its wild populations for establishment of in situ field gene bank. These surveys were guided by our in-house data analysis and GIS-supported ecogeographic mapping which identified potential areas of its wild occurrences based on interpretation of its mapped locations and the correlated ecological parameters like rainfall and altitude range.

This resulted in locating wild population of *Saraca asoca* in the forests at Kollur in Udupi district of Karnataka. Thus, the establishment of a Medicinal Plant Conservation Area (MPCA) was undertaken in collaboration with the State Forest Department of Karnataka. This MPCA is located close to the famous Mookambika temple and is spread over 300 hectares. Along with *Saraca asoca*, more than 20 species of threatened red-listed plants and around 200 species of other wild medicinal plant species occurring in the MPCA are also being conserved.

The Medicinal Plant Conservation Area (MPCA) established at Kollur, for long-term in situ conservation of wild gene pool of *Saraca asoca*, has been an important highlight of the pioneering medicinal plant conservation program initiated by FRLHT in southern India. Similar efforts, for other threatened medicinal plants of southern India, have resulted in the establishment of Anappadi MPCA (Kerala) to conserve *Utleria salicifolia* Bedd. ex Hook.f., Kulamavu MPCA (Kerala) for *Coscinium fenestratum* (Goetgh.) Colebr., and Nambikoil MPCA, KMTR for *Decalepis arayalpathra* (J. Joseph and V. Chandras) Venter (Plates 5.2 and 5.3).

Plate 5.2 (**a**) Kollur MPCA, Karnataka – *Saraca asoca.* (**b**) Kulamavu MPCA, Kerala – *Coscinium fenestratum*

Plate 5.3 (**a**) Anappadi MPCA, Kerala – *Utleria salicifolia*. (**b**) Nambikoil MPCA, Tamil Nadu – *Janakia arayalpathra*

5.4.1.2 Ex Situ Conservation

FRLHT also established several ex situ conservation sites to complement in situ conservation. Ex situ conservation was undertaken to improve livelihood and enhance the use through the establishment of Medicinal Plant Conservation Parks (MPCPs) (Fig. 5.5). It comprises of nurseries, establishment of living collections of a limited number of specimens of the medicinal plants collected, and promotion of kitchen herbal gardens/home herbal gardens (KHGs/HHGs) (Singh et al. 2008).

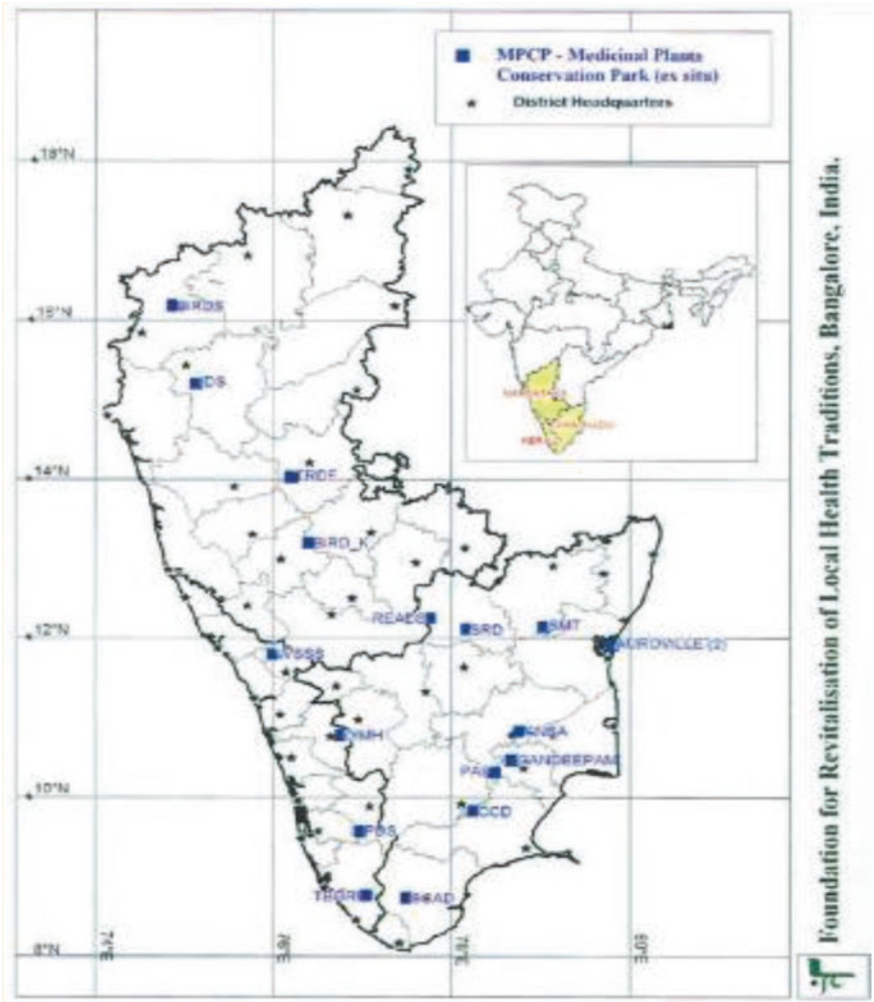

Fig. 5.5 The location of the Medicinal Plant Conservation Parks (MPCPs) established. (Source: Singh et al. 2008)

5.4.1.3 Medicinal Plant Development Areas (MPDAs)

A total of 12 MPDAs were established into four broad models: (1) eco-restoration of natural vegetation, (2) establishment of new mixed cropping systems, (3) enrichment of existing farming systems, and (4) integration of medicinal plants with joint forest management (Singh et al. 2008).

5.4.1.4 Conservation Action for Threatened Plant Species of India

The communities across the country have been conserving sacred groves as part of their traditional nature conservation practices. As a result, the threatened medicinal plants also are conserved in their habitats. For faunal species, several initiatives have been taken by the Government of India by means of prioritization of threatened species and conservation actions, whereas there are handful of such cases for plant species, such as Sessa in Arunachal Pradesh which is also known as "Orchid Paradise" conserving orchids. The sanctuary harbors about 200 species of orchids of subtropical types comprising the genera like *Dendrobium, Bulbophyllum*, *Coelogyne*, *Eria*, *Phaius*, *Liparis*, etc. The sanctuary is unique in the country in having six new species of orchids and seven species of saprophytic orchids (http://www.sfri.nic.in/sessa.htm). Pure strands of Rhododendron are conserved in Barsey Rhododendron Sanctuary situated in West Sikkim district, Sikkim. (http://www.sikkimforest.gov.in/docs/IBA/sk1.pdf). Ex situ conservation is another important form of conserving the threatened plant species; it is achieved by establishing field germplasm banks such as ICAR-NBPGR National Genebank and institutional botanical gardens such as the National Botanical Research Institute (NBRI) Botanic Garden, Lucknow; Jawaharlal Nehru Tropical Botanical Garden, Thiruvananthapuram, Kerala; ethnomedicinal garden, the University of Trans-disciplinary Health Sciences and Technology (TDU), Bangalore, to conserve medicinal and taxonomically important plants.

Endemic and endangered orchid *Ipsea malabarica* (Reich 2014) J. D. Hook. was saved from extinction through rapid clonal propagation and encapsulation; 50 plantlets were reintroduced into Vellarimala (at 1300 m height) of the Western Ghats of Kerala (Martin 2003).

The Government of India has taken up special efforts to conserve threatened medicinal plants. In recent years, the Department of Biotechnology, Government of India, Phase –I, has recovered half a dozen of plant species in the Western Ghats and over 100 plant species for the entire country in the second phase. For example, reintroduction and restoration of *Hubbardia heptaneuron* Bor, a monospecific, critically endangered, and endemic species in 16 Ghat regions at 108 locations covering from Jog falls in the South to Malshej Ghat in the north, and over 5000 individuals have been established so far in the Western Ghats (Yadav et al. 2009).

One of the threats for *Semecarpus kathalekanensis* is conversion of swamps into areca-nut gardens (Chandran et al. 1999). The Forest Department has declared one of the sites as an in situ conservation spot (Dasappa and Jagathram 2000). *Semecarpus kathalekanensis* existed only in four isolated populations with less than 100 breeding individuals in the wild, making it a critically endangered one. All these populations were restricted to about 25 km^2 around Jog falls in Uttara Kannada district. Through re-introduction, about 5000 individuals have been planted in locations as far as Kodagu and Kerala (Vasudeva et al. 2003). However, there is still a great need to effectively check all human interference and the invasive weeds to these populations.

Two hundred and fifty tissue-cultured individuals of *Ceropegia fantastica*, a critically endangered and endemic species, were successfully reintroduced in 18 native locations in the Western Ghats (Chandore et al. 2010). Similar attempts have been made in the second phase to recover a number of critically endangered species in the entire country (Ravikanth et al. 2018).

All these activities are largely mentored and executed by the Ministry of Environment, Forests, and Climate Change and Ministry of Agriculture, GoI. DBT has undertaken several plant species-specific recovery programs targeting 156 highly threatened species of the country during the past three decades. These species belong to 101 genera and 64 families and comprise 50 herbs, 42 trees, 24 orchids, 14 shrubs, 14 climbers, 3 bamboos, 3 palms, 3 rattans, 2 cycads, and 1 tree fern. One of the most important mega network programs of DBT entitled "Preventing extinction and improving the conservation status of threatened plants through application of biotechnological tools" was initiated during 2012 that successfully conserved 100 threatened species of India. This program took an integrated approach for species conservation such as resolving the taxonomic dispute, preparing herbarium records, establishing field germplasm bank, population characterization, distribution mapping, reclassification of threat status, reproductive biology, molecular characterization, bioactive compound profiling, standardization of micropropagation and macropropagation protocols and multiplication, and reintroduction in natural habitats.

The Department of Biotechnology, Government of India, in the last decade has supported studies on population characterization and distribution mapping, reproductive biology studies for identifying regeneration bottlenecks, molecular profiling, phytochemical profiling for species conservation, standard micropropagation protocol, standard macropropagation protocol, and reintroduction of threatened species. These species have been systematically listed by Sarojkumar Barik et al. (2018).

5.5 Medicinal Plants of Conservation Concern (Red Listed) in Trade

The increasing annual consumption level of wild, herbal raw-drug collection accompanied by general habitat degradation has caused a decline in wild population of many medicinal plant species. The dwindling wild populations of these species have become a cause of serious concern from the conservation and utilization point of view. Many of these Red-listed medicinal plant species continue to be in active commercial trade putting further pressure on their wild resources. Selected threatened and traded medicinal plants are shown in Plate 5.1.

The consolidated inventory of medicinal plant species in commercial demand, worked out under this study, includes 100 species that have been assessed as "Red Listed." The 100 Red-listed medicinal plant species are shown in Table 5.3.

Table 5.3 List of 100 species of conservation concern in commercial demand for herbal raw drugs

S.N.	Botanical name	Family	Habit	Threat category assigned
1	*Aconitum chasmanthum* Stapf *ex* Holmes	Ranunculaceae	Herb	CR
2	*Aconitum heterophyllum* Wall. *ex* Royle	Ranunculaceae	Herb	CR
3	*Justicia beddomei* (C.B. Clarke) Bennet	Acanthaceae	Shrub	CR
4	*Aquilaria malaccensis* Lam.	Thymelaeaceae	Tree	CR
5	*Arnebia benthamii* (Wall. *ex* G. Don) I. M. Johnst.	Boraginaceae	Herb	CR
6	*Arnebia euchroma* (Royle) I. M. Johnst.	Boraginaceae	Herb	CR
7	*Atropa acuminate* Royle *ex* Lindl.	Solanaceae	Herb	CR
8	*Betula utilis* D. Don	Betulaceae	Tree	CR
9	*Chlorophytum borivilianum* Santapau & R.R.Fern.	Anthericaceae	Herb	CR
10	*Cochlospermum religiosum* (L.) Alston	Cochlospermaceae	Tree	CR
11	*Commiphora wightii* (Arn.) Bhandari	Burseraceae	Shrub	CR
12	*Coscinium fenestratum* (Gaertn.) Colebr.	Menispermaceae	Climber	CR
13	*Cycas circinalis* L.	Cycadaceae	Tree	CR
14	*Dactylorhiza hatagirea* (D. Don) Soo	Orchidaceae	Herb	CR
15	*Embelia ribes* Burm. f.	Myrsinaceae	Climber	CR
16	*Gentiana kurroo* Royle	Gentianaceae	Herb	CR
17	*Holostemma annulare* (Roxb.) K. Schum.	Asclepiadaceae	Climber	CR
18	*Illicium griffithii* Hook.f. & Thomson	Magnoliaceae	Tree	CR
19	*Lilium polyphyllum* D. Don	Liliaceae	Herb	CR
20	*Litsea glutinosa* (Lour.) C.B. Rob.	Lauraceae	Tree	CR
21	*Malaxis muscifera* (Lindl.) Kuntze	Orchidaceae	Herb	CR
22	*Nardostachys jatamansi* (D. Don) DC.	Valerianaceae	Herb	CR
23	*Panax pseudoginseng* Wall.	Araliaceae	Herb	CR
24	*Picrorhiza kurroa* Royle *ex* Benth.	Scrophulariaceae	Herb	CR
25	*Pterocarpus marsupium* Roxb.	Fabaceae	Tree	CR
26	*Pterocarpus santalinus* L. f.	Fabaceae	Tree	CR
27	*Pueraria tuberosa* (Roxb. *ex* Willd.) DC.	Fabaceae	Climber	CR
28	*Rauvolfia serpentine* (L.) Benth. *ex* Kurz	Apocynaceae	Herb	CR
29	*Saraca asoca* (Roxb.) W.J. de Wilde	Caesalpiniaceae	Tree	CR
30	*Saussurea costus* (Falc.) Lipsch.	Asteraceae	Herb	CR
31	*Saussurea obvallata* (DC.) Edgew.	Asteraceae	Herb	CR
32	*Sinopodophyllum hexandrum* (Royle) T.S.Ying	Podophyllaceae	Herb	EN
33	*Smilax glabra* Roxb.	Smilaceae	Climber	EN
34	*Swertia chirayta* H. Karst.	Gentianaceae	Herb	EN

(continued)

Table 5.3 (continued)

S.N.	Botanical name	Family	Habit	Threat category assigned
35	*Symplocos racemose* Roxb.	Symplocaceae	Tree	EN
36	*Taxus wallichiana* Zucc.	Taxaceae	Tree	EN
37	*Aconitum palmatum* D. Don	Ranunculaceae	Herb	EN
38	*Aconitum heterophylloides* (Brühl) Stapf	Ranunculaceae	Herb	EN
39	*Aconitum ferox* Wall. *ex* Ser.	Ranunculaceae	Herb	EN
40	*Aconitum lethale* Griff.	Ranunculaceae	Herb	EN
41	*Acorus calamus* L.	Acoraceae	Herb	EN
42	*Alpinia calcarata* (Haw.) Roscoe	Zingiberaceae	Herb	EN
43	*Angelica glauca* Edgew.	Apiaceae	Herb	EN
44	*Asparagus racemosus* Willd.	Liliaceae	Climber	EN
45	*Boswellia serrata* Roxb. *ex* Colebr.	Burseraceae	Tree	EN
46	*Bunium persicum* (Boiss.) B. Fedtsch.	Apiaceae	Herb	EN
47	*Celastrus paniculatus* Willd.	Celastraceae	Climber	EN
48	*Chlorophytum arundinaceum* Baker	Liliaceae	Herb	EN
49	*Chonemorpha fragrans* (Moon) Alston	Apocynaceae	Climber	EN
50	*Cinnamomum wightii* Meisn.	Lauraceae	Tree	EN
51	*Rothea serrata* (L.) Steane & Mabb. [=*Clerodendrum serratum* (L.) Moon]	Verbenaceae	Shrub	EN
52	*Coptis teeta* Wall.	Ranunculaceae	Herb	EN
53	*Decalepis hamiltonii* Wight & Arn.	Periplocaceae	Climber	EN
54	*Dendrobium fugax* Rchb.f. [=*Flickingeria fugax* (Rchb.f.) Seidenf.]	Orchidaceae	Herb	EN
55	*Dendrobium nobile* Lindl.	Orchidaceae	Herb	EN
56	*Didymocarpus pedicellatus* R.Br.	Gesneriaceae	Herb	EN
57	*Dioscorea deltoidea* Wall. *ex* Griseb.	Dioscoreaceae	Climber	EN
58	*Dysoxylum malabaricum* Bedd. *ex* C.DC.	Meliaceae	Tree	EN
59	*Entada pursaetha* DC.	Mimosaceae	Liana	EN
60	*Ephedra gerardiana* Wall. *ex* C.A. Mey.	Ephedraceae	Herb	EN
61	*Fritillaria cirrhosa* D. Don [*=Fritillaria roylei* Hook.]	Liliaceae	Herb	EN
62	*Fumaria indica* (Hausskn.) Pugsley	Fumariaceae	Herb	EN
63	*Garcinia pedunculata* Roxb. *Ex* Buch.-Ham.	Clusiaceae	Tree	EN
64	*Gloriosa superba* L.	Liliaceae	Climber	EN
65	*Gymnema sylvestre* R. Br. *Ex* Schult.	Asclepiadaceae	Climber	EN
66	*Habenaria intermedia* D. Don	Orchidaceae	Herb	EN
67	*Homalomena aromatica* (Spreng.) Schott	Araceae	Herb	EN
68	*Hyoscyamus niger* L.	Solanaceae	Herb	EN
69	*Juniperus polycarpos* K. Koch	Cupressaceae	Shrub	EN

(continued)

Table 5.3 (continued)

S.N.	Botanical name	Family	Habit	Threat category assigned
70	*Jurinea dolomiaea* Boiss.	Asteraceae	Herb	EN
71	*Leptadenia reticulate* (Retz.) Wight & Arn.	Asclepiadaceae	Climber	EN
72	*Luffa echinata* Roxb.	Cucurbitaceae	Climber	EN
73	*Manilkara hexandra* (Roxb.) Dubard	Sapotaceae	Tree	EN
74	*Meconopsis aculeate* Royle	Papaveraceae	Herb	EN
75	*Mesua ferrea* L.	Clusiaceae	Tree	EN
76	*Michelia champaca* L.	Magnoliaceae	Tree	EN
77	*Mucuna pruriens* (L.) DC.	Fabaceae	Climber	EN
78	*Nervilia aragoana* Gaudich.	Orchidaceae	Herb	EN
79	*Nilgirianthus ciliates* (Nees) Bremek.	Acanthaceae	Herb	EN
80	*Nothapodytes nimmoniana* (J. Graham) Mabb. [=*Mappia foetida* (Wight) Miers]	Icacinaceae	Tree	EN
81	*Operculina turpethum* (L.) Silva Manso	Convolvulaceae	Climber	EN
82	*Oroxylum indicum* (L.) Benth. *ex* Kurz	Bignoniaceae	Tree	EN
83	*Desmodium oojeinense* (Roxb.) H. Ohashi	Fabaceae	Herb	EN
84	*Paris polyphylla* Sm.	Liliaceae	Herb	EN
85	*Piper longum* L.	Piperaceae	Herb	EN
86	*Piper nigrum* L.	Piperaceae	Climber	EN
87	*Plectranthus barbatus* Andrews [=*Coleus forskohlii* (Poir.) Briq.]	Lamiaceae	Herb	EN
88	*Plumbago indica* L.	Plumbaginaceae	Herb	EN
89	*Polygonatum cirrhifolium* (Wall.) Royle	Polygonaceae	Herb	EN
90	*Rheum austral* D. Don	Polygonaceae	Herb	EN
91	*Rheum moorcroftianum* Royle	Polygonaceae	Herb	EN
92	*Rhododendron anthopogon* D. Don	Ericaceae	Shrub	EN
93	*Salacia reticulate* Wight	Hippocrateaceae	Shrub	EN
94	*Santalum album* L.	Santalaceae	Tree	EN
95	*Sterculia urens* Roxb.	Sterculiaceae	Tree	EN
96	*Stereospermum tetragonum* DC.	Bignoniaceae	Tree	EN
97	*Tecomella undulata* (Sm.) Seem.	Bignoniaceae	Tree	EN
98	*Trichopus zeylanicus* Gaertn.	Trichopodaceae	Herb	EN
99	*Zanthoxylum armatum* DC.	Rutaceae	Shrub	EN
100	*Zanthoxylum rhetsa* (Roxb.) DC.	Rutaceae	Shrub	EN

Source: Goraya and Ved (2017)

It is interesting to note that nearly half of the species assessed as "critically endangered" are sourced from the Himalayan region. One-fourth of the Red-listed species are trees, and another quarter comprise of shrubs and large climbers. Some of the species enlisted above, like *Fumaria indica,* seem to be commonly growing in landscapes outside forests. However, the wild populations of these species have drastically declined due to high demand and loss of their habitats to rapid urbanization and degradation. Species like *Piper longum* and *Piper nigrum*, which are under extensive cultivation, are fast losing their wild germplasm. The germplasm is very important to conserve their genetic base for their long-term survival for development of newer varieties (Plates 5.4, 5.5, and 5.6).

These species require urgent management interventions for their conservation, sustainable availability for the herbal sector, and continuous cash income for thousands of wild gatherers. The Government of India has notified some of these species

Plate 5.4 Some of the endemic and threatened medicinal plants. (**a**) *Aconitum heterophyllum.* (**b**) *Amentotaxus assamica*. (**c**) *Ephedra gerardiana*. (**d**) *Nardostachys grandiflora*. (**e**) *Rheum emodi*. (**f**) *Swertia chirayita*. (Photo courtesy: Dr. K.Ravikumar)

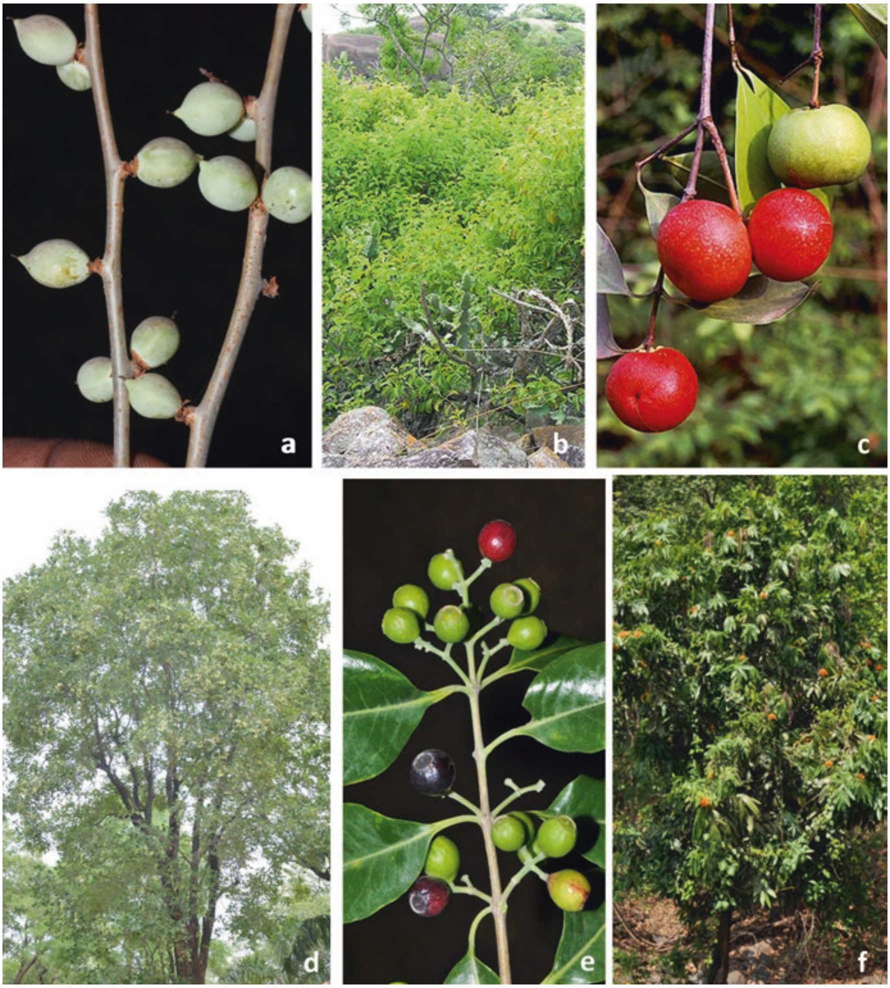

Plate 5.5 Threatened and traded medicinal Plants of conservation concern. (**a**) *Commiphora wightii*. (**b**) *Decalepis hamiltonii*. (**c**) *Garcinia indica*. (**d**) *Pterocarpus santalinus*. (**e**) *Santalum album*. (**f**) *Saraca asoca*. (Photo courtesy: Dr. K.Ravikumar)

under Section 38 of the Biological Diversity Act-2002, and their wild harvest and trade is prohibited. Some of these species have also been registered under "Negative List of Exports." However, what is required is to put these species in "Action Lists" for proactive action toward their conservation, building of their wild populations, developing sustainable harvest practices, and rooting of these practices in the local communities which are usually associated with their wild harvest.

Plate 5.6 Threatened and traded medicinal plants of conservation concern. (**a**) *Aegle marmelos.* (**b**) *Chlorophytum borivilianum*. (**c**) *Cinnamomum wightii*. (**d**) *Oroxylum indicum*. (**e**) *Myristica malabarica*

5.6 Wild Medicinal Plants of Conservation Concern Currently Being Traded in High Volumes

The current study on assessment of trade of botanical drugs in our country has revealed that herbal raw drugs pertaining to 242 plant species are in significant trade, i.e., the annual demand for each of these botanicals exceeds 100 MT per year. One hundred and seventy-three of these species are sourced almost entirely from the wild of which 114 species are found mainly or entirely in Indian forests. It is important to examine each of these 114 species to assess the impact of their trade and the resulting lessons for the management and conservation of these valuable forest resources accordingly. This requires a reliable and rapid assessment of conservation status of each of these species recorded in high volume trade (Goraya and Ved 2017).

In order to draw lessons for developing informed management responses for these wild resources, a tabulation has been prepared enlisting 49 threated medicinal plants which have also been recorded in high volume trade.

The 49 threatened medicinal plants recorded in high volume trade are shortlisted for developing informed management responses. These 49 medicinal plant species assessed as "threatened" in one or more states of India are *Aconitum ferox* Wall. *ex* Ser., *Aconitum heterophyllum* Wall. *ex* Royle, *Aquilaria malaccensis* Lam., *Berberis aristata* DC., *Bergenia ciliata* (Haw.) Sternb., *Buchanania lanzan* Spreng., *Boswellia serrata* Roxb. ex Colebr., *Celastrus paniculatus* Willd., *Chlorophytum tuberosum* (Roxb.) Baker, *Cinnamomum sulphuratum* Nees, *Cinnamomum tamala* (Buch.-Ham.) T. Nees & Eberm., *Commiphora wightii* (Arn.) Bhandari, *Coscinium fenestratum* (Gaertn.) Colebr., *Decalepis hamiltonii* Wight & Arn., *Embelia ribes* Burm. f., *Embelia tsjeriam-cottam* (Roem. & Schult.) A. DC., *Ephedra gerardiana* Wall. ex C.A. Mey., *Garcinia indica* (Thouars) Choisy, *Gymnema sylvestre* R. Br. ex Schult., *Holostemma annulare* (Roxb.) K. Schum., *Jurinea dolomiaea* Boiss., *Litsea glutinosa* (Lour.) C.B. Rob., *Mesua ferrea* L., *Nardostachys jatamansi* (D. Don) DC., *Operculina turpethum* (L.) Silva Manso, *Oroxylum indicum* (L.) Benth. *ex* Kurz, *Picrorhiza kurroa* Royle *ex* Benth., *Pseudarthria viscida* (L.) Wight & Arn., *Pterocarpus marsupium* Roxb., *Pterocarpus santalinus* L.f., *Rauvolfia serpentina* (L.) Benth. *ex* Kurz, *Rheum emodi* Wall., *Rheum moorcroftianum* Royle, *Rhododendron anthopogon* D. Don, *Rubia cordifolia* L., *Santalum album* L., *Saraca asoca* (Roxb.) W.J. de Wilde, *Saussurea costus* (Falc.) Lipsch., *Schrebera swietenioides* Roxb., *Smilax glabra* Roxb., *Sterculia urens* Roxb., *Strobilanthes ciliata* Nees, *Swertia chirayta* H. Karst. *Symplocos racemosa* Roxb., *Taxus wallichiana* Zucc., *Valeriana hardwickii* Wall., *Valeriana jatamansi* Jones, and *Vateria indica* L. Forest managers and policy makers also need to ensure that appropriate action is taken for the conservation of our valuable wild medicinal resources.

5.7 Conclusion

There is need for the taxonomist not only to document the floristic data (i.e., taxonomical character) but also to undertake ground truthing and document the population and distribution of the important threatened medicinal plants. Mapping using ecological niche modeling (ENM) will facilitate the study of their intra-genetic and inter-genetic variations. The genetic diversity of the species is conserved by studying plant population and their molecular characterization.

For critically endangered, endangered, and vulnerable medicinal plants, it is very important to identify factors responsible for depleting species. Similarly, reproductive biology should be rigorously studied for these species with regeneration problems. To take up such studies, it is very important to establish long-term monitoring sites to study the regeneration status, reproduction biology, phenology of the species, and effect of climate change on the survival of the species.

As these species are unable to regenerate on their own, there is need to standardize propagation techniques. The mass multiplied should be reintroduced into the wild suitable habitat of the species.

Assessment of the threatened medicinal plants in all the states of the country by means of CAMP workshops will ensure prioritization of species and thus help to take up informed conservation action programs. Research studies on these species will further aid in understanding their biology, taxonomy, distribution, and threat factors as well as ascertain their conservation.

The in situ (field gene banks) sites can also be used as study areas to understand identifying factors responsible for depleting species and the reproductive biology of the species and accordingly aid toward their recovery and long-term conservation. The detailed studies undertaken at these sites, on demography of priority species, can also be fed into the working (management) plan prescriptions of the forest divisions. All nursery network and seed banks linked to these in situ conservation areas can connect such field gene banks to users. It is very important to involve community and Forest Departments for the successful conservation programs.

Research on the threatened plants in India with special focus on their identified factors such as their rarity, reproductive bottleneck feature, and their demographic, as well as their environmental stochasticity leading to declining populations and eventual extinction can be of great help to policy makers and scientists. This essentially requires a comprehensive account of the conservation status of threatened plants and their spatial distribution patterns in India which will be vital in prioritizing plant species and taking steps for their conservation accordingly.

5.7.1 Way Forward

- Conduct Conservation Assessment and Management Priorization (CAMP) in every state and generate the list of respective state threatened medicinal plants.
- Establish in situ MPCAs to conserve the threatened medicinal plant species and revisit the old MPCAs to know the health status of these MPCAs.
- Regularly train sensitive forest staff on identification of the medicinal plants with special focus on threatened medicinal plants. They being custodians of forests can contribute in documenting the factors of threat and in research as well as conservation action program.
- Establish long-term monitoring sites for the observation of the threatened medicinal plants – life cycle, phenology, intrinsic and extrinsic factors, anthropogenic pressure, regeneration status, and population.
- Undertake genetic diversity studies across the range of distribution of the threatened species to conserve the in situ site with the highest genetic diversity.

- Use of GIS and ENM to capture the possible distribution range of the threatened medicinal plant species.
- Promote plant conservation and monitoring in various biogeographic zones of India, there is need to initiate an all India coordinated program on monitoring and restoration of highly threatened plants. The program would need the involvement of leading institutions and taxonomists, State Forest Departments (SFDs), universities, and volunteers. This would help in the following ways: (i) establishment of linkages between the field botanists and frontline staff of SFDs; (ii) restoration, habitat improvement, protection, and monitoring of threatened taxa on priority basis; and (iii) strengthening biodiversity conservation in various biogeographic zones of the country.
  - Undertake monitoring of MPCAs at regular intervals to monitor if the conservation and management interventions had the intended effects.
  - Carry out monitoring to (i) assess trends in populations size and structure, (ii) to detect changes in size and structure that may indicate a demographically unstable population, (iii) to assess trends in population genetic diversity, (iv) to determine effects of altering habitat disturbances (e.g., management interventions) on the plant populations, and (v) to provide data for modelling population trends.
  - Study the effect of vegetational succession as a consequence of hands-off' approach on medicinal plant population in forests where their occurrence may depend on degraded state of forest.
- Develop cadre of barefoot taxonomist by training them on plant identification, ecological studies, and field data documentation. Since there is shortage of taxonomist, these barefoot taxonomists will assist the taxonomists, foresters, and researchers in conservation action program.
- No conservation program is successful without involving community; thus students and community should be trained on medicinal plants, on threatened plants, and about their conservation.
- Encourage cultivation of the threatened medicinal plants to avoid dependency on the wild sources.

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Chapter 6
Biotechnological Interventions for Conservation and Multiplication of Threatened Medicinal Plants

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Abstract The conservation of rare, endangered and threatened plants is a global concern and increased attention is diverted towards this goal. With the realization to have a holistic approach for the conservation of genepool of threatened species, ex situ conservation using in vitro techniques has gained momentum along with the protection of the same in its natural habitats. The developments in molecular biology and biotechnology have enabled the use of in vitro techniques for the collection, conservation and use of plant genetic resources. In vitro conservation techniques using tissue culture have been massively adopted for the propagation as well as conservation of germplasm of threatened plants in the form of slow growth cultures. Slow growth cultures of many threatened species are maintained at national gene banks for their short- to medium-term conservation. The emerging techniques of cryopreservation and concepts of DNA banking further added to the holistic approach of conservation of the risk species. Biochemical profiling has enabled the identification of important secondary metabolites in endangered species, and plant cell culture techniques will aid in their large-scale production so that the collection of the material from the wild can be prevented. These modern techniques will act as a complementary mechanism which will aid to carry out further molecular studies on the endangered species or already extinct species. The selection of appropriate strategy depends on the type of the species, its status in natural habitat and its reproductive behaviour. The applicability and feasibility of the methods chosen and its cost-effectiveness will also decide the success of conservation. Thus, a balance of all available methods is advisable to ensure that the long-term goals are achieved. In this chapter an attempt has been made to review the state of art in these species in relation with multiplication and conservation using biotechnological means.

Keywords Rare endangered and threatened plants · In vitro · Cryopreservation · DNA Banking

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6.1 Introduction

India being one of the hotspots of biodiversity harbours rich diversity of medicinal plants. It is recorded that around 8000 plants are used for medicinal purposes out of which 800 are used by the industry and only less than 20 species are produced by cultivation There is a recent surge globally in the demand of herbal medicines owing to the side effects attributed to modern drugs, higher cost of modern medicines, limited accessibility and availability. This increased demand is causing indiscriminate harvest from wild creating a drastic loss of biodiversity. In India, the unsustainable collection of medicinal plants from the wild for commercial purpose and habitat destruction due developmental activities stand high among the human-induced causes of depletion of medicinal plant population. Increased exploitation of wild collections has led 120 medicinal plants into rare or endangered category in India. The RET (rare, endangered and threatened) medicinal plants which have been identified by IUCN are to be conserved by both in situ and ex situ methods. In situ conservation alone is not sufficient to meet the challenges of saving endangered species. Ex situ conservation of medicinal plant species is a complementary conservation strategy for the genetic diversity of prioritized medicinal plants. It is especially desirable in case of species where wild populations have dwindled to critical levels. Ex situ conservation can facilitate to conserve a species of high importance in controlled conditions and again reintroduce it into the wild. The recent development in biotechnology has come up as a boon in the area of conservation biotechnology. Biotechnological tools are important to select, multiply and conserve the critical genotypes of medicinal plants. The application of biotechnology for conserving threatened plants includes the use of tissue culture for micropropagation, in vitro conservation strategies for collection and short-, medium- and long-term conservation and also DNA banks to conserve the genomic resources. Tissue culture has emerged as a promising technique to obtain genetically pure elite populations under in vitro conditions rather than have in different populations. In vitro conservation is especially important for vegetatively propagated and for non-orthodox seed plant species. In vitro techniques can be used right from the germplasm collection of RET medicinal plants to their propagation and conservation. Furthermore, in vitro technique can be used for recovering and regenerating the wild population and also facilitates molecular and ecological research in threatened species and its habitat. In this chapter we will discuss the different biotechnological techniques used for the collection, conservation and multiplication of RET medicinal plants.

6.2 In Vitro Collection Techniques

Germplasm collection is the first step to acquire the plant material for adopting conservation strategies. In the case of rare and threatened plant species, extra care needs to be taken while collecting because there may be only few individuals of a given species in specific areas and their seed collection may be restricted. In such a case, in vitro collecting of tissues would be recommended than removing whole plants. The removal of small amounts of tissue should not harm in situ populations. Further, this will also increase the sampling efficiency. In the case of vegetatively propagated species, recalcitrant seed species and species with sterile seeds, in vitro collecting broadens the possibilities for collecting living tissues. In vitro material can be dispatched internationally with fewer restrictions, even though it is still subject to import permits and phytosanitary certificates. Use of in vitro collecting has been reported for crop plants (Pence et al. 2002; Silvana Alvarenga et al. 2002) and for the collection of buds and leaves of threatened plants (Pence et al. 2002).

Factors to be considered during in vitro collecting of plant tissue are:

1. Nature of the explant to be collected

   The explants selected should be strong enough to withstand surface sterilization. Young, growing meristematic tissues are generally used for initiating cultures.
2. Size of the explant

   Superficial damage should be minimized as far as possible, but the opportunity should also be taken to eliminate external tissues that are very dirty, infected or damaged.
3. Time of collection

   It is always desirable to collect the explants at optimal environmental conditions as compared to the extremes.
4. Soil residues and presence of diseased tissue

   The explant collected needs to be washed immediately with sterile water to remove the soil particles adhered and also scrap off the diseased tissue if any.
5. Sterilization technique to be used

   Sodium dichloroisocyanurate (SDICN) has proved to be a reliable sterilizing agent (Parkinson et al. 1996; Niedz and Bausher 2002) and has been used effectively at Royal Botanical Garden, Kew, to initiate plants from many taxonomic groups into axenic culture. Phytotoxicity of the compound is low and appears to act preferentially on old leaves and cut surfaces, making it ideal for use where plant material is limited (Parkinson et al. 1996).
6. Nutrient medium

   Nutrient medium should be selected according to the purpose. If the collection is meant for micropropagation purpose, then to encourage tissue development (e.g. to stimulate embryo germination or the growth of axillary buds), then appropriate growth regulators must be added to the medium. If the development must be suspended for long-term conservation, then a minimal medium or medium containing growth retardents must be used. A medium may also contain antimicrobial additives to retard the growth and destructive effects of bacteria and fungi. A liquid medium is more accessible for the inoculum, but it is less effective in retarding the growth of contaminating microorganisms. Moreover, containers with liquid medium must not leak.
7. Conditions of storage light, temperature and humidity

   An illumination of 16 h a day and 8 h at night is satisfactory for shoot proliferation and a temperature of 25 °C is optimal for the growth (Table 6.1).

Table 6.1 Endangered US species for which in vitro collecting has been used (Pence et al. 2002)

Sl. no.	Species	Tissue	Results
1	*Aconitum noveboracense* Gray & Coville	Bud	Shoot cultures established
2	*Astragalus cremnophylax*	Leaf and bud	Callus lines established
3	*Clematis socialis* Kral	Bud	Shoot cultures established
4	*Hedeoma todsenii* Irving	Bud	Shoot cultures established
5	*Lobelia boykinii*	Leaf and bud	Shoot cultures established
6	*Schoenocrambe suffrutescens*	Bud	Shoot cultures established
7	*Mespilus canescens* [Phipps]	Bud	Callus lines established

6.3 In Vitro Techniques for Conservation

In vitro conservation refers to the conservation of germplasm under defined nutrient conditions in an artificial environment in the form of in vitro cultures. The culture systems may be in the form of shoots, meristems, embryos, plantlets, callus or cell suspension. In vitro conservation can be effectively used for multiplication as well as conservation of endangered taxa. For vegetatively propagated species, recalcitrant seed species and species with sterile seeds, in vitro conservation is the only reliable method for long-term conservation. The properties required for a successful in vitro conservation system as defined by Grout (1990) are:

The ability of the biological system to

1. Minimize the growth and development in vitro
2. Maintain viability of the stored material at the highest possible level along with the minimum risk of genetic stability
3. Maintain full developmental and functional potential of the stored material when it is returned to physiological temperatures
4. Make significant savings in labour input, materials and commitment of specialized facilities

In vitro conservation is achieved through plant tissue culture technique. Plant tissue culture (PTC) refers to the culturing of plant cell or tissue in vitro under sterile conditions for rapid multiplication. This makes use of the totipotent property of the cell, which is the ability of any plant cell to grow into a whole plant when provided with suitable nutrient medium and environmental conditions. Plant tissue culture has many advantages over conventional methods of vegetative propagation listed as follows (Mathur 2013):

- Only a small amount of tissue is required to regenerate millions of clonal plants in a year.
- In vitro stock can be quickly proliferated as it is season independent.
- Rapid multiplication of superior clones can be carried out throughout the year, irrespective of seasonal variations.
- Multiplication of disease and virus free plants.
- It is a-cost effective process as it requires minimum growing space.
- Long-term storage of valuable germplasm possible.

Once the cultures have been established and multiplied in sufficient number, an effective method for conservation is required. The main aim of in vitro conservation is to increase the subculture interval which can be accomplished in two ways: by maintaining cultures under normal growth or by subjecting them to growth limiting strategies. Conservation for short to medium duration is achieved by subjecting materials to slow growth, and cryopreservation is the technique used to conserve the germplasm in a suspended growth stage for an indefinite time period. Cultures maintained under normal growth and slow growth conditions refer to active germplasm collection whereas cultures maintained under suspended growth by cryopreservation constitute the in vitro base collection (Rajasekharan and Sahijram 2015).

6.3.1 Micropropagation for Conservation of Threatened Medicinal Plants

Using tissue culture methods, micropropagation of many important threatened medicinal plants has been taken up to promote its multiplication as well as conservation. Micropropagation refers to the mass production of plant propagules from any part of the plant or cell. Micropropagation and cloning of plant tissue based on different explants are commonly used to conserve different endangered plants (Pathak and Abido 2014). It enables fast, season independent, continuous multiplication, maintenance and conservation of rare and endangered plants by using any plant parts as explant source (Sarasan et al. 2006; Chandra et al. 2010). Steps in micropropagation include:

(a) Initiation of culture – from an explant like shoot tip on a suitable nutrient medium. Initial shoot development can occur either directly from explant or through indirect way of callus-mediated de-differentiation of shoot initials.
(b) Multiple shoots formation from the cultured explant.
(c) Rooting of in vitro developed shoots.
(d) Transplantation – transplantation to the field following acclimatization.

Several rare and endangered plant species can be quickly and successfully propagated and conserved from a minimum plant material and with low impact on wild population (Branka et al. 1997). During the last three decades, in vitro techniques had been used to conserve and re-establish many threatened medicinal plants by means of media optimization and supplementation of plant growth regulators. Micropropagation, using somatic embryo and shoot tip culture techniques, assists many crop improvement programmes, and these methods are being used for the conservation of endangered medicinal plant species. Tissue culture techniques have been applied typically when traditional methods of propagation have either failed or proved inadequate. Thus, in vitro propagation of endangered plants enables rapid and efficient multiplication of at risk species which have a limited reproductive capacity and exist in threatened habitats (Fay 1992).

In vitro propagation and regeneration systems have been developed for IUCN red-listed medicinal plants with anticancer activity such as *Gymnema sylvestre*, *Leptadenia reticulata*, *Saussurea involucrata*, *Caralluma bhupenderiana*, *Zeyheria montana*, *Psoralea corylifolia*, *Gloriosa superba*, *Swertia chirayita* and *Nilgirianthus ciliatus* (Table 6.2) (Rameshkumar et al. 2017).

Table 6.2 Application of in vitro propagation for conservation of endangered medicinal plants

Species	Status	Explant	Reference
Allium wallichii	Threatened, medicinal	Seedling explants	Wawrosch et al. (2001)
Buchanania lanzan	Vulnerable, medicinal	Seedling explants	Shende and Rai (2005)
Celastrus paniculatus	Rare, medicinal	Stem	De Silva and Senarath (2009)
Ceropegia candelabrum	Endangered medicinal	Axillary bud multiplication	Beena et al. (2003)
Decalepis arayalpathra	Endangered, medicinal	Nodal explants	Sudha et al. (2005)
Embelia ribes	Threatened, medicinal	Nodal explant	Preetha et al. (2012)
Adhatoda vasica	Rare, medicinal	Petiole	Mandal and Laxminarayana (2014)
Baliospermum montanum	Endangered, medicinal	Nodal explant	Sasikumar et al. (2009)
Vitex trifolia	Endangered, medicinal	Nodal explant	Hiregoudar et al. (2006)
Curcuma caesia	Endangered, medicinal	Rhizome bud	Shahinozzaman et al. (2013)
Curculigo orchioides	Endangered, medicinal	Meristem tip	Bhavisha and Yogesh (2003)
Saussurea lappa	Endangered, medicinal	Shoot tip	Johnson et al. (1997)
Atropa acuminata	Endangered, medicinal	Petiole and nodal explant	Maqbool et al. (2016)
Trichopus zeylanicus sub sp. *travancoricus*	Rare, medicinal	Shoot tip	Krishnan et al. (1995)
Psoralea corylifolia	Rare, medicinal	Seedling explants Seeds	Saxena et al. (1997) Verma et al. (2012)
Rauwolfia serpentina	Endangered, medicinal	Shoot tips, nodal explant	
Coleus forskohlii	Endangered, medicinal	Nodal explant	Sharma et al. (1991)
Gentiana kurroo Royle	Endangered, medicinal	Apical meristem	Kaushal et al. (2014)
Picrorhiza kurroa	Endangered, medicinal	Nodal explant	Jan et al. (2010)

(continued)

Table 6.2 (continued)

Species	Status	Explant	Reference
Nothapodytes foetida (Wight)	Endangered, medicinal		
Tylophora indica	Endangered, medicinal	Nodal explants Shoot tips	Faisal et al. (2007) Sellathurai and Rathinavel (2012)
Drosera indica	Vulnerable, medicinal	Shoot tips	Kottapalli and Majeti (2007)
Garcinia indica	Threatened, medicinal	Immature seeds	Joshi et al. (2015)
Moringa oleifera	Endangered medicinal	Nodal explant	Marfori (2011)
Podophyllum hexandrum	Endangered medicinal	Root segments	Sultan et al. (2006)
Gloriosa superba	Endangered medicinal	Sprouts from tubers	Custers and Bergervoet (1994)
Pterocarpus santalinus	Endangered medicinal	Nodal explant	Prakash et al. (2006)
Santalum album	Threatened medicinal	Nodal explant	Peeris and Senarath (2015)
Valeriana jatamansi	Endangered medicinal	Shoot buds	Kaur et al. (1999)
Dorema ammoniacum	Rare, medicinal	Hypocotyl segment	Irvani et al. (2009)
Gymnema sylvestre	Threatened, medicinal	Nodal explant	Shah et al. (2013)
Holostemma adakodien	Rare, medicinal	Axillary bud multiplication	Martin (2002)
Elaeocarpus sphaericus (Rudraksha)	Threatened, medicinal	Nodal explant	Saklani et al. (2015)

Gymnema sylvestre is an important medicinal plant with high pharmaceutical value for manufacturing drugs for diabetes, asthma, eye complaints, etc. The species is now threatened with extinction due to its unscrupulous harvest from its natural habitat to meet the demand of pharmaceutical industry. Shah et al. (2013) developed in vitro propagation procedure for the species including four steps, viz. culture establishment, shoot multiplication, rooting and hardening. The study recommended a four-step micropropagation procedure for in vitro production of *G. sylvestre* plants on a commercial scale to meet the requirement of pharmaceutical industries and save the species from extinction (Fig. 6.1).

Paederia foetida L. (Family Rubiaceae) is a medicinally important climbing vine used by traditional medical practitioners in Bangladesh for the treatment of rheumatism, intestinal disorders and liver inflammation. This herb contains iridoid glycosides, sitosterol, stigmasterol, alkaloids and volatile oils. The plant has got a high

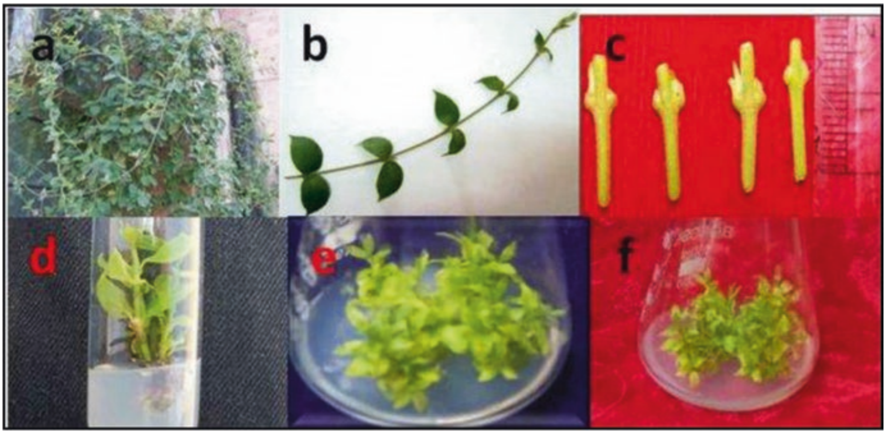

Fig. 6.1 Explant collection, culture establishment and shoot multiplication in *Gymnema sylvestre* R. Br.; (**a**) mother plant, (**b**) a twig, (**c**) nodal explants, (**d**) the in vitro culture establishment and (**e**–**f**) the in vitro shoot multiplication

commercial demand in Bangladesh because of which it is endangered in its natural habitat. Alam et al. (2010) established an in vitro propagation method to rescue it from extinction as well as to promote its conservation. Nodal explants from young shoot apices were used to produce multiple shoots. Multiple shoots (2.53 shoots/explant) resulted as a cluster when the media was prepared with [BAP (1.0 mg/l) + kinetin (0.5 mg/l)] to observe the synergism of auxin and cytokinin. Micro-shoots, when excised and cultured on ½ MS medium enriched with indole-3-butyric acid (0.3 mg/l), showed the best root induction percentage (85%). In vitro raised plantlets were then transplanted to earthen pot media after proper hardening. The survival percentage was 90%. The study reported that this method of clonal propagation is effective for producing plantlets in large quantities and conservation of this medicinal herb to meet the requirements of traditional medical practitioners in Bangladesh. Behera et al. (2018) also took up similar study for micropropagation of *Paederia foetida* using nodal explants and came up with similar results. The biochemical fidelity was assessed by evaluating the antioxidant activities of leaves of both field grown mother plant and micropropagated plants. Further, genetic fidelity of the micropropagated plants with that of the mother plant was assessed by inter simple sequence repeats markers, and their true-to-type nature was confirmed on the basis of monomorphic banding profile (Fig. 6.2; Table 6.3).

6.3.1.1 Factors Affecting In Vitro Cultures

The nature of explants selected for micropropagation is very critical in determining the in vitro regeneration capacity of endangered medicinal plants. There is a wide range of explants which can be used to initiate in vitro cultures of rare and threatened medicinal plants. The type of explants includes shoot tips, rhizomes, florets,

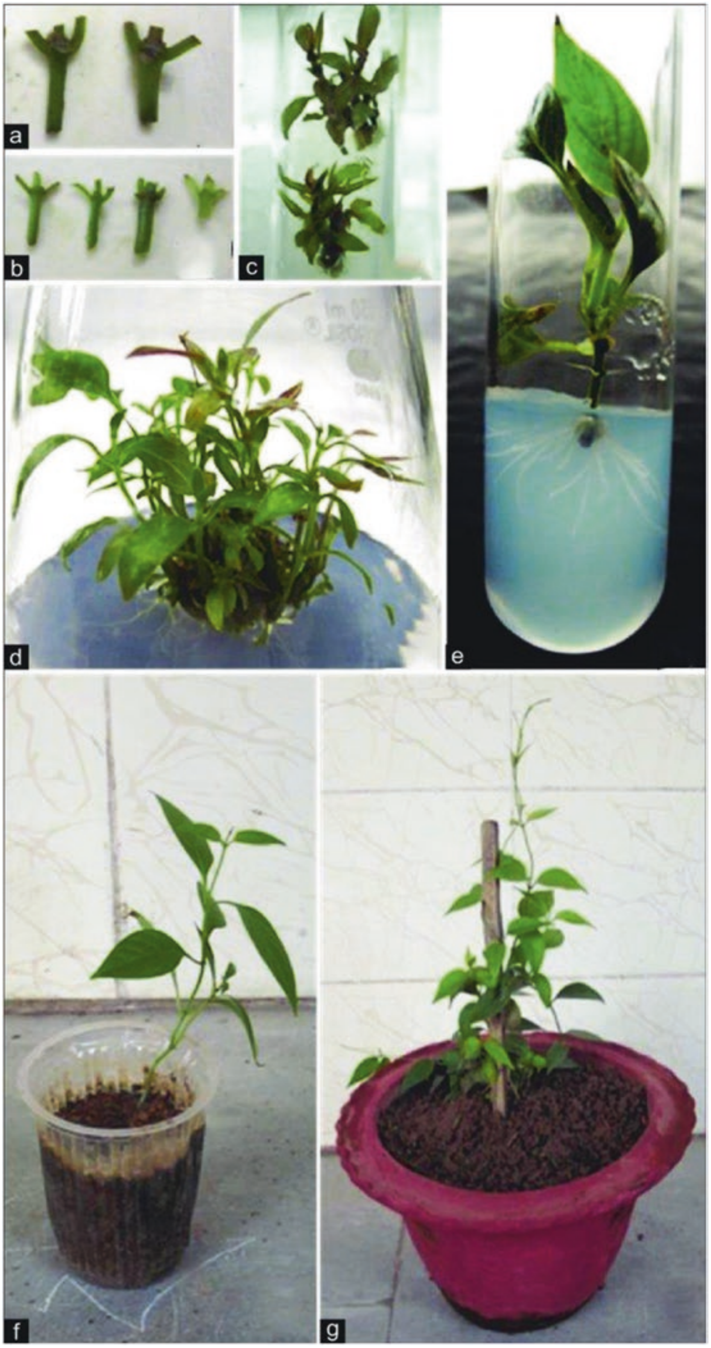

Fig. 6.2 (**a**) Nodal explants excised from young growth flush of naturally grown *Paederia foetida* plant, (**b**) axenic nodal segments derived from primary in vitro shoots, (**c**) shoot proliferation from mature nodal explants on Murashige and Skoog's (MS) supplemented with 3.0 mg/l N 6benzyl-aminopurine (BAP), (**d**) proliferation of multiple shoots from axenic nodal explant on MS augmented with 3.0 mg/l BAP medium, (**e**) rooting of in vitro regenerated shoot on ½ MS medium after 25 days of culture, (**f**) an acclimatized plant in soil:sand (1:1) substrate (**g**) acclimatized plant of *P. foetida* in clay pot. (From Beherea et al. 2018)

Table 6.3 In vitro regeneration conditions for selective IUCN RED LISTED medicinal plants

Sl. no.	Plant species	In vitro regeneration conditions	In vitro rooting conditions
1	*Gymnema sylvestre*	MS medium supplemented with 1 mg l^{-1} BA + 0.5 mg l^{-1} KIN + 0.1 mg l^{-1} NAA + 100 mg l^{-1} malt extract + 100 mgl^{-1} citric acid found 84% of regeneration after 30 days of light incubation	Half strength MS medium containing 3.0 mg l^{-1} IBA showed 50% of rooting after 50 days of light incubation
2	*Leptadenia reticulata*	MS medium supplemented with 0.25 mg l^{-1} BA + 0.25 mg l^{-1} KIN showed 90% of regeneration after 28 days of light incubation	MS medium containing 2.0 mg l^{-1} IBA + 200 mg l^{-1}activated charcoal showed 87% of rooting after 28 days of light incubation
3	*Saussurea involucrata*	MS medium supplemented with 10 μM BA + 2.5 μM NAA showed 66% of regeneration after 40 days of light incubation	Half strength MS medium containing 2.5 μM IAA showed 87% of rooting after 28 days of light incubation
4	*Caralluma bhupenderiana*	MS medium supplemented with 8.87 μM BA + 2.85 μM IAA + 100 mg l^{-1} ascorbic acid showed 93% of regeneration after 42 days of light incubation	Half strength MS medium containing 2.69 μM NAA showed 88% of rooting after 25 days of light incubation
5	*Zeyheria montana*	¼ strength MS medium containing 0.1 mg l^{-1} BA + 0.5 mg l^{-1} GA3 showed 70% of in vitro regeneration	¼ strength MS medium + 1.5 mg l^{-1} IBA showed 65% of rooting
6	*Psoralea corylifolia*	MS medium supplemented with 12 μM BA + 10 μM NAA + 15 μM Kn showed 95% of regeneration	½ strength MS medium + 2.5 μM IBA showed 95% of efficient rooting
7	*Gloriosa superba*	MS medium supplemented with 2 mg l^{-1} BA + 0.5 mg l^{-1} NAA showed 76.6% of regeneration	½ MS medium containing + 1 mg l^{-1} NAA + 0.5 mg l^{-1} IBA showed 66.6% of rooting
8	*Swertia chirayita*	MS medium supplemented with 2 mg l^{-1} BA + 0.1 mg l^{-1} IAA showed 83.0% of regeneration	MS medium alone showed 80.0% of rooting
9	*Nilgirianthus ciliatus*	MS medium supplemented with 3 mg l^{-1} BA + 0.1 mg l^{-1} IAA showed 93.2% of regeneration	½ MS medium containing + 1 mg l^{-1} IBA showed 82.2% of rooting

From Rameshkumar et al. (2017)

nodal segments, embryos or even roots. There have been many studies indicating the success of using specific explants for micropropagation and in vitro regeneration. The threatened medicinal plant *Decalepis hamiltonii* was successfully multiplied in vitro using shoot tip culture, as reported by Giridhar et al. (2005). In *Swertia chirayta,* again a threatened medicinal plant, roots were used as explants for micropropagation (Wawrosch et al. 1999). Chang et al. (2000) developed tissue culture protocol for propagation of a rare medicinal plant, *Lilium speciosum* var. *gloriosoides*, from young floret. Huang et al. (2000) have developed in vitro propagation system for *Limonium wrightii,* a rare medicinal plant from shoot tip, leaf and inflorescence node explants, but shoot tips were more responsive. Rapid micropropagation was observed in *Scopolia parviflora* by using rhizome as explants.

Composition of the culture media also affects the in vitro growth of plant tissues. Different types of growth media are available for plant tissue culture, but Murashige and Skoog (MS) medium is commonly used. The composition of growth medium includes a carbon source, macro- and micro-nutrients, vitamins, growth regulators and other organic substances. Growth hormones regulate various physiological and morphological processes in plants and are also known as plant growth regulators (PGRs) or phytohormones. Hormones can also be added into cultures to improve plant growth and to enhance metabolite synthesis. In *Alpinia galanga*, explants from rhizome buds were cultured on Murashige and Skoog (MS) medium supplemented with 6-benzylaminopurine (BAP) alone (0–5 mg/l) and a combination of BAP (0–5 mg/l) and indole 3-acetic acid (IAA) (0–2 mg/l). It was found that MS medium supplemented with a combination of 5.0 mg/l BAP and 2.0 mg/l IAA and 3.0 mg/l BAP and 0.5 mg/l IAA produced the highest mean number of shoots per explant as compared to other concentrations (Singh et al. 2014). In vitro multiplication and conservation of *Commiphora wightii*, an endangered medicinal species, was taken up with an objective to study the influence of growth regulators on the regeneration ability. Out of the 40, single as well as combinations of BAP (6-benzylaminopurine), Kn (Kinetin), NAA(naphthalene acetic acid), IBA(indole butyric acid) and GA_3 used, only three combination treatments of BAP (3 mg/l) with IBA (0.1–0.3 mg/l) induced shoot development in node and shoot tip cultures (Tejovathi et al. 2011). Different concentrations of growth hormones were tested for rapid clonal propagation of *Berberis lycium* Royle (Berberidaceae), an endangered medicinal shrub. Among different concentrations of hormones tried, 6-benzyl aminopurine with GA3 and 3% sucrose proved to be the best for shoot induction using cotyledonary node explants obtained from germinated seeds (Dhar et al. 2012). Multiple shoots were obtained from the meristem tip culture of *Curculigo orchioides* Gaertn. on MS medium supplemented with BA. The shoots were rooted either on half strength of MS basal medium or on the one supplemented with NAA (Wala and Jasrai 2003). Martin (2002) has shown that the cytokinin BAP has the strong effect with respect to the multiplication of axillary buds in rare medicinal plant *Holostemma ada-kodien.* Lal et al. (1988) observed the rapid proliferation rate in *Picrorhiza kurroa* using kinetin at 1.0–5.0 mg/1. Shoot regeneration in *Rauvolfia serpentina* was highest (75%) in BAP + IAA and usage of GA3 provided better result for elongation of shoot. Highest shoot regeneration (95%) results of *Psoralea corylifolia* were obtained on MS medium containing BAP with NAA, and KN in BAP was found to be best for shoot multiplication (Pandey et al. 2013). Proliferation of *Rauvolfia serpentina* shoots was achieved when cultured on MS medium supplemented with thidiazuron (TDZ) (0.1_2.5 mmol/L) although with low regeneration response and few number of shoots per explant. Maximum callus induction (100%) was achieved when *Thymus persicus* cultured on MS medium was fortified with NAA and KN. The highest frequency of shoot multiplication (96%) was observed with BAP and NAA. The maximum number of rootlets was induced on half-strength MS medium with IBA (Bakhtiar et al. 2016).

6.3.1.2 Somatic Embryogenesis and Organogenesis

Somatic embryogenesis refers to the development of somatic embryo from a single somatic cell or tissue. Somatic embryogenesis and organ development through organogenesis from various cultures of explants are the most commonly used technique applied to regenerate several endangered plants for the purpose of conservation (Sadeq et al. 2014). There is direct as well as indirect somatic embryogenesis. In direct somatic embryogenesis, plants develop directly from explants without any callus formation (mass of unorganized cells) whereas dedifferentiation of callus to produce plants occurs in indirect somatic embryogenesis. It has great application in the rapid multiplication of endangered medicinal plants (Table 6.4).

Genetically homogenous plants with uniform contents of secondary metabolites can be obtained by in vitro propagation of plants either by somatic embryogenesis or shoot organogenesis. There have been many reports where wild plants and micropropagated plants were tested for their ability to produce secondary metabolites. Since the production of secondary metabolites is generally higher in differentiated tissue, there are attempts to cultivate shoot cultures and root cultures for the production of medicinally important compounds. In endangered medicinal plants like *Rauvolfia micrantha* (Sudha and Seeni 1996), *Rotula aquatica* (Martin 2003) and *Aconitum atrox* (Nautiyal 1986) in which either seed viability is very low and or low germination has been reported, biotechnological methods are becoming increasingly important for their conservation .

Table 6.4 List of endangered plants regenerated through somatic embryogenesis

Plant species	Plant type	Explant used	Multiplication	Reference
Artemisia vulgaris	Restricted	Leaf	Organogenesis	Borzabad et al. (2010)
Baliospermum montanum	Threatened	Nodal bud	Shoot differentiation	Sasikumar et al. (2009)
Eleutherococcus senticosus	Endangered	Hypocotyl explants	Somatic embryogeneis, plant regeneration	Choi et al. (1999)
Heliotropium kotschyi	Endangered	Node	Shoot organogenesis	Sadeq et al. (2014)
Lilium ledebourii	Endangered	Bulb scale	Somatic embryogenesis and plant regeneration	Bakhshaie et al. (2010)
Psoralea corylifolia	Endangered	Hypocotyl segments	Somatic embryogenesis	Sahrawat and Chand (2001)
Rauvolfia serpentina	Endangered	Leaf	Somatic embryogenesis and plant regeneration	Singh et al. (2009)
Turbinicarpus pseudomacrochele	Endangered	Medullar tissue discs	Somatic embryogenesis and plant regeneration	Munoz and Garay (1996)
Woodfordia fruticosa	Rare	Shoot cuttings	Organogenesis	Krishnan and Seeni (1994)

6.3.2 In Vitro Technique for Medium-Term Conservation

This approach makes use of tissue culture technique to conserve the germplasm for medium duration. Tissue culture is the culture and maintenance of plant cells, tissue or organs in sterile nutritionally and environmentally supportive conditions in vitro. This technique allows the storage of biological material from several months to 2–3 years without subculture. This is achieved by reducing the growth rate of the plants either by modifying the components of the culture medium or by modifying the environmental conditions. Culture medium is altered by diluting the mineral elements, reducing concentration of sugar, changing the concentration of growth regulators or by addition of osmotically active compounds (Engelmann 2011). Sometimes, the explants are covered with paraffin or mineral oil overlay is also practiced to reduce the growth of plants. Modifications of the environmental condition are done by decreasing the temperature together with reducing the light intensity or keeping the cultures in complete darkness. Modifications in gaseous environment, desiccation and/or encapsulation are other possible options of reducing growth rate. The most frequently used combination of physical and chemical factors involves decrease of temperature, reduction of mineral elements and carbon source concentration in the medium and the use of low light intensity (Holobiuc et al. 2007). The optimum temperature for medium-term conservation is usually from 4 °C to room temperature. However, tropical plant species are often cold sensitive and have to be stored in the range of 15–20 °C or even higher, depending on their sensitivity. Therefore, the procedure to enable extending subculture periods will mainly focus on modifying the chemical composition of culture medium. Slow growth can also be achieved by encapsulating the explants (shoot buds or somatic embryos) to produce artificial seeds. The aim of slow growth is to reduce the subculturing interval under the multiplication procedure. There are various factors which affect the efficiency on in vitro slow growth cultures including type of explants used, their physiological state, type of culture vessel, volume and type of closures, etc. In vitro slow growth storage technique is routinely used for medium-term conservation of numerous species both from tropical and temperate origin and many endangered species. Slow growth conditions are most suitable for the conservation of valuable germplasm, the material required being readily available for regeneration, multiplication and distribution. The technique has the benefit of limiting the number of subcultures, making significant savings in labour input and reducing the risk of mutations, compared to germplasm, under normal growth conditions. In tropical and subtropical plant species, this technique seems to work well probably due to their inherent property of growing at higher temperature. In most of the species of temperate region, optimum subculture period could be extended only to 5 months. The added advantage of this approach is that mostly the cultures can be visibly assessed for viability and can readily be brought back to fresh culture medium to produce plants on demand (Rajasekharan and Sahijram 2015).

Bacopa monnieri L. (brahmi) is a renowned Indian medicinal plant with high commercial value for its memory revitalizer potential. Demand for this herb has increased due to its memory-enhancing property coupled with anticancer property and thus threatening its existence in the wild. Since it is predominantly a vegetatively propagated plant, seed propagation is difficult because of insufficient seed availability and short seed viability. Sharma et al. (2016) standardized in vitro clonal propagation method by enhanced axillary branching as a tool for medium term in vitro conservation of this species. Single node explants, cultured on Murashige and Skoog's medium supplemented with BA (0.2 mg/L), exhibited shoot and root proliferation without callus formation. The in vitro raised plants showed 80% survival. The study showed that the shoots could be conserved for 12 months with high survival and genetic stability on the same medium. The protocol optimized in this study has been applied for culture establishment, shoot multiplication and medium-term conservation of several *Bacopa* germplasm, procured from different agro-ecological regions of India. The slow growth technique was applied to conserve germplasm of *Garcinia indica* (Malik et al. 2005). In vitro conservation of *Coleus forskohlii* Briq. by slow growth technique was achieved by employing osmotic regulators (sorbitol and mannitol). 3 M mannitol showed best performance for reduced or slow growth of the culture (Dube et al. 2011).

Rajasekharan and Ganeshan (2010) successfully established slow growth cultures of 22 species of threatened medicinal plants of South India in MS medium supplemented with various concentrations of BA. Ninety-eight percentage of culture establishment was obtained with nodal and shoot tip explants when they were exposed to low-light intensity coupled with reduction in temperature. Reduced media concentration induced fast growth within 1 month, which remains more or less uniform up to a period of 3 months. In this way, the cultures were stored from 1 month to 1 year depending on the species (Table 6.5).

6.3.3 Long-Term Conservation Through Cryopreservation

Cryopreservation is the technique used for long-term conservation of plant genetic material. Cryopreservation is the maintenance of living cells, tissues organs and microorganisms at ultra low temperature (usually that of liquid nitrogen, −196 °C). Under this ultra low temperature, all the metabolic activities of the cell get arrested and the cells will not undergo any genetic changes. This allows the storage of germplasm under suspended growth for very long period of time. Cryopreservation is extremely helpful method to conserve rare, endangered, threatened plant species (Dussert et al. 1997; Zhao et al. 2008; Paunescu 2009). There occurs a wide range of tissues which are amenable to cryopreservation, viz. seeds, pollen, zygotic/somatic embryos, embryonic axes, embryogenic cell suspension, meristems or shoot tip cultures and winter buds. Advantages of this technique are that the cryopreserved cells are stored in a small volume, require very limited maintenance and samples are not continuously exposed to the risks of contamination and operator errors, due to frequent manipulations of the plant material. The principle behind cryopreservation is to bring the cells or tissues to a zero metabolism stage by sub-

Table 6.5 Details of in vitro establishment and conservation of threatened medicinal plant of South India using slow growth technique (Rajasekharan and Ganeshan 2010)

Sl. no.	Species	Multiplication media	Subculture frequency in months	Hardening status
1	*Aegle marmelos*	½ MS + 0.5 mg/L BAP	3	Earthen pots
2	*Aristolochia indica*	½ MS + 2 BAP	4	No success in hardening
3	*Alpinia galanga*	MS 2 mg/L BAP and 0.1 mg/L NAA	2	Established in field
4	*Artocarpus heterophyllus*	½ MS + 1 BAP	3	Established in field
5	*Bacopa monnieri*	MS + 1 BAP	2	Established in field
6	*Centella asiatica*	MS BASAL	1	Established in field
7	*Cissus quadrangularis*	½ MS BASAL	1	Earthen pots
8	*Citrus*	MS + 0.5 BAP	2	No success in hardening
9	*Coleus forskohlii*	½ MS + 1 BAP	1	Established in field
10	*C. zeylanicus*	MS BASAL	1	Established in pot
11	*Curculigo orchioides*	MS BASAL	4	Established in pot
12	*Cyclea peltata*	½ MS + 2 BAP	2	No success in hardening
13	*Decalepis hamiltonii*	MS + 0.5 BAP	2	No success in hardening
14	*Dioscorea bulbifera*	MS BASAL	2	Established in field
15	*Hemidesmus indicus*	½ MS + 0.5 BAP	2	No success in hardening
16	*Holarrhena antidysenterica*	½ MS 1 BAP	2	Established in field
17	*Lippia nodiflora*	½ strength and full strength MS	2	Established in field
18	*Rauvolfia serpentina*	½ MS + 2 BAP	2	Established in field
19	*Ocimum*	MS BASAL	1	Established in pot
20	*Vitex negundo*	½ MS + 2 BAP	2	Established in field
21	*Tylophora indica*	½ MS + 1 BAP	4	Established in field
22	*Wedelia chinensis*	½ strength and full strength MS	2	Established in field

jecting them to ultra low temperature in the presence of cryoprotectants. The initial and most important step of cryopreservation is dehydration of the explant to reduce the water content to prevent freezing injury. There are two types of cryopreservation: one is classical technique, which involves freeze-induced dehydration, while new techniques are based on vitrification. Classical freezing includes pregrowth of samples, cryoprotection, slow cooling (0.5–2 °C/min) to a determined prefreezing temperature (usually around −40 °C, rapid immersion of samples in liquid nitrogen (LN), storage, rapid thawing and recovery. Classical cryopreservation techniques have been successfully applied to undifferentiated culture systems such as cell suspensions and calluses (Kartha and Engelmann 1994; Withers and Engelmann 1998) and apices of cold-tolerant species (Reed and Uchendu 2008). The second type of cryopreservation is vitrification-based procedures like desiccation, encapsulation-dehydration, vitrification, encapsulation-vitrification, droplet freezing, etc. Here, cell dehydration is performed by exposure of samples to concentrated cryoprotective media and/or air desiccation. This is followed by rapid cooling. Vitrification-based procedures are used mainly for organized tissues like shoot tips and embryos.

When compared with crop plants, only limited studies are conducted on the cryopreservation aspects of rare and endangered species. Low temperature storage has been reported to be effective for cell cultures of medicinal and alkaloid producing plants such as *Rauvolfia serpentina*, *Digitalis lanata*, *Atropa belladonna* and *Hyoscyamus spp* (Bajaj 1988). Two cryopreservation techniques (encapsulation-dehydration and encapsulation-vitrification) were applied for in vitro conservation of *Ziziphora tenuior* L, a rare species with a promising medicinal potential that grows wild in the southern part of Jordan. In the encapsulation-dehydration experiment, the results revealed that 40% of the cryopreserved shoot tips survived when they were dehydrated chemically on 0.75 M sucrose in MS supplemented media for 1 day and exposed to air dehydration for 6 h. In the encapsulation-vitrification experiment, the highest survival (37.5%) and recovery (10%) percentages of the cryopreserved shoot tips were obtained when the encapsulated shoot tips were pretreated for 60 min with the loading solution before being exposed to PVS_2 vitrification solution and LN. Sharma and Sharma (2003) demonstrated the successful cryopreservation of shoot tips of *Picrorhiza kurroa,* an endangered medicinal plant, using the vitrification technique. The protocol involved the preculture of shoot tips at 4 °C for 2 days on hormone-free MS medium containing 5% DMSO followed by immersing in PVS_2 solution for 15 min at 0 °C. After vitrification, the shoot tips are directly immersed in liquid nitrogen. The protocol enabled to conserve this threatened species for a long period of time. Using encapsulation-dehydration technique, shoot tips of the rare and endangered species *Cosmos atrosanguineus* was successfully cryopreserved with 100% survival and 35% shoot regeneration (Wilkinson et al. 2003). In vitro shoot tips of *Dioscorea deltoidea* Wall., an endangered medicinal plant, were successfully cryopreserved by Mandal and Dixit (2007) using the vitrification and the encapsulation-dehydration techniques with subsequent high-frequency plant regeneration. Using vitrification regeneration up to 83%, regeneration was recorded, while using encapsulation-dehydration, the highest regeneration frequency recorded was 76% . Study showed that the cryopreserved shoot tips maintained their viability and an unaltered level of regeneration capability after up to 1 year of storage in LN.

Suk et al. (2009) studied cryopreservation of adventitious roots of *Panax ginseng*, the source of commercially produced ginsenosides using desiccation and vitrification technique. When only desiccation was applied, the survival was <14% regardless of the composition of the preculture medium or the explant origin. Callus formation was frequently observed after cryopreservation. In contrast, vitrification showed 90% survival and 32.5% root formation efficiency after cryopreservation. These cryopreserved root tips were used to re-establish adventitious root cultures in flasks and bioreactors. During the initial phase, lower biomass production was recorded as compared to the control after fourth subculturing. However, biomass accumulations did not differ between control and regenerated roots at the end of the sixth subculturing period. Production of triol and diol ginsenosides in the bioreactor cultures was also enhanced after cryopreservation, by 41.0% and 89.8%, respectively. These results suggested that the vitrification method is successful for cryopreservation of *Panax ginseng* adventitious roots.

6.4 Bioactive Metabolite Profiling for Conservation of High-Value Threatened Medicinal Plants

Medicinal plants are valued for their bioactive metabolites which are used as a source of drugs by the traditional healers and pharmaceutical companies. This high demand has threatened the existence of many important medicinal plants due to their indiscriminate harvesting. Conservation of such commercially important species is necessary to ensure sustainable supply of bioactive compounds to the drug industry. Biochemical analysis of medicinal plants for identifying superior germplasm for large-scale cultivation has been a successful strategy for the conservation of many threatened commercially important medicinal plants (Venkatasubramanian et al. 2018). Encouraging cultivation of such plants with high-value bioactive compounds is an important step in conservation action. Bioactive metabolite profiling will help to identify the major active ingredients present in the species, its quantity and quality and thus identifies the superior germplasm. Many a times, the plant part harbouring the active content will be essential for the survival of the plant like the roots or rhizomes or bark or whole plant; in such case, biochemical analysis helps to identify other plant parts or species or sources that have the same bioactive molecules and, therefore, can be used as substitutes. In a study for formulating conservation strategy for threatened medicinal plants by Venkatasubramanian et al. (2018), bioactive metabolite profiling of six threatened medicinal plant species, viz. *Aconitum balfourii*, *Aconitum heterophyllum*, *Podophyllum hexandrum*, *Picrorhiza kurroa*, *Berberis aristata* and *Embelia ribes*, was taken up. The study helped to identify the superior populations of each species in terms of bioactive compound and thus helped in prioritization of the genotypes for conservation. The approach proved to be effective for bringing back the species from the verge of extinction and plant cell cultures can be initiated as alternative for controlled production and supply of secondary metabolites, when the phytochemical of interest is known.

6.4.1 Plant Cell Cultures

When an important biochemical compound is found in the endangered or threatened medicinal plant, intensive cell culture is a practical alternative to wild collections of such plant material. It is believed that any substance of plant origin can be produced by cell cultures. Thus, it should be possible to achieve the synthesis of a wide range of compounds such as alkaloids, flavonoids, steroids, terpenoids, glycosides, etc., i.e. a total of several hundreds with complex chemical structures using plant cell culture technology (Smetanska 2008). Plant cell tissue culture is carried out with unorganized cells that are present as suspensions of individual cells, loose aggregates or sometimes as an immsbi1ized mass of cells (Scott and Dougall 1987). Callus cultures provide new means for the production of secondary metabolites on a commercial scale even from the rarest plants (Vanisree et al. 2004). There has been considerable interest in plant cell culture as a potential alternative to traditional agriculture for the industrial production of secondary metabolites (Dicosmo and Misawa 1995). The amount of secondary metabolites produced by plant cell cultures can be even higher than in the parent plants (Rao and Ravishankar 2002). Plant cell cultures have the following advantages over conventional agricultural production, viz. independent of geographical and environmental variations, defined production system with continuous supply of products, uniform quality, yield, rapidity of production and production of novel compounds. In recent years, various plant cell culture systems have been exploited for the enhancement of high-value metabolites (Rao and Ravishankar 2002). Callus culture has been used in number of medicinal plants to harvest different types of secondary metabolites including alkaloids, saponins, flavonoids and terpenes (Andrijany et al. 1999). In a study conducted by Kumar et al. (2014) in *Swertia chirayita*, it was found that the amount of secondary metabolites was found significantly higher in in vitro plantlets and callus compared to in vivo plantlets. They correlated higher heavy metal accumulation and secondary metabolite production in in vitro as compared to in vivo plantlets supporting that they play regulatory role in influencing the plant secondary metabolism. In *Rauwolfia serpentine* Shetty et al. (2014) developed in vitro method for induction of callus and hairy roots from explants to produce secondary metabolites. Hairy roots were induced from leaf explants and these leaf explants were infected with *Agrobacterium rhizogenes* to induce hairy roots for the production of secondary metabolites in large scale. Taxol (plaxitaxol) obtained from the bark of the Taxus tree is one of the most promising anticancer agents known due to its unique mode of action on the micro tubular cell system. Owing to the enormous commercial demand of taxol, the scarcity of Taxus tree and the costly synthetic process (Cragg et al. 1993; Suffness 1995), production of Taxol by cell cultures is one of the most explored areas of plant cell cultures. Fett-Neto et al. (1995) have studied the effect of nutrients and other factors on paclitaxel production by *T. cuspidata* cell cultures (0.02% yield on dry weight basis). Srinivasan et al. (1995) have studied the kinetics of biomass accumulation and paclitaxel production by *T. baccata* cell suspension cultures. Parc et al. (2002) reported production of taxoids by callus cultures from selected Taxus genotypes. Factors influencing stability and recovery of paclitaxel from suspension cultures and the media have been studied in detail by Nguyen et al. (2001).

6.5 DNA Banking

DNA banking is a potential method for the conservation of genetic resources by way of conserving their genomic DNA or conserving the plant tissue for extracting the genomic DNA at low temperatures. DNA conservation is useful for those species that cannot be conserved using traditional in situ and ex situ approaches. For many species like vegetatively propagated plants as well as endangered and threatened group of plants which are at high risk in the wild, DNA storage may prove useful for conserving the genetic diversity of their populations in the short term. The approach to store plant tissues and genomic extracts is suitable for enabling the storage of large numbers of samples securely, efficiently and cheaply (Brown et al. 1997). The stable nature of DNA in cold storage adds to the advantage of DNA banking. The availability of stored DNA can help to develop conservation focused studies on the extremely rare and endangered species. For species that have declined since the collection of DNA material, the available DNA samples will allow access to information on pre-decline levels of genetic diversity (Rabiya 2000). A DNA bank makes readily available the raw material for molecular research. Conservation of DNA ensures that the complete genetic information about the species or the family is not lost even if the species becomes extinct. The implementation of this technology on rare and endangered plant species may help in revival of their previous genes and their products which have been disappeared or inactivated in natural habitat. The main limitation of DNA bank with respect to conservation is that whole plants cannot be directly reconstituted from DNA. The genetic material must first be introduced artificially, through transformation or transduction using plasmids or liposomes, back into somatic cells that can then be grown into whole plants in in vitro culture.

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Chapter 7
In Vitro Multiplication and Conservation of Threatened Medicinal Plants of Western Ghats of South India

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Abstract Propagation of medicinal plants today is a promising alternative and counterpoint to wild collection, enabling preservation of natural genetic variability and survival of rare, endemic and endangered species, and it also provides quality raw material for pharmaceutical industries. Biotechnological methods like in vitro propagation technique hold tremendous potential for the production of high-quality plant-based medicines, which is an effective tool to conserve plant genes and guarantee the survival of the desired genotype, emphasised to make use of small units (cells and tissues) without losing their mother plant, thereby taking the pressure off from the waning wild populations and deriving a large number of plants in a very short time. Micropropagation protocols have worked out for many plant species cultured in vitro to provide macro – and micro-mineral nutrients, vitamins, source of carbohydrates under appropriate environmental conditions (light intensity, photoperiod and temperature) and plant growth regulators required to obtain high regeneration rates. In addition to the in vitro regeneration, germplasm conservation, reinforcement of genetic diversity and eco-rehabilitation of the waning medicinal plant taxa, it is very important to conserve and augment the resource supply. This chapter offers a brief insight into the status of micropropagation and mass multiplication strategies of elite genotypes, zygotic embryo cryopreservation of medicinal tree species and exploitation and utilisation of this technology for the conservation and ecorestoration of threatened or over-exploited medicinal plants in the tropical and subtropical regions of the Western Ghats, India.

Keywords In vitro · Conservation · Threatened medicinal plants · Multiplication

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Abbreviations

Auxin	Plant growth regulator assembling IAA in physiological activity
Axenic	Aseptic
BA	Benzylaminopurine
Callus	Disorganised meristematic or tumour-like mass of plant cells
Cytokinin	Plant growth regulator stimulating cell division and resembling kinetin in physiological activity. Mainly N_6 substituted aminopurine compounds
Explant	Excised fragment of plant tissue or organ used to initiate a tissue culture
IAA	Indole-3- acetic acid
IBA	Indole-3-butyric acid
JNTBGRI	Jawaharlal Nehru Tropical Botanic Garden and Research Institute
Meristem	Apical meristem culture; explant consisting only of apical dome tissue distal to the youngest leaf primordium
MS	Murashige and Skoog (1962) medium
MSL	*Mean* sea level
PGRs	Plant growth regulators
RET	Rare, endangered and threatened (RET) plants
NAA	Naphthalene acetic acid
SH	Schenk and Hilderbraandt (1972) medium

7.1 Introduction

The World Health Organization has estimated that more than 80% of the world population in developing countries depends primarily on herbal medicine for basic health care (Vines 2004; Peter et al. 2005; Krishnan et al. 2011), which accelerates the growth of herbal medicines in developed countries also. Subsequent global preference towards herbal medicine has advanced the expansion of plant-based pharmaceutical industries. Approximately two-thirds of the different medicinal plant species in use are collected from the wild, and in India, only 10% of medicinal species used commercially are cultivated. There is a growing concern about diminishing populations, loss of genetic diversity, extinctions and habitat degradation. Overexploitation and/or destructive harvesting to meet such demands, in fact, threatens the survival of many rare species (Krishnan et al. 2011; Tasheva and Kosturkova 2010). Confronted by such unprecedented genetic erosion and disappearance of species and ecosystems, conservation of natural resources assumes paramount urgency. In this perspective, micropropagation/in vitro clonal propagation techniques using shoot tip and nodal segments are indispensable to achieve mass multiplication and conservation of an endangered or threatened medicinal plant species within short period and limited space.

The interest in in vitro mass propagation of medicinal plants has distinctly increased as the method involves only organised meristems, allowing the recovery of genetically stable and true-to-type progenies, which is a major boon over the conventional methods of propagation. The advantages of micropropagation in medicinal taxa described by many authors (Krishnan et al. 2011; Eric et al. 2011; Sarasan et al. 2011; Mathe et al. 2015) are as follows: (i) In general, clonally propagated plants will have identical phytochemical profile independent of regional or seasonal variations. (ii) In many species, in vitro derived plantlets produced higher amount of desired compound than the normal plants. (iii) Usually multiple shoot cultures show stability of growth and secondary metabolite production characteristic to mature plants. (iv) In vitro shoots are used in the large-scale production of secondary metabolites. (v) In vitro shoots are also used for the long-term conservation and exchange of plant genetic resources.

It is also recommended to clone sufficient number of propagules collected from one source population to copy maximum genetic diversity (McGlaughlin et al. 2002), ensuring a self-sustained population of endangered species with full genetic diversity which is essential to salvage them from extinction (Falk et al. 2001; Eric et al. 2011; Sarasan et al. 2011). This route is seldom preferred by conservationists of India, but Jawaharlal Nehru Tropical Botanic Garden and Research Institute is one of the pioneer institutions to experiment with biotechnology-mediated curation of the waning medicinal plant taxa, which are employed over several countries in traditional system of medicine and in modern pharmaceutical industry through micropropagation, cryopreservation and recovery of the same through reintroduction into selected forest segments of the Western Ghats, India, thereby conserving and augmenting the resource supply.

7.2 In Vitro Propagation of Medicinal Plants Through Organogenesis

The development of reliable in vitro protocols is of great importance for conservation of threatened species by virtue of producing uniform planting material for offsetting the presence on the natural populations especially for medicinal plants. Application of both embryo and tissue culture facilitates rescuing the target species from the brink of extinction and establishment of viable populations in nature, contributing to eventual removal of them from the Red list. In vitro propagation protocols have been established for several thousand plant species, and many authors have reported encouraging results of plant regeneration from shoot tip and axillary meristems in medicinal plants like *Catharanthus roseus, Cinchona ledgeriana* and *Digitalis* spp., *Rehmannia glutinosa*, *Isoplexis canariensis* (Paek et al. 1995; Perez-Bermudez et al. 2002), *Oroxylum indicum* (Dalal and Rai 2004), *Ginkgo biloba* (Tommasi and Scaramuzzi 2004), *Curcuma longa* (Prathanturarug et al. 2003), *Dendrobium candidum* (Shiau et al. 2005), *Curcuma zedoaria* (Loc et al. 2005), *Murraya koeningii* (Rout 2005b), *Euphorbia nivulia* (Martin et al. 2005), *Clitoria ternatea* (Rout 2005a), *Tylophora indica* (Faisal et al. 2007) *Decalepis arayalpathra* (Sudha et al. 2005), *Tinospora cordifolia* (Raghu et al. 2006; Gururaj et al. 2007), C*urculigo orchioides* (Bhavisha and Jasrai 2003; Francis et al. 2007), *Glycyrrrrhiza glabra* (Vadodaria et al. 2007), *Swertia chirata* (Balaraju et al. 2009), *Picrorhiza kurroa* (Sood and Chauhan 2009), *Momordica tuberosa* (Aileni et al. 2009), *Withania coagulans* (Jain et al. 2009), *Ceropegia spiralis* (Murthy et al. 2010), *Aloe vera* (Singh and Sood 2009), *Aristolochia indica* (Soniya and Sujitha 2006), *Aristolochia tagala* (Animesh et al. 2007), *Rauvolfia serpentina* (Baksha et al. 2007), *Asparagus racemosus* (Nishritha and Sanjay 2008), *Vitex negundo* (Noman et al. 2008), *Baliospermum montanum* (Sasikumar et al. 2009), *Utleria salicifolia* and *Hemidesmus indicus* (George et al. 2010) and *Rubia cordifolia* (Radha et al. 2011), *Echinops spinosissimus* (Pan et al. 2003), *Elettaria cardamomum* (Nadganda et al. 1983; Bajaj et al. 1993), *Eleutherococcus koreanum* (Park et al. 2005), *Garcinia indica* (Malik et al. 2005), *Gloriosa superba* (Arumugam and Gopinath 2012), *Gynura procumbens* (Chan et al. 2009), *Hoslundia opposita* (Prakash and Van Staden 2007), *Hypericum perforatum* (Danova et al. 2012; Savio et al. 2012), *Labisia pumila* (Hartinie and Jualang 2007), *Leptadenia reticulata* (Kalidass et al. 2008), *Mollugo nudicaulis* (Nagesh and Shanthamma 2011), *Ornithogalum ulophyllum* (Ozel et al. 2008), *Ocimum gratissimum* (Gopi et al. 2006), *Peganum harmala* (El-Tarras et al. 2012), *Phyllanthus urinaria* (Kalidass and Mohan 2009), *Picrorhiza kurroa* (Jan et al. 2010), etc.

Micropropagation using seedling shoot culture has also been reported in *Camptotheca acuminata* (Liu and Li 2001), *Helleborus niger* (Seyring 2002), *Ophiorrhiza mungo* (Jose and Satheeshkumar 2004), *Origanum sipylum* (Oluk and Ali 2009) *Quercus semecarpifolia* (Sushma et al. 2008) and *Psidium guajava* (Shah et al. 2008), etc.

A number of reviews have been published on micropropagation, in vitro production of secondary metabolites and on field cultivation of medicinal plants; however, they do not provide the pragmatic standing of the protocol and scale-up production of plants that demonstrates the pilot-scale cultivation or continuous survival in the field. During the last two decades, various medicinal plants in threatened category which currently has high demand in pharmacuetical sectors have been sucessfully propagated and re-established in JNTBGRI by means of media optimization with supplementation of plant growth regulators and sucessful field establishment. Different regeneration pathways such as somatic embryos, callus-mediated shoot regeneration, direct regeneration without callus phase or with different explant sources including axenic seedlings were critically analysed in different species (Table 7.1) like Rauvolfia serpentina*, Rauvolfia micrantha, Justicia gingiana, Celastrus paniculatus, Trichopus zeylanicus, Nothapodytes nimmoniana, Decalepis arayalpathra, Piper barberi, Piper trichostachyon, Utleria salicifolia, Aristalochia tagala, Holostemma ada–kodien, Anaphyllum wightii, Coleus forskohlii,* Kaempferia galanga*,* Helminthostachys zeylanica *and Baliospermum montanum,* etc., to get optimum shoot multiplication (Fig. 7.2a–h), in vitro rooting and successful field establishment (Krishnan et al. 2011). Scale-up production and pilot-scale

Table 7.1 In vitro propagation protocols standardised in medicinal plants of the Western Ghats, India

Species (family)	Biome	Explant	Shoot proliferation medium with PGRs	Rooting medium and percentage establishment (%)	Explant and medium for subculture/mass multiplication
[a]*Mahonia leschenaultii* (Berberidaceae)	Palani Hills, Kodaikanal	Shoot tip/node	SH + 1BA + 0.02IAA	MS + 1 IBA (72)	Node MS + 0.5BA + 0.01IAA
[a]*Heracleum candolleanum* (Apiaceae)	Peerumedu (Kerala) and Palani Hills (Kodaikanal)	Shoot tip/node	MS+ 1BA	MS + 1 IBA (77)	Shoot tip/node MS + 0.5BA
[a]*Acorus calamus* (Acoraceae)	Prakashapuram (Kodaikanal) and Munnar (Kerala)	Rhizome with axillary bud	MS + 1BA + 0.5NAA	MS + 1 IBA (90)	Axillary bud/rhizome bud MS + 0.5BA + 0.2NAA
Kaempferia galanga (Zyngiberaceae)	Kallar Reserve Forest, Trivandrum (Kerala)	Rhizome With axillary buds	MS + 1BA+ 0.1NAA	0.2 IBA (85)	Axillary bud/rhizome bud MS + 1BA + 0.1NAA
[a]*Rubia cordifolia* (Rubiaceae)	Karadipara, Munnar (Kerala)	Shoot tip/node	MS + 1BA + 0.5IAA	1 IBA (84)	Node/shoot tip MS + 0.5BA
Coleus forskohlii (Lamiaceae)	Salem, Tamil Nadu	Shoot tip/node	MS + 1BA	1 IAA (98)	Node MS + 0.5BA
Rauvolfia serpentina (Apocynaceae)	Kanyakumari, Nilgiri Hills (Tamil Nadu)	Shoot tip/node	MS + 0.5BA + 0.1IAA	MS+ 1IBA (80)	Node/shoot tip MS + 0.5BA + 0.1 IAA
Rauvolfia micrantha (Apocynaceae)	Kanyakumari, Nilgiri Hills (Tamil Nadu)	Shoot tip/node	MS + 1BA + 0.5INAA	MS+ 1IBA (90)	Node/shoot tip MS + 0.5BA + 0.1 IAA
Justicia gingiana (Acanthaceae)	Malapuram, Thiruvananthapuram (Kerala), Coimbatore, Kanyakumari (Tamil Nadu)	Shoot tip/node	MS + 1BA + 0.2 IAA	MS + 0.5 IBA (95)	Node MS + 1BA + 0.2IAA

(continued)

Table 7.1 (continued)

Species (family)	Biome	Explant	Shoot proliferation medium with PGRs	Rooting medium and percentage establishment (%)	Explant and medium for subculture/mass multiplication
Curcuma longa (Zyngiberaceae)	All districts in Kerala	Rhizome with axillary bud	MS + 3BA	Rooting was spontaneous in all the treatments (99)	Shoot tip/node MS + 1BA
Helminthostachys zeylanica (Ophioglossaceae)	Kannur, Malappuram, Thiruvananthapuram (Kerala)	Rhizome bud	WPM + 1 BA	WPM + 1 BA (69)	Shoot bud WPM + 1 BA
[a]*Myristica malabarica* (Myristicaceae)	Kuzhathupuzhaand Sendurnai forests, Kerala	Shoot tip/ node	MS + 1BA + 0.2 NAA	MS + 0.5 IBA (75)	Node/MS +1BA
Curcuma aromatica (Zyngiberaceae)	Palakkad, Kasaragode, Wayanad, Thrissur, Pathanamthita, Kollam, Idukki, Thiruvananthapuram, Kozhikode (Kerala)	Rhizome with axillary bud	MS + 3BA	Rooting was spontaneous in all the treatments (99)	Shoot tip/node MS +1BA
Trichopus zeylanicus (Dioscoraceae)	Southern Western Ghats, Kollam, Thiruvananthapuram (Kerala)	Rhizome with axillary bud	MS + 2BA + 0.5NAA	MS + 0.5 IBA (80)	Node MS + 0.5BA
Celastrus paniculatus (Celastraceae)	Palakkad, Idukki, Malapuram, Kannur, Thrissur, Wayanad, Kozhikode (Kerala)	Shoot tip/ node	MS + 1BA	MS + 0.2 IBA (90)	Node MS + 0.5BA
Nothapodytes nimmoniana (Icacinaceae)	Idukki (Kerala)	Shoot tip/ node	MS + 1BA	MS + 0.2 IBA (90)	Node MS + 0.5BA
Decalepis arayalpathra (Periplocaceae)	Kallar reserve Forest, Bonacaud Forest (Kerala)	Shoot tip/ node	MS + 0.5BA	MS + 1IBA (80)	Node MS + 0.5BA
Piper barberi (Piperaceae)	Southern Western Ghats, Palakkad, Thiruvananthapuram, Idukki, Kollam, Thrissur, Wayanad	Shoot tip/ node	MS + 1BA	MS + 0.5BA (89)	Node MS + 0.5BA

Species (family)	Biome	Explant	Shoot proliferation medium with PGRs	Rooting medium and percentage establishment (%)	Explant and medium for subculture/mass multiplication
Piper trichostachyon (Piperaceae)	Palakkad, Idukki, Pathanamthitta, Kollam, Wayanad (Kerala)	Shoot tip/node	MS + 1BA	MS + 0.5BA (85)	Node MS + 0.5BA
Utleria salicifolia (Periplocaceae)	Southern Western Ghats, Palakkad, Idukki (Kerala)	Shoot tip/node	MS + 0.5BA	MS + 1IBA (80)	Node MS + 0.5BA
Baliospermum montanum (Euphorbiaceae)	All districts in Kerala, Coorg, Chikmagalur, Karnataka	Shoot tip/node	MS + 0.5BA	MS + 1IBA (80)	Node MS + 0.5BA
Holostemma ada-kodien (Asclepidaceae)	All districts in Kerala	Shoot tip/node	MS + 0.5BA	MS + 1IBA (90)	Shoot tip/node MS + 0.5BA

[a]Reintroduced in the natural forest segments of the tropical and subtropical regions of Southern Western Ghats, India

cultivation trials of mericlones regenerated from rhizome bud/axillary bud explants were explored (Krishnan et al. 2011) in rhizomatous plants like *Curcuma longa, Curcuma aromatica, Kaempferia galangal* and *Acorus calamus* (Fig. 7.3). Different species (*Mahonia leschenaultii, Heracleum candolleanum,* Acorus calamus*, Rubia cordifolia and Myristica malabarica*), which are employed over several countries in pharmaceutical industry, are also critically examined by the author for conservation through micropropagation and recovery of the same through reintroduction into selected forest segments of the Western Ghats, India.

Quick and large-scale production of clonal plants through in vitro regeneration of single node/shoot tip/ axillary bud explants, and its subsequent shoot proliferation obtained in *M. leschenaultii, H. candolleanum, A. calamus, R. cordifolia and M. malabarica* can be portrayed as the best example of how in vitro protocols increase the rate of multiplication over hundred-fold in comparison to the conventional methods. This eases the ways of obtaining explants for deliberate establishments of species into an area and or habitat where it has become extirpated (Tables 7.1 and 7.2).

Successful in vitro regeneration procedure in *Mahonia leschenaultii Nutt.*, an endemic small tree of the Western Ghats with excellent source of berberine having antitumour properties, was achieved through multiple axillary shoot formation in single node cultures. A synergistic combination of 1.0 mgl^{-1}BA and 0.02 mgl^{-1}IAAin Schenk and Hildebrandt (SH) induced the maximum number (5.9) of axillary shoot formation which were relatively high (75%) when the explant collected during May–June and fifth node from the top of the growing shoots were used. Repeated subculture of the nodes from shoot cultures at 5–6 week intervals in medium supplemented with reduced concentrations of the growth regulators (0.5mgl^{-1} BA, 0.01 mgl^{-1} IAA) through at least 10 passages enabled consistent production of 6–7 shoots (Fig. 7.1a) per node at 92% success rate without loss of vigour, growth and morphological abnormalities. Shoots of 3–6 cm were rooted in vitro in the presence of 1.0 mgl^{-1} IBA (Fig. 7.1b) and hardening in the mist house at 76–78%, and this rooted plants were established in a potting medium of river sand and top soil (1:1) under constant mist irrigation. The plants reared in the nursery for 5–8 weeks were successfully transferred into the natural forest segment of the institute's campus (MSL 200 m) revealed an establishment frequency of 78.75 after 18 months (Radha

Table 7.2 Medicinal plants of the Western Ghats micropropagated through direct shoot regeneration with experimental trials conducted for restoration/translocation in forest habitats

Species	Establishment in native/alien localities (%)	Observed period (months)
Acorus calamus	90/85	36
Heracleum candolleanum	85/90	48
Mahonia leschenaultii	80/75	24
Rubia cordifolia	90/85	36
Myristica malabarica	90/80	24

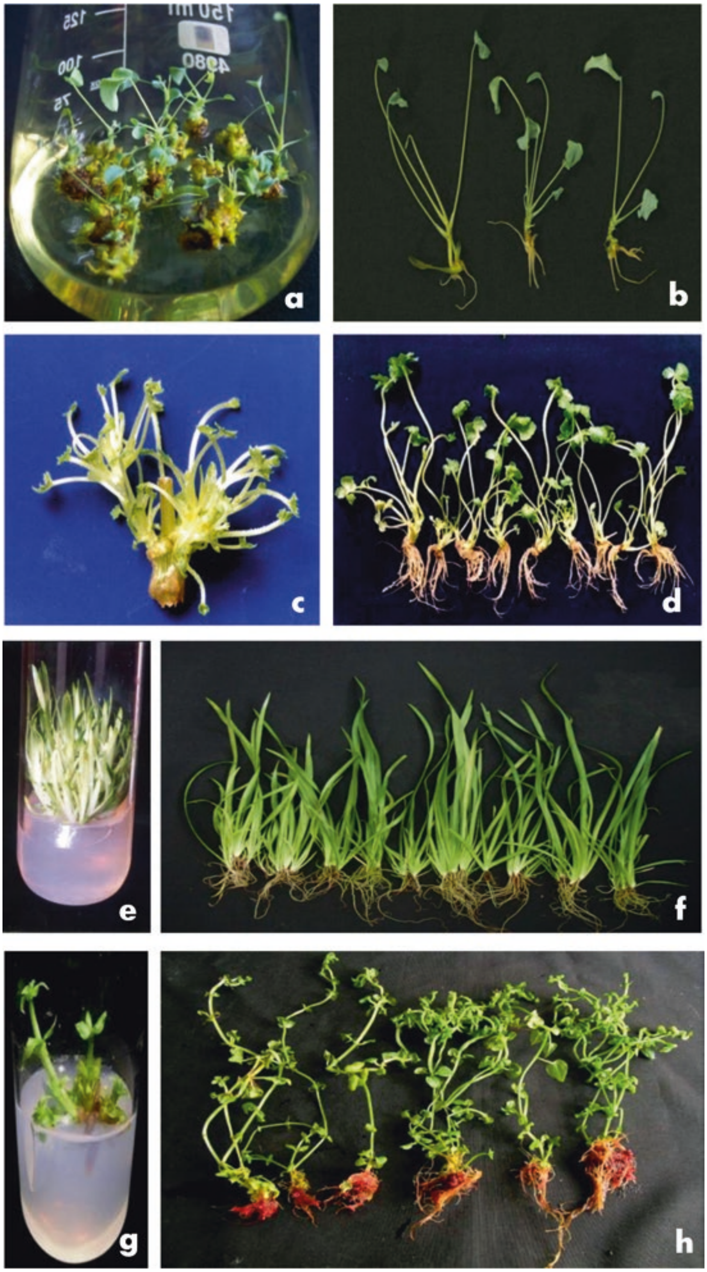

Fig. 7.1 In vitro shoot proliferation and rhizogenesis in threatened medicinal plants of the Western Ghats, India. (**a**) Shoot proliferation from the nodal explants (SH + 0.5 BA and 0.01 IAA) in *M. leschenaultii*. (**b**) Rooted meiclones of *M. leschenaultii* (SH medium + 1 IBA). (**c**) Shoot proliferation from the nodal explants (MS + 1 BA) in *H. candolleanum*. (**d**) Rooted mericlones of *H. candolleanum* (MS medium + 1 IBA), (**e**) Shoot proliferation from the nodal explants (MS + 1 BAP and 0.5 NAA) in *A. calamus*. (**f**) Rooted mericlones of *A. calamus* (MS medium + 1 IBA). (**g**) Shoot proliferation from the nodal explants (MS +1 BA and 0.2 IAA) in *R. cordifolia*. (**h**) Rooted mericlones of *R. cordifolia* (MS + 1 IAA)

et al. 2013). Conventional vegetative propagation of this small tree distributed along the margins in high-altitude evergreen forests between 1600 and 2400 m in the southern Western Ghats is slow while outright clearing of the natural stands due to increased human inhabitation and conversion into hill crop areas especially in the Palani Hills and Nilgiri Hills of Western Ghats posing danger to its survival. Perusal of the literature also revealed very little information on tissue culture of this species, though in vitro propagation of berberine-rich *Berberis thunbergii* (Karthu and Hakala 1991), high berberine-producing cells of *Coptis japonica* (Sato and Yamada 1984) and bioproduction of berberine in callus tissues of *Thalictrum minus* (Ikuta and Hokawa 1982) and cell cultures of *Coscinium fenestratum* (Nair et al. 1992) are reported. The ready availability of micropropagated systems as demonstrated in *M. leschenaultii* may spur economic cultivation of the species for future industrial raw material supply, if it is developed as an economic crop for the extraction of berberine.

High-frequency microcloning of *Heracleum candolleanum* (wight&Arn.) Gamble., an important medicinal plant endemic to India with limited geographical distribution recorded across the Western Ghats of Karnataka (Bababudan Hills of Chikmagalur), Kerala (Peermade) and Tamil Nadu (Palani Hills in an altitude range of 1500–2300 m) regions of southern India and considered vulnerable/global, was established through callus-free axillary meristem cultures on Murashige and Skoog (MS) medium supplemented with cytokinin alone (1.0 mgl^{-1} BA); a maximum of 9.8 shoots (Fig. 7.1c) were formed in the nodal explants. Shoots were multiplied by routine periodic subcultures through 6-week intervals and 1.0 mgl^{-1} IBA favoured the development of 4.24 roots within 5 weeks of culture (Radha 2011) and rooted plants of *H. candolleanum* preferred a mixture of river sand, soil and farmyard manure (1:1:1). Micropropagated plantlets transplanted into forest segments in the institute's campus (MSL 200 m) followed by their growth characteristics free of abnormalities confirm their utility in conservation through revegetation of the denuded forest segments in the Western Ghats.

In the process of efficient shoot proliferation from axillary bud explants of *Acorus calamus* L., (vulnerable, semi-aquatic perennial) a combined influence of BA and IAA (Fig. 7.1e), 13.9 resulted in the production of shoots after an incubation of 30 days. Each bud thus raised rooted profusely (~14 roots with 80%) in medium supplemented with 1.0 mgl^{-1} (Fig. 7.1f) of any of the said auxin type (IBA,IAA,NAA) to produce 13 plantlets. On recurrent subculture, fresh flush of shoots raised more than 15 plants after every 30 days from the mother culture, resulting in the stocking of approximately 115 plants (Fig. 7.3) at the end of the first subculture in contrast to the published results (Rani et al. 2000; Anu et al. 2001). Formation of aromatic rhizome was first noticed in the 10 months after field transfer and then onwards rhizome continued to grow under the soil in length and breadth simultaneously producing aerial leaves from the nodes. The repeated cultivation of rhizomes of shoots at 8–9 month intervals in specially prepared bed of soil and mud (5 × 5 m) favoured profusion of shoots and production of rhizome, the useful part of the plant containing essential oil; these processes would be better achieved through bulk supplies of propagules for planting in diverse localities through biotechnology-mediated multiplication than by conventional means. This is despite the fact that agro technology for cultivation of *Acorus calamus*is already developed using rhizome cuttings. Demand for Sweet flag in the world market is growing in pharmaceutical industry as production of syrups, balms and medicated candies; it is also used in combination with Basil, Brahmi and other herbs as popular health supplement for memory booster, immunity enhancer and tonic, and its smell makes calamus essential oil valued in the perfume industry.

The highly traded medicinal plant *Rubia cordifolia* Linn. (Manjishtha/Indian Madder) contains substantial amounts of anthraquinone especially in the roots; plants distributed sparsely in the lower hills of Indian Himalayas in the North and Western Ghats in the south showed remarkably efficient in vitro shoot regeneration and rooting capacity, both of which are significantly influenced by the varying concentrations of the different plant growth regulators. The optimum number of shoots obtained was 5.9 and 5.2 per explant in 2 weeks on the medium supplemented with 1mgl^{-1}BA and 0.02 mgl^{-1} IAA in nodes (Fig. 7.1g) and split vertical halves of the node, respectively. Shoot multiplication was rapid and consistent for four subcultures with 0.5mgl^{-1}BA. The best root induction (98%) and survival was achieved on 1 mgl^{-1} IBA followed by 1 mgl^{-1} IAA (Fig. 7.1h). Micropropagated plants displayed normal phenotypes in ex situ conditions with 89% survival. These plantlets can be used to replenish declining populations in the wild, for the extraction of bioactive compounds and reducing pressure on wild stocks (Radha et al. 2011).

Nodal explants of germinated axenic seedlings of *Myristica malabarica* Lam., a threatened tree species, when introduced into half MS medium with 1.5 mgl^{-1} BA and 0.2 mgl^{-1} NAA with activated charcoal (1 gm) induced multiple shoot formation (Table 7.1). Sprouting of axillary buds on the lower nodes (mature nodes) of the seedlings was obtained with the addition of 5 gl^{-1} adenine sulphate. Supplementation of the medium with auxin was essential for rooting of adventitious shoots (1.0 mgl^{-1} IBA). More importantly, the investigations prove beyond doubt the efficacy of shoot regeneration from axillary bud explants of plants raised from the seedlings and zygotic embryos with cotyledons and successful field establishment (90%) (Figs. 7.2 and 7.3).

7.2.1 Conservation Through Micropropagation and Ecorestoration

The establishment of a plant species as a stable component of a plant community is widely regarded as the most desirable process of species conservation and will be achieved only through reintroduction of micropropagated plants into its native habitat. Many authors (Wochok 1981; Maunder 1992; Fay 1992, 1994; Frankel et al. 1995; Wyse and Sutherland 2000; Eric et al. 2011) have emphasised the importance of this critical requirement for rare plant conservation. Falk et al. (1996, 2001) stress

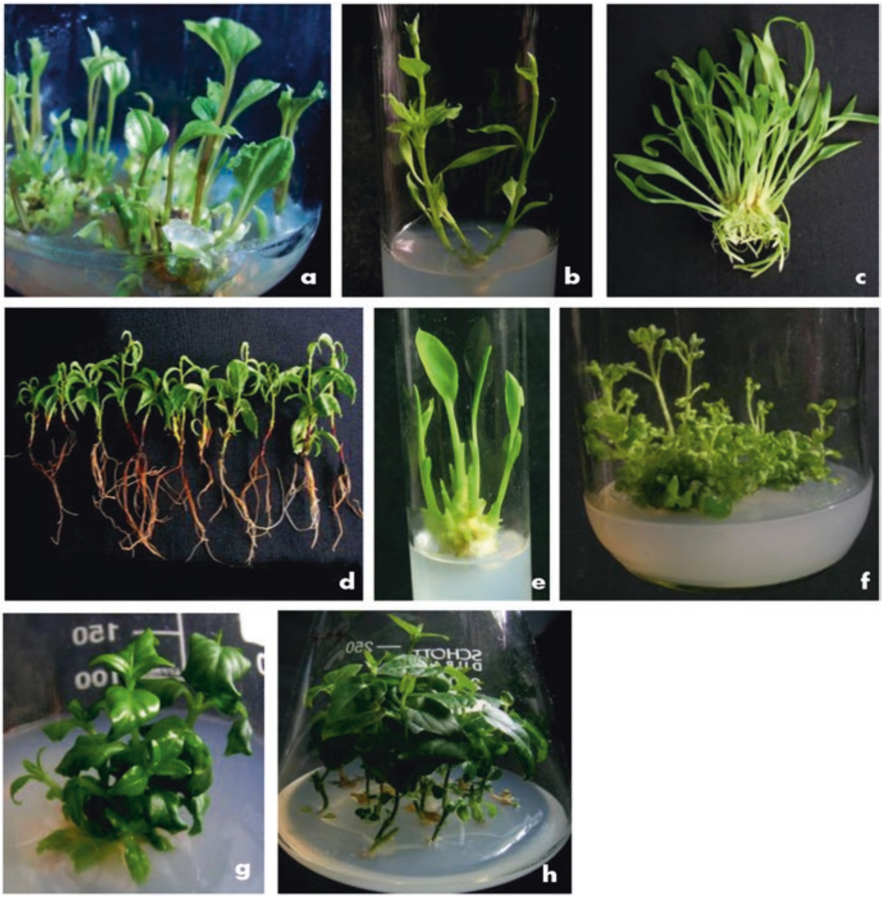

Fig. 7.2 In vitro shoot proliferation in threatened medicinal plants of the Western Ghats, India. (**a**) *Trychopus zeylanicus.* (**b**) *Decalepis arayalpathra.* (**c**) *Kaempferia galanga.* (**d**) *Celastrus paniculatus.* (**e**) *Curcuma longa.* (**f**) *Coleus forskohlii.* (**g**) *Rauvolfia serpentina.* (**h**) *Holostemma ada-kodien*

Axillary bud of field grown plants —6 weeks→ Multiple shoot induction (13.9 shoots) —6 weeks→ First subculture (115 shoots)

Multiplication of plants by second subculture within 6 weeks = 1548 plants in 6 months from one axillary bud

Fig. 7.3 Rate of multiplication of *A. calamus* by tissue culture

the importance of conservation strategies, involving in situ and ex situ preservation as well as reintroduction. Reintroduction/ecorestoration is the deliberate establishment of individuals of RET species into an area and/or habitat where it has become extirpated with the specific aim of establishing a viable self-sustaining population for conservation purposes. In fact, the goal of reintroducing endangered species is to reverse decline in the distribution and abundance that have been caused directly or indirectly by human activities. The intention is to ascertain self-sustaining populations that retain the genetic diversity necessary to undergo evolutionary change (McGlaughlin et al. 2002). Many species reintroduced into its native habitats have been growing well and the technology has already been successfully demonstrated by many authors in *Paphiopedilum rothschildianum* (Grell et al. 1988), *Bletia urbana* (Rubulo et al. 1989), *Ipsea malabarica* (Gangaprasad et al. 1998; Martin 2003), *Calophyllum apetalum* (Lakshmi and Seeni 2003), *Blepharistemma membranifolia* (Lakshmi and Seeni 2001), *Decalepis arayalpathra* (Gangaprasad et al. 2005), *Vanda coerulea* (Seeni and Latha 2000), *Vanda spathulata* (Decruse et al. 2003), *Syzygium travancoricum* (Anand 2003), Bulgaria golden root (Tasheva and Kosturkova 2010), *Ceropegia fantastica* (Chandore et al. 2010) and *Rhododendron ponficum* (Almeida et al. 2005). As a part of our continued efforts to conserve rare, endangered and endemic plants of conservation value through in vitro propagation and reintroduction, experimental ecorestoration of mericlones of five medicinal plants, *Mahonia leschenaultia* (Palani Hills of Kodaikanal), *Heracleum candolleanum* (Palani Hills of Kodaikanal), *Acorus calamus* (Palani Hills of Kodaikanal), *Rubia cordifolia* (Karadipara, Munnar) *and Myristica malabarica* (Sendurnai forest ranges), was successfully attempted during 2000–2016. About 100–500 plants were reintroduced into their native (Table 7.1) or alien habitats (forest patches of institute campus) recorded 75–90% establishment after 1–2 years (Table 7.2). Plants reintroduced into forest segments of the Western Ghats with favourable microclimatic conditions performed better with high-percentage establishment and profuse growth as evidenced from formation in quick succession of new leaves in relation to that of the plants in the institute campus (Krishnan et al. 2011; Radha 2011; Radha et al. 2013). Periodical monitoring of the establishment of reintroduced plants after 5 years also showed promising response of growth, flowering and seed set. Overall, this study comprising the development of an in vitro propagation protocol, mass propagation and recovery of the plants through reintroduction into native and alien habitats together provides a comprehensive package for conservation and sustainable utilisation of all the experimental species. The establishment of viable populations in sites (forest patches of institute) other than their natural habitats (translocation) is also desirable as it facilitates the survival of the species in more than one ecologically conducive site.

7.2.2 Ex Situ Conservation Through Cryopreservation

Long-term ex situ storage of plant germplasm is of increasing importance, both for maintaining the genetic diversity of species with existing human use and for the preservation of species threatened with extinction in the wild; seed banks have long been used for this purpose. Most of the agricultural species are desiccation tolerant or orthodox seeds, which are often viable for many years, and their longevity can be increased further by storing the seeds at a very low temperature (−196 °C), in liquid nitrogen (LN). In contrast, number of species have desiccation sensitive or recalcitrant seeds; tropical timber, fruit and plantation crops as well as species from several threatened habitats fall into this category (Berjack et al. 2011; Noor et al. 2011). Recalcitrant seeds are generally short lived, are often large with considerable quantities of fleshy endosperm and cannot be stored intact using traditional methods of drying. In vitro conservation offers alternative techniques for the long-term preservation of this plant germplasm, consisting of slow growth techniques and cryopreservation. While slow growth is for short- to medium-term storage, cryopreservation of zygotic embryos, shoot tip/meristems and pollen play a major role in the long-term conservation of tropical plants with recalcitrant seeds. Seeds of many species are too large to be frozen directly, so desiccation technique is mainly employed for freezing embryos and embryonic axes (Engelmann 2004) which has been confirmed for the investigations regarding cryopreservation of woody species like *Myristica malabarica, Nothapodytes nimmoniana, and Celastrus paniculatus* (Radha et al. 2006, 2010a, b) at JNTBGRI (Table 7.3). In *N. nimmoniana* and *C. paniculatus*, excised zygotic embryos subjected to simple desiccation under laminar air flow for 60 min reduced moisture content to 19.6 and 31.8, 60 to 66% of them regenerated into whole plants upon LN storage. Excised zygotic embryos of *M. malabarica*, *M dactyloides* and *M. undapine* subjected to desiccation for 120 min are also suitable for cryopreservation to get 56–65% whole plant regeneration, but the desiccation trials on *C. fenestratum* is not promising, as cryopreserved embryos recorded only 34% germination against 56% in desiccation control (60 min). Experiments suggest that isolated embryos of *C. apetalum* are tolerant to 2 hrs desiccation with 53% survival. However, successful cryostorage was achieved

Table 7.3 Zygotic embryo cryopreservation protocols standardised for medicinal trees of tropical and subtropical regions of Western Ghats, India

Species	Category of seeds	Desiccation time (min.)	Moisture content after desiccation (%)	Percentage survival after LN storage
Myristica malabarica	Recalcitrant	120	31.7	60
Myristica dactyloides	Recalcitrant	120	39.9	65
Myristica undapine	Recalcitrant	120	39.8	56
Nothapodytes nimmoniana	Intermediate	60	19.6	60
Coscinium fenestratum	Intermediate	60	37.3	34
Celastrus paniculatus	Recalcitrant	60	31.8	66
Calophyllum apetalum	Recalcitrant	120	20.6	53

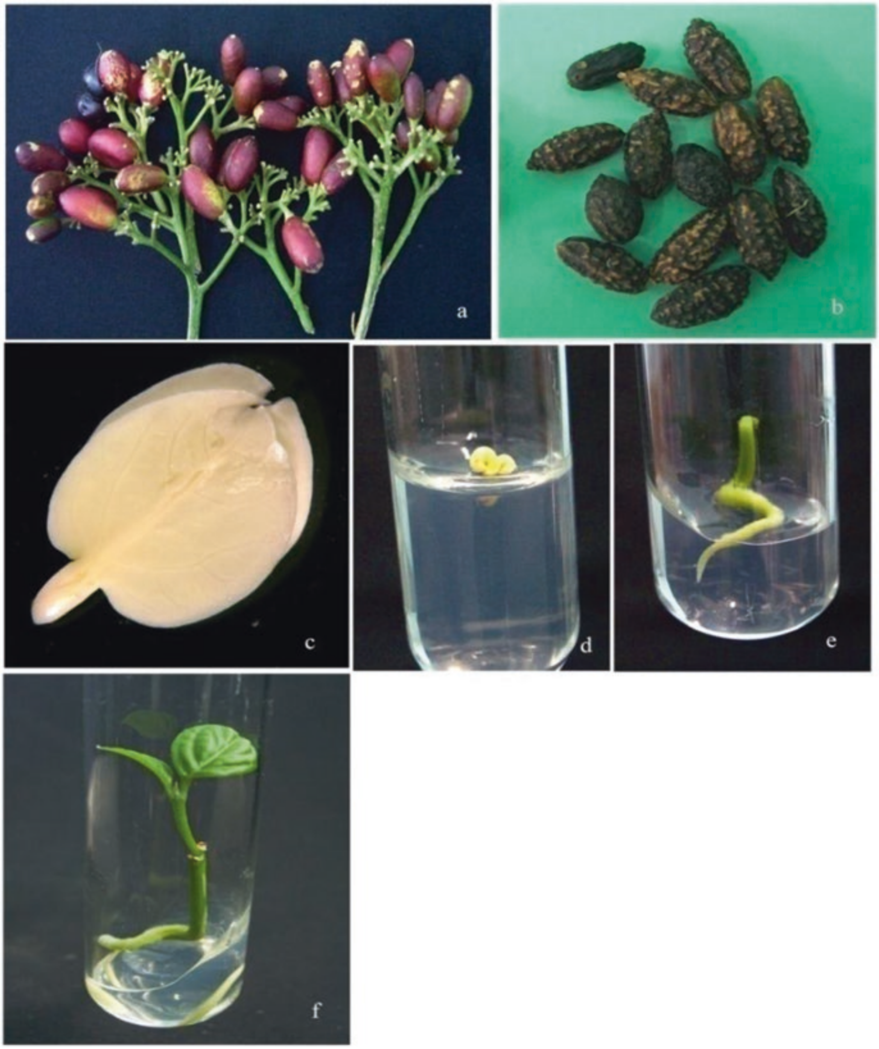

Fig. 7.4 Plant regeneration from cryopreserved embryos of *Nothapodytes nimmoniana.* (**a**) Fruits. (**b**) Seeds. (**c**) Embryo. (**d**) Cryopreserved embryo showing germination. (**e**) Cryopreserved embryo developed into seedling (30 days). (**f**) Cryopreserved embryo developed into seedling (60 days)

for most of the dehydration-tolerant seeds (Figs. 7.4, 7.5, and 7.6); this situation together with an ease to develop independent plants in vitro from embryonic axes may provide an effective technique for the long-term conservation of desiccation sensitive woody medicinal plants of the Western Ghats (Radha et al. 2010a, b; Krishnan et al. 2011).

Fig. 7.5 Plant regeneration from cryopreserved embryos of *Myristica malabarica.* (**a**, **b**) Fruit and seed from two accessions. (**c**) Embryo of two accessions. (**d**–**h**) Cryopreserved embryo showing germination and development. (**i**–**k**) Fruit, zygotic embryo, and germinating LN treated zygotic embryo of *Myristica dactyloides*

7.3 Conclusion

The efficiency of regimented micropropagation system, rehabilitation, understanding the phenomenon of seed recalcitrance and comprehensive cryopreservation practices are thus proved effective for the conservation and ecorestoration of threatened medicinal plants of the tropical and subtropical regions of Western Ghats, India. However, the reintroduction carried out in these published work of our laboratory is on an experimental scale. In order to realise ecological restoration, extended planting of plantlets in more than one locality is a mandate. Sufficient numbers of

Fig. 7.6 Plant regeneration from cryopreserved embryos of *Celastrus paniculatus.* (**a**) Fruits. (**b**) Ripened fruits. (c) Embryo. (**d**) Germination of LN treated embryo. (**e**) Cryopreserved embryo developed into seedling (30 days). (**f**) Cryopreserved embryo developed into seedling (60 days)

propagules are recommended to be cloned from one source population to mirror utmost genetic diversity, fortifying a self-sustained population of endangered species with the precise genetic diversity essential to extricate them from extinction. The exact number of plants that needs to be reintroduced varies with species and heterogeneity of the source population. In addition, the experiments with optimisation of scale-up production in different culture vessels including airlift bioreactors can support in the development of more effective propagation and storage technologies. In fact, most of the pharmaceutically important medicinal plants have not been micropropagated on large scale or reintroduced more than one region which is a major glitch in the current scenario. As the extinction pressures are increasing, it is important that priority species are identified for scaling up of shoot cultures and establishment of demonstration stage cultivation at pilot-scale level to conserve and commercialise production of therapeutic plants utilising all the premier biotechnological tools available.

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Chapter 8
In Vitro Conservation and Cryopreservation of Threatened Medicinal Plants of India

Neelam Sharma, Ruchira Pandey, and R. Gowthami

Abstract Plants are the most important source of medicines and an array of plant species are employed for medicinal purposes – in allopathy, in traditional systems of medicine, and in tribal and folk practices. In most of the cases (~80%), these are collected from naturally occurring wild populations. The growing realization about the adverse side effects of allopathic drugs and "*Back to Nature*" trend since early 1980s have led to sudden upsurge of herbal medicines leading to escalating pressure on the pharmaceutical industry. However, the pressure of ever-increasing human population and ruthless clearing of forests and agricultural land have led to the depletion of naturally occurring genetic resources, a rich source of future pharmaceutical and other useful products. Though an issue of global concern, conservation of germplasm of wild threatened medicinal species is challenging. Realizing the urgent need to conserve the germplasm of a large number of rare and endangered species of medicinal plants, there has been significant thrust on their conservation by appropriate ex situ conservation approaches. With the application of biotechnological (in vitro) approaches to conventional approaches of conservation, it is expected that many rare and threatened medicinal plant species will be conserved in the near future. During the last three decades, in vitro methods have been used for propagation of many rare and endangered medicinal plants for sustainable utilization and evaluation of their potential uses. The present chapter attempts to assess the current status of application of various in vitro approaches for the conservation of threatened medicinal plants of India.

Keywords In vitro conservation · Cryopreservation · Threatened medicinal plants

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8.1 Introduction

The alarming rate of species extinction in recent times has led to approximately 34,000 out of a total of 270,000 plant species in existence be considered as endangered (IUCN 1998). India ranks tenth among the plant – rich nations of the world and fourth among the countries of Asia (Natesh 1999; Natesh and Mohan Ram 1999). The country also ranks sixth for having the largest number of threatened plant species (Hilton-Taylor 2000). According to the IUCN designed Conservation Assessment and Management Plan (CAMP) methodology, about 112 species from southern India, 74 species from Northern and Central India, and 42 species from the high altitude of Himalayas are threatened in the wild (Sharma et al. 2005; Krishnan et al. 2011, see Sharma et al. 2010a, b; Sharma and Pandey 2013, 2015a). Recently, in the updated IUCN Red List – 2015, another 44 medicinal plants have been added. In this update, from among Indian medicinal plants, 10 have been categorized as critically endangered, 16 as endangered, and 18 as vulnerable (Dhyani and Dhyani 2016).

Growing demand of pharmaceutical industries and unsustainable harvesting from the wild coupled with absence of organized cultivation efforts besides destruction and degradation of ecosystem itself have been identified as major causes of endangering some of the high-value medicinal plant taxa. It is important to note that pressure of unsustainable harvesting, of already drastically shrunk natural populations of some of the species, has been so great that they are hardly able to regenerate themselves.

The aforementioned figures indicate that conservation measures for the germplasm of such rare, elite, and endangered species of medicinal value is a matter of urgency. Both in situ and ex situ methods are well known and have been applied for the conservation of red-listed plants. However, the choice of one or the other technique, or a combination of both, depends on the particular genera/species, and both are complementary to each other. The ultimate objective of conservation of wild occurring plants of medicinal value will yield greatest sustainable benefit to present generation while ensuring its potential to meet the needs and aspirations of future generations.

Among various ex situ strategies, tissue culture techniques have played significant role in propagation and conservation of threatened medicinal plants. Publications on this aspect are available since 1980s. To assess the status of conservation, literature was surveyed and reviewed for the in vitro propagation and conservation of threatened medicinally important species of India.

8.2 Conservation Strategies

Conservation of species diversity can be accomplished both in situ (in native) and ex situ (in repositories and research laboratories). Being a naturally adopted process of conservation, in situ conservation is the best natural method while ex situ method is more effective and more scientific because of its way of conservation (Chandel et al. 1996). It is emphasized that in situ conservation is an ideal approach to be followed since it not only helps preservation of flora, fauna, and microorganisms but also enables continued evolution. Though in situ conservation is the best method of conservation, it may not always be feasible due to limitation of resources and accessibility of the area. Also it is unlikely that pressures on land would permit more than 4% of the geographical area to be set aside as protected area (Natesh 1999). Ex situ conservation, on the other hand, involves a higher degree of protection and isolation of germplasm (Withers 1991) and can ensure reliable supply on a sustainable basis of threatened species linked with cultivation of some targeted species that are in greater demand (Chandel and Sharma 1996). Ex situ conservation includes botanical gardens, herbal gardens, arboreta, "sacred groves," and gene banks (field, seed, and in vitro/cryo). Among the ex situ methods, seeds, being natural perennating organs of plants, can be maintained for relatively long periods, at low temperatures in seed gene banks. However, conventional seed storage, the most easy and popular strategy, can only be applicable for threatened medicinal species with adequate seed production. A number of these species do not set seeds and in several species the seeds may be sterile, recalcitrant, or otherwise unsuitable for storage. Since most of the collections are made from the wild, first and foremost limitation is quantity of seeds for conservation. Seed dormancy, nonuniform maturity of seeds, and lack of literature on germination/propagation/storage behavior are some of the other constraints faced while working with seed conservation.

Maintenance of clonal material in the field gene banks is not only costly but difficult due to lack of information on propagation method of many threatened taxa. Therefore, conservation through in vitro approaches is the viable alternative (Chandel et al. 2000; Sharma and Pandey 2013) for such plant species.

In vitro conservation strategies offer advantages over in vivo methods in being amenable to rapid multiplication and storage of large germplasm in a relatively small space and under disease-free conditions, away from vagaries of nature. The progress achieved in past three decades in adopting in vitro techniques in conservation of threatened medicinal plants will be the focus of discussion in the present chapter.

8.2.1 The Need of In Vitro Conservation

Ninety percent of all the accessions of various crop plants held in gene banks world over are conserved as seeds at low temperature after partial desiccation. In many threatened and medicinal species, this conventional method is not applicable due to the following reasons:

- Inadequately documented mode of propagation and storage potential of propagules
- Lack of information on germination behavior
- Inadequate seed production/availability
- Short seed viability
- Vegetative mode of propagation

- Unsustainable method of collection at a particular time of growth
- Perennial species with long regeneration period
- Root/rhizome being source of active principle
- Erosion of genetic resources due to biotic and abiotic stresses

Thus, it is imperative to conserve germplasm in the form of in vitro cultures, and it appears to be the most promising option to ensure safe conservation with their genetic fidelity intact. Maintenance of plant species that cannot be stored as true seeds using in vitro techniques provides an effective system for establishing both active and base germplasm collections (Fay 1994). The application of in vitro technique can also facilitate exchange and be applied for elimination of diseases including viruses.

8.3 In Vitro Approaches

In vitro conservation program mainly includes development of in vitro multiplication, slow growth, and cryopreservation protocols. Requirement of infrastructure facility along with availability of trained skilled personnel and linkage with herbal garden/farmers field are the essential prerequisite for an in vitro conservation program. The main advantage of in vitro clonal propagation lies in generation of large amount of material in a short span from a minimum starting material. The technique also offers a great potential by virtue of mass cloning of elite types to meet ever-increasing demand of quality material for cultivation as well as for secondary metabolite production. It also helps in genetic manipulation of medicinal plants for enhanced active ingredients (Bhat et al. 2012). In plants such as *Aristolochia* spp., *Picrorhiza* spp., *Curculigo orchioides, Dioscorea* spp., and *Rauvolfia serpentina*, wherein clonal propagation by vegetative means is inadequate/slow, the method is particularly beneficial. The technique of in vitro propagation can be used for conservation of threatened medicinal plants in two ways: (i) use of in vitro multiplication technique in species with reproductive problems and/or with extremely low population to increase number of individuals and (ii) the development of an in vitro storage technique which is particularly useful when conservation of seeds is not possible (Sharma and Pandey 2015a).

Various in vitro approaches applicable for conservation and sustainable utilization of threatened medicinal plants are discussed below.

8.3.1 In Vitro Collection

In vitro collection, the initiation of tissue culture in the field, is one technique that has been gaining importance in conservation program. The technique involves taking the minimal lab equipment (a box to hold instruments and culture medium, a bottle of sterilant, etc.) for collection and initiation of cultures at the collecting site and using simplified inoculation techniques. Either partial or full sterilization of tissue is done on site, and tissue is transferred to containers of medium for transport back to the lab. In vitro collection technique has been used to collect a variety of plant tissues including seeds, embryos, and vegetative tissues (apical buds, nodal segments, leaf tissues) (Pence 1999). Successfully used in the germplasm collection of cotton, cassava, and coconut, and reported also for the collection of two rare endangered wetland species – *Lobelia boykinii* and *Rhexia aristosa* in the USA (Pence and Engelmann 2011) – the technique has definite potential in the in vitro conservation of threatened genetic resources including wild relatives that grow in remote, difficult-to-approach areas or those that have large seeds with high moisture content, low viability, and short life.

8.3.2 In Vitro Propagation

During the past decades, there has been a great interest and progress in in vitro propagation of medicinal plants. The techniques applicable for threatened medicinal plants are discussed in the following sections.

8.3.2.1 In Vitro Propagation Using Seeds

Germination of seeds and seedling growth in some species can be greatly increased by the use of in vitro methods, where no or low germination is achieved using conventional techniques, due to dormancy or specific requirements needed for germination. Removal of the testa from seeds of these species under sterile conditions can greatly increase the percentage germination and allow the successful establishment of plants *ex vitro*. In some species such as *Aconitum heterophyllum* (Nandi et al. 2016), *Commiphora wightii* (Kumar et al. 2006), *Podophyllum hexandrum*, and *Saussurea lappa* (Bhojwani et al. 1989), these techniques have been used for initiating cultures for in vitro propagation (see Table 8.1).

8.3.2.2 In Vitro Propagation Using Vegetative Material

The stages critical to successful micropropagation of a species are described below and illustrated in Fig. 8.1.

Donor Plant Selection and Preparation of Explant

Explant quality and subsequent responsiveness in vitro is significantly influenced by the phytosanitary conditions of the donor plant (Debergh and Maene 1981). It is desired that stock plants are maintained under clean, controlled environment that allows active growth but reduces the probability of diseases. Additionally, use of

Table 8.1 Status of in vitro propagation of threatened medicinal plants of India

Sl. no.	Plant species	Explant	Mode of multiplication	Multiplication medium	Rooting medium	Establishment in soil (%)	Reference
1.	*Abutilon ranadei*	Nodes	Direct shoot	MS + 3.0 mg/l BAP + 0.5 mg/l GA	½ MS + 1.5 mg/l IBA + 1.0 mg/l BAP	82	Patil et al. (2017)
	Abutilon ranadei	Leaves	Callus mediated organogenesis	MS + 1.5 mg/l Kn + 0.4 mg/l NAA	½ MS + 1.5 mg/l IBA + 1.0 mg/l BAP	82	Patil et al. (2017)
2.	*Aconitum heterophyllum*	Nodal segments	–	MS + 0. 5 mg/l BAP + 0.25 mg/l NAA	MS + 1.0 mg/l IAA	–	Jabeen et al. (2006)
	Aconitum heterophyllum	Excised explants middle portions of cotyledon	Callus mediated organogenesis	MS + 25.0 μM NAA + 5.0 μM BAP	–	–	Nandi et al. (2016)
	Aconitum heterophyllum	Excised explants outer portions of cotyledon	Callus mediated organogenesis	MS + 25.0 μM NAA + 5.0 μM BAP	–	–	Nandi et al. (2016)
	Aconitum heterophyllum	Shoot tip, leaves		MS + 1.0 mg/l 2,4-D + 0.5 mg/l Kn	MS + 1.0 mg/l IBA	–	Giri et al. (1993)
3.	*Acorus calamus*	Rhizome explants	Multiple shoots	¼ MS + 5.7 μM IAA + 2.4 μM IBA	MS + 1.0 mg/l IBA	90	Ahmed et al. (2010)
	Acorus calamus	Rhizome bud	Multiple shoots	MS + 2.0 mg/l Kn + 0.05 mg /l NAA	MS + 1.2 mg/l IBA	–	Ahmed et al. (2010)
	Acorus calamus	Rhizome tip	–	MS + 2.0 mg/l Kn + 0.05 mg /l NAA	MS + 1.0 mg/l IBA	80	Ahmed et al. (2007)
	Acorus calamus	Rhizome bud	Multiple shoots	MS + 8.87 μM BA + 5.37 μM NAA	MS basal	90–95	Anu et al. (2001)
	Acorus calamus	Apical meristem	Multiple shoots	MS + 1.0 mg/l BAP	MS basal	75	Hettiarachchi et al. (1997)
4.	*Adhatoda beddomei*	Stem node explants	Axillary proliferation	SH + 1.0 mg/l BAP + 0.2 mg/l IAA	Liquid medium + 0.2 mg/l IBA or IAA	95	Sudha and Seeni (1994)
5.	*Alpinia calcarata*	Rhizome bud	Axillary shoot proliferation	MS + 1.5 mg/l Kn + 0.5 mg/l BAP	½ MS + 0.5 mg/l IBA	87–90	Asha et al. (2012)

	Alpinia calcarata	Rhizomatous bud	–	MS + 5.0 μM BAP + 10 μM Kn + 2.5 μM NAA	MS + 5.0 μM BAP + 10 μM Kn + 2.5 μM NAA	–	Bhowmik et al. (2016)
6.	*Aristolochia indica*	Leaf	Callus mediated organogenesis	MS + 0.8 mg/l BAP + 0.5 mg/l NAA	MS + 0.8 mg/l NAA	+	Pramod and Jayaraj (2012)
	Aristolochia indica	Nodal explants	Callus mediated organogenesis	MS + 0.8 mg/l BAP + 0.5 mg/l NAA	MS + 0.8 mg/l NAA	+	Pramod and Jayaraj (2012)
	Aristolochia indica	Shoot tip and nodal segments	Axillary shoot multiplication	MS + 3.0 mg/l 6-BA + 1.0 mg/l NAA	MS + 1.0 mg/l IBA	+	Theriappan et al. (2010)
	Aristolochia indica	Nodal explants	Callus mediated organogenesis	MS + 1.0 mg/l BAP + 2.5 mg/l NAA	MS + 1.0 mg/l Kn	–	Siddique et al. (2006a)
	Aristolochia indica	Axillary shoots	Multiple shoots	MS + 2.5 mg/l Kn + 1.0 mg/l BAP	MS + 1.0 mg/l Kn	–	Siddique et al. (2006b)
	Aristolochia indica	Shoot tip and nodal segments	Adventitious directly from leaf bases and internodes + organogenesis	MS + 5.0 mg/l 2iP	MS + 1.0 mg/l IBA	100	Soniya and Sujitha (2006)
	Aristolochia indica	Shoot tip, nodal segment	Regeneration via axillary and adventitious shoots	MS + 0.54 μM NAA + 13.31 μM BA	White's medium + 2.46 μM IBA	85	Manjula et al. (1997)
	Aristolochia indica	Internodal segment	Callus mediated organogenesis	MS + 2.69 μM NAA + 1.0 mg/l PG	–	–	Manjula et al. (1997)
	Aristolochia indica	Leaf segment	–	MS + 13.31 μM BA + 50 mg/l AC	–	–	Manjula et al. (1997)
7.	*Aristolochia tagala*	Nodal segments and shoot tips	Axillary bud proliferation and direct organogenesis	MS + 1.0 mg/l BAP	MS + 2.0 mg/l IBA	100	Rajanna and Shailaja Sharma (2015)
	Aristolochia tagala	Apical bud	–	MS + 3.0 μM BAP + 0.5 μM Kn + 0.1% AC	MS + 1.5 μM IAA + 1.5 μM Kn + 0.5 μM BAP	+	Remya et al. (2016)
8.	*Asparagus racemosus*	Seedlings (epicotyledonary node)	Multiple shoot	MS + 13.93 μM/l Kn + 5.70 μM/l IAA	–	–	Jat et al. (2014)

(continued)

Table 8.1 (continued)

Sl. no.	Plant species	Explant	Mode of multiplication	Multiplication medium	Rooting medium	Establishment in soil (%)	Reference
	Asparagus racemosus	Nodal explants	Callus mediated organogenesis	MS + 1.0 mg/l IBA + 1.0 mg/l BAP	½ MS + 1.5 mg/l IBA + 1.0 mg/l IBA + 0.1 mg/l BAP	83	Patel and Patel (2015)
	Asparagus racemosus	Single node segments		MS + 3.69 μM 2iP + 3% sucrose	½ MS + 1.61 μM/l NAA + 0.46 μM Kn + 98.91 μM AS + 500 mg/l malt extract + 198.25 μM Phloroglucinol + 3% sucrose	100	Bopana and Saxena (2008)
	Asparagus racemosus	Nodal segments	Adventitious shoot bud regeneration and callus	MS + BA 3.0 mg/l + 0.5 mg/l NAA	½ MS + NAA	75	Kumar (2009)
	Asparagus racemosus	Nodal segment	–	MS + 0.5 mg/l BA	MS + 0.1 mg/l NAA	60	Pant and Joshi (2009)
9.	*Atropa acuminata*	Shoot tips and nodal explants	Axillary shoot proliferation	MS + 1.0 mg/l BAP + 1 mg/l IBA	RT + 1.0 mg/l IBP	80	Ahuja et al. (2002)
	Atropa acuminata	Petiole	Callus mediated organogenesis	MS + 5.0 mg/l BAP	MS + 0.5 mg/l IBA	80	Maqbool et al. (2016)
	Atropa acuminata	Nodal explants	Callus mediated organogenesis	MS + 2.0 mg/l BAP	MS + 0.5 mg/l IBA	80	Maqbool et al. (2016)
	Atropa acuminata	Leaf explants.	Callus mediated organogenesis	MS + 3.0 mg/l BAP + 2.0 mg/l IAA	–	–	Maqbool et al. (2014)
10.	*Bacopa monnieri*	Nodal segments, internodes, leaf	Adventitious shoots	MS + 2.2 μM BA	MS + 4.9 μM IBA	100	Tiwari et al. (2001)
	Bacopa monnieri	Leaves, stem segments	Adventitious shoots	MS + 2.0 μM BA + 0.2% gelrite	–	–	Shrivastava and Rajani (1999)
	Bacopa monnieri	Leaf segments, node, internode	Adventitious shoots	MS basal	MS basal	97	Mathur and Kumar (1998)

	Bacopa monnieri	1. Internodal segments 2. Leaf 3. Flower buds	Callus mediated organogenesis multiple shoot formation	1. and 2. MS + 1.0 mg/l Kn + 0.1 mg/l IAA 3. MS + 0.1 mg/l 2iP + 0.1 mg/l IAA	Rooting on same medium	–	Tejavathi and Shailaja (1999)
	Bacopa monnieri	Nodal segments	Axillary shoots	MS + 0.2 mg/l BA	Same medium	80–100	Sharma et al. (2007a), Sharma and Pandey (2013)
11.	*Blepharestemma membranifolia*	Nodes	Organogenesis	–	–	–	Laksmi and Seeni (2001)
12.	*Calophyllum apetalum*	Shoot tip and single node explants	Organogenesis	MS + 4.4 µM BAP	¼ MS + 9.8 µM IBA	56	Lakshmi and Seeni (2003)
13.	*Celastrus paniculatus*	Nodal segments, shoot tips and leaf discs	Callus mediated organogenesis	MS + 5.0 µM BAP + 0.5 µM IAA	MS + 5.6 µM IAA + 9.6 µM IBA	80	Silva and Senarath (2009)
	Celastrus paniculatus	Nodal explants	Multiple shoots	MS + 0.5 mg/l BAP + 0.1 mg/l NAA	½ MS + 0.5 mg/l IAA	91	Senapathi et al. (2013)
	Celastrus paniculatus	Nodal segments	Shoot multiplication	MS + 0.5 mg/l BAP + 0.1 mg/l IAA	Ex vitro rooting shoots were treated with 300 mg /l IBA for 3 min	95	Phulwaria et al. (2013)
14.	*Ceropegia attenuata*	Nodal explants	Multiple shoots	MS + 13.31 µM BA	½ MS + 2.46 µM IBA	85	Chavan et al. (2011)
15.	*Ceropegia evansii*	Nodal explants	Shoot multiplication	MS + 4.0 mg/l BA + 0.3 mg/l IAA	½ MS + 1.0 mg/l IBA	90	Chavan et al. (2015)
16.	*Ceropegia fantastica*	Nodal explants	Multiple shoots	MS + 1.5 mg/l BA	MS + 1.0 mg/l IBA	65	Chandore et al. (2010)
17.	*Ceropegia jainii*	Nodal explants	Multiple shoots	MS + 0.1 µM BA + 1.0 µM IBA	½ MS + 0.18 µM IBA + 6% sucrose	80	Patil (1998)

(continued)

Table 8.1 (continued)

Sl. no.	Plant species	Explant	Mode of multiplication	Multiplication medium	Rooting medium	Establishment in soil (%)	Reference
18.	*Ceropegia maccannii*	Leaves	–	MS + 7.5 μM BA + 3% sucrose + 0.8% agar	½ MS + 5% sucrose + 0.5 μM IBA	–	Nikam et al. (2008a)
19.	*Ceropegia mahabalei*	Nodal explants	Axillary multiplication	MS + 5.0 μM BA	Liquid MS medium + 1.0 μM NAA	88	Nikam et al. (2012)
	Ceropegia mahabalei	Tubers	–	MS + 0.1 mg/l BAP	–	–	Deshmukh (2010)
20.	*Ceropegia media*	Nodal explants	–	MS + 5.0 μM BA	Liquid MS medium + 1.0 μM NAA	88	Nikam et al. (2012)
21.	*Ceropegia noorjahanae*	Nodal explants	Axillary bud proliferation	MS + 2.0 mg/l BAP	½ MS + 1.0 mg/l IBA	85	Chavan et al. (2014)
	Ceropegia noorjahanae	Axillary bud	Multiple shoots	MS + 0.5 mg BA + 0.3 mg Kn	–	–	Kedage et al. (2006)
22.	*Ceropegia odorata*	Node, internode,	–	MS + 7.5 μM BA + 3% sucrose + 0.8% agar	½ MS + 5%sucrose + 0.5 μM IBA	80	Nikam et al. (2008b)
23.	*Ceropegia panchganiensis*	Single nodal explants	Callus mediated organogenesis	MS + 13.31 μM BA + 2.69 μM NAA	½ MS + 7.36 μM IBA	85	Chavan et al. (2013)
24.	*Ceropegia sahyadrica*	Axillary shoot	Multiple shoots	MS + 10 μM BA	MS + 6.0 mg/l spermine + 5% sucrose	–	Nikam and Savant (2007)
	Ceropegia sahyadrica	Nodes and internodes	Callus mediated organogenesis	MS + 7.5 μM Kn	0.5–2.0 μM IAA or NAA	–	Nikam and Savant (2009)
25.	*Ceropegia santapaui*	Cotyledonary nodes and cotyledons	Callus mediated organogenesis	MS + 2.5 mg/l BA + 0.4 mg/l IBA	–	–	Chavan et al. (2014)
26.	*Chlorophytum borivilianum*	Single Juvenile shoot bud	*In vitro* shoot multiplication	MS + 2.0 mg/l BAP + 0.2 mg /l NAA	MS + 2.0 mg/l IBA	95	Chauhan et al. (2016)
	Chlorophytum borivilianum	Young shoot bases	*In vitro* shoot multiplication	MS + 3.0 mg/l BA	MS + 1.0 mg/l IAA + 3.0 mg/l NAA	–	Jauhari et al. (2014)

	Chlorophytum borivilianum	Young shoot bases	*In vitro* clonal multiplication	MS + 22.2 μM BA	MS + 3/4-strength inorganic and organic constituents + 9.8 μM IBA	67	Purohit et al. (1994)
	Chlorophytum borivilianum	Young shoot buds	*In vitro* clonal multiplication	MS + 22.2 μM BA + 3% sucrose	MS + 3/4-strength inorganic and organic constituents + 9.8 μM IBA	87–90	Dave et al. (2003)
27.	*Cinnamomum tamala*	Immature embryos	Callus mediated organogenesis	MS + 3% sucrose + 3.0 μM Kn	MS + 3.0 μM NAA	70	Deb et al. (2014)
28.	*Commiphora wightii*	Hypocotyl region	Somatic embryogenesis	Modified MS + 0.5 g/l AC + 10% sucrose	–	–	Kumar et al. (2006)
	Commiphora wightii	Nodal	Forced axillary branching	MS + 17.8 μm BA + 18.6 μM Kn + 100 mg/l glutamine + 10 mg/l thiamine HCL + 0.3% AC	MS + IAA + IBA in dark transfer to low salt medium + AC	100	Barve and Mehta (1993)
29.	*Coleus forskohlii*	Nodal segments, shoot tips	Axillary shoots	MS + 2.0 mg/l Kn + 1.0 mg/l IAA	MS + 1.0 mg/l IAA	–	Sharma et al. (1991)
	Coleus forskohlii	Shoot tip & bud initials of nodal segments	Axillary shoots	MS + 2.0 mg/l BA	MS basal	–	Sen and Sharma (1991)
	Coleus forskohlii	Shoot tips	Axillary shoots	MS + 0.57 μM IAA + 0.46 μM Kn	–	–	Bhattacharya and Bhattacharya (2001)
	Coleus forskohlii	Mature leaves	Callus mediated organogenesis	MS + 4.6 μM Kn + 0.54 μM NAA	½ MS	–	Reddy et al. (2001)
	Coleus forskohlii	Node	Callus mediated organogenesis	MS + 0.2 BAP	MS + 2.5 IAA	70	Verma et al. (2012)

(continued)

Table 8.1 (continued)

Sl. no.	Plant species	Explant	Mode of multiplication	Multiplication medium	Rooting medium	Establishment in soil (%)	Reference
30.	*Coptis teeta*	Hypocotyl	Callus mediated organogenesis	½ MS + 4.6 μM Kn	½ MS + 4.9 μM IBA	60	Tandon and Rathore (1992)
31.	*Costus speciosus*	Rhizome sections	Micropropagation	B_5 + 5.0 μg/l TRIA	B_5 + 2.0 μg/l TRIA	100	Malabadi et al. (2005)
	Costus speciosus	Zygotic embryos	Multiple shoots leading to rhizome formation	SH + 250 mg/1 Casamino acids (CA)	–	+	Roy and Pal (1991)
	Costus speciosus	Shoot tips	Rhizome	MS + 0.5 mg/l BAP or 1 mg/l Kn + 15 mg/l AdS + 1 mg/l IAA	SH + 1 mg/l IAA	+	Chaturvedi et al. (1984)
	Costus speciosus	Single axillary buds	Shoot multiplication	MS + 10 μM AdS + 1 μm NAA + 50 g/l sucrose + 7 μM BAP	MS + 10 μM AdS + 1 μM NAA + 50 g/l sucrose +7 μM BAP	95	Punyarani and Sharma (2010)
	Costus speciosus	Pseudostem	Shoot proliferation	MS + 0.05 mg/l BAP	MS + 0.1 mg/l IBA	75	Robinson et al. (2009)
32.	*Curculigo orchioides*	Rhizomes and leaves	Direct organogenesis in leaf explants	MS + 2.0 mg/l 2,4-D	MS + 2.0 mg/l 2,4-D	82.5	Prajapati et al. (2003)
	Curculigo orchioides	Rhizome buds, shoot base	Multiple shoots	MS + 0.2 mg/l BA + 2.5 mg/l spermidine	MS basal	86	Sharma et al. (2007b)
	Curculigo orchioides	Apical meristem	Multiple shoots	MS + 1.5 mg/l BA + 100 mg/l ads + 0.25 mg/l IBA + 3% sucrose	½ MS + 0.25 mg/l IBA + 2% sucrose	+	Francis et al. (2007)
33.	*Decalepis arayalpathra*	Single node	Organogenesis	MS + 2.22 μM BA + 0.24 μM 2iP	½ MS + 1.07 μM NAA	86	Sudha et al. (2005)
	Decalepis arayalpathra	Nodal explants	–	MS + 0.5 mg/l BAP	MS + 1.5 mg/l IAA	84	Gangaprasad et al. (2005)

34.	*Decalepis hamiltonii*	Shoot tip	Callus mediated organogenesis	Agar-based MS + 4.9 µM 2iP	MS + 9.8 µM IBA	–	Giridhar et al. (2005)
	Decalepis hamiltonii	Cotyledonary explants	Callus mediated organogenesis	MS + 1.0 mg/l BA + 0.1 mg/l GA3	½ MS + 0.4 mg/l IBA	97.5	Samydurai et al. (2016)
35.	*Digitalis lanata*	Shoot tips	Axillary shoots	LS + 1.0 mg/l BAP + 0.1 mg/l IAA	LS + 1/5 of normal nitrogen + 0.5 mg/l IBA	–	Erdei et al. (1981)
	Digitalis lanata	Shoot tips	Axillary shoots	MS + (5×10^{-6} M) BAP + (5×10^{-5} M) IAA	MS + (5×10^{-5}M)IBA	–	Schoner and Reinhard (1982)
36.	*Dioscorea bulbifera*	Nodal segments	–	MS + 1.0 mg/l BA	–	+	Forsyth and van Staden (1982)
	Dioscorea bulbifera	Nodal segments		MS + 0.25 mg/l Kn + 0.25 mg/l NAA	MS + 0.15 mg/l NAA	–	Mandal et al. (2000), Sharma et al. (2009, 2014)
37.	*Dioscorea deltoidea*	Tuber	Callus medicated embryogenesis	SH + 2.0 mg/l BAP + 0.5 mg/l IAA + 0.1 mg/l 2, 4-D	–	–	Sharma and Chaturvedi (1989)
	Dioscorea deltoidea	Nodal segments	Multiple shoots	MS + 1.5 mg/l BAP + 0.5 mg/l IBA	MS + 1.0 mg/l IAA	–	Kumar et al. (2017)
	Dioscorea deltoidea	Nodal segments	Callus medicated embryogenesis	RT + 1.0 mg/l BAP + 0.2 mg/l NAA	RT + 1.0 mg/l IAA	–	Kumar et al. (2017)
38.	*Dioscorea floribunda*	Node and internode segments	Axillary shoots	MS + modified White's medium + 2,4-D or NAA + BAP or Kn	½ MS basal + 0.5 mg/l NAA	70	Sengupta et al. (1984)
	Dioscorea floribunda	Nodal segments	Indirect organogenesis	MS + 0.25 mg/l Kn + 0.25 mg/l NAA	MS + 0.15 mg/l NAA	–	Mandal et al. (2000)
39.	*Dioscorea prazeri*	Nodal explants and axillary buds	Multiple shoots	MS + 0.5 mg/l BAP + 0.01 mg/l NAA	MS + 0.5 mg/l BAP + 0.01 mg/l NAA	–	Thankappan and Patell (2011)

(continued)

Table 8.1 (continued)

Sl. no.	Plant species	Explant	Mode of multiplication	Multiplication medium	Rooting medium	Establishment in soil (%)	Reference
40.	*Entada pursaetha*	Cotyledonary explants	Adventitious shoot organogenesis	MS + 5.0 mg/l BAP + 0.5 mg/l NAA	½ MS + 2.0 mg/l IBA	70	Vidya et al. (2005)
41.	*Fritillaria roylei*	Bulb scale	–	MS + 5.0 μM Kn + 2.0 μM NAA	–	–	Joshi et al. (2007)
42.	*Gentiana kurroo*	Nodal segments and shoot tips	Axillary branching	MS + 8.9 μM BA + 1.1 μM NAA	½ MS + 6% sucrose	90–100	Sharma et al. (1993)
43.	*Gloriosa superba*	Leaves, non-dormant corm bud auxillary bud, nodal portion and seeds	Calli and multiple shoot formation	MS + 4.52 μM 2,4-D + 13.30 μM BAP	–	–	Ade and Rai (2011)
	Gloriosa superba	Nodal explants	Root tuber induction	MS + 3.0 mg/l NAA + 1.0 mg/l TDZ	½ MS + 3.0 mg/l NAA	90	Madhavan and Joseph (2010)
	Gloriosa superba	In vitro root tuber	Shoot initiation and multiplication	MS + 2.0 mg/l Kn + 1.0 mg/l NAA	½ MS + 3.0 mg/l NAA	–	Madhavan and Joseph (2010)
	Gloriosa superba	Non dormant tubers	In vitro tuberization	MS basal	Modified MS basal	–	Ghosh et al. (2007)
	Gloriosa superba	Apical and axillary buds of young sprouts	Shoot multiplication	MS + 1.5 mg/l BA + 0.5 mg/l NAA + 15% coconut water + 2.0 g/l AC	½ MS + 1.0 mg/l IBA + 0.5 mg/l IAA	85–90	Hassan and Roy (2005)
44.	*Gymnema sylvestre*	Apical buds	Shoot multiplication	MS + 4.44 μM BAP + 4.64 μM Kn + 3% sucrose	½ MS + 2.85 μM IAA	80	Sharma and Bansal (2010)
	Gymnema sylvestre	30 day old seedling axillary node explants	Multiple shoots	MS + 1.0 mg/l BA + 0.5 mg/l Kn + 0.1 mg/l NAA + 100 mg/l ME + 100 mg/l citric acid	½ MS + 3.0 mg/l IBA	+	Komalavalli and Rao (2000)

	Gymnema sylvestre	Node	Enhanced axillary sprout	MS + 5.0 mg/l BA + 0.2 mg/l NAA	½ MS	75	Reddy et al. (1998)
	Gymnema sylvestre	Seedling	Organogenesis	MS + 1.0 mg/l BA + 0.5 mg/l IAA + 100 mg/l vitamin B_2 + 100 mg/l citric acid	½ MS	–	Devi and Srinivasan (2008)
45.	*Hemidesmus indicus*	Bud	*In vitro* clonal propagation	MS + 0.1 mg/l NAA + 2.0 mg/l BAP	MS + 1.5 mg /l IBA	–	Saha et al. (2003)
	Hemidesmus indicus	Nodal explants	Axillary bud culture	MS + 1.15 µM Kn + 0.054 µM NAA	MS basal + 1.15 µM Kn + 7.35 µM IBA	70	Patnaik and Debata (1994)
	Hemidesmus indicus	Leaf/stem segments	Organogenesis	MS + 2.0 mg/l NAA + 0.5 mg/l Kn	½ MS basal	+	Sarasan et al. (1994)
	Hemidesmus indicus	Axillary shoots	Callus mediated organogenesis	MS + 1.0 mg/l NAA + 2.5 mg/l Kn	MS + IBA + Kn	+	Siddique and Bari (2006)
	Hemidesmus indicus	Nodes (0.5 cm)	Multiplication upto 25 passages	½ MS + 2.22 µM BA + 1.07 µM NAA	¼ MS + 9.8 µM IBA	96	Sreekumar et al. (2000)
	Hemidesmus indicus	Roots segment (0.5 cm)	–	MS + 4.44 µM BA + 2.69 µM NAA	–	–	Sreekumar et al. (2000)
46.	*Holostemma annulare*	Chlorophyllus root segments (3–4 cm)	Adventitious shoots	MS + 0.2 mg/l BA	½ MS basal	80	Sudha et al. (2000)
47.	*Holostemma ada-kodien*	Nodal segment	Axillary sprouting	MS + 2.0 mg/l BAP + 0.5 mg/l IBA	½ MS + 0.05 mg/l IBA	90	Martin (2002)
	Holostemma ada-kodien	Leaf, internode	Somatic embryogenesis	MS + 1.0 mg/l 2,4-D	50% embryos maturation and conversion	90	Martin (2003)
48.	*Kaempferia galanga*	Rhizome	Plantlet	0.75 MS + 12 µM BA + 3.0 µM NAA	0.75 MS + 12 µM BA + 3.0 µM NAA	+	Shirin et al. (2000)
	Kaempferia galanga	Rhizome tip and lateral bud	Organogenesis and multiple shoot regeneration	MS + 2.0 mg/l BA + 0.2 mg/l NAA	MS + 1.0 mg/l IBA	81	Kalpana and Anbazhagan (2009)

(continued)

Table 8.1 (continued)

Sl. no.	Plant species	Explant	Mode of multiplication	Multiplication medium	Rooting medium	Establishment in soil (%)	Reference
	Kaempferia galanga	Rhizome tip and lateral bud	Multiple shoots	MS + 1.0 mg/l BA + 0.1 mg/l NAA	Modified MS + 0.2 mg/l IBA	85	Rahman et al. (2005)
	Kaempferia galanga	Rhizome with vegetative buds	Callus induced embryogenesis	MS + 0.1 mg/l BA + 1.0 mg/l NAA	MS basal	+	Vincent et al. (1992a)
	Kaempferia galanga	Axillary buds	Axillary shoots	MS + 13.9 μM Kn + 2.2 μM BA	MS + 13.9 μM Kn + 2.2 μM BA	90	Vincent et al. (1992b)
	Kaempferia galanga	Rhizome buds	Multiple shoots	MS + 0.57 μM IAA + 4.65 μM Kn	MS + 6–9% sucrose + 22.2 μM BAP or 23.25 μM Kn	80–90	Chirangini et al. (2005)
	Kaempferia galanga	Microshoots	Microshoots mediated microrhizome	MS + 22.2 μM BA or 23.25 μM Kn + 6/9% sucrose	MS + 6–9% sucrose + 22.2 μM BAP or 23.25 μM Kn	80–90	Chirangini et al. (2005)
	Kaempferia galanga	Rhizome buds	Multiple shoots	MS + 2.69 μM NAA + 2.85 μM BAP	MS + 6–9% sucrose + 22.2 μM BAP or 23.25 μM Kn	80–90	Chirangini et al. (2005)
49.	*Litsea glutinosa*	Nodal explants	Multiple shoots	MS + 2.0 mg/l IAA + 3.0 mg/lBAP	MS + 1.0 mg/l IAA + 2.0 mg/l IBA	15	Tiwari et al. (2015)
50.	*Mesua ferrea*	Apical and axillary buds	Apical and axillary buds	WPM + 4.6 μM Kn + 4.3 μM BAP	Pulse treatment of 9800 μM IBA		Jadhav and Deodhar (2015)
51.	*Mucuna pruriens*	Nodal explants	Multiple shoots	½ MS + 5.0 μM BA + 0.5 μM NAA	MS + 1.0 μM IBA	90	Faisal et al. (2005)
52.	*Nardostachys jatamansi*	Petiole	Callus mediated organogenesis	MS + 3.0 mg/l NAA + 0.25 mg/l Kn	MS + 1.0 mg/l IAA or IBA	–	Mathur (1992)
53.	*Panax pseudoginseng*	Rhizome explants	Callus mediated organogenesis	MS + 2.5 mg/l BAP + 2.5 mg/l 2,4-D	½ MS + 1.0 mg/l GA_3	70	Kharwanlang et al. (2016)
54.	*Picrorhiza kurroa*	Nodal segment	Shoot multiplication	MS basal medium	MS + 1.0 mg/l	+	Sharma et al. (2010a, b)

	Picrorhiza kurroa	Leaf explant	Shoot multiplication	MS + 2.0 mg/l Kn + 0.50 mg/l IBA	MS + 1.0 mg/l	+	Sharma et al. (2010a, b)
	Picrorhiza kurroa	Cotyledonary node, shoot tip	Multiple shoots	MS + 1.0 µM BAP	MS + 0.5 µM NAA	85–92	Chandra et al. (2004)
	Picrorhiza kurroa	Nodal segments	Multiple shoots	Ms + 1.0 µM BAP	MS + 1.0 /2.5 µM IBA	65	Chandra et al. (2006)
	Picrorhiza kurroa	Shoot tips	Adventitious shoots	MS + 3.0–5.0 mg/l Kn	MS + 1.0 mg/l NAA	–	Lal et al. (1988)
	Picrorhiza kurroa	Shoot tips	Axillary branching	MS + 0.22 mg/l BAP	MS + 0.1 mg/l NAA	–	Sharma et al. (1995), Upadhyay et al. (1989), Sharma and Sharma (2003)
	Picrorhiza kurroa	Nodal segment from germinated seed and mature plant	Callus mediated organogenesis	MS + 0.2 mg/l NAA	MS + 0.4 mg/l NAA	–	Jan et al. (2010)
55.	*Picrorhiza scrophulariiflora*	Rhizome, shoot tips	Multiple shoots	WPM + 0.44 µM BAP	WPM + 5.3 µM NAA	90	Bantawa et al. (2010)
56.	*Plectranthus barbatus*	Leaf explants	–	MS + 1.5 mg/l Kn + 2.0 mg/l BAP + 1.0 mg/l NAA	–	–	Thangavel et al. (2011)
57.	*Plumbago indica*	Nodal segments, leaf segments	Multiple shoot buds or callus	MS + 2.0 mg/l BA + 1.0 mg/l IAA	¼ MS + 0.5 mg/l IAA + 0.75% sucrose	90	Bhadra et al. (2009)
	Plumbago indica	Encapsulated clump of shoots	4–6 plantlets/bead	MS + 2.0 mg/l BAP + 3% sucrose	MS + 1.0 mg/l putrescine	–	Bhattacharyya et al. (2007)
	Plumbago indica	Nodal segments	Adventitious shoot multiplication	MS + 3.0 mg/l BA + 0.1 mg/l IAA	–	–	Chetia and Handique (2000)

(continued)

Table 8.1 (continued)

Sl. no.	Plant species	Explant	Mode of multiplication	Multiplication medium	Rooting medium	Establishment in soil (%)	Reference
58.	*Podophyllum hexandrum*	Zygotic embryo	Somatic embryogenesis via callus	MS + 2.0 μM BA + 0.5 μM IAA	–	–	Arumugam (1989), Arumugam and Bhojwani (1990)
	Podophyllum hexandrum	Zygotic embryo	Multiple shoots	MS + 1.0 μM BAP + 1.0 μM IAA	–	–	Nadeem et al. (1996)
	Podophyllum hexandrum	Zygotic embryo	Somatic embryogenesis	MS + 5.0 μM NAA + 0.5 μM BAP	–	–	Nadeem et al. (1996)
59.	*Rauvolfia serpentina*	Axillary buds	Multiple shoot formation	MS + 1.0 mg/l BAP + 0.1 mg/l NAA	MS + 1.5 mg/l NAA	–	Mathur et al. (1987)
	Rauvolfia serpentina	Shoot tips	–	MS + 2.0 mg/l BA + 0.5 mg/l NAA	MS + 0.5 mg/l NAA + 2.0 mg/l Kn	–	Mukhopadhyay et al. (1991)
	Rauvolfia serpentina	Nodal segments & shoot apices	Callus mediated organogenesis	0.5–0.1 mg/l BAP + 0.1 mg/l NAA	–	–	Sarkar et al. (1996)
	Rauvolfia serpentina	Axillary meristems	–	4.44 μM BA + 0.54 μM NAA	0.54 μM NAA	–	Roja and Heble (1996)
	Rauvolfia serpentina	Shoot tips & lateral buds	–	MS + 1.5 mg/l BA + 0.5 mg/l NAA	MS + 1.0 mg/l IBA + 1.0 mg/l IAA	–	Roy et al. (1996)
	Rauvolfia serpentina	Nodal segments	Multiple shoot formation	MS + 1.0 mg/l BA + 0.1 mg/l NAA	MS + 1.5 mg/l NAA	–	Sharma and Chandel (1992a), Sharma et al. (2007c), Sharma and Pandey (2013)
60.	*Rheum emodi*	Shoot tips	–	MS + 2.0 mg/l BAP + 1.0 mg/l IBA	–	90–95	Lal and Ahuja (1993)

61.	*Saraca asoca*	Shoot tip, nodal explants and intermodal explants	Shoot organogenesis	MS + 0.5 mg/l BAP	MS + 4.0 mg/l IBA	40	Subbu et al. (2008)
62.	*Saussurea lappa*	Shoot tips	Callus mediated organogenesis	MS + 5.0 µM BA + 3.0 µM GA_3	MS + 1.0 µM NAA	–	Arora and Bhojwani (1989), Sharma et al. (1995)
	Saussurea lappa	Shoot tips	–	MS + 0.45 µM TDZ	MS + 1.07 µM NAA	–	Johnson et al. (1997)
63.	*Shorea tumbuggaia*	In vitro germinate seedlings	Multiple shoots	MS + 6.66 µM BAP + 0.454 µM TDZ	½ MS + 2.24 µM IBA	70	Shukla and Sharma (2015)
64.	*Swertia chirayita*	Root explants	–	MS + 3.0 µM BAP	½ MS	–	Wawrosch et al. (1999)
	Swertia chirayita	Nodal explants	Axillary multiplication	MS + 4.0 µM BA + 1.5 µM 2iP	MS + 1.0 µM NAA + 500 mg AC	94	Joshi and Dhawan (2007)
	Swertia chirayita	Leaves	Callus mediated	MS + 2.5 mg/l BA + 0.1 mg/l Kn	½ MS + 400 mg/l AC + 0.1 mg/l NAA	70–80	Shailja (2017)
65.	*Tinospora cordifolia*	Nodal segments	Axillary shoots	MS + 5.0 µM BA + 150 µM glutamine	½ MS + 0.5 µM IBA	100	Mishra et al. (2010)
	Tinospora cordifolia	Mature nodes	Axillary shoots	WPM + 8.87 µM BA	½ MS + 2.85 µM IAA	80	Raghu et al. (2006)
	Tinospora cordifolia	Mature nodes	Axillary shoots	MS + 2.22 µM BA + 4.65 µM Kn	½ MS + 2.85 µM IAA	80	Raghu et al. (2006)
66.	*Tylophora indica*	Leaf explants	Adventitious shoots	MS + 5.0 µM Kn	MS + 0.5 µM IBA	–	Faisal et al. (2007)

(continued)

Table 8.1 (continued)

Sl. no.	Plant species	Explant	Mode of multiplication	Multiplication medium	Rooting medium	Establishment in soil (%)	Reference
	Tylophora indica	Leaf segment	Axillary buds	MS + 5.0 mg/l BAP + 0.5 mg/l NAA + 100 mg/l ascorbic acid	MS + 1.0 mg/l IAA	90–100	Sharma and Chandel (1992b)
	Tylophora indica	Adventitious shoots	Axillary shoots	½ MS + 5 μM Kn	MS + 0.5 μM IBA	+	Faisal and Anis (2003)
67.	*Utleria salicifolia*	Nodal segments	–	MS + 0.5 mg/l BAP	–	–	Gangaprasad et al. (2003)
68.	*Valeriana wallichii*	Shoot tips & axillary buds	Axillary shoots	MS + 5.0 mg/l Kn or BAP 1.0 mg/l NAA	MS + 5.0 mg/l BAP + 1.0 mg/l IAA	–	Mathur et al. (1988)
	Valeriana wallichii	Apical & axillary meristems	Callus mediated organogenesis	MS + 1.0 mg/l Kn + 0.25 mg/l NAA	Same as shoot multiplication medium	–	Mathur and Ahuja (1991)
	Valeriana wallichii	Node		MS + 2.5 mg/l Kn	+MS0	100	Verma et al. (2012)
69.	*Zanthoxylum rhetsa*	Nodal explants	Multiple shoots	MS + 2% sucrose + 10 mg/l TDZ	Rooted *ex vitro* by pretreatment with 1 mg /l catechol for ½ h	–	Augustine and D'Souza (1997)

2,4-d 2,4-dichlorophenoxy acetic acid, *2ip* 2-isopentenyl adenine, *ABA* abscisic acid, *AP* acid phosphatase, *B5* Gamborg, medium, *BAP* 6-benzyl aminopurine, *AC* activated charcoal, *IAA* indole-3-acetic acid, *IBA* indole-3-butyric acid, *Kn* kinetin, *MS* Murashige and Skoog medium, *NAA* naphthalene Acetic Acid, *NAOH* sodium hydroxide, *SH* Schenk and Hildebrandt medium, *TDZ* thiadiazon, *mg* milligram, *cm* centimeter, *μM* micromolar, *M* molar, *ppm* parts per million, *g* gram, *W/v* weight/volume, *L* liter, *RT* Revised tobacco medium, *TRIA* Triacontanol, (–) Not mentioned, (+) Successful

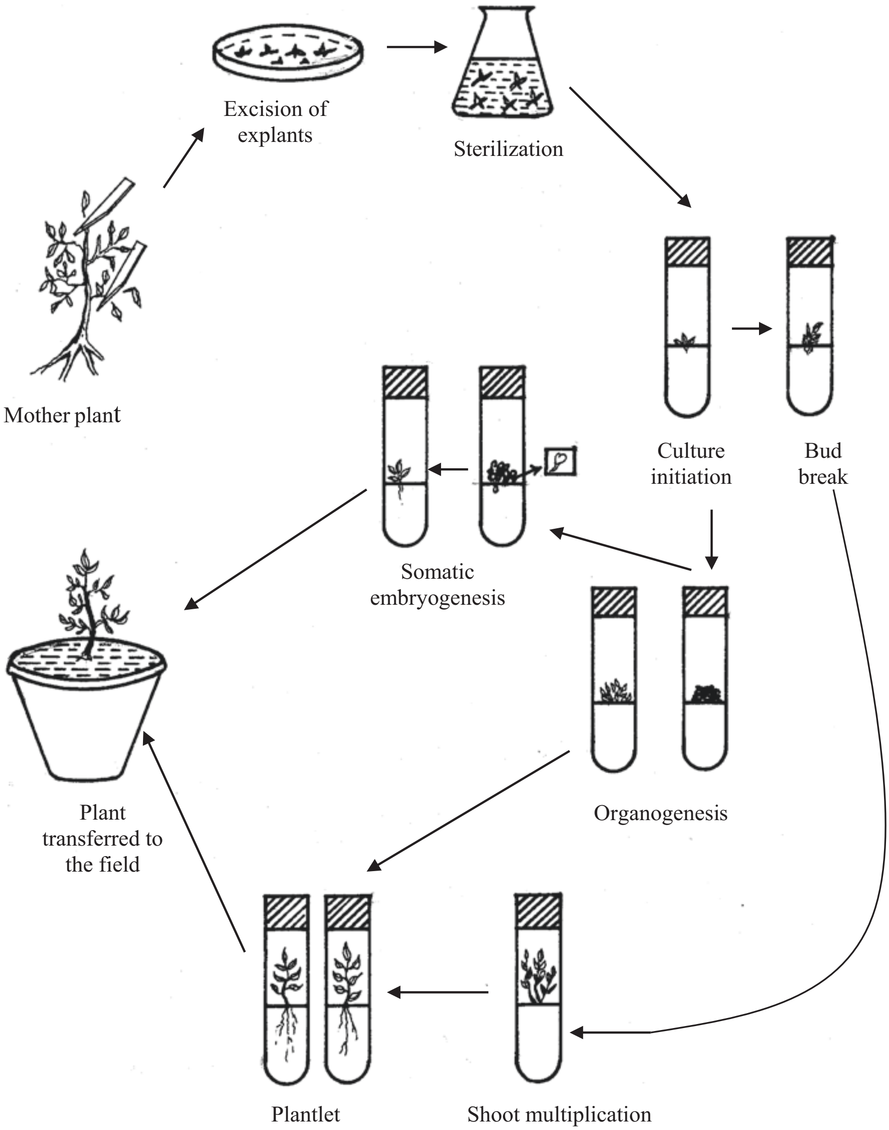

Fig. 8.1 Diagrammatic representation of in vitro propagation techniques applicable to threatened medicinal plants of India

antibiotic sprays, lowering of relative humidity, and use of drip irrigation may also reduce the risk of contamination. However, in case of threatened plants, it may not always be possible to grow the plants due to lack of information regarding their growth requirements and/or nonavailability/simulating conditions for favorable growth keeping in view the diverse forms and ecological niche of these plants. Many a times, limited number of propagules collected from threatened areas may limit options, but lead to culture establishment.

Establishment of Aseptic Cultures

Aseptic establishment of in vitro explants is an essential prerequisite for successful application of tissue culture technology. The decisive factors for successful in vitro establishment include physiology of mother plants, selection of explants, sterilization techniques, culture medium, etc. (Sharma and Pandey 2015b). In case of rare, endangered and threatened (RET) species, availability of limited propagules coupled with lack of information on in vitro techniques adds to the difficulty in initial establishment. In most of the cases, desirable explants have been terminal buds or nodal segments from seedlings or from field-grown plants. The primary explants may range in size from 0.1 mm to about 1 cm. Nodal segments have been used in *Bacopa monnieri* (Sharma et al. 2007a, 2016), *Commiphora wightii* (Barve and Mehta 1993), *Gentiana kurroo* (Sharma et al. 1993), *Rauvolfia serpentina* (Mathur et al. 1987; Sarkar et al. 1996; Sharma and Chandel 1992a), and *Valeriana wallichii* (Mathur et al. 1988; Sharma et al. 2000) and in many others (see Table 8.1). Proliferation of apical buds have also been achieved in plants like *Aconitum heterophyllum* (Giri et al. 1993), *Digitalis lanata* (Erdei et al. 1981), *Picrorhiza kurroa* (Lal et al. 1988; Upadhyay et al. 1989), and *Rheum emodi* (Lal and Ahuja 1993).

After washing with various surfactants and detergents, surface sterilization of explants is carried out using various sterilants (mercuric chloride, sodium hypochlorite, etc.); type and duration of sterilant treatment needs to be standardized for each species (Sharma 1995a). After sterilization, the explants are implanted aseptically on to the culture medium. Continuous monitoring of cultures is done to screen microbial contamination. Various factors affecting successful establishment of explants include time of sterilization, position of explant on the stem, explant size, and polyphenol oxidation. Among the various culture media formulated, Murashige and Skoog's (Murashige and Skoog 1962) basal medium is the most frequently used medium. Cytokinins and auxins are added to the medium to enhance shoot development. The type and concentration of growth regulators is dependent on the species, genotype, explant, and mode of propagation (Table 8.1). For a new species, the media can be tailored using information from previous reports or that which has been applied to related species. As mentioned above, sometimes number of propagules available for initiation of cultures is limited (2–3). Devising the sterilization protocol in such cases is very crucial and depends on calculated risk based on the experience of the researcher. The practice to start with mild sterilization (0.01–0.05% mercuric chloride for 2–10 min) treatment using a media with low concentrations of growth regulators (0–0.05 mg/l) has been excellent strategy experienced by authors.

Multiplication of Propagule

Once the explant has been established in culture, depending on the species and cultural conditions, regeneration can be achieved by (1) enhanced axiliary shoot proliferation, (2) de novo formation of adventitious shoots, or (3) somatic embryogenesis. Figure 8.1 illustrates various stages of propagation. Preferably, shoot tips or nodal segments are cultured on a nutrient medium containing specific combination of cytokinin and/or auxin to stimulate bud break. Other propagules such as leaf segments and roots can also be used for propagation in cases where either the propagules are not available or are in limited number or not responding to in vitro technique.

The type of regeneration is largely dependent on combination of growth regulators. A high concentration of cytokinin usually stimulates continued multiplication of axillary or adventitious shoots, whereas a high auxin:cytokinin ratio is generally stimulatory for callus induction. Auxin like 2,4-D in combination with high nitrogen is conducive for somatic embryogenesis (Sharma 1995b). Although basic media formulation tends to be constant, extensive experimentation is required to standardize for combination of growth regulators for each species. Various combinations of growth regulator reported for in vitro propagation of a number of medicinally important threatened plants of India, as depicted in Table 8.1.

Currently, the most frequently used micropropagation method for conservation as well as commercial production utilizes enhanced axiliary shoot proliferation from cultured meristems. Proliferation of axillary buds is achieved by repeated subculturing of propagules onto fresh shoot multiplication medium, which varies from species to species. Multiple shoot cultures are divided into smaller clusters, individual shoot tips, or nodal segments that serve as propagules for further proliferation. Sharma et al. (1993) reported a 15-fold increase in number of shoots in *Gentiana kurroo* using nodal segments and shoot tips as explants on MS basal medium containing 8.9 μM BA and 1.1 μM NAA. As many as 40 shoots per 8 weeks were observed in cultures of *Rauvolfia serpentina* on MS basal medium containing 1.0 mg/l BAP + 0.1 mg/l NAA (Mathur et al. 1987). In vitro multiplication of *Orchis latifolia,* a threatened medicinal orchid, was achieved on MS medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA using sprouted buds as explants (Sharma and Chandel 1996). Occasionally, corm-like structures were also produced on the same medium (see Table 8.1).

Organogenesis from explants results in de novo formation of both shoot and roots. It starts with distinct organization of shoot and/or root meristem within the explant or from callus. Callus-mediated shoot regeneration has been reported in *Aristolochia* sp. (Siddique et al. 2006a), *Crocus sativus* (Ahuja et al. 1993; Plessner et al. 1990), *Podophyllum hexandrum* (Bhojwani et al. 1989), *Nardostachys jatamansi* (Mathur 1992), *Saussurea lappa* (Arora and Bhojwani 1989), *Swertia chirata* (Shreshta and Joshi 1992), etc. In our laboratory, direct adventitious shoots regeneration in *Curculigo orchioides* was obtained using leaf segment explants (Sharma et al. 2009).

Somatic embryogenesis results in the formation of a bipolar embryonic structure either directly in the explant or from callus, depending upon the type of explant, composition of culture medium, and subculture regime (Sharma 1995b). As illustrated in Table 8.1, somatic embryogenesis has been reported in a few threatened species such as *Aconitum heterophyllum* (Giri et al. 1993), *Crocus sativus* (Ahuja et al. 1994), *Commiphora wightii* (Kumar et al. 2006), *Dioscorea deltoidea* (Sharma and Chaturvedi 1989), *Holostemma ada-kodien* (Martin 2003), and *Podophyllum hexandrum* (Arumugam 1989; Arumugam and Bhojwani 1990). However, in most of the cases, the success is limited to induction of somatic embryos except In *H. ada-kodien* (40 embryos were obtained per 10 mg callus and there was 50% embryo maturation and conversion with 90% success in *ex vitro* transfer) (Martin 2003).

Plantlet Establishment, Elongation, and Rooting

This stage is characterized by arrest of rapid multiplication and establishment of fully developed plantlets. It involves shoot elongation, root formation, and when required formation of storage organs. Rooting of shoots is achieved using auxins like IAA, IBA, and NAA, singly or in combination, or by transferring to growth regulator-free medium (see Table 8.1). In *Picrorhiza kurroa,* Upadhyay et al. (1989) reported rooting in 89% of the cultures on MS basal medium containing 0.1 mg/l NAA while in the same species Lal et al. (1988) reported inclusion of 1.0 mg/l NAA to be beneficial for rooting response. In *Rheum emodi,* rooting was observed on medium containing 1 mg/l IBA (Lal and Ahuja 1993). Reduction in concentration of salts of the medium is also known to increase rooting response. Most of the cultures of *Gentiana kurroo* rooted on ½ MS basal medium containing 6% sucrose (Sharma et al. 1993).

Acclimatization

The quality of regenerated plants to be transferred to in vivo conditions is critical to the success of any in vitro propagation protocol. Plantlets exhibiting abnormalities such as hyperhydricity or apex necrosis should be discarded. Complete plantlets after removal of adhering media are transferred to field following a gradual hardening. This involves a stepwise shift in temperature, light and humidity regime of the plant. In *Valeriana wallichii,* simulated optimum temperature (20 ± 2 °C), humidity conditions (60–70% RH), and 14 h photoperiod in a growth chamber resulted in 100% establishment of plantlets during the period from March to September which is otherwise considered not suitable for transferring plants as the temperature during this period is very high for plantlet survival (Mathur et al. 1988). Success rate of transfer out is higher in tropical plant species compared to those of temperate/high-altitude region (Table 8.1). This may be due to difficulty/limitation in simulating conditions required for hardening of temperate plants, though success of varying degrees has been reported in *Picrorhiza* (Chandra et al. 2004, 2006) while in a number of tropical RET species (both at NBPGR and TBGRI, and in literature), plantlets transferred directly to pots and maintained at high humidity/mist chambers (without any pretreatment procedure) exhibited 70–100% survival and establishment (Krishnan et al. 2011; Sharma and Pandey 2013).

During last three decades, in vitro techniques have been increasingly applied for multiplication of threatened medicinal plants. As evident from Table 8.1, in vitro propagation has been reported with varying degree of success in more than 70 threatened medicinal plants of India. It is emphasized that although somatic embryogenesis and adventitious callogenesis result in faster multiplication, this approach is not preferred for conservation. Existing meristematic material (meristem/shoot tip) is the explant of choice for vegetative propagation, as the fidelity of germplasm is more likely to be maintained. High multiplication rate, reported in some species, for example, in *Aristolochia, Bacopa* (129 shoots – explant leaf; Tiwari et al. 2001), and *Gymnema* may, have advantages for raising plants for nurseries for reintroduction and commercial plantings.

8.3.3 In Vitro Conservation

As discussed above, clonal multiplication leading to regeneration of plantlets through tissue culture is an important prerequisite for in vitro conservation. It is to re-emphasize that though the rate of shoot multiplication varies from 3.5 fold/3 weeks in *Saussurea lappa* (Arora and Bhojwani 1989) to as high as 150 shoots every 4 months in *Coleus forskohlii* (Sen and Sharma 1991), for conservation, very high multiplication rate is not desirable. The mode of regeneration is preferred to be through axillary sprouting, e.g., *Coleus forskohlii* (Sharma et al. 1991; Sen and Sharma 1991) and *Gentiana kurroo* (Sharma et al. 1993) in order to ensure genetic stability of the conserved plants.

The main objective in developing an in vitro conservation method is to reduce the frequent demand of subculturing. In vitro cultures can be conserved under normal growth conditions or subjected to growth-limiting conditions for short- to medium-term conservation whereas cryopreservation, i.e., conservation under suspended growth, offers the potential solution for long-term conservation. This can be achieved by using one or a combination of the techniques described in the following sections.

8.3.3.1 Normal Growth

Cultures can be stored virtually indefinitely under standard culture conditions provided nutrients are supplied continuously and accidents avoided. This method is preferred for naturally slow growing culture systems and for cultures for which there is no other method of choice. In the case of unorganized cultures, there is a risk of loss of regeneration capacity and the progressive accumulation of variant genotypes. Also this method is laborious and abounds with risks of genetic alterations with time or loss due to error or contamination. The advantages of the method include saving inputs on low-temperature facility (particularly for developing countries), avoid stress-induced variability, and ensure ready availability of material for multiplication and distribution. Shoot cultures of *Rauvolfia* can be maintained without recourse to any growth inhibitory treatment, on a simple tissue culture medium (MS medium supplemented with 1 mg/l BAP) for 12–24 months at 25 °C (Gautam et al. 2000; Sharma et al. 2000). In our laboratory, maintenance of large number of threatened species such as *Bacopa monnieri*, *Costus speciosus, Curculigo orchioides, Picrorhiza kurroa, etc.,* on a single medium has been a significant achievement.

8.3.3.2 Slow Growth

The principle of this method is that the growth of the culture is reduced significantly leading to increased subculture interval. Slow growth strategies allow the cultures to be held for 1–2 years under tissue culture conditions without subculture, depending on species (Table 8.2). This is achieved by any of following methods used singly or in combination.

Table 8.2 Status of in vitro conservation of threatened medicinal plants of India

Plant species	Culture system	Strategy	Period of conservation	Institute/Country of conservation	Reference
Acorus calamus	Shoot culture	MS + 1.0 mg/l BAP, 20 °C	12 months	Sri Lanka	Hettiarachchi et al. (1997)
Bacopa monnieri	Nodal segments	MS + 0.2 mg/l BA covered with mineral oil	6–24 months	NBPGR, India	Sharma et al. (2012)
Bacopa monnieri	Shoot cultures	Polypropylene caps (PP) at 25 °C	12 months	NBPGR, India	Sharma et al. (2007a, c)
Bacopa monnieri	Multiple shoot clumps	½ MS + 2% sucrose; polypropylene caps	20 months	Center for Medicinal Plant Research, India	George et al. (2007)
Bacopa monnieri	Encapsulated shoot tips	In a vial without nutrient medium at 25 °C	6 weeks	NBPGR, India	Sharma and Pandey (2013)
Baliospermum montanum	Shoot tip cuttings	½ MS + 1.33 μM BA + 1% agar, polypropylene caps at 25 °C	12 months	TBGRI, India	Krishnan et al. (2011)
Coleus forskohlii	Axillary shoots	Polypropylene caps at 25 °C	18 months	NBPGR, India	Sharma et al. (1995), Chandel and Sharma (1996, 1997)
Curculigo orchioides	Shoot cultures	Polypropylene caps at 25 °C	8–12 months	NBPGR, India	Sharma et al. (2009)
Dioscorea spp.	Shoot cultures	MS + 0.15 mg/l NAA at 25 ± 2 °C or MS + 0.05 mg/l BAP + 0.1 mg/l NAA	10–12 months	NBPGR, India	Sharma et al. (2014)
Gentiana kurroo	Axillary shoots	Low temperature (LT) 10 °C	11 months	NBPGR, India	Sharma et al. (1995)
Gentiana kurroo	Axillary shoots	LT 4 °C	30 months	NBPGR, India	Sharma (2001)
Gloriosa superba	In vitro root tuber	MS + 3 mg/l NAA + 1 mg/l TDZ	24 months	India	Madhavan and Joseph (2010)
Hemidesmus indicus	Shoot cultures	½ MS + 2% sucrose; polypropylene caps	18–22 months	Center for Medicinal Plant Research, India	George et al. (2010)
Holostemma annulare	In vitro nodes	½ MS + 2% mannitol; polypropylene caps caps; 25oC	12 months	TBGRI, India	Krishnan et al. (2011)
Kaempferia galanga	Shoot cultures	Polypropylene caps at 25 °C	12 months	NBPGR, India	Sharma et al. (2000)
Orchis latifolia	Shoots	LT 10 °C	10 months	NBPGR, India	Sharma and Chandel (1996)
Picrorhiza kurroa	Axillary shoots	LT 5 °C in dark	10 months	NBPGR, India	Upadhyay et al. (1989)

Picrorhiza kurroa	Axillary shoots	25 °C osmoticum	9 months	NBPGR, India	Chandel et al. (2000)
Picrorhiza kurroa	Axillary shoots	LT 10 °C osmoticum	16 months	NBPGR, India	Sharma et al. (1995, 2000)
Podophyllum hexandrum	Somatic embryogenesis	LT, 5 °C	7 months		Arumugam and Bhojwani (1990)
Rauvolfia serpentina	Axillary shoots	MS + 4.44 µM BA + 0.54 µM NAA 15 °C	15 months	NBPGR, India	Sharma and Chandel (1992a)
Rauvolfia serpentina	Axillary & adventitious shoots	PP, osmoticum 25 °C and 15 °C	9 and 15–20 months, respectively	NBPGR, India	Sharma et al. (1995), Chandel et al. (1996)
Rauvolfia serpentina	Shoot cultures	Minimal media	18–24 months	NBPGR, India	Chandel et al. (1996), Sharma et al. (2007c)
Rauvolfia serpentina	Root cultures	25 °C	16 years with periodic subculture	NBRI, India	Chaturvedi et al. (1991)
Saussurea lappa	Axillary & adventitious shoots	LT, 4 °C	12 months	Delhi University, India	Arora and Bhojwani (1989), Bhojwani et al. (1989)
Saussurea lappa	Axillary & adventitious shoots	LT, 4 °C & 10 °C	15 months	NBPGR, India	Sharma et al. (1995, 2000), Gautam et al. (2000)
Tylophora indica	Axillary shoots	LT 15 °C & 25 °C	12 months	NBPGR, India	Chandel and Sharma (1996), Sharma et al. (1995)

- Reduction in temperature and/or light
- Use of minimal growth media or osmotica in the medium
- Addition of growth retardants in the medium
- Culture tube enclosures
- Modification of gaseous environment reduction of oxygen pressure
- Desiccation and Encapsulation/Induction of storage organs

Reduction in Temperature and/or Light

The principle behind this is that incubation at temperature or light intensity lower than that required for optimum growth would reduce or decrease the metabolic activities, thereby restricting the growth of the plants. The subculture period is prolonged without any significant injuries. In *Picrorhiza kurroa,* shoot cultures have been stored for 10 months at 5 °C in dark, with 70% survival. Thus, conserved shoots on transfer to 25 °C multiplied at rates comparable to the cultures maintained under normal conditions (Upadhyay et al. 1989). *Gentiana kurroo,* another threatened medicinally important species, has been conserved for up to 30 months at 4 °C (Sharma 2001). In two tropical species, *B. monnieri* and *R. serpentina,* shoot cultures were successfully conserved for over 15 months at 15 °C, while 10 °C and 5 °C were deleterious for growth of cultures (Sharma and Chandel 1992a; Sharma et al. 2016). Successful results of low temperature have also been obtained in many species (Table 8.2) such as S*aussurea lappa* (Arora and Bhojwani 1989; Gautam et al. 2000), *Picrorhiza kurroa* (Sharma et al. 2000), and *Podophyllum hexandrum* (Bhojwani et al. 1989).

The light intensity can be reduced by 60% from standard requirement. Reduction of light along with temperature has extended shelf life of cultures in *Saussurea lappa*. According to Arora and Bhojwani (1989), shoot cultures of *Saussurea* could be successfully stored at 4 °C in dark for 12 months with 100% viability (Sharma et al. 1995).

This method is very simple and may be applicable to a wide range of genotypes. It is to emphasize that for tropical species, low temperature for conservation varies from 15 to 18 °C while for temperate/alpine species, the ideal temperature for conservation is 4–10 °C. However, maintenance of reduced temperature for long term may be difficult and expensive in tropical regions.

Use of Minimal Growth Media or Osmotica in the Medium

Different modifications of culture medium can be made in order to reduce growth. Altering the mineral contents or carbon source, either as nutrient factor or as osmotic factor, can have a marked effect on growth rate. In *Rauvolfia serpentina*, on half-strength medium and full-strength medium without hormones, the cultures showed 35–50% survival rates after 6 months of storage at 25 °C (Sharma and Chandel 1992a, b). Similarly, use of half MS was shown to be beneficial in conservation of shoot cultures in *Decalepis arayalpathra* and *Holostemma annulare* (see Krishnan et al. 2011). Use of sucrose at an increased (5–10%) or decreased (0.5–2%) dose proved beneficial in storing shoot cultures of many species such as *Hemidesmus* sp., *Utleria salicifolia* (George et al. 2010), and *Gentiana kurroo* (Neelam Sharma).

Inclusion of non-metabolizable, inert sugar alcohol particularly mannitol and sorbitol in the range of 3–6% (w/v) have been quite effective in restricting the growth of many plant species and prolonging subculture period. Though reported to extend subculture duration in *Holostemma annulare* and *Gentiana kurroo*, there is no documented information regarding its effective use in case of threatened plant species.

Use of Growth Retardants

These compounds reduce the overall growth rate of the in vitro plantlets and thereby enhance the subculture interval. Growth retardants such as maleic hydrazide (MH), abscisic acid (ABA), n-dimethylaminosuccinamic acid, transcinnamic acid (TCA), chlorocholine chloride (phosphon-D), daminozide (B 995) (DASA), and cycocel (CCC) have been reported to extend subculture period to 6–12 months. However, the optimal level of growth retardants required for a particular system needs to be determined. Use of growth retardants for slowing down the growth of cultures is generally avoided, as they are known to cause genetic alterations. To the best of our knowledge, there is no report of use of growth retardants in threatened medicinal plants. However, reduction of growth regulator concentrations in multiplication media has been beneficial in extending subculture period in *Bacopa monnieri*, *Coleus forskohlii, Curculigo orchioides,* and *Picrorhiza kurroa* (Tables 8.2 and 8.3).

Culture Tube Enclosures

Types of enclosure of the culture vessel have been shown to influence the rate of evaporation of the medium. The replacement of cotton plugs with polypropylene caps as enclosures has been known to increase the storage period. Germplasm of various medicinal species is conserved for more than two decades using the polypropylene caps at in vitro repository of NBPGR (Mandal et al. 2000). This is one of the most easy, simple, cost-effective, and safe technique when used singly and/or in combination with other techniques.

Shoot cultures of *Bacopa monnieri*, *Coleus forskohlii, Curculigo orchioides, Gentiana kurroo*, *Rauvolfia serpentina*, etc., have been conserved for 10–24 months at 25 °C without requiring any intermittent subculturing (Krishnan et al. 2011; Sharma and Chandel 1992a, b; Sharma et al. 1995, 2000, 2016) by use of polypropylene caps instead of cotton plugs (Table 8.2). The increase in storage duration is attributed to reduction in evaporation of water from the medium in the culture tubes.

Modification of the Gaseous Environment Reduction of Oxygen Pressure

The growth of in vitro cultures is influenced by the composition and atmospheric pressure inside the culture vessel. Growth reduction can be achieved by reducing the quantity of oxygen available to the culture. The simplest method consists of

Table 8.3 Status of in vitro cryopreservation of threatened medicinal plants of India

Plant species	Propagule cryopreserved	Cryopreservation technique	Regrowth of cryopreserved propagule	Reference
Bacopa monnieri	*In vitro* shoot tips	Vitrification	20% regrowth	Sharma et al. (2009)
Dioscorea bulbifera	*In vitro* shoot tips	Encapsulation-dehydration	Plantlet regeneration	Malaurie et al. (1998)
Dioscorea bulbifera	Somatic embryos/ embryogenic tissue	Encapsulation-dehydration	Regeneration of plants	Mandal (1999), Mandal et al. (2009)
Dioscorea deltoidea	*In vitro* shoot tips	Encapsulation dehydration/ Vitrification	Shoot regeneration	Mandal (2003), Mandal and Dixit (2000)
Dioscorea deltoidea	*In vitro* shoot tips	Vitrification	40–60% regrowth	Sharma et al. (2014), Sharma and Pandey (2015a)
Dioscorea floribunda	*In vitro* shoot tips	Encapsulation-dehydration	75% survival; 25% regeneration	Mandal et al. (2000), Mandal and Ghosh (2007)
Dioscorea floribunda	*In vitro* shoot tips	Vitrification	87% survival; 30% regeneration	Mandal et al. (2000), Mandal and Ghosh (2007)
Holostemma annulare	*In vitro* shoot tips	Pregrowth; encapsulation-dehydration	54.2% regeneration	Decruse et al. (1999, 2004), Decruse and Seeni (2002)
Kaempferia galanga	*In vitro* shoot tips	Pregrowth; vitrification	50% regeneration	Krishnan et al. (2011)
Kaempferia galanga	Somatic embryos	Pregrowth; desiccation	42.8% regeneration	Krishnan et al. (2011)
Picrorhiza kurroa	*In vitro* shoot tips	Cold hardening pregrowth; vitrification	70% survival, regeneration up to 35%	Sharma and Sharma (2003)
Rauvolfia serpentina	Nodal segments	Pregrowth; vitrification	66% regeneration	Ray and Bhattacharya (2008)

covering the explants with paraffin, mineral oil, or liquid medium. However, some of the problems associated with decreased oxygen concentration encountered are hyperhydricity of explants during storage and partial or complete necrosis. In *Bacopa monnieri,* shoot cultures could be conserved for 24–36 months using mineral oil overlay (Sharma et al. 2012). Although viewed as a promising technique, it is yet to prove its worth in conservation of other medicinal plants.

Desiccation and Encapsulation/In Vitro Induction of Storage Organs

Encapsulation of somatic embryos/shoot tips/ axillary buds in calcium alginate has emerged as a promising strategy for in vitro storage of germplasm. Once the tissue is encapsulated, it can be dehydrated to a suitable level using osmotic agent (sucrose) and air-drying treatments and then subjected to either slow growth or cryopreservation. In *Bacopa monnieri*, encapsulated shoot tips/nodal segments were successfully conserved in a cryovial without any medium for up to 6 months (Sharma et al. 2016). This technique once developed can contribute significantly to the conservation of germplasm diversity, as it requires minimum inputs and infrastructure.

Though advocated as a promising strategy to increase the storage period in plant species, in vitro induction of storage organs has been reported only in *Kaempferia galanga* (Chirangini et al. 2005) and *Gloriosa superba* (Ghosh et al. 2007; Madhavan and Joseph 2010) (see Table 8.2).

8.3.3.3 Suspended Growth

Cryopreservation, a method in which explants are suspended at ultra low temperature (−196 °C) in liquid nitrogen (LN2) to facilitate the arrest of metabolic activities, offers the potential for long-term conservation.

Cryopreservation is the preferred and only option for the long-term storage of clonal germplasm and for genetic variants with special medicinal or industrial value, recalcitrant seeds, rare germplasm, disease-free plants, pollen, and embryogenic cultures (Bajaj 1995; Benson 1999; Engelmann 2000; Reed 2008). It involves storage of germplasm in the form of buds, shoot tips, meristems, embryos, and cells at the temperature of LN_2 (−196 °C) or in vapor phase above LN_2. A classical cryopreservation technique involves pregrowth, cryoprotection, freezing, storage, thawing, and recovery. Figure 8.2 illustrates the various steps and techniques that can be used for cryopreservation of germplasm per se.

Successful cryopreservation of in vitro cultures can be achieved by using either slow cooling, encapsulation-dehydration, or vitrification techniques, which differ in their dependence on precisely controlled cooling. Cryoprotectant mixtures (such as PVS_2 or PVS_3) are used to protect the tissue against the desiccation injury. Encapsulation-dehydration technique involves dehydration of encapsulated explants followed by air-drying before freezing. The technique of vitrification includes treatment with a highly concentrated solution of cryoprotectant mixture followed by

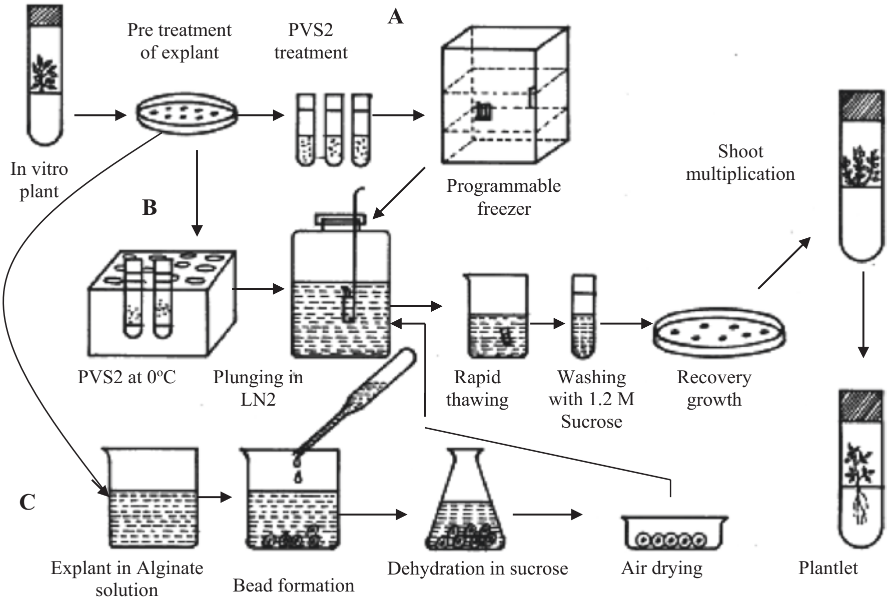

Fig. 8.2 Schematic representation of steps and techniques for cryopreservation of shoot tips/meristems. The explant (ca 1.0 mm) dissected from in vitro shoots is pregrown on a medium containing supplement with low concentration of cryoprotectant such as 5% DMSO (dimethyl sulfoxide) at low temperature for 2 days and then subjected to cryopreservation by any of the following techniques:

A. Slow cooling: the explant is subjected to slow cooling (0.5–2.0 °C/min) in a cryoprotectant solution at a controlled rate (0.2–2.0 °C/min) to a determined prefreezing temperature (usually around –40 °C) in a programmable freezer followed by rapid immersion in LN2

B. Vitrification: it involves cryoprotectant treatment [e.g. PVS2 (Plant Vitrification Solution 2 of Sakai et al. 1990)] for precise time before plunging in LN2

C. Encapsulation: dehydration that involves encapsulation of explant in calcium alginate, dehydration in high sucrose solution followed by air drying under laminar air flow before freezing in LN2

The cryopreserved explant is subjected to rapid thawing by plunging the cryovials in warm water at 40 °C, washed with high sucrose solution (1.2 M) and then implanted on to recovery medium

rapid cooling. The most critical step in the latter two protocols is the dehydration step in contrast to freezing step in classical protocols. The cryopreserved explant is then subjected to thawing at 40–45 °C/25 °C before implanting onto regrowth medium following washing with high sucrose solution for recovery growth. The regenerated plants can then be multiplied by repeated subculture on shoot multiplication media.

Despite significant success with shoot tips/meristems of a large number of crop species such as garlic, yams, cassava, *Solanum* spp. wasabi orange, and lily (Mandal et al. 2000), information on the cryopreservation of shoot tips of threatened medicinal plants is limited (Decruse et al. 1999; Mandal 2000; Chandel et al. 1996) (Table 8.4). However, extensive research is required with respect to optimization of various steps

Table 8.4 Genetic stability studies in threatened medicinal plants of India

Plant species	Culture system	Strategy	Response	Reference
Alpinia calcarata	Regenerated plants	Molecular [Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR)] analyses	No variability in the *in vitro* multiplied plantlets	Bhowmik et al. (2016)
Bacopa monnieri	Regenerated plants	Molecular (RAPD analysis)	No change observed in regenerants	Ceasar et al. (2010)
Bacopa monnieri	Regenerated plantlets from conserved cultures	Molecular (RAPD), Biochemical (HPLC) and Morphological analyses	No variation was observed	Sharma et al. (2012)
Bacopa monnieri	Regenerated plantlets from cryopreserved shoot tips	Molecular (RAPD), Biochemical (HPLC) and Morphological analyses	No variation was observed	Sharma et al. (2017)
Celastrus paniculatus	Regenerated plants	Molecular (RAPD) analysis	No polymorphism was detected in regenerated plants and the mother plan	Phulwaria et al. (2013)
Coleus forskohlii	Regenerated plants	Cytological analysis	Seven plants showed diploids while three did not	Sen and Sharma (1991)
Coleus forskohlii	6-month -old regenerated plants	Chemical analysis	No change in forskolin content	Sharma et al. (1991)
Dioscorea bulbifera	Explant cryopreserved derived in vitro plantlets	Molecular (RAPD) Biochemical (HPLC) and Morphological analyses	Cryopreserved derived plants maintained genetic stability	Dixit et al. (2003)
Dioscorea deltoidea	Explant cryopreserved derived in vitro plantlets	Biochemical (HPLC) and Morphological analyses	Cryopreserved derived plants maintained genetic stability	Dixit-Sharma et al. (2005)
Dioscorea floribunda	Explant cryopreserved derived in vitro plantlets	Molecular(RAPD) Biochemical (HPLC) and Morphological analyses	Cryopreserved derived plants maintained genetic stability	Ahuja et al. (2002)
Holostemma ada-kodien	In vitro regenerants	Molecular (RAPD) analysis	In vitro raised plants were monomorphic and similar to that of the mother plant.	Tuppad et al. (2017)
Picrorhiza kurrooa	In vitro regenerants	Molecular (RAPD) analysis	Genetic stability of plants derived from encapsulated microshoots following 3 months of storage	Mishra et al. (2011)

(continued)

Table 8.4 (continued)

Plant species	Culture system	Strategy	Response	Reference
Picrorhiza kurrooa	*In vitro* regenerants	Molecular (RAPD and ISSR) analyses	No significant differences observed in regenerants and mother plant, but notable differences observed among three adventitious shoots regenerated from three calli	Rawat et al. (2013)
		Phytochemical study (HPLC)	Tissue culture raised plants showed higher secondary metabolite (picrotin and picrotoxinin) as compare to mother plant	
Rauvolfia serpentina	*In vitro* regenerants	Electrophoretic analysis	No change observed in regenerants	Chandel et al. (1996)

of cryopreservation protocol for individual species. Success has been achieved to varying degrees with respect to cryopreservation and plantlets regeneration from in vitro shoot tips of *Bacopa, Dioscorea* spp., and *Picrorhiza kurroa* at NBPGR (Mandal et al. 2000; Sharma and Pandey 2015a; Sharma and Sharma 2003; Sharma et al. 2011, 2017) and in *Holostemma annulare* at TBGRI (Decruse et al. 1999, 2004; Decruse and Seeni 2002). Cryopreservation protocol with subsequent plant regeneration has been developed for shoot tips of *B. monnieri*, *Dioscorea deltoidea*, and *D. floribunda.* Application of vitrification and encapsulation-dehydration techniques resulted in high-frequency regeneration (up to 50%) from cryopreserved explants of yams (Mandal 2000, 2003; Mandal and Ghosh 2007). Plantlets thus generated could be successfully transferred to soil in *Bacopa* and *Dioscorea* spp. (Sharma et al. 2009, 2011; Mandal et al. 2000; Mandal 2003). Removal of NH+ ions (ammonium nitrate) from culture medium during preparative procedures in *Holostemma annulare* and cold hardening of shoots (22 °C/5 °C) in *Picrorhiza kurroa* enhanced post-thaw recovery and success of cryopreservation (Decruse et al. 1999, 2004; Decruse and Seeni 2002; Sharma et al. 2011; Sharma and Sharma 2003). Application of cryopreservation for long-term conservation (cryobanking) of germplasm of threatened medicinal plants has not yet been reported. However, the use of cryopreservation is limited to small laboratory collections, and its use on a large scale is currently exceptional.

8.4 Genetic Stability

Monitoring of genetic stability is an important aspect of in vitro slow growth and cryopreservation. The techniques of monitoring stability will depend on the need, type, and nature of species and its economic product (see Chandel et al. 1996). In literature, there is limited information on the topic, and the available information deals mainly with comparisons between *in vitro* regenerants and their putative parents (Table 8.4) (Chandel et al. 1996; Sharma and Pandey 2013, 2015a). For example in *B. monnieri*, shoot cultures conserved using mineral oil overlay exhibited maintenance of genetic stability as assessed by morphological, biochemical (Bacoside) and molecular analyses (RAPD and ISSR) (Sharma et al. 2012). Further studies using morphological, molecular, and biochemical parameters indicated maintenance of genetic stability after in vitro slow growth and cryopreservation in *B. monnieri* and *Dioscorea* spp. (Ahuja et al. 2002; Dixit et al. 2003; Sharma et al. 2017).

Owing to the diverse nature of threatened medicinal plants coupled with the fact that these are less worked out for active principle, it is practically difficult to devise general strategy for screening for active principle (secondary metabolites), which is the important parameter for their conservation. This, however, is one of the thrust areas which need attention to ensure conservation for sustainable utilization.

8.5 Conservation: Practical Application

8.5.1 In Vitro Gene Bank

In India, experiments on in vitro slow growth and cryopreservation of plants were initiated in early 1980s at few universities and research centers (Bhojwani 1981; Bhojwani et al. 1989; Chaturvedi et al. 1982). However, most of these works were supported financially by ad hoc projects and/or meant for Ph.D. dissertation. Thus, these works could not be continued for long or be expanded to the desired extent. In 1986, concerted research efforts on various aspects of in vitro slow growth and cryopreservation were initiated with launching of a special project "National Facility for Plant Tissue Culture Repository" (NFPTCR) at ICAR-NBPGR jointly by Indian Council of Agricultural Research (ICAR) and Department of Biotechnology (DBT) to carry out in vitro conservation of "problem crops," difficult to conserve by conventional means including medicinal, aromatic, and rare/threatened plant species. Today, renamed as Tissue Culture and Cryopreservation Unit (TCCU), the facility at ICAR-NBPGR has become an acknowledged center for its significant contribution in comprehensive in vitro conservation of various plant species (about 50) of both tropical and temperate nature (Ashmore 1997; Mandal et al. 2000). Later, the Department of Biotechnology, the nodal agency for the G-15 GEBMAP (Gene Banks for Medicinal and Aromatic Plants) project in India, established a network of four national gene banks at ICAR-NBPGR Tropical Botanic Garden and Research Institute (TBGRI), Central Institute of Medicinal and Aromatic Plants (CIMAP), and Regional Research Laboratory (RRL) Jammu under the G-15 GEBMAP program for medicinal and aromatic plants. This has not only given better focus and thrust especially on collection and conservation of medicinally important threatened species but also helped in consolidating the ongoing efforts in the country (Chandel et al. 2000; Natesh 1999, 2000; Sharma and Pandey 2013).

At ICAR-NBPGR, New Delhi, India, 110 accessions comprising 20 species are maintained as shoot cultures in the in vitro gene bank. Some of these include *Acorus calamus, Chlorophytum borivilianum, Holostemma ada-kodien, Gentiana kurroa, Costus speciosus, Curculigo orchioides, Rauvolfia* spp., *Tylophora indica, Swertia chirayita* and *Valeriana sp*.

In the in vitro bank at TBGRI, Thiruvananthapuram, India, 20 accessions belonging to 18 medicinal plant species are maintained as shoot cultures and include *Acorus calamus, Adhatoda beddomei, Alpinia calcarata, Celastrus paniculatus, Geophila reniformis, Holostemma annulare, Rauvolfia micrantha, R. serpentine*, and *Utleria salicifolia* (JNTBGRI 2012–2014).

Regarding in vitro conservation at gene bank at CIMAP, Lucknow, India, 10 RET species, namely, *Picrorhiza kurroa*, *Rauvolfia serpentina*, *Rheum emodi*, and *Valeriana wallichii*, are maintained, as shoot cultures, while *Aconitum heterophyllum*, *Nardostachys grandiflora*, and *Panax* spp. are maintained as callus/somatic embryos (Kumar et al. 1999).

8.5.2 Conservation Through Reintroduction

Reintroduction is the re-establishment of plants of an endangered species into an area suitable for its growth or from where it has become threatened. The attraction of in vitro propagation lies in its ability for rapid regeneration of plantlets and their establishment. Such plants produced in large numbers can also be reintroduced in nature especially in case of rare or threatened plant species. The idea is to establish a self-sustaining population for conservation purposes. Taking the lead from the success of the technology in a number of species including *Vanda* spp. (Seeni and Latha 2000; Decruse et al. 2003) and *Syzygium travancorium* (Anand 2003), efforts were made at TBGRI for reintroduction of 8 medicinal plants including the endemic/threatened species of Western Ghats such as *Decalepis arayalpathra, Mahonia leschenaultia, Heracleum candolleanum, Calophyllum apetalum*, and *Blepharistemma membranifolia* (Krishnan et al. 2011). Through successful reintroduction with 78–95% establishment after 1–2 years, the reintroduction carried out at TBGRI on the experimental scale needs to be extended to more plants in more than one locality. Consequently, a national program on recovery of endangered taxa, initiated by the DBT, Government of India, has given considerable boost to the necessity of saving endangered species through the use of in vitro propagation technology. However, the impact of such restoration on ecosystem needs further study.

8.6 Conclusion

Development of efficient methods for germplasm conservation of medicinal plants is a high priority for many countries including India in view of the rapid depletion of these valuable resources from their natural habitat. The future of the herbal and pharmaceutical industry depends on the continuous supply of medicinal and aromatic plant's materials. Conservation of the genetic diversity of medicinal and aromatic plants germplasm will not be guaranteed without a proper conservation program. A network approach similar to that initiated by DBT under the umbrella of G-15 project may be adopted and linked to ICAR-NBPGR, the nodal agency at national level for conservation of various crop plants including medicinal and aromatic plants. First and foremost prerequisite of any such program is prioritization of species keeping in view the availability of resources and infrastructure.

The urgent need to conserve the germplasm of rare, elite, and endangered species of medicinal plants by using aforementioned in vitro techniques, especially those of recalcitrant types, is the priority issue that has emerged from above cited facts. Against this backdrop, application of in vitro conservation methods holds the key for the continued availability and sustainable utilization of these overlooked resources.

However, before applying in vitro conservation and cryopreservation techniques to a new system, it is essential to develop new methods or optimize standard protocols for specific plant species and/or tissue types. A large number of species are recalcitrant to in vitro manipulations. Notwithstanding the limitation of material availability and technical-know-how of propagation for the large number of species, significant advances have been made in the past three decades in the in vitro conservation of threatened medicinal plants in India (Chandel et al. 1996; Sarasan et al. 2006; Sharma and Pandey 2013). Advocated as a promising tool for long-term germplasm conservation, cryopreservation of medicinal and aromatic plants is still at an experimental stage. In medicinal plants, it is highly essential to retain quality and quantity of secondary metabolites for the sustainable utilization on long-term basis. This is another thrust area needing immediate attention. The developments in plant cell and tissue culture techniques have also provided an alternative to whole plant cultivation for obtaining several plant-derived chemicals. *Agrobacterium tumefaciens*-mediated genetic transformation studies in *Picrorhiza kurroa* (Bhat et al. 2012) and use of elicitors in *Bacopa monnieri* (Jauhari et al. 2019) open up new avenues for conducting further research on aspects related to enhancing secondary metabolites. Using in vitro methods, the compounds can be obtained under precise controlled conditions unaffected by seasonal conditions within a short period of time.

Conservation biotechnology has opened up new vistas by offering new tools not only for conservation of medicinal plants but also in the assessment and monitoring of biodiversity. With the application of biotechnology to conventional approaches of conservation, it is expected that more number of threatened medicinal species will be conserved and investigated for their potential medicinal values. With the advent of biotechnology, conservation of DNA and/or gene libraries offers additional strategy for conservation. As a means of conserving a portion of the gene pool, this technique is particularly useful for threatened species especially for those species where no other strategy is applicable.

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Chapter 9
Geospatial Technologies for Threatened Medicinal Plant Conservation

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Abstract This chapter presents the applications of geospatial technology in managing threatened medicinal plant genetic resources for conservation. It ensures integrated approach to use geospatial technologies (remote sensing, geographic information system, global positioning system and information system) in precise way to map, quantify and predict threatened taxa and diversity-rich areas besides other applications in the forestry sector. The Indian sub-continent is endowed with unique combination of habitats, ecosystems and medicinal plants of economic importance, which together make up rich and diverse forest genetic resources. The relative abundance and richness of medicinal plant species is another criterion to measure the degree of diversity. Management of threatened medicinal plant genetic resources (MPGR) at national level involves collation of enormous data and its analysis crucial to the effectiveness of its organizational process and also adding extensively to the value of natural resources. Innovations in geospatial technology are underutilized in the management of plant

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genetic resources (PGR) including those of threatened medicinal plant genetic resources (MPGR) in India and many other countries around the world. Geospatial technology and Geographical Information system (GIS) technology could help better meet the challenges and facilitate enhanced decision support including planning colletcing, conserving and managing threatened MPGR. Herbal medicines continue to play significant role in country's health sector, as evidenced by activities of Ayush Ministry. Hence sustainable management of threatened MPGR is of atmost important for the country's health security. In this paper the potential of using geospatial technologies in the management of threatened MPGR are highlighted.

Keywords Conservation · GIS · Geospatial technology · Remote sensing · Threatened medicinal plants

9.1 Geospatial Technologies

Technology relating to the collection or processing of data that is associated with location is known as geospatial technology. Common examples of geospatial technologies are

(a) *Global Positioning System* (GPS): A satellite-based geolocation system that functions throughout the globe and accessible to common man through handheld units, viz., GPS units. These units record a location point associated with all observations and help with data management. Researchers could easily revisit the exact site where the threatened medicinal plant taxa located for long-term research. Also, GPS allows co-researchers to verify the results. A network of U.S. Department of Defence satellites can give precise coordinate locations to researchers with proper receiving handheld equipment. The autonomous Indian Regional Navigation Satellite System (IRNSS) with an operational name of NAVIC (NAVigation with Indian Constellation) with seven satellites in space segment launched during 2013–2017. NAVIC provides accurate real-time positioning and timing services and would be made operational by Indian Space Research Organisation (ISRO) from early 2018 for the two levels of service, viz., the 'standard positioning service' (civilian use) and a 'restricted service' for authorised users.

(b) *Remote Sensing*: The process of imagery and data collected from space or airborne camera and sensor platforms is known as remote sensing, the technology of acquiring information about an object or target from space without any physical contact with it. Some commercial satellite image providers now offer images showing details of one meter or even much smaller, making these images appropriate for monitoring, planning and deploying humanitarian and other needs or interventions.

In remote sensing, sun light is the source of energy and electro-magnetic radiation (EMR) is the medium of interaction to the earth's objects. The sun energy reached on the earth and some part of them absorbed, reflected, transmitted or emitted by the object/target and reflected energy recorded by the satellite sensor and stored in digital form for further image analysis. Remote sensing represents a technology for synoptic acquisition of spatial data and the extraction of specific/selected area information. Remote sensing satellite data combined with ground-truthing provides valuable information on different threatened species by using temporal (time interval) digital data. Remote sensing has the capability to collect real-time data of the same area of the earth's surface at different periods of time, which is one of the most important features in satellite technology. Spectral features (reflectance value of a particular object/species) may change over time, and these changes can be detected by collecting and comparing multi-date satellite data using ratio vegetation index (RVI) and normalized difference vegetation index (NDVI). Since spectral characteristics (reflectance value) of different threatened species vary at the same period of observations that could be used to locate their availability (Singh et al. 2002). Thus, remote sensing coupled with other geospatial technologies could be used for developing suitable strategies for conservation of threatened taxa and developing medicinal plant conservation areas.

(c) *Internet Mapping Technologies*: Software programs like Google Earth and web features like Microsoft Virtual Earth are revolutionizing the way geospatial data is viewed and shared. The developments in user interface are also making such technologies available to a wider audience whereas traditional GIS have been reserved for specialists and those who invest time in learning complex software programs.

(d) *Geographical Information System (GIS)*: A geographic information system (GIS) integrates hardware, software and data for capturing, managing, analysing and displaying all forms of geographically referenced information. GIS allows us to view, understand, question, interpret and visualize data in many ways that reveal relationships, patterns, and trends in the form of maps, globes, reports and charts (www.esri.com). Thus, a GIS is a database management system that can simultaneously handle data representing spatial objects and their attribute data.

In other words, geographic information system (GIS) is a database management system which effectively stores, retrieves, manipulates analyses and displays spatial information of both cartographic and thematic origin. GIS is a computer-based system which can handle large volumes of spatial data related to threatened medicinal plants availability, distribution and conservation derived from a variety of sources such as field surveys, characterization and evaluation, documentation, aerial surveys, space remote sensing, in addition to the already existing maps and reports. This involves bringing together diverse information from a variety of sources on a common platform, viz., forestry.

Major components of GIS are data input, data encoding, data management,data analysis and manipulation, Data presentation or output (Fig. 9.1). Any data that can be mapped has both locational and non-locational characteristics. For example, a feature may exist at an X, Y location and possess an attribute, Z. The attributes can be both qualitative (land use, soil, etc.) or quantitative (altitude, plant population, etc.), and it may sometime vary with time (temperature, land use, population, etc.). These three components, viz., location, attribute and time, represents the content of most GIS and it is applicable to forestry GIS also.

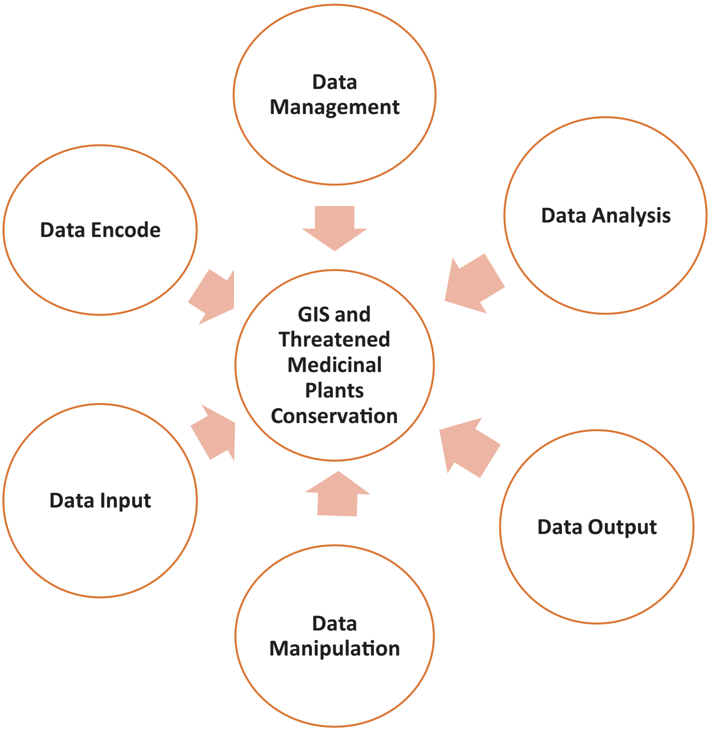

Fig. 9.1 Major components of geographic information system (GIS) for management of threatened medicinal plants

9.2 Medicinal Plant Wealth of India

India has a rich and varied heritage of plant biodiversity, encompassing a wide spectrum of habitats ranging from tropical rain forests to alpine vegetation and from temperate forests to coastal wetlands (Gautam 2004). The Indian sub-continent is one of the eight centres of origin (Vavilov 1951) and is one of the 12 mega diversity centres of the world with 11.9% of the world flora. India is endowed with rich plant genetic resource (PGR) wealth of 49,219 higher plant species including 5725 endemic species belonging to 141 genera under 47 families (Nayar 1980). Of these endemic species, 3500 are found in the Himalayan region and 1600 in the Western Ghats (Arora 1991). Out of 17,000–18,000 species of flowering plants recorded in India, more than 7000 are used for medicinal purposes in AYUSH system of medicine (Ayurveda, Unani, Siddha & Homoeopathy). Habitat-wise classification showed that about 33% are trees, 32% herbs, 20% shrubs, 12% creepers and 3% others (Kumar and Jnanesha 2016). An analysis of distribution of MAP in natural habitat showed that about 70% of India's MAPs are found in tropical forests of Western and Eastern Ghats, the Vindhyas, Chotta Nagpur plateau, Aravalis and the Himalayas. Studies also showed that a large percentage of known medicinal and aromatic plants occur in the dry and moist deciduous vegetation area compared to evergreen and temperate regions. Threatened medicinal plants of India are listed in Table 9.1. The Medicinal plants

Table 9.1 Threatened medicinal plants in India

S.No	Name of the plant	Family	Distribution	Status
1	*Abutilon ranadei* Woodr. *et* Stapf	Malvaceae	Maharashtra	E or presumed extinct
2	*Acacia campbellii* Arn.	Fabaceae	Andhra Pradesh	R
3	*Acer caesium* Wall. ex Brandis	Aceraceae	Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh	V
4	*Acer hookeri* Miquel var. *majus* Pax	Aceraceae	West Bengal	E
5	*Acer oblongum* Wall. *ex* DC. var. *membranaceum* Banerji	Aceraceae	Uttar Pradesh	E
6	*Acer oblongum* Wall. *ex* DC. var. *microcarpum* Hiern	Aceraceae	Arunachal Pradesh	E
7	*Acer osmastonii* Gamble	Aceraceae	West Bengal	E
8	*Acer sikkimense* Miq. var. *serrulatum* Pax	Aceraceae	Arunachal Pradesh	E
9	*Achyranthescoynei* Sant.	Amaranthaceae	Maharashtra	R
10	*Aconitum deinorrhizum* Stapf	Ranunculaceae	Jammu & Kashmir, Himachal Pradesh	V
11	*Aconitum falconeri* Stapf var. *latilobum* Stapf	Ranunculaceae	Himachal Pradesh	V
12	*Aconitum ferox* Wall. ex Seringe	Ranunculaceae	Himachal Pradesh, Sikkim	V
13	*Acranthera grandiflora* Bedd.	Rubiaceae	Tamil Nadu	E
14	*Acranthera tomentosa* R. Br. ex Hook. f.	Rubiaceae	Meghalaya, Assam, Nagaland	V
15	*Acrocephalus palniensis* Mukherjee	Lamiaceae	Tamil Nadu	I
16	*Acronema pseudotenera* Mukh.	Apiaceae	Sikkim	I
17	*Actinodaphne bourneae* Gamble	Lauraceae	Tamil Nadu	E
18	*Actinodaphne lanata* Meisner	Lauraceae	Tamil Nadu	E
19	*Actinodaphne lawsonii* Gamble	Lauraceae	Tamil Nadu, Kerala	R
20	*Adiantum soboliferum* Wall. ex Hook.	Adiantaceae	Assam, Nagaland	Possibly Extinct
21	*Adinandra griffithii* Dyer	Ternstroemiaceae	Meghalaya	E
22	*Aerva wightii* Hook. f.	Amaranthaceae	Tamil Nadu	I
23	*Aglaia talbotii* Sundararaghavan	Meliaceae	Karnataka, Goa	V

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
24	*Albertisiamecistophylla* (Miers) Forman	Menispermaceae	Assam, Meghalaya	I or Possibly Extinct
25	*Albizia thompsonii* Brandis	Fabaceae	Andhra Pradesh, Tamil Nadu, Orissa	R
26	*Allium stracheyi* Baker	Alliaceae	Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh	V
27	*Allophylus concanicus* Radlk.	Sapindaceae	Maharashtra	R
28	*Alniphyllum fortunei* (Hemsl.) Makino	Styracaceae	Arunachal Pradesh	R
29	*Ammania desertorum* Blatt. & Hallb.	Lythraceae	Gujarat, Rajasthan	R
30	*Amomum microstephanum* Baker	Zingiberaceae	Tamil Nadu, Kerala	R
31	*Amorphophallus longistylus* Kurz ex Hook. f.	Araceae	South Andaman Island	R
32	*Amorphophallus oncophyllus* Prain ex Hook. f.	Araceae	South Andaman Island	R
33	*Ampelocissus helferi* (Lawson) Planch.	Vitaceae	Andaman Islands	V
34	*Anaphalis barnesii* C.E.C. Fischer	Asteraceae	Kerala	E
35	*Aneilema glanduliferum* Joseph *et* Rolla Rao	Commelinaceae	Arunachal Pradesh	V
36	*Angelica nubigena* (Clarke) Mukh.	Apiaceae	Sikkim	I
37	*Anisochilus argenteus* Gamble	Lamiaceae	S. India	V
38	*Anisochilus wightii* Hook. f.	Lamiaceae	Tamil Nadu	R
39	*Anoectochilus nicobaricus* Balakr. *et* P. Chakarab.	Orchidaceae	Great Nicobar Island	E
40	*Anoectochilus rotundifolius* (Blatt.) Balakr.	Orchidaceae	Tamil Nadu	E or possibly extinct
41	*Anoectochilus tetrapterus* Hook. f.	Orchidaceae	Manipur	V or possibly endangered
42	*Anogeissus sericea* Brandis var. *nummularia* King ex Duthie	Combretaceae	Gujarat, Punjab, Rajasthan	R
43	*Antistrophe serratifolia* (Bedd.) Hook. f.	Myrsinaceae	Tamil Nadu, Kerala	R
44	*Aphyllorchis gollani* Duthie	Orchidaceae	Uttar Pradesh (Tehri Garhwal)	E or possibly extinct

45	*Aphyllorchis parviflora* King & Pantl.	Orchidaceae	Uttar Pradesh (Garhwal)	R
46	*Aponogeton appendiculatus* van Bruggen	Aponogetonaceae	Kerala, Tamil Nadu.	I
47	*Aponogeton satarensis* R. Sundararaghavan, A.R. Kulk. & S.R. Yadav	Aponogetonaceae	Maharashtra	V
48	*Archineottia microglottis* (Duthie) Chen	Orchidaceae	Uttar Pradesh (Garhwal)	R
49	*Arenaria curvifolia* Majumdar	Caryophyllaceae	Uttar Pradesh (Garhwal)	E
50	*Arenaria ferruginea* Duthie ex Williams	Caryophyllaceae	Uttar Pradesh (Kumaon)	E
51	*Arenaria thangoensis* Smith	Caryophyllaceae	Sikkim	V
52	*Argostemma khasianum* Clarke	Rubiaceae	Meghalaya	I
53	*Artabotrys nicobaricus* D. Das	Annonaceae	Nicobar Islands	R
54	*Asparagus jacquemonti* Baker	Asparagaceae	Maharashtra, Kerala	I
55	*Asparagus rottleri* Baker	Asparagaceae	Deccan Peninsula	I or insufficiently known
56	*Aspidopteris canarensis* Dalz.	Malpighiaceae	Karnataka, Kerala, Maharashtra.	R
57	*Aspidopteris oxyphylla* (Wall.) Juss.	Malpighiaceae	Meghalaya	I
58	*Aspidopteris tomentosa var. hutchinsonii (Haines) Srivastava*	Malpighiaceae	Orissa	R
59	*Athyrium atratum* Bedd.	Athyriaceae	Manipur	E
60	*Athyrium duthei* (Bedd.) Bedd.	Athyriaceae	North West and Eastern Himalaya	V
61	*Atunatravancorica* (Bedd.) Kosterm.	Chrysobalanaceae	Travancore hills	I
62	*Barleria gibsonioides* Blatt. *et* McC.	Acanthaceae	Maharashtra	R
63	*Begonia aborensis* Dunn	Begoniaceae	Arunachal Pradesh	R
64	*Begonia aliciae* C.E.C. Fischer	Begoniaceae	Kadalar Valley and Nilgiri Hills, Souther W. Ghats	E
65	*Begonia anamalayana* Bedd.	Begoniaceae	Southern W. Ghats	E
66	*Begonia brevicaulis* DC.	Begoniaceae	Meghalaya	E or possibly extinct

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
67	*Begonia burkillii* Dunn	Begoniaceae	Arunachal Pradesh	R
68	*Begonia canarana* Miq.	Begoniaceae	South West India, Western Ghats	E or possibly extinct
69	*Begonia cordifolia* (Wight) Thw.	Begoniaceae	South Deccan Paninsula, Western Ghats	R
70	*Begonia lushaiensis* C.E.C. Fischer	Begoniaceae	Mizoram	R
71	*Begonia phrixophylla* Blatt. *et* McC.	Begoniaceae	Maharashtra	R
72	*Begonia rubella* Buch.-Ham. ex D. Don	Begoniaceae	Sikkim	R
73	*Begonia rubrovenia* var. *meisneri*Clarke	Begoniaceae	Meghalaya	R
74	*Begonia satrapis* Clarke	Begoniaceae	West Bengal, Sikkim	R
75	*Begonia scintillans* Dunn	Begoniaceae	Arunachal Pradesh	I
76	*Begonia scutata* Wall. ex DC.	Begoniaceae	West Bengal, Sikkim	R
77	*Begonia subpeltata* Wight	Begoniaceae	South Deccan Peninsula, Western Ghats (Malabar)	R
78	*Begonia tessaricarpa* Clarke	Begoniaceae	Assam	I
79	*Begonia trichocarpa* Dalz.	Begoniaceae	Western Ghats, South West India	V
80	*Begonia watti* Clarke	Begoniaceae	Nagaland	E or possibly extinct
81	*Begonia wengeri* C.E.C. Fischer	Begoniaceae	Mizoram	I
82	*Belosynapsis kewensis* Hassk.	Commelinaceae	Tamil Nadu	E
83	*Belosynapsis vivipara* (Dalz.) Sprague *et* Fischer	Commelinaceae	Sahyadri Hills, Western Ghats	V
84	*Bentinckia condapanna* Berry ex Roxb.	Arecaceae	Kerala	R
85	*Bentinckia nicobarica* (Kurz) Becc.	Arecaceae	Nicobar Islands	E
86	*Berberis affinis* G. Don	Berberidaceae	Uttar Pradesh (Kumaon)	R
87	*Berberis apiculata* (Ahrendt) Ahrendt	Berberidaceae	Himachal Pradesh	R
88	*Berberis huegeliana* Schneid.	Berberidaceae	Jammu & Kashmir	I
89	*Berberis kashmiriana* Ahrendt	Berberidaceae	Jammu & Kashmir	R

90	*Berberis lambertii* Parker	Berberidaceae	Uttar Pradesh (Kumaon)	V or E
91	*Berberis osmastonii* Dunn	Berberidaceae	Uttar Pradesh (Garhwal)	R
92	*Bhidea burnsiana* Bor	Poaceae	Maharashtra, Karnataka	R
93	*Bombax insigne* Wall. var. *polystemon* Prain	Bombacaceae	Narcondam Islands	I
94	*Bridelia kurzii* Hook. *f.*	Euphorbiaceae	Andaman & Nicobar Islands	V
95	*Buchanania beriberi* Gamble	Anacardiaceae	Kerala	E
96	*Buchanania platyneura* Kurz	Anacardiaceae	Andaman & Nicobar Islands	I
97	*Bulbophyllum acutiflorum* A. Rich.	Orchidaceae	Tamil Nadu	R
98	*Bulbophyllum albidum* Hook. f.	Orchidaceae	Tamil Nadu	R
99	*Bulbophyllum aureum* (Hook. f.) J.J. Sm.	Orchidaceae	Kerala	R
100	*Bulbophyllum elegantulum* (Rolfe) J.J. Sm.	Orchidaceae	Karnataka	V
101	*Bulbophyllum kaitiense* (Wight) Reichb. f. Duthie	Orchidaceae	Nilgiris	V
102	*Bulleyia yunnanensis* Schltr.	Orchidaceae	Arunachal Pradesh, West Bengal	R
103	Bunium nothum (Clarke) Mukh.	Apiaceae	S. India (Nilgiri)	Possibly extinct
104	*Calamus dilaceratus* Becc.	Arecaceae	Andaman Islands	R
105	*Calamus inermis* T. Anders.	Arecaceae	Sikkim	E
106	*Calamus nagbettai* Fernandez *et* Dey	Arecaceae	Karnataka	V
107	*Calanthe alpina* Hook. f. ex Lindl.	Orchidaceae	Uttar Pradesh (Garhwal)	R
108	*Calanthe anthropophora* Ridley	Orchidaceae	Meghalaya	E
109	*Calanthe mannii* Hook. f.	Orchidaceae	Arunachal Pradesh, Uttar Pradesh (Garhwal), Sikkim	R
110	*Calanthe pachystalix* Reichb. f. ex Hook. f.	Orchidaceae	Himachal Pradesh, Uttar Pradesh (Mussoorie)	E
111	*Campanula alphonsii* Wall. ex DC.	Campanulaceae	Nilgiri and Pulney Hills, Western Ghats	R
112	*Campanula wattiana* Nayar *et* Babu	Campanulaceae	Himachal Pradesh, Uttar Pradesh	R

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
113	*Campylanthus ramosissimus* Wt.	Scrophulariaceae	Gujarat	R
114	*Capparis cinerea* Jacobs	Capparaceae	Manipur	I
115	*Capparis diversifolia* Wight &Arn.	Capparaceae	Tamil Nadu	V
116	*Capparis fusifera* Dunn	Capparaceae	Kerala, Tamil Nadu	R
117	*Capparis pachyphylla* Jacobs	Capparaceae	North East India	I
118	*Capparis rheedii* DC.	Capparaceae	Tamil Nadu, North Kanara	R
119	*Capparis shevaroyensis* Sundararaghavan	Capparaceae	Tamil Nadu	V
120	*Carex christii* Boeck.	Cyperaceae	Tamil Nadu	I or possibly extinct
121	*Carex fuscifructus* Clarke	Cyperaceae	Assam	I
122	*Carex kingiana* Clarke	Cyperaceae	Sikkim	I
123	*Carex munroi* Clarke	Cyperaceae	Himachal Pradesh	I
124	*Carex pseudoaperta* Kuekenth.	Cyperaceae	Tamil Nadu	I
125	*Carex repanda* Clarke	Cyperaceae	Meghalaya	Ex
126	*Carex vicinalis* Boott	Cyperaceae	Tamil Nadu	I
127	*Carum villosum* Haines	Apiaceae	Bihar	Possibly extinct
128	*Catamixis baccharoides* Thoms.	Asteraceae	Uttar Pradesh (Garhwal)	V
129	*Cayratia pedata* (lam.) Juss. ex Gagnepain var. *glabra* Gamble	Vitaceae	Nilgiri	R
130	*Cayratia roxburghii* (Wight *et* Arn.) Gagnepain	Vitaceae	Kerala, Tamil Nadu	V
131	*Ceropegia angustifolia* Wight	Asclepiadaceae	Meghalaya	V
132	*Ceropegia arnottiana* Wight	Asclepiadaceae	Meghalaya	E or possibly extinct
133	*Ceropegia attenuata* Hook.	Asclepiadaceae	Maharashtra, Karnataka	R
134	*Ceropegia barnesii* Bruce *et* Chatterjee	Asclepiadaceae	S. India	E
135	*Ceropegia beddomei* Hook. f.	Asclepiadaceae	Kerala	E
136	*Ceropegia decaisneana* Wight	Asclepiadaceae	Kerala, Tamil Nadu	R

137	*Ceropegia evansii* McCann Wight	Asclepiadaceae	Maharashtra	V
138	*Ceropegia fantastica* Sedgw.	Asclepiadaceae	Karnataka, Goa	E or possibly extinct
139	*Ceropegia fimbriifera* Bedd.	Asclepiadaceae	Karnataka, Tamil Nadu	V
140	*Ceropegia hookeri* Clarke ex Hook. f.	Asclepiadaceae	Sikkim	E
141	*Ceropegia huberi* Ansari	Asclepiadaceae	Maharashtra	V
142	*Ceropegia jainii* Ansari *et* Kulkarni	Asclepiadaceae	Maharashtra	R
143	*Ceropegia lawii* Hook. f.	Asclepiadaceae	Maharashtra	E
144	*Ceropegia lucida* Wall.	Asclepiadaceae	Meghalaya, Assam, Sikkim	E or possibly extinct
145	*Ceropegia maccannii* Ansari	Asclepiadaceae	Maharashtra	R
146	*Ceropegia maculata Bedd.[C. parviflora Trimen]*	Asclepiadaceae	Tamil Nadu, Kerala	E or possibly extinct
147	*Ceropegia mahabalei* Hemadri *et* Ansari	Asclepiadaceae	Maharashtra	E
148	*Ceropegia metziana* Miq.	Asclepiadaceae	Karnataka, Tamil Nadu, Kerala	R
149	*Ceropegia noorjahaniae* Ansari	Asclepiadaceae	Maharashtra	R
150	*Ceropegia oculata* Hook. f.	Asclepiadaceae	Maharashtra	R
151	*Ceropegia odorata* Nimmo ex Hook. f.	Asclepiadaceae	Maharashtra, Rajasthan	E
152	*Ceropegia omissa Huber*	Asclepiadaceae	Tamil Nadu	E
153	*Ceropegia panchganiensis* Blatter *et* McC.	Asclepiadaceae	Maharashtra	E
154	*Ceropegia pusilla* Wight *et* Arn.	Asclepiadaceae	Karnataka, Tamil Nadu, Kerala	R
155	*Ceropegia rollae* Hemadri	Asclepiadaceae	Maharashtra	R
156	*Ceropegia sahyadrica* Ansari *et* Kulkarni	Asclepiadaceae	Maharashtra	R
157	*Ceropegia santapaui* Wadhwa *et* Ansari	Asclepiadaceae	Maharashtra	R
158	*Ceropegia spiralis* Wight	Asclepiadaceae	Andhra Pradesh, Karnataka, Tamil Nadu, Kerala	V

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
159	*Ceropegia thwaitesii* Hook.	Asclepiadaceae	Tamil Nadu, Kerala	V
160	*Ceropegia vincaefolia* Hook. *emend.* Ansari	Asclepiadaceae	Maharashtra	R
161	*Chaerophyllum orientalis (Clarke) Mukh*	Apiaceae	Nagaland, Arunachal Pradesh	I
162	*Chlorophytum borivilianum* Sant. *et* Fernand.	Liliaceae	Gujarat, Maharashtra	R
163	*Chondrilla setulosa* Clarke ex Hook. f.	Asteraceae	Jammu & Kashmir	R
164	*Christella clarkei* (Bedd.) Holtt.	Thelypteridaceae	West Bengal, Sikkim	V
165	*Christella kaumaunica* Holtt.	Thelypteridaceae	Uttar Pradesh (Kumaon)	V
166	*Christensenia assamica* (Griff.) Ching	Christenseniaceae	Assam	V
167	*Christiopteris tricuspis* (Hook.) Christ.	Polypodiaceae	West Bengal	I
168	*Chrysoglossum hallbergii* Blatt.	Orchidaceae	Peninsular India (Tamil Nadu)	I or insufficiently known
169	*Cissus spectabilis* (Kurz) Planch.	Vitaceae	Sikkim, West Bengal	E
170	*Clarkella nana* (Edgew.) Hook. f.	Rubiaceae	Western Himalaya	R
171	*Clematis apiculata* Hoook. f. *et* Thoms.	Ranunculaceae	Meghalaya	E
172	*Clematis bourdillonii* Dunn	Ranunculaceae	Kerala	V
173	*Clematis theobromina* Dunn	Ranunculaceae	Tamil Nadu	R
174	*Cleome burmanni* Wight *et* Arn.	Capparaceae	Tamail Nadu, Kerala	I
175	*Cleyera japonica* Thunb. var. *grandiflora* (Wall. ex Choisy) Kobuski	Theaceae	Meghalaya	R
176	*Codonopsis affinis* Hook. f. *et* Thoms.	Campanulaceae	West Bengal	R
177	*Coelachne minuta* Bor	Poaceae	Maharashtra	R
178	*Coelogyne mossiae* Rolfe	Orchidaceae	Peninsular India	V
179	*Coelogyne rossiana* Reichb. f.	Orchidaceae	Assam, Mizoram	V
180	*Coelogyne treutleri* Hook. f.	Orchidaceae	Sikkim	Possibly extinct
181	*Commelina hirsuta* (Wight) Clarke	Commelinaceae	Nilgiri and Pulney Hills	R
182	*Commelina indehiscens* Barnes	Commelinaceae	Karnataka, Kerala, Tamil Nadu	R

183	*Commelina tricolor* Barnes	Commelinaceae	Tamil Nadu	V
184	*Commelina wightii* Rolla Rao	Commelinaceae	Tamul Nadu	V
185	*Coptis teeta* Wall.	Ranunculaceae	Arunachal Pradesh	V
186	*Corybus purpureus* Joseph *et* Yog.	Orchidaceae	Meghalaya	R
187	*Corymborkis veratifolia* (Reinw.) Bl.	Orchidaceae	Tamil Nadu	R
188	*Corypha macropoda* Lindel ex Kurz	Arecaceae	South Andaman Island	R
189	*Coryphoteris didymochlaenoides* (Clarke) Holtt.	Thelypteridaceae	Meghalaya	R
190	*Cotoneaster buxifolius* Wall. ex Lindley	Rosaceae	Tamil Nadu	V
191	*Cotoneaster simonsii* Hort. ex Baker	Rosaceae	Sikkim	I
192	*Crinum eleonorae* Blatt. &McC.	Amaryllidaceae	Maharashtra	R
193	*Crotalaria clavata* Wight *et* Arn.	Fabaceae	Tamil Nadu	E
194	*Crotalaria digitata* Hook.	Fabaceae	Tamil Nadu	R
195	*Crotalaria filipes* Benth. var. *trichophora* (Benth. ex Baker) Cooke	Fabaceae	Maharashtra	R
196	*Crotalaria fysonii* Dunn var. *glabra* Gamble	Fabaceae	Tamil Nadu	E
197	*Crotalaria globosa* Wight *et* Arn.	Fabaceae	Tamil Nadu, Karnataka	R
198	*Crotalaria kodaiensis* Debberm. *et* Biswas	Fabaceae	Tamil Nadu	E
199	*Crotalaria longipes* Wight *et* Arn.	Fabaceae	Tamil Nadu	E
200	*Crotalaria lutescens* Dalz.	Fabaceae	Karnataka, Maharashtra	R
201	*Crotalaria meeboldii* Dunn	Fabaceae	Nagaland	I
202	*Crotalaria noveoides* Griff.	Fabaceae	Meghalaya	I
203	*Crotalaria peduncularis* Grah. ex Wight *et* Arn.	Fabaceae	Tamil Nadu, Kerala	R
204	*Crotalaria priesleyoides* Benth. ex Baker	Fabaceae	Tamil Nadu	R
205	*Crotalaria rigida* Heyne ex Roth	Fabaceae	Tamil Nadu, Karnataka	R
206	*Crotalaria sandoorensis* Bedd. ex Gamble	Fabaceae	Karnataka	E

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
207	*Crotalaria scabra* Gamble	Fabaceae	Tamil Nadu	R
208	*Crotalaria stocksii* Benth. ex Baker	Fabaceae	Maharashtra, Andaman Island	R
209	*Cryptocarya ferrarsii* King	Lauraceae	Middle Andaman Island	I
210	*Cryptocoryne cognate* Schott	Araceae	Maharashtra	I
211	*Cryptocoryne cognatoides* Blatt. &McC.	Araceae	Karnataka, Maharashtra	V
212	*Cryptocoryne tortuosa* Blatt. &McC.	Araceae	Maharashtra	E
213	*Cyananthus integra* Wall. ex Benth.	Campanulaceae	Tehri Garhwal	R
214	*Cyanotis burmanniana* Wight	Commelinaceae	Western coastal region	R
215	*Cyanotis cerifolia* Rolla Rao *et* Kammathy	Commelinaceae	Tamil Nadu	I
216	*Cyathea nilgirensis* Holtt.	Cyatheaceae	Southern India	E
217	*Cyathocline lutea* Law ex Wight	Asteraceae	Maharashtra, Karnataka	R
218	*Cycas beddomei* Dyer	Cycadaceae	Andhra Pradesh	V
219	*Cyclea debiliflora* Miers	Menispermaceae	Meghalaya	I or possiblyextinct
220	*Cyclea fissicalyx* Dunn	Menispermaceae	Kerala	Insufficiently known
221	*Cyclea watti* Diels	Menispermaceae	Nagaland	I or possibly extinct
222	*Cyclogramma squamaestipes* (Clarke) Tagawa	Thelypteridaceae	Sikkim	R
223	*Cymbidium eburneum Lindl.*	Orchidaceae	Arunachal Pradesh, Manipur, Meghalaya, Nagaland, Sikkim	V
224	*Cymbidium hookerianum* Reichb. f.	Orchidaceae	Uttar Pradesh (Kumaon), Arunachal Pradesh, Sikkim	V
225	*Cymbidium tigrinum* Parish	Orchidaceae	Nagaland	R
226	*Cymbidium whiteae* King &Pantl.	Orchidaceae	N.E. Himalaya, Sikkim	E
227	*Cynometra beddomei* Prain	Fabaceae	Kerala	I
228	*Cynometra bourdillonii* Gamble	Fabaceae	Karnataka	V

229	*Cynometra travancorica* Bedd.	Fabaceae	Karnataka	R
230	*Cyperus dwarkensis* Sahni *et* Naithani	Cyperaceae	Gujarat	R
231	*Cypripedium cordigerum* D. Don	Orchidaceae	Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh (Garhwal)	R
232	*Cypripedium elegans* Reichb. f.	Orchidaceae	Sikkim, Uttar Pradesh (Garhwal)	R
233	*Cypripedium himalaicum* Rolfe	Orchidaceae	Sikkim, Uttar Pradesh (Garhwal, Kumaon)	R
234	*Dalechampia stenoloba* Sundararaghavan *et* Kulkarni	Euphorbiaceae	Karnataka	R
235	*Decaschistia rufa* Craib	Malvaceae	Peninsular India	E
236	*Decaschistia trilobata* Wight	Malvaceae	Peninsular India	R
237	*Delphinium uncinatum* Hook. f. *et* Thoms.	Ranunculaceae	Jammu & Kashmir, Himachal Pradesh	V
238	*Dendrobium arachnites* Reichb. f.	Orchidaceae	India	V
239	*Dendrobium aurantiacum* Reichb. f.	Orchidaceae	Assam	E
240	*Dendrobium tenuicaule* Hook. f.	Orchidaceae	Middle Andaman Island	E
241	*Dendroglossa minutula* (Fee) Copel.	Polypodiaceae	Meghalaya	E
242	*Dennstaedtia elwesii* (Bak.) Bedd.	Dennstaedtiaceae	Sikkim	Possibly extinct
243	*Desmos viridiflorus* (Bedd.) Safford	Annonaceae	Tamil Nadu, Kerala	E
244	*Deyeuxia simlensis* Bor	Poaceae	Himachal Pradesh	Presumed extinct
245	*Dialium travancoricum* Bourd.	Fabaceae	Kerala	I
246	*Dicanthium armatum* (Hook. f.) Blatt. *et* McCann	Poaceae	Maharashtra	R
247	*Dicanthium compressum* (Hook. f.) Jain *et* Deshpande	Poaceae	Maharashtra	R
248	*Dicanthium maccannii* Blatt.	Poaceae	Maharashtra	V
249	*Dicanthium panchaganiensis* Blatt. *et* McCann	Poaceae	Maharashtra	R
250	*Dicanthium woodrowii* (Hook. f.) Jain *et* Deshpande	Poaceae	Maharashtra	V
251	*Dicliptera abuensis* Blatt.	Acanthaceae	Rajasthan	E

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
252	*Dicliptera ghatica* Sant.	Acanthaceae	Maharashtra	I
253	*Dicranopteris linearis* (Burm. f.) Underw. var. *sebastiana* Panigr. & Dixit	Dicranopteridaceae	Tamil Nadu	V
254	*Dictyospermum ovalifolium* Wight	Commelinaceae	Kerala	R
255	*Didiciea cunninghamii* King *et* Prainex King *et* Pantl.	Orchidaceae	Sikkim, Uttar Pradesh (Garhwal)	E
256	*Didymocarpus missionis* Wall. ex R. Br.	Gesneriaceae	Tamil Nadu	R
257	*Dimeria blatteri* Bor	Poaceae	Maharashtra	R
258	*Dimeria woodrowii* Stapf	Poaceae	Maharashtra, Goa, Karnataka	R
259	*Dioscorea deltoidea* Wall. ex Kunth	Dioscoreaceae	Kashmir to Assam	V
260	*Dioscorea rogersii* Prain& Burk.	Dioscoreaceae	Andaman Islands	I
261	*Dipcadi concanense* (Dalz.) Baker	Liliaceae	S. India	Possibly extinct
262	*Dipcadi maharashtrensis* Deb *et* Dasgupta	Liliaceae	Maharashtra	E
263	*Dipcadi minor* Hook. f.	Liliaceae	Deccan Plateau	I
264	*Dipcadi reidii* Deb *et* Dasgupta	Liliaceae	Western Himalaya	Presumed extinct
265	*Dipcadi saxorum* Blatt.	Liliaceae	Maharashtra	V
266	*Dipcadi ursulae* Blatt.	Liliaceae	Maharashtra	V
267	*Diplazium travancoricum* Bedd.	Athyriaceae	S. India	R
268	*Diplomeris hirsuta* (Lindl.) Lindl.	Orchidaceae	Uttar Pradesh (Kumaon), West Bengal	V
269	*Diplomeris pulchella* D. Don	Orchidaceae	Meghalaya, Arunachal Pradesh	V
270	*Drimia razii* Ansari	Liliaceae	Maharashtra	R
271	*Drynaria meeboldii* Rosenst.	Polypodiaceae	Manipur	V
272	*Drypetes andamanica* (Kurz) Pax &Hoffm.	Euphorbiaceae	S. Andaman Island	R
273	*Elaeagnus conferta* Roxb. ssp. *dendroidea*Servettaz	Elaeagnaceae	Meghalaya	E
274	*Elaeocarpus acuminatus* Wall. ex Mast.	Elaeocarpaceae	Meghalaya	R
275	*Elaeocarpus blascoi* Weibel	Elaeocarpaceae	Tamil Nadu	R
276	*Elaeocarpus gaussenii* Weibel	Elaeocarpaceae	Western Ghats	R

277	*Elaeocarpus munronii* (Wt.) Mast.	Elaeocarpaceae	Tamil Nadu, Karnataka, Maharashtra	R
278	*Elaeocarpus prunifolius* (C. Muell.) Mast.	Elaeocarpaceae	Meghalaya	R
279	*Elaeocarpus recurvatus* Corner	Elaeocarpaceae	Tamil Nadu	R
280	*Elaeocarpus venustus* Bedd.	Elaeocarpaceae	W. Ghats	V
281	*Elaphoglossum beddomei* Sledge	Elaphoglossaceae	Anamalai & Nilgiri Hills	R
282	*Elaphoglossum nilgiricum* Krajina ex Sledge	Elaphoglossaceae	Tamil Nadu	E
283	*Elaphoglossum stigmatolepis* (Fee) Moore	Elaphoglossaceae	Nilgiri Hills	V
284	*Eleiotis trifoliolata* Cooke	Fabaceae	Karnataka	R
285	*Eragrostis rottleri* Stapf	Poaceae	S. India	Presumed extinct
286	*Eremurus hamalaicus* Baker	Liliaceae	Jammu & Kashmir, Himachal Pradesh	R
287	*Eria albiflora* Rolfe	Orchidaceae	Tamil Nadu, Kerala, Karnataka	R
288	*Eria occidentalis* Seid.	Orchidaceae	Uttar Pradesh (Kumaon)	R
289	*Erinocarpus nimmonii* Graham	Tiliaceae	Maharashtra, Karnataka	R
290	*Eriocaulon humile* Moldenke	Eriocaulaceae	Maharashtra	V
291	*Eriochrysis rangacharii* Fischer	Poaceae	Tamil Nadu	Presumed extinct
292	*Eriolaena lushingtonii* Dunn	Sterculiaceae	Andhra Pradesh, Tamil Nadu	V
293	*Erysimum thomsonii* Hook. f.	Brassicaceae	Himachal Pradesh	R
294	*Eugenia argentea* Bedd.	Myrtaceae	Kerala	E or possibly extinct
295	*Eugenia discifera* Gamble	Myrtaceae	Tamil Nadu, Kerala	E
296	*Eugenia singampattiana* Bedd.	Myrtaceae	Tamil Nadu	E or possibly extinct
297	*Eulophia mackinnonii* Duthie	Orchidaceae	Uttar Pradesh, Madhya Pradesh	R
298	*Eulophia nicobarica* Balakr. & N.G. Nair	Orchidaceae	Nicobar Islands	E
299	*Euonymus angulatus* Wight	Celastraceae	Karnataka, Tamil Nadu, Kerala	E

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
300	*Euonymus assamicus* Blakelock	Celastraceae	Assam	E
301	*Euonymus serratifolius* Bedd.	Celastraceae	Tamil Nadu	E or possibly extinct
302	*Euphorbia katrajensis* Gage	Euphorbiaceae	Maharashtra	R
303	*Euphorbia panchganiensis* Blatt. & McCann	Euphorbiaceae	Maharashtra	R
304	*Ficus andamanica* Corner	Moraceae	South Andaman Island	R
305	*Fimbristylis stolonifera* Clarke	Cyperaceae	Meghalaya	R
306	*Flemingia gracilis* (Mukherjee) Ali	Fabaceae	Maharashtra, Karnataka	R
307	*Flickingeria hesperis* Seid.	Orchidaceae	Uttar Pradesh (Kumaon)	E
308	*Frerea indica* Dalz.	Asclepiadaceae	Maharashtra	E
309	*Garcinia cadelliana* King	Clusiaceae	South Andaman Island	I
310	*Garcinia kingii* Pierre ex Vesque	Clusiaceae	Andaman Island	I
311	*Garcinia manii* (King) Kosterm.	Clusiaceae	South Andaman Island	I
312	*Ginalloaandamanica* Kurz	Viscaceae	South Andaman Island	E
313	*Gleditsia assamica* Bor	Fabaceae	Arunachal Pradesh, Meghalaya, Nagaland	I
314	*Glycosmis macrocarpa* Wight	Rutaceae	Tamil Nadu, Kerala	R
315	*Glyphochloa divergens* (Hook.) Clayton	Poaceae	Karnataka	R
316	*Glyphochloa santapaui* (Jain *et* Deshpande) Clayton	Poaceae	Maharashtra	R
317	*Glyphochloa talbotii* (Hook. f.) Clayton	Poaceae	Goa, West coast of Peninsular India	V
318	*Gomphandra comosa* King	Icacinaceae	Andaman & Nicobar Islands	R
319	*Goniothalamus rhynchantherus* Dunn	Annonaceae	Tamil Nadu, Kerala	R
320	*Gymnema khandalense* Santapau	Asclepiadaceae	Maharashtra	R
321	*Habenaria andamanica* Hook. f.	Orchidaceae	South Andaman Island	R
322	*Habenaria barnesii* Summerh.	Orchidaceae	Tamil Nadu, Kerala	R
323	*Habenaria panchgeniensis* Sant. &Kapad.	Orchidaceae	Maharashtra	R

324	*Hedyotisalbonervia* Bedd.	Rubiaceae	Tamil Nadu	E
325	*Hedyotis barberi* (Gamble) Henry *et* Subramanyam	Rubiaceae	Tamil Nadu	V
326	*Hedyotis beddomei* Hook. f.	Rubiaceae	Kerala	E
327	*Hedyotis bourdillonii* (Gamble) Rolla Rao *et* Hemadri	Rubiaceae	Kerala	V
328	*Hedyotis brunonis* Merr.	Rubiaceae	West Bengal, Assam	R
329	*Hedyotis buxifolia* Bedd.	Rubiaceae	Tamil Nadu, Kerala	R
330	*Hedyotis cyanantha* Kurz	Rubiaceae	Tamil Nadu, Maharashtra, Karnataka	R
331	*Hedyotis eualata (Bedd. ex Gamble) Henry et Subramanyam*	Rubiaceae	Tamil Nadu, Kerala	R
332	*Hedyotis fruticosa* Linn.	Rubiaceae	Travancore	R
333	*Hedyotis hirsutissima* Bedd.	Rubiaceae	Tamil Nadu	Possibly extinct
334	*Hedyotis ramarowii* (Gamble) Rolla Rao *et* Hemadri	Rubiaceae	Tamil Nadu, Kerala	V
335	*Hedyotis scabra* Wall. ex Kurz	Rubiaceae	West Bengal, Assam, Arunachal Pradesh	R
336	*Hedyotis swersioides* Hook. f.	Rubiaceae	Tamil Nadu, Kerala	R
337	*Hedysarum astragaloides* Benth. ex Baker	Fabaceae	Jammu & Kashmir, Himachal Pradesh	R
338	*Hedysarum cachemirianum* Benth. ex Baker	Fabaceae	Jammu & Kashmir	R or possibly vulnerable
339	*Hedysarum microcalyx* Baker	Fabaceae	Jammu & Kashmir, Himachal Pradesh	V
340	*Helichrysum cutchicum* (C.B. Clarke) Rolla Rao *et* Deshpande	Asteraceae	Gujarat	R
341	*Helichrysum perlanigerum* Gamble	Asteraceae	Tamil Nadu	R
342	*Heliotropium calcareum* Stocks	Boraginaceae	Gujarat, Rajasthan, Maharashtra	R
343	*Heracleum jacquemontii* Clarke	Apiaceae	North West Himalaya	I
344	*Hildegardia populifolia* (Roxb.) Schott &Endl.	Sterculiaceae	Andhra Pradesh, Tamil Nadu	E
345	*Hippocratea andamanica* King	Hippocrateaceae	South Andaman Islands	R
346	*Hopea jacobi* Fischer	Dipterocarpaceae	Karnataka	R
347	*Hubbardia heptaneuron* Bor	Poaceae	Karnataka	Presumed extinct

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
348	*Hugonia belli* Sedgw.	Linaceae	Karnataka, Kerala	
349	*Humboldtia bourdilloni* Prain	Fabaceae	Tamil Nadu, Kerala	E
350	*Humboldtia decurrens* Bedd. ex Oliver	Fabaceae	Tamil Nadu	R
351	*Humboldtia laurifolia* Vahl	Fabaceae	Kerala	E
352	*Humboldtia unijuga* var. *unijuga* Bedd.	Fabaceae	Tamil Nadu, Kerala	E
353	*Huodendron biaristatum* (W.W. Sm.) Rehder	Styracaceae	Arunachal Pradesh	R
354	*Hydnocarpus macrocarpa* (Bedd.) Warb. ssp. *macrocarpa*	Flacourtiaceae	Tamil Nadu, Kerala	E
355	*Hydrocotyle conferta Wt.*	Apiaceae	Nilgiri&Pulney hills	R
356	*Hypoestes andamanensis* Thoth.	Acanthaceae	Andaman & Nicobar	V
357	*Hypoestes lanata* Dalz.	Acanthaceae	Maharashtra	R
358	*Ilex gardneriana* Wight	Aquifoliaceae	Peninsular India (Nilgiri)	Possibly extinct
359	*Impatiens anaimudica* Fischer	Balsaminaceae	Kerala	E or possibly extinct
360	*Impatiens johnii* E. Barnes	Balsaminaceae	Kerala	E or possibly extinct
361	*Impatiens macrocarpa* Hook. f.	Balsaminaceae	Kerala	E or possibly extinct
362	*Impatiens munnarensis* E. Barnes	Balsaminaceae	Kerala	E
363	*Impatiens neo-barnesii* Fischer	Balsaminaceae	Tamil Nadu	E
364	*Impatiens nilagirica* Fischer	Balsaminaceae	Tamil Nadu	E
365	*Impatiens pandata* E. Barnes	Balsaminaceae	Kerala	R
366	*Impatiens talbortii* Hook. f.	Balsaminaceae	Karnataka	R
367	*Indigofera barberi* Gamble	Fabaceae	Andhra Pradesh, Tamil Nadu	R
368	*Indigofera caerulea* Roxb. var. *monosperma*(Sant.) Sant.	Fabaceae	Gujarat, Rajasthan	R
369	*Indigofera constricta* (Thw.) Trimen	Fabaceae	Goa, Karnataka, Kerala	R

370	*Indopolysolenia wallichii* (Hook. f.) Bennet [*Polysoleniawallichii* Hook. f.]	Rubiaceae	Meghalaya	R
371	*Indotristicha tirunelveliana* Sharma, Karthi. & Shetty	Podostemonaceae	Tamil Nadu	R or V
372	*Inga cynometroides* (Bedd.) Bedd. ex Baker	Fabaceae	Kerala	I
373	*Inula kalapani* Clarke	Asteraceae	Meghalaya	R
374	*Inula racemosa* Hook. f.	Asteraceae	Jammu & Kashmir	V
375	*Iphigenia magnifica* Ansari *et* Rolla Rao	Liliaceae	Maharashtra, Karnataka	V
376	*Iphigenia sahyadrica* Ansari *et* Rolla Rao	Liliaceae	Karnataka	E
377	*Iphigenia stellata* Blatt.	Liliaceae	Maharashtra	V
378	*Ipomoea clarkei* Hook. f.	Convolvulaceae	Maharashtra	R
379	*Ipsea malabarica* (Reichb. f.) Hook. f.	Orchidaceae	Kerala	E
380	*Isachne borii* Hemadri	Poaceae	Maharashtra	R
381	*Isachne fischeri* Bor	Poaceae	Kerala	R
382	*Isachne lisboae* Hook. f.	Poaceae	Maharashtra, Karnataka	
383	*Isachne mysorensis* Raghavan	Poaceae	Karnataka	R
384	*Ischaemum raizadae* Hemadri *et* Billore	Poaceae	Maharashtra	R
385	*Isonandra stocksii* Clarke	Sapotaceae	Western Peninsular India	V
386	*Isonandra villosa* Wight	Sapotaceae	Tamil Nadu, Kerala, Andhra Pradesh	I
387	*Ixonanthes khasiana* Hook. f.	Ixonanthaceae	Meghalaya, Assam	V
388	*Jasminum unifoliolatum* Balakr. & N.G. Nair	Oleaceae	North Andaman Island	R
389	*Juncus sikkimensis* Hook. f.	Juncaceae	Sikkim	R
390	*Kalanchoe olivacea* Dalz.	Crassulaceae	Tamil Nadu	R
391	*Kalanchoe roseus* Clarke	Crassulaceae	Nagaland, Manipur	E
392	*Kendrickia walker* (Wight) Hook. f. ex Triana	Melastomataceae	Tamil Nadu	E
393	*Kingiodendron pinnatum* (Roxb. ex DC.) Harms	Fabaceae	Karnataka, Tamil Nadu, Kerala	R

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
394	*Korthalsia rogersii* Becc.	Arecaceae	South Andaman Island	R
395	*Lactuca benthamii* Clarke	Asteraceae	Jammu & Kashmir	E
396	*Lactuca cooperi* Anthony	Asteraceae	Sikkim	E
397	*Lactuca filicina* Duthie ex Stebbins	Asteraceae	Uttar Pradesh (Kumaon)	E
398	*Lactuca undulata* Ledeb.	Asteraceae	Jammu & Kashmir	E
399	*Lagerstroemia minuticarpa* Debberm. ex P.C. Kanjilal	Lythraceae	Assam, Sikkim	R
400	*Lastreopsis wattii* (bedd.) Tagawa	Aspidiaceae	Manipur	Presumed extinct
401	*Lepidagathis difusa* Clarke	Acanthaceae	Karnataka, Tamil Nadu	I
402	*Lepidagathis barberi* Gamble	Acanthaceae	Tamil Nadu	R
403	*Leucas angustissima* Sedgw.	Lamiaceae	Karnataka	R
404	*Leucas mukerjiana* Subba Rao *et* Kumari	Lamiaceae	Andhra Pradesh	E
405	*Ligusticum albo-alatum* Haines	Apiaceae	Bihar	Possibly extinct
406	*Lilium macklineae* Sealy	Liliaceae	Manipur	E
407	*Limnopoameeboldii* (Fischer) Hubb.	Poaceae	Kerala	V
408	*Lindsaea himalaica* Kramer	Lindsaeaceae	Eastern India	R
409	*Lindsaea malabarica* (Bedd.) Bak. ex Christ.	Lindsaeaceae	S. India, Madhya Pradesh	R
410	*Liparis biloba* Wight	Orchidaceae	Tamil Nadu	V
411	*Litsea leiantha* (Kurz) Hook. f.	Lauraceae	South Andaman Island	V
412	*Livistona jenkinsiana* Griff.	Arecaceae	Sikkim, Assam, Meghalaya, Arunachal Pradesh	E
413	*Lloydia himalensis* Royle	Liliaceae	Jammu & Kashmir, Himachal Pradesh, Sikkim	R
414	*Mackenziea caudata* (T. And.) Ramam.	Acanthaceae	Karnataka, Tamil Nadu	R
415	*Madhuca bourdillonii* (Gamble) H.J. Lam	Sapotaceae	Kerala	Possibly extinct
416	*Madhuca diplostemon* (Clarke) van Royen	Sapotaceae	Peninsular India	Insufficiently known

417	*Madhuca insignis* (Radlk.) H.J. Lam	Sapotaceae	Karnataka	Possibly extinct
418	*Malleola andamanica* Balakr. & Bhargava	Orchidaceae	Andaman Islands	E
419	*Mangifera andamanica* King	Anacardiaceae	South Andaman	V
420	*Marsdenia raziana* Yog. *et* Subr.	Asclepiadaceae	Karnataka	R
421	*Mecanopsis latifolia* (Prain) Prain	Papaveraceae	Jammu & Kashmir	V
422	*Mecodium levingei* (Clarke) Copel.	Hymenophyllaceae	Sikkim	R
423	*Melicope indica* Wight	Rutaceae	Tamil Nadu	V
424	*Memecylon flavescens* Gamble	Melastomataceae	Tamil Nadu	E
425	*Memecylon sisparense* Gamble	Melastomataceae	Tamil Nadu	I
426	*Metathelypteris decipiens* (Clarke) Ching	Thelypteridaceae	West Bengal, Meghalaya	R
427	*Meteoromyrtus wynaadensis* (Bedd.) Gamble	Myrtaceae	Tamil Nadu, Kerala	E
428	*Michelia punduana* Hook. f. *et* Thoms.	Magnoliaceae	Meghalaya, Nagaland	R
429	*Microschoenus duthiei* Clarke	Cyperaceae	Uttar Pradesh (Tehri Garhwal)	I
430	*Miliusa nilagirica* Bedd.	Annonaceae	Tamil Nadu	V
431	*Mitrastemon yamamotoi* (Makino) Makino	Mitrastemonaceae	Meghalaya	E
432	*Mitrephora andamanica* Thoth. & D. Das	Annonaceae	Andaman Islands	R
433	*Murdannia juncoides* (Wight) Rolla Rao *et* Kammathy	Commelinaceae	Thenmalai (Western Ghats)	R
434	*Murdannia lanceolata* (Wight) Kammathy	Commelinaceae	Tamil Nadu, Kerala	V
435	*Murdannia lanuginose* (Wall. ex Clarke) Bruckn.	Commelinaceae	Deccan Plateau, Sahyadri hills	R
436	*Nanothamnus sericeus* Thoms.	Asteraceae	Maharashtra, Karnataka	R
437	*Nardostachys grandiflora* DC.	Valerianaceae	Himachal Pradesh	V
438	*Nauclea gageana* King	Rubiaceae	Andaman islands	I
439	*Neanotis carnosa* (Dalz.) Lewis	Rubiaceae	Karnataka	I
440	*Neanotis oxyphylla* (G. Don) Lewis	Rubiaceae	Meghalaya	R
441	*Neanotis prainiana* (Talbot) Lewis	Rubiaceae	Karnataka	V

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
442	*Neottia inayatii* (Duthie) Beauv.	Orchidaceae	Jammu & Kashmir	R
443	*Neuracanthus neesianus* (Wight ex T. Anders.) Clarke	Acanthaceae	Tamil Nadu	E or possibly extinct
444	*Nilgirianthus circarensis* (Gamble) Bremek.	Acanthaceae	Andhra Pradesh, Orissa	R
445	*Nogra dalzellii* (Baker) Merr.	Fabaceae	Maharashtra, Karnataka	V
446	*Nogra filicaulis* (Kurz) Merr.	Fabaceae	Madhya Pradesh	E
447	*Nomocharis synaptica* Sealy	Liliaceae	Arunachal Pradesh	R
448	*Nothopegia aureo-fulva* Bedd. ex Hook. f.	Anacardiaceae	Tamil Nadu	E
449	*Oberonia brachyphylla* Blatt. & McCann	Orchidaceae	Karnataka, Kerala	R
450	*Ochreinauclea missionis* (Wall. ex G. Don) Ridsd.	Rubiaceae	Tamil Nadu, Kerala, Karnataka	V
451	*Oianthus deccanensis* Talb.	Asclepiadaceae	Maharashtra	E or possibly extinct
452	*Ophiorrhiza barnesii* Fischer	Rubiaceae	Kerala	Possibly Extinct
453	*Ophiorrhiza brunonis* Wight *et* Arn.	Rubiaceae	Tamil Nadu, Kerala, Karnataka	Presumed extinct
454	*Ophiorrhiza caudata* Fischer	Rubiaceae	Kerala	Presumed extinct
455	*Ophiorrhiza gracilis* Kurz	Rubiaceae	Nagaland	I
456	*Ophiorrhiza griffithii* Hook. f.	Rubiaceae	Nagaland	I
457	*Ophiorrhiza hispida* Hook. f.	Rubiaceae	Meghalaya, Assam	E
458	*Ophiorrhiza incarnata* Fischer	Rubiaceae	Kerala	E
459	*Ophiorrhiza lurida* Hook. f.	Rubiaceae	Sikkim, West Bengal, Manipur	R
460	*Ophiorrhiza pykarensis* Gamble	Rubiaceae	Nilgiri Hills	Possibly extinct
461	*Ophiorrhiza radicans* Gardn.	Rubiaceae	Kerala	Possibly extinct
462	*Ophiorrhiza subcapitata* Wall. ex Hook. f.	Rubiaceae	Meghalaya	E
463	*Ophiorrhiza tingens* Clarke ex Fischer	Rubiaceae	Meghalaya, Assam, Tripura, Nagaland	V
464	*Ophiorrhiza wattii* Fischer	Rubiaceae	Meghalaya, Nagaland, Manipur	E
465	*Oreopteris elwesii* (Bak.) Holtt.	Thelypteridaceae	Sikkim	R

466	*Orophea uniflora* Hook. f. &Thoms.	Annonaceae	Tamil Nadu, Karnataka	R
467	*Palaquium bourdillonii* Brandis	Sapotaceae	Kerala	I
468	*Panax pseudo-ginseng* Wall.	Araliaceae	E. Himalaya	V
469	*Paphiopedilum druryi* (Bedd.) Stein	Orchidaceae	Kerala	E or possibly extinct
470	*Paphiopedilum fairrieanum* (Lindl.) Stein	Orchidaceae	Sikkim, Arunachal Pradesh	E
471	*Paphiopedilum hirsutissimum* (Lindl. ex Hook.) Stein	Orchidaceae	Meghalaya	R
472	*Paphiopedilum insigne* (Wall. ex Lindl.) Pfitz.	Orchidaceae	Meghalaya	V
473	*Paphiopedilum specerianum* (Reichb. f.) Pfitz.	Orchidaceae	Manipur	V
474	*Paphiopedilum venustum* (Wall. ex Sims.) Pfitz.	Orchidaceae	Meghalaya, Sikkim	V
475	*Paphiopedilum villosum* (Lindl.) Stein	Orchidaceae	Mizoram	V
476	*Paphiopedilum wardii* Summerh.	Orchidaceae	Arunachal Pradesh	E
477	*Paracautieya bhatii* Smith	Zingiberaceae	Karnataka	V
478	*Pauia belladonna* Deb *et* Dutta	Solanaceae	Arunachal Pradesh	R
479	*Pavetta hohenackeri* Brem.	Rubiaceae	Tamil Nadu	V
480	*Pavetta oblanceolata* Brem.	Rubiaceae	Kerala	I or possibly extinct
481	*Pavetta wightii* Hook. f.	Rubiaceae	Tamil Nadu	Possibly extinct
482	*Peucedanum anamallayense* Clarke	Apiaceae	Tamil Nadu	R
483	*Phaeanthus malabaricus* Bedd.	Annonaceae	Kerala	V
484	*Phalaenopsis speciosa* Reichb. f.	Orchidaceae	Andaman & Nicobar Islands	E
485	*Phlebophyllum jeyporense* (Bedd.) Bremekamp	Acanthaceae	Madhya Pradesh, Orissa, Andhra Pradesh	E
486	*Phoenix rupicola* T. Anders.	Arecaceae	Sikkim, West Bengal, Arunachal Pradesh, Meghalaya	R
487	*Pholidota wattii* King *et* Pantl.	Orchidaceae	Arunachal Pradesh, Assam	R

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
488	*Phyllanthus narayanaswamii* Gamble	Euphorbiaceae	Andhra Pradesh	E
489	*Phyllanthus talbotii* Sedgw.	Euphorbiaceae	Karnataka	R
490	*Picrorhiza kurrooa* Royle ex Benth.	Scrophulariaceae	Jammu & Kashmir to Sikkim	V
491	*Pimpinella evoluta* (Clarke) Mukh.	Apiaceae	Nagaland (Naga Hills)	Possibly extinct
492	*Pimpinella flaccida* Clarke	Apiaceae	Nagaland	I
493	*Pimpinella katrajensis* Rao *et* Hemadri	Apiaceae	Maharashtra	R
494	*Pimpinella pulneyensis* Gamble	Apiaceae	S. India (Pulney hills)	Possibly extinct
495	*Pimpinella tirupatiensis* Balakr. *et* Subram.	Apiaceae	Andhra Pradesh	E
496	*Pimpinella tongloensis* Mukh.	Apiaceae	West Bengal	E
497	*Pimpinella wallichii* Clarke	Apiaceae	Sikkim	E
498	*Pinanga andamanensis* Becc.	Arecaceae	Andaman Islands	R
499	*Pinanga manii* Becc.	Arecaceae	Nicobar & South Andaman Islands	V
500	*Piper barberi* Gamble	Piperaceae	Southern Western Ghats	R
501	*Pittosporum eriocarpum* Royle	Pittosporaceae	Uttar Pradesh (Garhwal, Kumaon)	I
502	*Plectranthusbishopianus* Gamble	Lamiaceae	Tamil Nadu	Possibly extinct
503	*Plectranthus bourneae* Gamble	Lamiaceae	Tamil Nadu	I
504	*Pleione lagenaria* Lindl.	Orchidaceae	Meghalaya	Presumed extinct
505	*Poeciloneuron pauciflorum* Bedd.	Bonnetiaceae	Travancore, Tirunelveli hills	I
506	*Pogostemon atropurpureus* Benth.	Lamiaceae	Tamil Nadu, Kerala	R
507	*Pogostemon nilagiricus* Gamble	Lamiaceae	Nilgiri hills of Western Ghats	E
508	*Pogostemon paludosus* Benth.	Lamiaceae	Tamil Nadu	E
509	*Pogostemon travancoricus* Bedd. var. *travancoricus*	Lamiaceae	Kerala	R
510	*Pollia pentasperma* Clarke	Commelinaceae	Meghalaya, Nagaland	I
511	*Polyalthia rufescens* Hook. f. &Thoms.	Annonaceae	Tamil Nadu, Kerala	R
512	*Polycarpaea diffusa* Wight & Arn.	Caryophyllaceae	Tamil Nadu	V

513	*Polypodioides wattii* (Bedd.) Ching	Polypodiaceae	Eastern India	R
514	*Polyzygus tuberosus* Dalz.	Apiaceae	Maharashtra, Karnataka	R
515	*Popowia beddomeana* Hook. f. &Thoms.	Annonaceae	Tamil Nadu, Kerala	R
516	*Prismatomeris andamanica* Ridley	Rubiaceae	South Andaman Islands	I
517	*Pronephrium thwaitesii* (Hook.) Holtt.	Thelypteridaceae	Kerala	V
518	*Pseudocyclosorous gamblei* Holtt. & Grimes	Thelypteridaceae	Nilgiri&Palni Hills	E
519	*Pseudocyclosorous griseus* (Baker) Holtt. & Grimes [*Neprodium griseum* Bake]	Thelypteridaceae	Kerala, Tamil Nadu	E
520	*Pseudoglochidion anamalayanum* Gamble	Euphorbiaceae	Tamil Nadu	I
521	*Pseuduvaria prainii* (King) Merr.	Annonaceae	Andaman & Great Nicobar Islands	R
522	*Psychotria aborensis* Dunn	Rubiaceae	Arunachal Pradesh	R
523	*Psychotria andamanica* Kurz	Rubiaceae	Andaman & Nicobar Islands	R
524	*Psychotria globicephala* Gamble	Rubiaceae	Tamil Nadu	E
525	*Psychotria pendula* Hook. f.	Rubiaceae	South Andaman Islands	I
526	*Psychotria tylophora* Kurz	Rubiaceae	Nicobar islands	Possibly extinct
527	*Ptenopetalum radiatum (W.W. Sm.) Mukh.[Pimpinella radiata W.W. Sm.]*	Apiaceae	Sikkin	I
528	*Pternopetalum senii* Deb *et* Dutta	Apiaceae	Arunachal Pradesh	R
529	*Pterospermum reticulatum* Wight &Arn.	Sterculiaceae	Karnataka, Kerala, Tamil Nadu	R
530	*Puccinellia kashmiriana* Bor	Poaceae	Jammu & Kashmir, Himachal Pradesh	R
531	*Pueraria bella* Prain	Fabaceae	Arunachal Pradesh	R
532	*Pyrenaria khasiana* R.N. Paul	Theaceae	Meghalaya	I
533	*Renanthera imschootiana* Rolfe	Orchidaceae	Manipur, Nagaland, Mizoram	E
534	*Rhopalocnemis phalloides*Jungh.	Balanophoraceae	Meghalaya, Arunachal Pradesh, Sikkim	R
535	*Rhynchoglossum lazulinum* Rao & Joseph	Gesneriaceae	Arunachal Pradesh	R

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
536	*Rhynchosia beddomei* Baker	Fabaceae	Karnataka	R
537	*Rhynchosia velutina* Wight *et* Arn.	Fabaceae	Tamil Nadu	V
538	*Rhynchospora submarginata* Keukenth.	Cyperaceae	Kerala	I
539	*Rotala ritchiei* (C.B. Clarke) Koehne	Lythraceae	Maharashtra	V
540	*Rubia edgeworthii* Hook. f.	Rubiaceae	Western Himalaya	V
541	*Rubia himalayensis* Klotzsch	Rubiaceae	Jammu & Kashmir	V
542	*Sageraea grandiflora* Dunn	Annonaceae	Kerala	E, possibly extinct
543	*Salacia beddomei* Gamble	Celastraceae	Tamil Nadu, Kerala	R
544	*Salacia jenkinsii* Kurz	Celastraceae	Assam	E
545	*Salacia malabarica* Gamble	Celastraceae	Karnataka, Kerala	E or possibly extinct
546	*Santapaua madurensis* Balakr. &Subram.	Acanthaceae	Tamil Nadu	E
547	*Sapria himalayana* Griff.	Rafflesiaceae	Arunachal Pradesh, Manipur, Meghalaya	R
548	*Saussurea bracteata Decne.*	Asteraceae	Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh	R
549	*Saussurea clarkei* Hook. f.	Asteraceae	Jammu & Kashmir	R
550	*Saussurea costus* (Falc.) Lipschitz	Asteraceae	Jammu & Kashmir, Himachal Pradesh, Uttar Pradesh	E
551	*Schizachyrium paranjpyeanum* (Bhide) Raizada *et* Jain	Poaceae	Maharashtra, Karnataka	R
552	*Scilla viridis* Blatt. *et* Hallb.	Liliaceae	Maharashtra	E
553	*Scleria alta* Boeck.	Cyperaceae	Assam, Meghalaya	I
554	*Scutellaria andamanica* Prain	Lamiaceae	Andaman Islands	R
555	*Selaginella adunca* A. Br. ex Hieron.	Selaginellaceae	North West Himalayas	E
556	*Selaginella cataractrum* Alston	Selaginellaceae	South India	E
557	*Senecio kundaicus* Fischer	Asteraceae	Tamil Nadu	E

558	*Senecio mayurii* Fischer	Asteraceae	Karnataka	R
559	*Senecio mishmi* Clarke	Asteraceae	Meghalaya	V or I
560	*Senecio rhabdos* Clarke	Asteraceae	Nagaland, Manipur	R
561	*Seshagiria sahyadrica* Ansari *et* Hemadri	Asclepiadaceae	Maharashtra	R
562	*Silene khasiana* Rohrb.	Caryophyllaceae	Meghalaya	I
563	*Silene kumaonensis* Williams	Caryophyllaceae	Uttar Pradesh (Garhwal)	R
564	*Silene kunawarensis* Royle	Caryophyllaceae	Jammu & Kashmir, Himachal Pradesh	R
565	*Silene vagans* Clarke	Caryophyllaceae	Nagaland	I
566	*Smilax wightii* A. DC.	Smilacaceae	Tamil Nadu	R
567	*Smithia agharkarii* Hemadri	Fabaceae	Maharashtra	R
568	*Sphaeropteris albosetacea (Bedd.) Tryon*	Cyatheaceae	Nicobar Islands	V
569	*Sphaeropteris crinita (Hook.) Tryon*	Cyatheaceae	Tamil Nadu, Kerala	E
570	*Stenogramme himalaica* (Ching) K. Iwats.	Thelypteridaceae	North West Himalaya	V
571	*Stephania andamanica* Diels	Menispermaceae	South Andaman	I
572	*Sterculia khasiana* Debbarman	Sterculiaceae	Meghalaya	Presumed extinct
573	*Strobilanthes dupenii* Bedd. ex Clarke	Acanthaceae	Peninsular India (Anamalais)	I
574	*Strobilantheshallbergii* Blatter	Acanthaceae	Rajasthan	E
575	*Synotis simonsii* (Clarke) Jeffrey *et* Chen	Asteraceae	Assam	I
576	*Syzygium andamanicum* (King) Balakr.	Myrtaceae	Andaman Islands	I
577	*Syzygium bourdillonii* (Gamble) Rathakr. *et*N.C. Nair	Myrtaceae	Kerala	E or possiblyextinct
578	*Syzygium courtallense* (Gamble) Alston	Myrtaceae	Tamil Nadu	E
579	*Syzygium gambleanum* Rathakr. *et* Chitra	Myrtaceae	Tamil Nadu	E
580	*Syzygium manii* (King) Balakr.	Myrtaceae	Middle Andaman Island	R

(continued)

Table 9.1 (continued)

S.No	Name of the plant	Family	Distribution	Status
581	*Syzygium palghatense* Gamble	Myrtaceae	Kerala	E or possiblyextinct
582	*Syzygium travancoricum* Gamble	Myrtaceae	Kerala	E
583	*Taeniophyllum andamanicum* Balakr. & Bhargava	Orchidaceae	Andaman Islands	E
584	*Tarenna agumbensis* Sundararaghavan	Rubiaceae	Karnataka	V
585	*Tephrosia barberi* Drumm.	Fabaceae	Tamil Nadu	R
586	*Tephrosiacalophylla* Bedd.	Fabaceae	Tamil Nadu, Karnataka	R
587	*Tephrosia jamnagerensis* Sant.	Fabaceae	Gujarat	R
588	*Tephrosia wynaadensis* Drumm.	Fabaceae	Kerala	R
589	*Teucrium plectranthoides* Gamble	Lamiaceae	Tirunelveli hills, Western Ghats	V
590	*Thalictrum dalzellii* Hook.	Ranunculaceae	Karnataka, Maharashtra	I
591	*Thottea barberi* (Gamble) Ding Hou	Aristolochiaceae	Tirunelveli	V
592	*Toxocarpus beddomei* Gamble	Asclepiadaceae	Tamil Nadu, Kerala	R
593	*Toxocarpus longistigma* (Roxb.) Wight & Arn. ex Steud.	Asclepiadaceae	Andhra Pradesh	E
594	*Toxocarpus palghatensis* Gamble	Asclepiadaceae	Kerala	V
595	*Trachycarpus takil* Becc.	Arecaceae	Uttar Pradesh	R
596	*Trivalvaria kanjilalii* D. Das	Annonaceae	Meghalaya	E
597	*Typhonium incurvatum* Blatt. &McC.	Araceae	Maharashtra	R
598	*Urginea cogesta* Wight	Liliaceae	S. India	E
599	*Urginea polyphylla* Hook. f.	Liliaceae	Deccan Peninsula	Presumed extinct
600	*Utleria salicifolia* Bedd.	Periplocaceae	Tamil Nadu, Kerala	E
601	*Uvaria eucincta* Bedd. ex Dunn	Annonaceae	Orissa	E
602	*Uvaria nicobarica* Raiz. &Sahni	Annonaceae	Great Nicobar island	R
603	*Vanasushava pedata* (Wight) Mukh. *et* Const.	Apiaceae	S. India (Shevagherry, Palni and Anamalis Hills)	R
604	*Vanda coerulea* Griff. ex Lindl.	Orchidaceae	North East India	R

605	*Vanda wightii* Reichb. f.	Orchidaceae	Tamil Nadu	Possibly extinct
606	*Vanilla wightiana* Lindl.	Orchidaceae	Tamil Nadu, Kerala	R
607	*Vateria macrocarpa* B.L. Gupta	Dipterocarpaceae	Kerala	R
608	*Vernonia andamanica* Balakr. & N.G. Nair	Asteraceae	North Andaman	R
609	*Vernonia multibracteata*Gamble	Asteraceae	Kerala	E
610	*Vernonia pulneyensis* Gamble	Asteraceae	Pulney Hills	E
611	*Vernonia recurva* Bedd. ex S. Moore	Asteraceae	Tamil Nadu	E or possiblyextinct
612	*Vigna khandalensis* (Sant.) Raghavan & Wadhwa	Fabaceae	Maharashtra	R
613	*Viscum mysorense* Gamble	Loranthaceae	Karnataka	I
614	*Wallichia triandra* (Joseph) S.K. Basu	Arecaceae	Arunachal Pradesh	R
615	*Weisneria triandra* (Dalz.) Micheli	Alismataceae	Maharashtra, Goa	R
616	*Wendlandia andamanica* Cowan	Rubiaceae	Andaman Islands	E
617	*Wendlandia angustifolia* Wight ex Hook. f.	Rubiaceae	Tamil Nadu	Presumed extinct
618	*Willisia selaginoides* (Bedd.) Warm. ex Willis	Podostemonaceae	Kerala	R
619	*Youngia silgiriensis* Bebcock	Asteraceae	Nilgiri Hills and Sispara of Western Ghats	E
620	*Zeuxine pulchra* King *et* Pantl.	Orchidaceae	Sikkim, Meghalaya	E or possibly extinct

Source: Nayar and Sastry (1990); bsienvis.nic.in/database/red listed plants diversity is distributed in the following biogeographic zones of India: Trans Himalayan, Himalayan, Indian deserts, semi-arid areas, Western Ghats, Gangetic Plains, northeast India, islands, and coasts. Medicinal plants are not only a major resource base for the traditional medicine and herbal industry but also provide livelihood and health security to a large segment of Indian population. About 1178 species of medicinal plants are estimated to be in trade of which 242 species have annual consumption levels in excess of 100 metric tons/year. Conservation of such threatened and vulnerable species is highly warranted (Ramanatha Rao and Arora 2004).

9.3 Geospatial Technology as a Tool for Managing Medicinal Plant Genetic Resource Conservation

Many medicinal plant species are threatened by overexploitation, habitat destruction and lack of proper management practices. GIS applications can contribute significantly to the call for the improved understanding and monitoring of threatened medicinal plants, a component of biodiversity. Results obtained from spatial analysis allow the formulation and implementation of more targeted, and hence more effective, conservation strategies. Outputs from spatial studies can provide critical information on the diversity present in specific geographic areas and can be used for various purposes, for example, to evaluate the current conservation status of threatened species and to prioritise areas for conservation. Spatial information, combined with available characterization and/or evaluation data, has been proven useful for effective gene bank management. GIS tools, which allow one to carry out complex analyses combining different (spatial) data sources and generate clear maps, facilitate the uptake of outcomes by responsible authorities and encourage the development and implementation of conservation policies (Guarino et al. 2002). In recent years, technological advances and the growing availability of computers and GPS (global positioning system) receivers have led to the increased application of GIS analysis.

The induction of modern technologies of geospatial tools like remote sensing, geographic information system and global positioning system have provided very powerful methods of surveying, identifying, classifying, mapping, monitoring, characterizing and tracking changes in the composition of species and distribution of several threatened species genetic resources in nature. Geographical information system has been successfully used for managing genetic resources by many researchers in the past (Adair et al. 2006; Aggelopoulou et al. 2010; Gixhari et al. 2012; Chang et al. 2015; Chong et al. 2017; Hijmans et al. 2000; Miller and Knouft 2006; Parra-Quijano et al. 2012; Sankaran and Ehsani 2011; Scheledeman and van Zonneveld 2010; van Zonneveld et al. 2011).

Geospatial technology and GIS could be effectively used in
Ecogeographic survey for locating diversity in threatened medicinal plant taxa
Planning field exploration and collecting of threatened taxa
Design and management of in-situ conservation sites/sanctuaries
Managing medicinal plants conservation areas
Monitoring national parks, botanical gardens and sanctuaries
Site identification for threatened taxa evaluation and regeneration
Identification of climate suitable sites for reintroduction/cultivation
Identify threatened species, species-rich areas and vegetation types that are not represented or under represented
Developing niche models of threatened medicinal plant taxa
Threatened taxa inventory, monitoring and assessment
Threatened taxa assessment and plant health management
Site suitability assessment
Threatened taxa database creation

In the forest genetic resource management, geo-referencing technique is helpful to record passport information, potential/trait-specific/threatened taxa and wild plant germplasm collection sites and to identify gaps in collections. It is also helpful in predicting their habitat which may change in near future, particularly due to climate change. Priority may be assigned to those areas for exploration. Available passport/genebank data on threatened medicinal plant taxa needs to be analysed using modern tools and techniques along with environmental variables for their categorization and efficient use. Geospatial technology essentially involves acquisition of real-time satellite data integrating, analysing, managing and depicting geospatial information for use in management, planning and decision-making. Geospatial technology could play an important role in genetic resource management through mapping of collected diversity, prediction of diversity-rich areas and locating other suitable sites using different climate/crop models. Geospatial technology also helps in delineation of diversity-rich areas, their spatial distribution pattern and variability under the influence of biophysical factors and climatic conditions. The greater the variety of habitat types (phytogeographic zones) the better the diversity of medicinal plant genetic resources and mitigating risk of extinction of genetic resources from ever-changing climate. GIS is an integrated geospatial system where database consists of observations on spatially distributed features, objects, which are definable in space as points, lines and polygons (Burrough 1986; Heywood et al. 2002). Utility of GIS in management of plant genetic resources (PGR) has been recognized through various studies (Jones et al. 1997; Hijmans et al. 2000; Hijmans and Spooner 2001; Guarino et al. 2002).

Sustainable management of threatened medicinal plants is of interest as increasing population and rapid technological strides are putting enormous pressure on this country's health security. Management of such resources at national level generates and uses enormous data, and the analysis of these data is crucial to the effectiveness of forest genetic resource management process and can add significant value to the medicinal plant taxa. As data is geo-referenced, it can be analysed and linkages between other external geo-referenced data sources could be established using GIS and other spatial technologies. Brief description of the role of GIS in germplasm exploration and collection, conservation and documentation is provided below.

9.3.1 Threatened Taxa Germplasm Collection

GIS can be effectively used in preparing maps of collection sites, distribution maps of species, gap analysis and analysing diversity-rich pockets etc. GIS can also be used to link passport database with district and state map layers to analyse what has been explored and collected from where and what the gaps are in terms of areas to be explored and the need for collection of germplasm (Semwal and Ahlawat 2015). Thus, to plan future exploration programmes which are trait-specific/region-specific GIS can be an effective strategy. Mapping spatial distribution of target threatened species which are involved in knowledge systems can be effectively carried out using GIS. DIVA-GIS tools have been widely used for eco-geographic mapping of collection sites, diversity distribution of crops and wild species (Hijmans et al. 2000; Jones et al. 1997). GIS-based grid mapping technique is used to know the diversity-rich areas, variability assessment and occurrence of trait-specific germplasm of selected taxa in different parts of the world (Hijmans et al. 2000; Hijmans et al. 2001; Guarino et al. 2002). A grid of definite size is assigned on the map to the points representing collected germplasm. The grid size depends on the size of geographical area. At country level (large geographical area), a grid of 1° × 1° (111.32 × 111.32 km) size, while for states/sub-divisions (smaller area), the grid of 0.2º × 0.2 º to 0.8º × 0.8 º size (Semwal et al. 2013), may be used depending upon the area and diversity. GIS and other specialized computer program (e.g., FloraMap) along with associated data can be used to map the predicted distribution of plant species or areas of possible climatic adaptation of organisms in the wild (Jones et al. 2002). Also, GIS can play an important role in the management of large and complex genetic resource datasets (Guarino et al. 1999; Semwal and Ahlawat 2015). Apart from the prediction of natural distributions and gaps, GIS can be used to check the quality of large datasets, predicting climatic adaptation in other regions, identifying groups of germplasm accessions (ecotypes) with distinct climatic adaptations and comparing climatic adaptation among priority groups of accessions (Greene et al. 1999; Jones et al. 2001). Guarino (1995) discussed the use of GIS in developing strategies for collecting germplasm. For example, collection regions can be mapped to identify areas with desired eco-geographic attributes such as acid soils or climate extremes (Hart et al. 1996). An eco-geographic survey using remotely sensed data can assist in acquiring a broad base sample of genetic diversity by optimizing sampling from many different environments. Potential collection areas can be identified having similar environmental envelopes as germplasm of known value. Eco-geographic representation of collections can be assessed by overlaying collection sites on maps such as climate, soil, topography and ecosystem for effective planning of future exploration programmes in the country. GIS tools can be useful in the identification of areas for reintroduction and restoration of highly threatened plant species as well (Quested et al. 2014).

9.3.2 Diversity Analysis Using DIVA-GIS

DIVA-GIS is a software tool for diversity analysis. It helps in managing genetic resources, understanding and comprehending the distribution of diversity on the geographical scale and facilitates identifying gaps in germplasm collections. GIS studies done at different time intervals/periods in the same location can provide information on change in genetic diversity of target species/crops/areas. DIVA-GIS is a technology that supports the analysis of exploration, evaluation, gene bank and herbarium databases to elucidate genetic, ecological and geographic patterns in the distribution of crops and wild species. It is designed to assist the PGR curators and biodiversity managers to map the range of distribution in the species (Hijmans et al. 2000). DIVA-GIS mapping may be effectively used for diversity analysis, identifying gaps in collection and loss of diversity, developing new strategies for conservation and sustainable utilization, particularly in the wake of recent international developments related to food and nutritional security. For example, diversity analysis of *Hemidesmus indicus* accessions from Rayalaseema region of Andhra Pradesh, India using DIVA-GIS approach has been demonstrated (Venkateswaran et al. 2018). The analysis unravels that *Hemidesmus indicus* accessions from Chittoor district of Andhra Pradesh possessed high diversity index for leaf traits indicating diversity-rich pockets in the region (Fig. 9.2).

GIS mapping has been successfully used in assessing diversity and in identifying areas of high diversity in *Phaseolus* bean (Jones et al. 1997), coconut (Bourdeix et al. 2005), wild potatoes (Hijmans and Spooner 2001), wild *Arachis* (Jarvis et al. 2003), horsegram (Sunil et al. 2008), *Jatrophacurcas* (Sunil et al. 2009), linseed (Sivaraj et al. 2009, 2012), sesame (Spandana et al. 2012), blackgram (Babu Abraham et al. 2010), piper (Parthasarathy et al. 2008), *Canavalia* fatty acids (Sivaraj et al. 2010), medicinal plants (Varaprasad et al. 2007) and agro-biodiversity (Varaprasad et al. 2008).

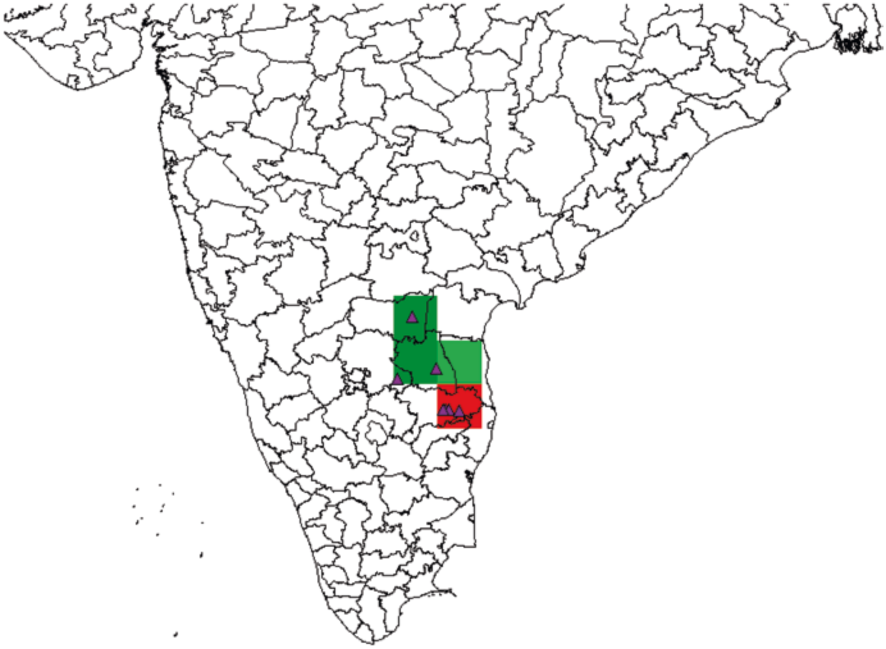

Fig. 9.2 Diversity analysis grid map generated for leaf traits (leaf length, width, thickness and pigments) in Hemidesmus germplasm (high-diversity region indicated as red grid)

9.3.3 Ecological Niche Modelling for Threatened Medicinal Plants Using Geospatial Technology – A Case Study

Continued pressure on forest land, food insecurity and required adaptation to climate change have made modelling of future sustainable forest ecosystems development increasingly important. The concept of sustainable forestry involves producing quality timber andnon-timber forest products in an environmentally benign, and socially and economically acceptable way. To comply with these principles of sustainable forestry, one must introduce the threatened taxa where they are best suited, which require a thorough environmental suitability analysis. Environmental suitability is an important aspect which has a direct impact on the survival and productivity of the threatened medicinal plants. Environmental suitability analysis is a prerequisite for sustainable medicinal plant production. An important component in this is ecological niche modelling. Various modelling tools are used to support the decision making and planning in sustainable forestry. Ecological simulation models are research tools usually applied in assessing the relationship between taxa and environmental factors. They have been shown to be efficient in determining the response of medicinal plant taxa to changes in weather and climate. Several studies have been undertaken using GIS for ecological niche modelling in horticultural crops. We discuss here ecological niche model construction using geospatial technology (GIS) by taking *Madhuca insignis*, a globally threatened (Critically endangered) and endemic medicinal tree taxa of Western Ghats.

Madhuca insignis (Radlk.) H.J. Lam: The critically endangered species *Madhuca insignis*(Radlk) H.J. Lam belongs to the Sapotaceae family, originated from an Indo-Malayan genus. This taxon is classified as 'Extinct' by the IUCN Red List, but has been recently rediscovered after a long gap of 120 years from Udupi District of Karnataka. It has been reported to occur near the banks of the water bodies mainly associated with *Garcinia, Lagerstroemia* and other riparian species in regions of Dakshina Kannada, Udupi Districts of Karnataka and Kasaragod District of Kerala, overall endemic to India. *M. insignis* has been documented as a source of firewood and green manure; traditional uses involve consumption of fruits studied to have very high Total Soluble Solids (TSS) content and usage of oil from seeds as medicine by the local communities. This species calls for individual attention not because of its recognised uses but because it is deemed to be under threat of imminent extinction due to varying reasons such as climate change and increased anthropogenic activities. Conservation of this species in its natural habitats is highly warranted due to its economic value and other factors. There is a need for reintroduction of the species in suitable natural habitats. By using GIS and species modelling approaches, sustainable and the most suitable areas for the adaptability of this critically endangered, endemic species of Western Ghats could be identified. Distribution areas of endangered crop species can be increased by bringing new areas under forest vegetation cover. Site suitability is an important factor to determine the productivity of the species (Parthasarthy et al. 2007). Suitability maps are useful to determine areas which proved successful in growing a particular species (Parthasarthy et al. 2007). Several site suitability models have been used extensively to evaluate the potential impact of climate change on shifts in the production and growing regions of various crop species (Easterling et al. 1993; Rosenzweig et al. 1995; Tubiello et al. 2000, 2002). Species prediction models include EcoCrop (EC), Maximum Entropy (MaxEnt), Crop Niche Selection in Tropical Agriculture (CaNaSTA), Decision Support System for Agrotechnology Transfer (DSSAT), etc. These are the most appropriate models to use in the assessment of suitability of various areas for threatened species reintroduction/cultivation. MaxEnt model for the critically endangered *Madhuca insignis* constructed on presence records is presented in Fig. 9.3. Warmer colours (red and orange) indicate the potential regions for reintroduction of *Madhuca insignis*. Based on the current climatic conditions, it could be effectively reintroduced in the Western Ghats region and a pocket in Andaman Islands. Thus, conservation of threatened taxa is possible through using geospatial technology.

Maximum entropy (MaxEnt) is considered the most accurate model that performs extremely well in predicting occurrences in relation to other common approaches (Elith et al. 2006; Hijmans and Graham 2006), especially with incomplete information. MaxEnt is a niche modelling method that has been developed involving species distribution information based only on known presences. MaxEnt is a niche modelling method and was selected to model potential current and future

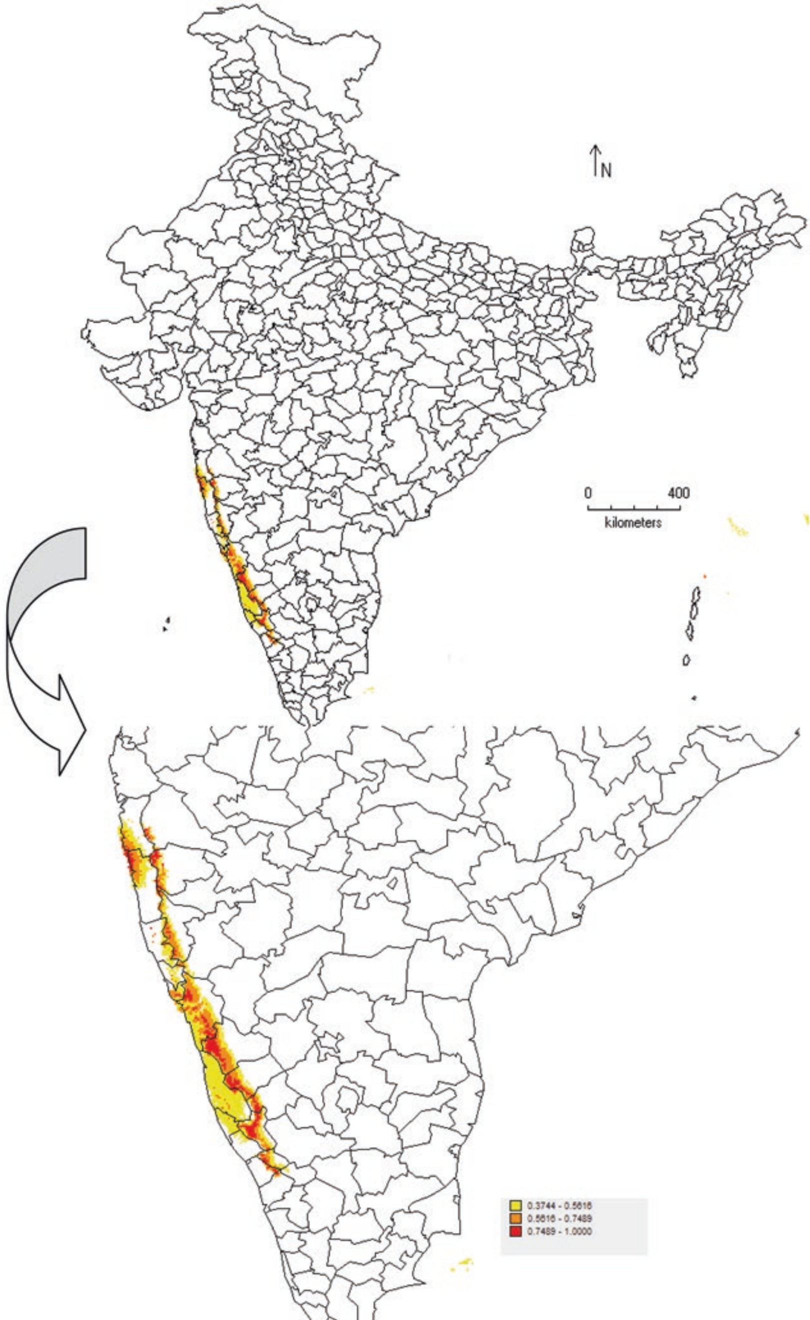

Fig. 9.3 Ecological niche model generated for *Madhuca insignis* using geospatial technology

distribution of a crop. MaxEnt has been successfully used by many researchers earlier to predict distributions such as stony corals (Tittensor et al. 2009), macrofungi (Wollan et al. 2008), seaweeds (Verbruggen et al. 2009), forests (Carnaval and Moritz 2008), rare plants (Williams et al. 2009) and many other species (Elith et al. 2006). Several articles describe its use in ecological modelling and explain the various parameters and measures involved (Phillips et al. 2004, 2006; Elith et al. 2011). It is the most adapted model to be used for horticultural crops including coffee and mango (Eitzinger et al. 2013).

9.3.4 Threatened Medicinal Plant Genetic Resource Conservation

Medicinal plants germplasm, the inter- and intraspecific variability of potentially useful genetic materials is an essential natural resource that provides insurance towards health security. A better understanding of genetic diversity and its distribution is essential for its conservation and use (Ramanatha Rao and Toby Hodgkin 2002). Decline in global biodiversity threatens plant diversity at the species level and within the species, at the genetic level. Complementary conservation strategies include protection of wild species and plant populations where they have evolved (in situ conservation), with the collection and preservation of inter- and intraspecific diversity in gene banks and botanical gardens (ex situ conservation). As habitat degradation and destruction are increasing, ex situ conservation regarded as the process of cultivating and naturalizing endangered species outside of their original habitats has become a practical alternative (Meilleur and Hodgkin 2004). Ex situ genetic resource collections maintain germplasm in the form of seed or live plants, representing current, obsolete and primitive cultivars including threatened wild species collected or augmented from around the world. This material is conserved but is also available to a broad scientific community for basic research and development into crop cultivars (Greene and Hart 1996; Greene et al. 1999). In situ conservation, which is considered as the method of conserving endangered species in their wild habitats, is promising in protecting indigenous species and maintaining natural communities along with their intricate network of relationships. GIS has proved valuable in natural resource management (e.g., land use planning, watershed management, etc.), and however, it is less used for managing horticultural crop germplasm conservation. GIS can be effectively used for genetic resource conservation as summarized in the following areas:

- Design and management of on-farm in situ conservation sites
- Identifying gaps in ex situ collections
- Development of protocols for the propagation of target species in ex situ collections
- Development of core sets.
- Utilization of existing ex situ collections

GIS can be used to understand ex situ collections present in the gene bank and utilizing the same for various purposes in the country. Geographical information system, climate change models, geographical distribution data of crop plants and their wild relatives could be used to predict the impact of a changing climate on conservation and use of forest genetic resources (FGR). GIS has a major role to play in assessing the genebank collections (database) to draw distribution maps and identifying gaps in collections of threatened medicinal plant taxa.

9.3.5 Threatened Taxa Health management Towards Conservation

Geographical information system (GIS) is potentially very useful for managing species health especially in developing countries for reducing expenditure of plant protection. GIS technology can be effectively used in locating 'high risk' pockets, ecogeographic analysis for identifying diversity in pest pathogens, developing early warning systems, building risk assessment models, assisting in site-specific pest management systems (IPM), identification of hotspots, identification of medicinal plant conservation areas (MPCAs), which are relatively free from pest attack, etc. Climate data and distribution maps for pests and diseases can be overlapped using GIS to identify potential pest-free sites for regeneration/reintroduction of threatened germplasm (Guarino et al. 2002). GIS technology help researchers worldwide to assemble, store and retrieve large amounts of spatial data and other associated information related to integrated pest management for managing plant health problems. Thus, the technology allows researchers to manipulate, analyse and display the spatial patterns of variables (environment, economy, socio-cultural aspects, etc.) which are having direct and indirect influence in solving problems of crop health management. Thus, pest-free threatened taxa conservation areas could be identified using geospatial technology.

Thus, GIS can be effectively used in threatened medicinal plants health management asindicated below:
Locating 'high risk' regions, eco-geographic survey for identifying diversity including pests and pathogens
Develop early warning systems
Build risk assessment models of endangered taxa
Assist in site specific pest management systems
Planning field explorations for collecting pest free threatened medicinal plant taxa
Design and management of in-situ pest-free medicinal plant conservation sites/areas
'Hot spots' site identification for pests, medicinal plant taxa regeneration sites.
Precision Area Wide Pest Management

Drone technology provides an important innovation in medicinal plant species health management of threatened taxa. By attaching multispectral hyperspectral near infrared (NIR) camera to a drone, it is possible to map the health status of threatened taxa. Using the sensor technology, it is possible to determine the soil quality, composition and humidity of forest vegetation. Changes over a specific period could be mapped using drones. Targeted plant protection measures are managed with drone technology coupled with GIS and other geospatial technologies.

9.4 Role of National Medicinal Plants Board (NMPB) in Conservation of Medicinal Plants

National Medicinal Plants Board, New Delhi (NMPB) of India has initiated the use of remote sensing & GIS technologies in the creation of geospatial database of medicinal plants from ancillary data as well as from the field data. Digitization work of projects like Medicinal Plant Conservation Areas, Resource Augmentation, Joint Forest Management, Herbal Garden, Cultivation Schemes of National Ayush Mission, Manufactures & Traders Information is in the process of developing the geo-spatial database. Creation of medicinal plant buffer zones, development of digital atlas of medicinal plants and species distribution modelling of some selected medicinal plants as well as development of spectral signatures of some important medicinal plant species will be the advanced work of the board in near future to assess the conservation, development and sustainable management of medicinal plants. Further, NMPB, Ministry of Ayush, is in collaboration with Indian Institute of Remote Sensing (IIRS) Dehradun and Indian Space Research Organisation (ISRO) to initiate some joint project work in future to develop spectral signatures of some important medicinal plant species and also some web GIS-based applications for the medicinal plants application. The remote sensing and geographic information system (GIS) section is responsible for a variety of functions to assist the board in streamlining the environmental assessment process with special reference to medicinal plants (MPs). Remote sensing and GIS is the process through which digital maps and databases are linked and is used in environmental assessment that includes geospatial database creation, biodiversity conservation, ecological niche modelling, development of spectral signatures and environmental justice issues. The GIS map of medicinal plant conservation areas (72 sites) in India as provided by NMPB is depicted in Fig. 9.4.

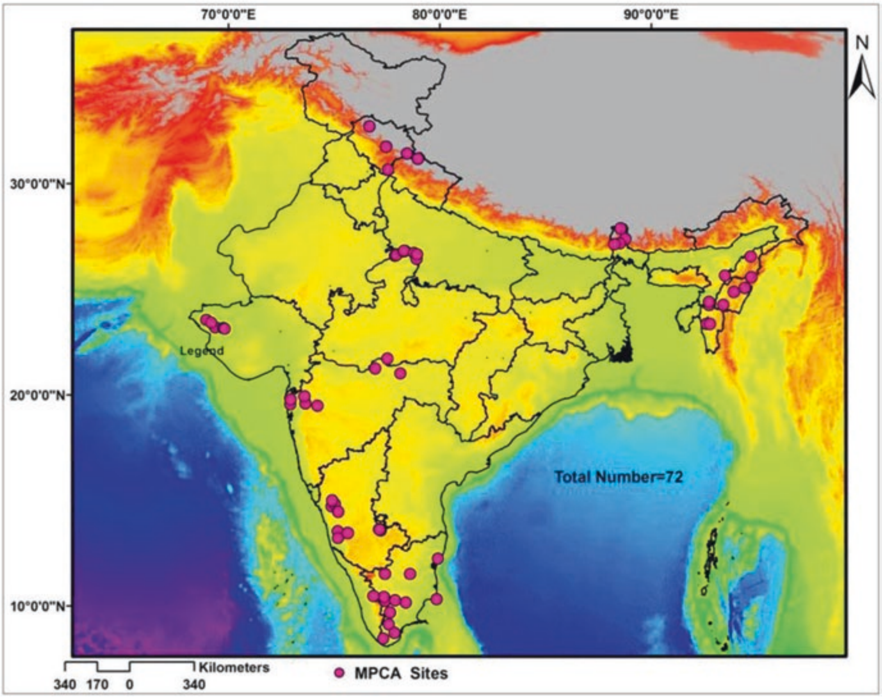

Fig. 9.4 GIS map showing medicinal plant conservation areas in India (source: NMPB, New Delhi)

9.5 Conclusion

Geospatial tools and species distribution models have been used in diversity distribution mapping and predicting suitable sites for future collection of threatened taxa, planning conservation strategies using data on collected germplasm and different climatic variables. Remote sensing satellite temporal data (time interval) in digital form can be used in impact assessment (by overlaying of different geospatial layers) studies, temporal changes to pinpoint status of collected threatened taxa diversity, find gaps and predict new areas for diversity collections. The article highlights application of geospatial technology in geo-referencing, diversity distribution and prediction mapping using plant genetic resources data. Geographical prediction of threatened plant distribution is important to genetic resource conservation planning and regional management decisions. Geospatial technologies are useful in predicting the spatial distribution of target threatened species. It assesses multiple interdependent abiotic factors, e.g., solar radiation, air temperature, precipitation and soil properties, that affect plant distribution, model the environmental niches of target plants and refine their distribution maps for conservation planning. Geospatial technology can be effectively used in locating hotspots and spread of pests and pathogens, developing early warning systems, building risk assessment models, assisting in site-specific protection measures, etc. It helps in the improvement of the present systems of acquiring and generating GIS resources data for effective conservation of threatened medicinal plants.

9.6 Future Thrusts

The geospatial technology in conjunction with the passport/herbarium/gene bank database could serve as a potential information treasure house to the scientific community in general and forestry in particular. In forestry studies geoinformatics technology could be of great use in the management of threatened medicinal plant genetic resources particularly in the following thematic area of studies

1. Integrated geospatial technology can be used in gap analysis, planning and execution of future exploration programme at national level for effective conservation and utilisation of threatened medicinal plants.
2. Threatened taxa passport data information, satellite data spectral signature and climate analogue tools could be used in diversity distribution mapping and prediction of diversity-rich areas for various threatened taxa.
3. Hyperspectral remote sensing can be used in distinguishing and identifying threatened taxa for effective conservation measures.
4. High spatial resolution (60 cm) satellite data can be used in mapping of disease symptoms in threatened species at fine grid level.
5. Ecological niche models/species distribution models for all the threatened taxa to be constructed for effective conservation in the changed climatic regime.

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Part III
Characterization and Evaluation of Threatened Medicinal Plants

Chapter 10
Threatened Medicinal Plants in the Western Ghats – Phytochemical Perspective

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Abstract The plant kingdom represents an extraordinary reservoir of molecules with a variety of astonishingly diverse structural features derived from complex biosynthetic steps. Medicinal plants are a rich bioresource of drugs for traditional systems of medicine and modern medicines, nutraceuticals and food supplements. Threatened medicinal plants have an important role in traditional herbal medicinal practices and are being widely exploited, leading to near extinction. The phytochemical profiling of threatened medicinal plants has potential application in identifying novel sources of bioactive compounds, identification of authentic plant material, in excluding the adulterants, in maintaining the quality and consistency of the herbal drug and in evolving suitable conservation strategies. Chemical profiling of the secondary metabolites can be achieved by conventional approaches involving extraction, separation and identification; by chromatographic and spectroscopic profiling such as HPLC, HPTLC, GC, UV-Vis, IR, NMR and MS; or by online separation, identification and quantitative evaluation using modern hyphenated techniques such as LC-MS, LC-MS/MS, LC-NMR and LC-MS/NMR. The Western Ghats hosts a number of medicinal plants and their wild relatives, mostly coming under threatened category, and the present chapter gives a brief outlook into the phytochemistry of selected threatened species belonging to *Garcinia, Myristica, Rauwolfia* and *Coscinium* from the Western Ghats.

Keywords Threatened medicinal plants · Western Ghats · Phytochemistry

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10.1 Introduction

Medicinal plants have been used by human kind from time immemorial for healing purposes, and the poisonous and healing nature of plants were tested through generations and thus evolved the traditional herbal medicinal systems. Traditional herbal medicinal system, the knowledge that developed over generations within various societies before the era of modern medicine, is still being practiced successfully and is more affordable and accessible to most of the population, especially for the rural communities world over (WHO 2013). More than 35,000 plant species have been reported to be used in various human cultures around the world for traditional medical purposes (Lewington 1993). In India, about 8000 species are of medicinal importance and 3000 species are used in the codified systems of Indian medicine such as Ayurveda, Siddha, Unani and Amchi (Ramawat and Goyal 2008). Most of the plants in traditional herbal medicinal sectors were collected from the wild, leading to threatened status for abound 1000 medicinal plant species.

Medicinal plants contain a large number of complex secondary metabolites that can act as drugs or can provide templates for enhanced bioactivities. More than 50% of all the drugs introduced worldwide can be traced to or were inspired by natural products. Approximately 75% of these drugs were discovered as a direct result of chemical studies focused on the isolation of active substances from plants used in traditional herbal medicine (Newman et al. 2000; Butler 2004; Cragg and Newman 2013). Discovery of the antimalarial compound artemisinin, isolated from *Artemisia annua,* was based on traditional Chinese herbal medicinal information. *Rauvolfia serpentina* has been used in the treatment of mental illness in India from age old, and reserpine isolated from *Rauvolfia serpentina* is a well-known tranquilizer and used effectively in control of high blood pressure (Sahu 1983). Salix tree has been used to relieve from gum pain; and the phenolic glycoside salicin, an effective analgesic and anti-inflammatory compound, was isolated from the bark of Salix tree. The oil of *Papaver somniferum* was used as an analgesic in ancient Mesopotamia; and morphine, a potent analgesic compound, was isolated from *P. somniferum*. *Adathoda vasica* has been widely used in Ayurveda for treating cold, cough, chronic bronchitis and asthma; and the alkaloids vasicine and vasicinone isolated from the plant possess remarkable bronchodilatory properties. The natives of the Amazon region used the bark of *Cinchona officinalis* to treat fevers, and quinine was isolated from the barks of *Cinchona officinalis* as an effective antimalarial drug. If it is assumed that 60,000 plant species have been screened to yield around 135 known drugs, then the remaining plant species could be expected to yield around 650 new drug candidates (Newman et al. 2003).

Though most of the drugs derived from plant resources are from tropical species, it is interesting to note that the tropical flora is least studied for the constituents or bioactivities (Farnsworth and Soejarto 1991). It is estimated that fewer than 5% of tropical forest plant species have been examined for chemical compounds and medicinal values (Zakrzewski 2002). The tropical rain forests of the Western Ghats of India, which extends from the west coast of peninsular India from the river Tapti in the north to Kanyakumari in the south, are one among the richest repositories of endemic biota in the world. With around 7500 plant species, of which about 1250 species being endemic, the Western Ghats is among the highly endemic areas of the world. Among the 36 global biodiversity hotspots, the Western Ghats occupies fifth position in the economic potential of its biological resources (Rajasekharan 2002). The medicinal and aromatic plant species, their intra-specific variants and wild relatives of important medicinal, food and spice crops in the forests of the Western Ghats produce a large number of potential molecules. Though least studied scientifically, most of the threatened species in the Western Ghats region are heavily exploited due to their allied nature to the closely related medicinal species of commercial interest (Ravikumar and Ved 2000; Santhoshkumar and Mathew 2018).

The current trend towards an increased exploitation of herbal medicines has resulted in overharvesting of medicinal plants, leading to high threat for the medicinal plants. Of the estimated 3,10,442 plant species, 27,514 species are threatened as per IUCN data (IUCN 2018). The International Union for Conservation of Nature (IUCN) and the World Wildlife Fund (WWF) have estimated that up to 60,000 higher plant species could become extinct or nearly extinct by the year 2050 if the current trends of utilization continue (Etkin 1998). Threatened medicinal plant species have become the focus of attention because they represent vanishing flora that need protection and because of their role as an essential commodity for health care (Pitman and Jorgensen 2002; Sharma and Thokchom 2014). An extinct plant means the compounds evolved in the plant during the 3 billion years are also extinct. The rich biodiversity is destroyed because it is undervalued and less understood of the potentiality. The chemical profiling of hitherto uninvestigated threatened medicinal plants can significantly contribute in understanding the potential utility of the plants and also create an awareness to conserve the threatened species from extinction. The present chapter elaborates the phytochemical approaches towards threatened medicinal plants and the chemical constituents of selected threatened species in the Western Ghats region, highlighting the potential of these species with respect to the high-value phytochemicals.

10.2 Role of Phytochemistry in the Field of Threatened Medicinal Plants

The miraculous effects of herbs, as experienced in the age-old herbal medicinal traditions such as Ayurveda, Sidha and Unani, were acquired through keen observation and experimentation over generations within various societies. Though the efficacy is validated, in most cases the constituent responsible for the activity and the mode of action of the drug remains obscure, which make the developments in herbal medicinal sector unacceptable for the modern scientific community. Also standardization and authentication are major hurdles that are yet to overcome in traditional herbal medicinal sector. An understanding of the constitution of the plants can definitely help in uncovering the mysteries around the miraculous herbs, and thus, the role of phytochemistry is highly significant in medicinal plants.

Unlike a modern drug that is generally a pure compound, medicinal plant or herbal drug formulation may have a number of constituents that may act synergistically, leading to a potential bioactivity. As elaborated in General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines (WHO 2000), the efficacy evaluation, quality control and standardisation of medicinal plants and herbal formulations are more difficult compared to modern drugs. The major application of phytochemical profiling of threatened species is in authentication and standardisation.

The systematics of plant species primarily depends on the analysis of reproductive morphological features. However, the use of distribution patterns of secondary metabolites is well established as a major tool to characterize, classify and describe plant taxa. The chemical fingerprinting could provide useful information with regard to population structure, species and phyletic relationships and evolutionary status of threatened species (Reynolds 2007). Chemical ecology is an interdisciplinary field between chemistry and biology, dealing with the role of chemical compounds in interactions between organisms. The chemical profiling can give valuable insights into the co-existence between plants and its associated biota, and the information can be effectively utilized in assessing population structure and evolving conservation strategies for threatened species (Rates 2001). Metabolite profiling of plants also contributes significantly in the fields of plant metabolite engineering, functional genomics and plant physiology, which are least applied with regard to the threatened species of the Western Ghats (Trethewey 2004).

10.3 Phytochemical Approaches for Threatened Medicinal Plants

The phytochemical approach for the analysis of a medicinal plant depends on the outcome expected. The phytochemical profiling aims at a targeted group of related metabolites, while phytochemical fingerprinting is generally non-targeted metabolic profiling, determining as many metabolites as possible without necessarily identifying or quantifying the compounds present (Castro-Puyana and Herrero 2013). In metabolic profiling, target metabolites are selected beforehand and are analysed using specific analytical methods. Through fingerprinting, relevant differences between the samples can be evaluated rather than identifying all the molecules present in the samples and is an easy step for quality control of herbal drugs.

Often a phytochemist has to extract, isolate, characterize and estimate certain compound from the plant material by the wise selection and skillful application of various tools, methods and techniques for extraction, separation, purification, identification and estimation of different constituents present in plants. A chromatographic profiling using TLC, HPTLC or HPLC provides qualitative information, while spectroscopic profiling using techniques such as UV-Vis., IR, NMR or MS provides structural information as well (Bhatia et al. 2015; Zhang et al. 2018).

Hyphenation of chromatographic separation and spectroscopic identification and quantification yield more comprehensive information on qualitative as well as quantitative characteristics. The huge data on metabolites has to be properly elaborated and treated using data processing steps involving multivariate statistical analysis to derive a comprehensible result (Castro-Puyana and Herrero 2013).

10.3.1 Conventional Phytochemical Techniques

For detailed phytochemical profiling of a hitherto uninvestigated medicinal plant, conventional techniques such as extraction, separation and characterisation are still being used extensively. Extraction is the process of separating the desired compounds from crude plant material using selective solvents, leaving behind the insoluble cellular materials. A good extraction procedure should bring all the compounds looking for into solution and causes little or no change in the nature of compounds and easy for further analysis (Handa et al. 2008; Zhang et al. 2018). The general techniques of plant extraction include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), hydrodistillation (water distillation, steam distillation, water and steam distillation), expression and enfleurage (cold fat extraction). In addition to conventional hot and cold extraction techniques, countercurrent extraction (CCE), ultrasound extraction (sonication), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), pressurised solvent extraction (PSE), headspace trapping, solid phase microextraction (SPME), microdistillation, thermo-microdistillation and molecular distillation are unconventional extraction methods (Kaufmann and Christen 2002; Zhang et al. 2018).

Once the extract is reduced to a comfortable volume, chromatography is the best method to separate compounds in pure state. On the phenomenological basis, chromatography can be defined as a molecular level separation technique, where the differential migration of solute compounds between stationary phase and mobile phase effect the separation. A chromatogram is the graphical or other presentation of the separated zones. Liquid chromatography is the widely used separation technique in plant chemistry, and conventional column chromatography (CC) has been elevated to flash chromatography (FC), medium pressure liquid chromatography (MPLC) and high pressure liquid chromatography (HPLC) and now reached ultra high-performance liquid chromatography (UHPLC) (Wu et al. 2013). Through the versatile, rugged, economic and rapid analytical features, capillary electrophoresis (CE) is emerging as a potential tool in phytochemical research. Convergence chromatography that uses supercritical fluid as the mobile phase is another interesting field, where the advantages of liquid chromatography and gas chromatography converge. The column chromatography separation of phytochemicals, which took several days and large columns, now takes less than few minutes with the advanced liquid chromatography methods.

Based on the chemical and physical properties of specific compounds, a number of different detection techniques including ultraviolet (UV) or photodiode array (DAD), fluorescence (FD), refractive index (RI), evaporative light scattering (ELSD), nuclear magnetic resonance (NMR) and mass spectrometry (MS) have been used for detecting the components in mixtures. Structure elucidation of the isolated pure compounds can be done through spectroscopic techniques such as UV-Vis, IR, NMR, Mass and X-ray (Prichystal et al. 2016). The final structure of a compound is arrived based on interpreting the data from each spectra. Though structure elucidation depends on skilful application of various spectroscopic techniques by the phytochemist, there has been a lot of progress made in automated structure elucidating algorithms that help the natural product chemist significantly.

10.3.2 Chromatographic Techniques

Advanced chromatographic fingerprinting and profiling has become one of the most powerful analytical methods for the quality control of herbal medicines (Fan et al. 2006). For qualitative or quantitative chromatographic profiling of plant extracts, high-performance thin layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), super critical fluid chromatography (SFC) and capillary electrophoresis (CE) are used.

10.3.2.1 High-Performance Thin Layer Chromatography (HPTLC)

HPTLC is the automated form of TLC that uses more efficient stationary phases for better separation, state-of-the-art instrumentation for precise sample application and development and software controlled evaluation. The initial cost for an HPTLC system as well as maintenance and costs per sample are comparatively low. The most preferred advantage of HPTLC over other chromatographic separations is the visual evaluation of separated spots on the plate that gives an appealing result. Further, the retention factor (R_f), colour of the spots and absorption spectra of the resolved spots, together with the derivatised profiles using different reagents, make HPTLC the most preferred chromatographic profiling technique for plant research (Reich and Schibli 2007).

10.3.2.2 High-Performance Liquid Chromatography (HPLC)

HPLC is the updated version of liquid chromatography (LC), with fine particles of up to 5 μm and operational pressures significantly higher than ordinary liquid chromatography. HPLC is a simple and popular method for the analysis of herbal medicines that provide qualitative as well as quantitative information of the plant constituents through retention time of individual peaks, area of the peaks and the absorption spectra of the resolved peaks (Fan et al. 2006).

10.3.2.3 Gas Liquid Chromatography (GLC)

GLC is the most dependent analytical technique in volatile chemical profiling of plants, especially of aromatic plants. GC provides an extraordinary resolution permitting the separation of structurally similar compounds, compared to HPLC. The nonvolatile constituents can be made into volatile by derivatisation techniques such as TMS and FAME (Wilson and Brinkman 2003; Pinho et al. 2009). The technique in combination with mass spectrometry is widely utilised in the analysis of aroma compounds from plant resources.

10.3.3 Spectroscopic Techniques

Compared to chromatographic profiling, spectroscopic profiling gives structural information as well. The major spectroscopic techniques used for chemical profiling are UV-Vis, IR, NMR and MS. UV-Vis spectroscopic profiling of extracts gives valuable information regarding the presence of characteristic compounds with chromophoric groups or conjugation (Ojeda and Rojas 2004). In certain cases, the analyte can be detected spectroscopically after chemical reactions. However, rather than profiling, UV-Vis scpectroscopic technique has been utilized for quantitative estimation. Infrared spectroscopy (IR) and near infrared (NIR) spectroscopy are fast, accurate and non-destructive analytical tools, used successfully for structure elucidation as well as for screening purposes (Cozzolino 2014). NMR spectroscopy is the most informative tool for structure elucidation in natural product chemistry, which is now being increasingly used for qualitative and quantitative evaluation, especially through multivariate analysis–based NMR spectroscopy (Bhatia et al. 2015). The sample processing for NMR is minimal while the method provides multiple means of identification and quantitative options (Markley et al. 2017). Mass spectrometry is perhaps the most widely relied analytical tool by a phytochemist for qualitative as well as quantitative information. In a mass spectrometer, in the ionization portion the sample in gaseous state was ionised and the ions were accelerated and separated in the mass analyser by their mass to charge ratios. Hard ionization techniques such as EI-MS and FAB-MS occur in gas phase and in vacuo while most of the soft ionisation techniques such as ESI and APCI occur in atmospheric pressure. The latest developments have made ionisation possible even in ambient conditions as in the case of DART and DESI, leading to analysis of crude plant material without extraction (Hajslova et al. 2011). The earlier sector field mass analysers that use deflection in electric and magnetic fields for the separation of the gas phase ions have now been replaced by various techniques such as quadrapole, ion trap, time of flight and orbitrap techniques, yielding better resolution; and the advances in mass spectrometry have now led to fg/ml detection limits in analytical field (Cai et al. 2018).

10.3.4 Hyphenated Analytical Techniques

Chromatographic fingerprint is mainly used as a qualitative method for authentication and quality control of plant extracts. Spectroscopic techniques such as UV-Vis, IR, NMR and MS are generally used for the characterisation of constituents. In hyphenated techniques, the mixtures are separated by chromatographic techniques such as HPLC or UPLC and a suitable detection technique or a combination of detection techniques such as UV, MS or NMR was used for the characterisation of the components. As crude plant extracts represent complex mixtures containing hundreds of constituents, it is quite often cumbersome to isolate each compound and characterise through a series of spectroscopic techniques individually. The hybrid technique avoids the tedious and time-consuming isolation process of pure constituents and allows a rapid structural determination of known plant constituents with only a minute amount of plant material. Such hyphenated analytical techniques have been recognised as a revolutionary breakthrough in the analysis and characterisation of phytochemicals (Sarker and Nahar 2012). The major hyphenated instruments are high-performance liquid chromatography-diode array detection (HPLC-DAD), gas chromatography-mass spectroscopy (GC-MS), capillary electrophoresis-diode array detection (CE-DAD), capillary electrophoresis/mass spectrometry (CE-MS), high-performance liquid chromatography-mass spectroscopy (HPLC-MS) and high-performance liquid chromatography-nuclear magnetic resonance spectroscopy (HPLC-NMR). With the full set of spectroscopic information obtained by hyphenated techniques such as LC/UV, LC/MS and LC/NMR, the phytochemist will be able to characterise rapidly the constituents of a given plant and to choose carefully which metabolites are to be isolated for in-depth structural or pharmacological studies.

10.3.4.1 Gas Chromatography-Mass Spectroscopy (GC-MS)

In gas chromatography-mass spectrometry (GC-MS), volatile compounds are separated by GC and then transferred online to the mass spectrometer for detection. The most widely used ionization method for GC-MS is electron impact (EI) ionization, and the common mass analyser is single quadrupole analyser. The hyphenated technique GC-MS with computerized library search facility can be regarded as the best single tool for the analysis of volatile chemicals from plants, especially essential oils. GC-MS has a pivotal role in flavour analysis of aromatic and spice plants. Several upgradation and specialisations such as SPME-GC, GC-IT, GC-TOF and GC-QqQ have developed in GC-MS instrumentation (Pinho et al. 2009).

10.3.4.2 Liquid Chromatography-Mass Spectroscopy (LC-MS)

In the hyphenated technique LC-MS, liquid chromatography (LC) acts as the best separation technique, whereas MS is the most sensitive method for structure determination. The hyphenated LC-MS technique offers qualitative as well as quantitative data conveniently and provides a significant advancement in the identification and dereplication process in phytochemistry (Wolfender et al. 1998; Wu et al. 2013). Quadrupole time-of-flight (Q-TOF) instrument is perhaps the most reliable tool for phytochemists, giving both accurate MS and MS/MS data directly from crude plant extracts in a single LC-MS analysis. However, different ionisation modes, interfaces and mass analysers are necessary to obtain a complete picture of the exact composition of plant metabolites through LC-MS. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) employs mass analysers in combination as in the case of triple quadruple (QqQ), quadrupole time-of flight (Q-TOF), ion trap-time-of-flight (IT-TOF) and orbitrap analysers and provides more accurate and detailed structural information (Castro-Puyana and Herrero 2013; Cai et al. 2018).

10.3.4.3 Liquid Chromatography-Nuclear Magnetic Resonance Spectroscopy (LC-NMR)

The coupling of LC with NMR spectroscopy is one of the most powerful methods, especially when LC/UV/MS data are insufficient for unambiguous characterisation of peaks. With technical improvements such as high field NMR and employment of detection cells with smaller volumes, it is possible to analyse nanogram quantities of eluted compounds from an analytical HPLC. LC-NMR generally uses ^{1}H NMR spectra or ^{1}H-^{1}H correlation experiments and very rarely ^{13}C NMR. Recent developments like cryoflow probes and post-column solid phase extraction and capillary separations by means of solenoidal microcoils (HPLC-SPE-Cap NMR) have offered high sensitivity to LC-NMR (Lambert et al. 2007). However, compared to UV or MS, NMR remains rather insensitive due to solvent interference (Wolfender et al. 1998).

10.3.4.4 Liquid Chromatography-Mass Spectrometry/Nuclear Magnetic Resonance Spectroscopy (LC-MS/NMR)

The complexity of the plant metabolites demands the combined use of several analytical platforms in tandem to detect maximum number of metabolites in plant samples. LC-MS/NMR is a recent development that combines the detailed structural information from NMR with the high sensitivity of MS. NMR and mass spectrometry are highly complementary, and combining the two techniques is likely to improve the overall quality of chemical profiling, by providing complete characterisation of the compound. The method gives comprehensive information required for the structure elucidation of a compound in a single chromatographic run (Marshall and Powers 2017; Gathungu et al. 2018).

10.3.5 Dereplication Studies Based on Phytochemical Prospecting

The discovery of novel bioactive natural products from plant resources is often confronted by isolation and characterisation of known phytochemicals (replication), and to alleviate this bottleneck in natural product research, efforts are now concentrated on dereplication or detecting known compounds before isolation and structure elucidation. Dereplication prevents the overexploitation of threatened species considerably for research purpose, and several such dereplication platforms are now available that combines natural product databases, structural features and open source cheminformatics tools (Hubert et al. 2017; Zani and Carroll 2017).

A review on the phytochemistry of selected threatened medicinal plants of the Western Ghats belonging to the genus *Garcinia, Myristica, Rauwolfia* and *Coscinium* is presented below. Detailed phytochemistry reported through extraction, isolation and characterisation was elaborated, followed by phytochemical prospecting using hyphenated analytical techniques.

10.4 *Coscinium fenestratum* (Gaertner) Colebr.

Coscinium fenestratum (Gaertner) Colebr. (Family: Menispermaceae), the dioecious, woody climber, is a threatened medicinal plant indigenous to the Indo-Malay region. In India, *C. fenestratum* is mostly found in the moist evergreen forest of the Western Ghats, upto around 750 m altitude (Mohanan and Sivadasan 2002). The wood is yellow in colour both externally and internally, implying the name *tree turmeric* or *daru haridra* to the climber (Tushar et al. 2008). *C. fenestratum* is a highly traded medicinal plant, and the destructive harvesting of the plant due to the huge demand in traditional medicinal sector has led to near extinction of the species. The plant is a slow growing climber and takes around 15 years to mature, and more than 80% of the wild population has been destroyed during the last 30 years (Ravikumar and Ved 2000) (Fig. 10.1).

C. fenestratum is the source of the important raw drug *daru haridra* in the Indo-China region, south India and Sri Lanka. The stem has been used in south India and Sri Lanka as a yellow dye and bitter tonic. Traditionally, dried stem and root are used for treating various vitiated conditions of kapha and vata, ulcers, jaundice, skin diseases, diabetes and fever (Warrier et al. 1994). The root bark is used to treat bleeding piles and excessive bleeding during menstruation and other gynaecological troubles and also against leucorrhoea, influenza, eye diseases and leishmaniasis (Caius 1992). *C. fenestratum* is an important ingredient of Ayurvedic formulations like Aswagandharishtam, Khadirarishtam, Anuthailam, Katakakhadiradi kashayam, Elaneer kuzhampu and Mahapanchagavyam (Nambiar et al. 2000).

Fig. 10.1 *Coscinium fenestratum*

Extracts of *C. fenestratum* roots and stem showed antioxidant, hypotensive, antihepatotoxic (Venukumar and Latha 2004), antiinociceptive (Chitra et al. 2004), antidiabetic (Punitha et al. 2005), antiplasmodial (Tran et al. 2003), hypolipidemic (Wongcome et al. 2007), antiproliferative and antimicrobial properties (Ueda et al. 2002). The fruit pulp extract showed significant antioxidant and anthelmintic activities (Das et al. 2018).

Protoberberine and aporphine alkaloids belonging to isoquinoline group are biosynthetically derived from tyrosine and exhibited a vast array of medicinal properties such as anti-inflammatory, antimicrobial, antileukemic and antitumor (Whitehouse et al. 1994; Qing et al. 2018). Among the isoquinoline alkaloids, benzylisoquinoline alkaloids showed more potent pharmacological activities. The benzylisoquinoline alkaloid berberine is the most explored and the major active compound with potent biological effects reported from all parts of *C. fenestratum* (Birdsall and Kelly 1997; Rojsanga and Gritsanapan 2005). It has been used to treat diabetes effectively and also to control high cholesterol and high blood pressure (Kong et al. 2004; Jun et al. 2008). The compound is reported to cause stronger heartbeats, which help people with heart conditions (Xie et al. 2011). The compound displayed inhibitory activity against phytopathogenic fungi and also showed remarkable antibacterial activity (Nair et al. 2005; Singburaudom 2015).

The berberine content in the stem of *C. fenestratum* has been analysed by chromatographic methods such as thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC), and estimated at around 1–2% (Rojsanga et al. 2006; Akowuah et al. 2014). Berberine has been identified as the most abundant compound in the species by validated LC-MS/MS analysis, and the maximum content was in the root (186.7 mg/g) followed by stem (173.9 mg/g) (Awantika et al. 2016).

Other protoberberine alkaloids isolated from the stem and roots of *C. fenestratum* were berberrubine, thalifendine, oxyberberine, oxypalmatine, (−)-8-oxotetrahy drothalifendine, (−)-8-oxoisocorypalmine, (−)-8-oxothaicanine (−)-8-oxo-3-hydroxy-2, 4, 9, 10- tetramethoxyberbine, 12,13-dihydro-8-oxoberberine,5,6,13, 13a-tetrahydro-9,10,dimethoxydibenzo-1,3-benzodioxolo quinalizine-8-one, berlambine, dihydroberlambine, (−)-8-oxocanadine and noroxyhydrastinine (Siwon et al. 1980; Pinho et al. 1992; Tran et al. 2003; Ali et al. 2008). Other phytoconstituents present in the stem include aliphatic compounds like ceryl alcohol, hentriacontane, palmitic acid and oleic acid, steroids like sitosterol and stigamasterol, and saponins (Malhotra et al. 1989; Siwon et al. 1980). Ecdysterone, an analogue of the insect moulting hormone and attributed with multifaceted bioactivities, has been reported from the stem (0.22%) and leaves (0.12%) (Sreejith et al. 2014).

Microwave-assisted extraction (MAE) and liquid chromatography hybrid ion trap time-of-flight mass spectrometry (LC/IT-TOF MS) analysis has led to the identification of two benzylisoquinoline alkaloids, three aporphine alkaloids, twelve quaternary protoberberine alkaloids, ten 8-oxoprotoberberine alkaloids, three tetrahydro protoberberine alkaloids and a steroid compound (Deevanhxay et al. 2009).

Ultra-performance liquid chromatography coupled to hybrid triple quadrupole/linear ion trap mass spectrometry (UPLC-ESI-MS/MS) in multiple reactions monitoring (MRM) mode was used to quantify the protoberberine and aporphine alkaloids such as isocorydine, jatrorrhizine, tetrahydropalmatine, tetrahydroberberine, palmatine, berberine, glaucine and magnoflorine in different parts of the species (Awantika et al. 2016) (Fig. 10.2).

Development of alternate viable sources of the active constituent berberine, such as from endophytic fungi, can alleviate the extent of threat to the species (Diana and Agastian 2013). Further, the application of advanced hyphenated analytical techniques such as LC-MS/MS and LC-MS/NMR gives exhaustive chemical profiling using minimal plant resources and also helps in dereplication.

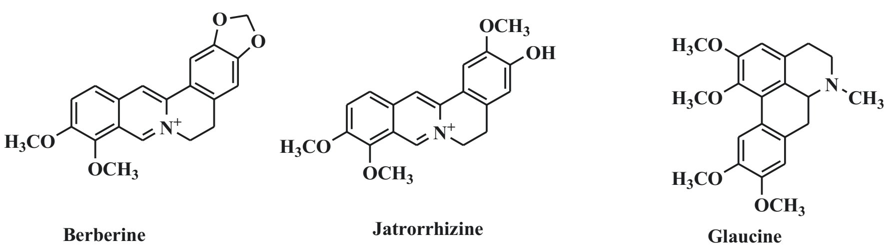


Fig. 10.2 Characteristic compounds reported from *Coscinium fenestratum*

10.5 *Garcinia* Species

The genus *Garcinia* L. (family: Clusiaceae), comprising about 250 species, is distributed in the pan-tropical regions with high species richness in South-East Asia and Africa (Shameer et al. 2016). In India, the genus is represented by 44 species and is distributed mainly in three phytogeographical zones viz., the Western Ghats, North East India and Andaman and Nicobar Islands. Ten *Garcinia* species were reported from the Western Ghats, of which 8 species are endemic to the region (Maheshwari 1964; Shameer et al. 2017; Singh 1993).

Garcinia trees have received considerable attention as sources of herbal medicines, gums, pigments, resins, waxes and edible fruits. Most of the species are cultivated mainly for their spice and fruits. Some *Garcinia* species are used for the extraction of gamboge, a golden-yellow coloured resin, which is used as colouring pigment and folk medicine. Gamboge is a rich source of polyprenylated caged benzophenones and xanthones (Chantarasriwong et al. 2010).

Garcinia fruits are a rich source of hydroxycitric acid (HCA), an anti-obesity compound. Fruit rinds of *G. gummi-gatta*, *G. indica* and *G. atroviridis* are the prime source of HCA (Jena et al. 2002). The fruit rinds of *G. gummi-gutta* and *G. indica* contain 20–30% HCA. *Garcinia* leaves also contain HCA in significant quantity, and an LC-MS analysis by Pandey et al reported that HCA content in the leaves of 11 *Garcinia* species is the highest in *G. indica* (120 mg/g leaf extract), followed by *G. gummi-gutta* (95 mg/g leaf extract) (Pandey et al. 2015).

Garcinia species are reported as a rich source of structurally diverse secondary metabolites such as xanthones, benzophenones and biflavonoids. Xanthones have restricted distribution in plant families, and among the 120 *Garcinia* species studied for the phytochemicals, 74 species have been reported with xanthones (Aravind et al. 2016a, b; Negi et al. 2013). The genus *Garcinia* is one of the rich sources of benzophenones, and 50 *Garcinia* species are reported to contain benzophenones (Aravind et al. 2016a, b). The polyisoprenylated benzophenones garcinol and isogarcinol are the active ingredients of nutraceutical products from *G. indica* and *G. gummi-gutta*. Guttiferone, a pharmaceutically important benzophenone isolated from *Garcinia* species, showed anti-HIV, trypanocidal and cytotoxic activities (Acuna et al. 2009). Biflavonoids, the dimers of flavonoids, also have limited distribution in the plant kingdom, and the genus *Garcinia* is a rich source of biflavonoids, with around 45 species reported to contain biflavonoids. Flavonoids, biphenyls, acylphloroglucinols, depsidones and triterpenoids are reported as minor constituents in *Garcinia* species (Aravind et al. 2016a, b). Volatile mono- and sesquiterpenoids and phenyl propanoids were also reported from *Garcinia* species (Rameshkumar et al. 2016a, b).

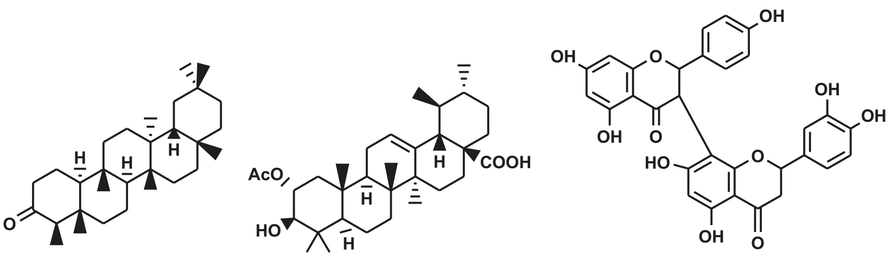
Among the ten *Garcinia* species reported from the Western Ghats, 8 species, viz, *G. gamblei*, *G. imberti, G. morella*, *G. indica*, *G. rubro-echinata, G. talbotii, G. travancorica* and *G. wightii* are coming under threatened category (Shameer et al. 2016, 2017; IUCN 2018). *G. gamblei* is a newly reported species and no reports are there on phytochemistry of the species (Shameer et al. 2017).

10.5.1 Garcinia imbertii *Bourd.*

Garcinia imbertii Bourd. has limited distribution in the forest regions of Agasthyamala Biosphere Reserve, South India. Phytochemical investigation of *G. imbertii* has led to the isolation of the biflavonoid morelloflavone and triterpenoids friedelin, 2α-hydroxy-3β-acetoxy-urs-12-en-28-oic acid and the steroid stigmasterol (Rameshkumar et al. 2016a, b). HPTLC estimation showed 0.76% w/w of morelloflavone in the stem bark and 2.2% w/w of the triterpenoid friedelin in the leaves (Rameshkumar et al. 2016a, b) (Figs. 10.3 and 10.4).

The analysis of the leaves of *G. imbertii* using QqQ LIT-MS/MS technique showed 22 compounds comprising benzophenone (garcinol), xanthones (mangostin and gambogic acid), flavonoids (isoorientin, epicatechin, orientin, isovitexin, vitexin, luteolin, kaempferol-3-O-rutinoside, quercetin, kaempferol, apigenin), biflavonoids (fukugiside, GB-2, GB-1, GB-1a, amentoflavone) and phenolic acids (protocatechuic acid, caffeic acid, ferulic acid, vanillic acid) (Pandey et al. 2015). The plant was also a potential source of essential oils and the volatile chemical studies by GC-MS revealed caryophyllene derivatives as the predominant compounds in the essential oils from leaf, bark and fruits (Rameshkumar et al. 2016a, b).

Fig. 10.3 *Garcinia imbertii*



Friedelin 2α-Hydroxy-3β-acetoxy-urs-12-en-28-oic acid Morelloflavone

Fig. 10.4 Major compounds isolated from *Garcinia imbertii*

10.5.2 Garcinia rubro-echinata *Kosterm.*

Garcinia rubro-echinata Kosterm. is an endemic tree species of the forest regions of Agasthyamala Biosphere Reserve, south India, and is an allied species of *G. echinocarpa* reported from Sri Lanka (Kostermans 1977). Phytochemical analysis of the leaves of *G. rubro-echinata* yielded the triterpenoid friedelin, the flavonoids naringenin, apigenin and (−)-epicatechin, the biflavonoids podocarpusflavone A and amentoflavone and the dihydrochalcone phloretin and phloretin-4'-O-β-D-glycoside (Menon et al. 2018). HPTLC estimation showed 0.21% (w/w) amentoflavone and 0.18% (w/w) friedelin in *G. rubro-echinata* leaf (Menon et al. 2018) (Figs. 10.5 and 10.6).

UHPLC-QqQLIT-MS/MS analysis of the leaf methanol extract of *G. rubro-echinata* has identified 24 compounds comprising benzophenone (garcinol), xanthones (mangostin and gambogic acid), flavonoids (isoorientin, epicatechin,

Fig. 10.5 *Garcinia rubro-echinata*

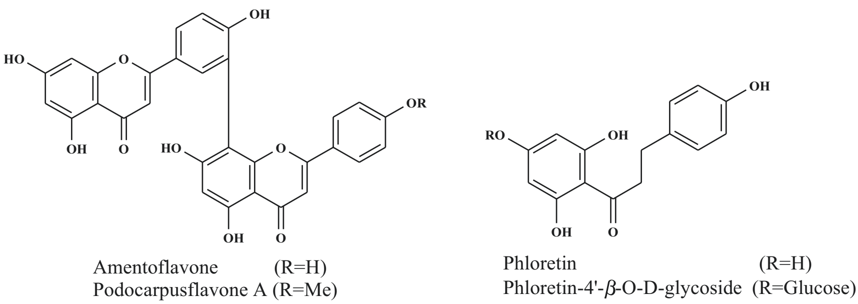


Fig. 10.6 Biflavonoids and dihydrochalcones isolated from *G. rubro-echinata*

orientin, isovitexin, vitexin, luteolin, kaempferol-3-O-rutinoside, quercetin, kaempferol, apigenin), biflavonoids (fukugiside, GB-1, amentoflavone), phenolic acids (protocatechuic acid, caffeic acid, ferulic acid, vanillic acid), terpenoids (ursolic acid and betulinic acid), hydroxycitric acid and its derivative hydroxycitric acid lactone (garcinia acid) (Pandey et al. 2015). GC-MS analysis of the essential oil of *G. rubro-echinata* leaf revealed the ubiquitous sesquiterpenes β-caryophyllene and the isomeric compound α-humulene as the major volatile constituents (Rameshkumar et al. 2016a, b).

10.5.3 Garcinia indica *(Thouars) Choisy*

Garcinia indica (Thouars) Choisy is an endemic species of India coming under threatened category and distributed in the tropical rain forests of the Western Ghats, ranging from Konkan southward to Mysore, Coorg and Wayanad (Jena et al. 2002). The species is well known for its food and medicinal values; and a variety of products such as bioactive acids, nutraceuticals, fats and condiments are derived from the species. The dried fruit rind of *G. indica* is widely used as a substitute for tamarind and also as a pink and purple food colouring agent (Jayaprakasha and Sakariah 2002; Kaur et al. 2012). The anthocyanins cyanidin-3-glucoside and cyanidin-3-sambubioside are the major pigments present in fruit rind (Baliga et al. 2011). Kokum butter is another important product obtained from the seeds of *G. indica*, which finds application in cosmetic products and in chocolates (Baliga et al. 2011; Maheshwari and Reddy 2005) (Fig. 10.7).

Detailed phytochemical investigation *G. indica* has led to the isolation of the biflavonoids fukugetin and volkensiflavone from the heartwood (Cotterill and Scheinmann 1977) and benzophenones isogarcinol, garcinol, and 14-deoxyisogarcinol from the fruits (Kaur et al. 2012) (Fig. 10.8).

Fig. 10.7 *Garcinia indica*

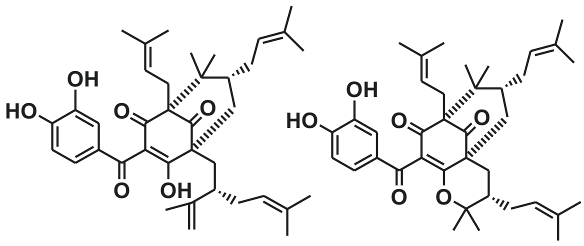


Fig. 10.8 Major benzophenones isolated from *G. indica*

LC-MS/MS analysis revealed the presence of the benzophenones xanthochymol and isoxanthochymol (Chattopadhyay and Kumar 2006). Quantitative analysis by UHPLC-QqQLIT-MS/MS has identified 26 compounds in the leaf methanol extract of *G. indica,* and hydroxy citric acid and garcinia acid were found as the major constituents (Pandey et al. 2015). GC-MS analysis has identified β-caryophyllene, α-humulene and α-selinene as the major compounds in the leaf essential oil of *G. indica* (Rameshkumar et al. 2016a, b).

10.5.4 Garcinia travancorica *Bedd.*

Garcinia travancorica Bedd.*,* with limited distribution in the forest regions of Agasthyamala Biosphere Reserve, comes under vulnerable category (IUCN 2018). The detailed phytochemical investigation of the leaves of *G. travancorica* yielded the polyisoprenylated benzophenones, 7-epi-nemorosone and garcinol along with the biflavonoids GB-1a, GB-1, GB-2, morelloflavone and morelloflavone-7-O-β-D-glycoside (fukugiside) (Anu Aravind et al. 2015). *G. travancorica* leaves were found as a rich source of the biflavonoid glycoside morelloflavone-7”-O-β-D-glycoside (7.12% w/w) by a validated HPTLC method (Anu Aravind et al. 2015) (Fig. 10.9).

Qualitative screening of secondary metabolites present in the leaves, fruits and stem bark methanol extracts of *G. travancorica* using HPLC-QTOF-MS resulted in the identification of 23 compounds including eight biflavonoids (morelloflavone, GB-1, GB-1a, GB-2a, GB-2, fukugiside, xanthochymusside and GB-1a glucoside), two acids (hydroxycitric acid and hydroxycitric acid lactone), nine xanthones (α-mangostin, γ-mangostin, garciniaxanthone E, 1,5-dihydroxy-3-methoxyxanthone, 4-(1,1-dimethylprop-2-enyl)-1,3,5,8-tetrahydroxy-xanthone, garcinone A, garcinone B, garcinone C and polyanxanthone C) and four polyisoprenylated benzophenones (gambogenone, aristophenone A, garcinol and garciyunnanin A) (Aravind et al. 2016a, b) (Fig. 10.10).

G. travancorica was also found as a rich source of essential oils, and the aliphatic hydrocarbon n-undecane was identified as the major volatile compound in leaf, stem bark and fruit by GC-MS analysis (Rameshkumar et al. 2016a, b). n-Undecane

Fig. 10.9 *Garcinia travancorica*

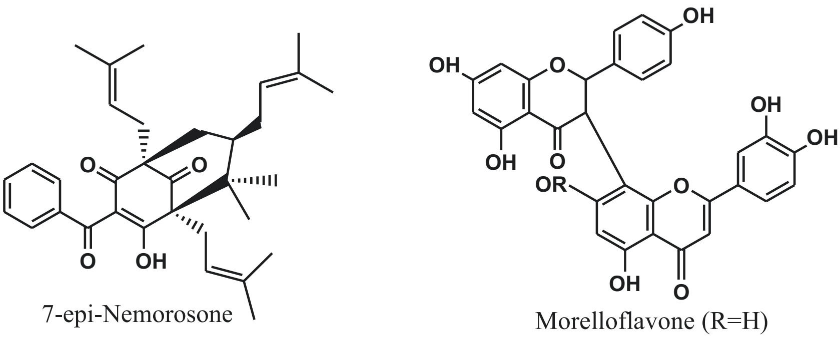


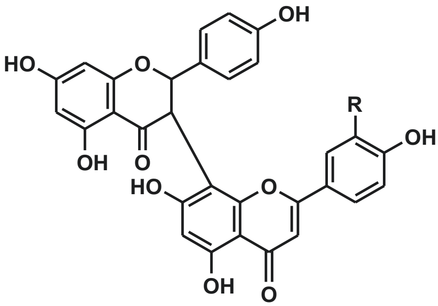
Fig. 10.10 Benzophenone and biflavonoids isolated from *Garcinia travancorica*

is the major pheromone found in Dufour's gland of the ant *Camponotus obscuripes* (Formicinae), and also reported to possess pheromone-type character which attracts flies, moths and ants (Schiestl et al. 2000). The mutualism in any possible ant–plant interaction needs to be studied on a chemical ecological basis.

10.5.5 Garcinia talbotii *Raizada ex Santapau*

Garcinia talbotii Raizada ex Santapau is an endangered and endemic tree species of the Western Ghats, and the species is closely allied to *Garcinia spicata* Wight and Arn. (Raizada 1960; Shameer et al. 2016). The biflavonoids talbotaflavone and morelloflavone had been isolated from the root of *G. talbotii* (Joshi and Viswanathan 1970). UHPLC-QqQLIT-MS/MS quantitative analysis of the leaves of *G. talbotii* showed the biflavonids GB-1 and GB-2 as predominant compounds in the species

Fig. 10.11 *Garcinia talbotii*



Talbotaflavone (R=H)
Morelloflavone (R=OH)

Fig. 10.12 Biflavonoids isolated from *Garcinia talbotii*

followed by the xanthone mangostin (Pandey et al. 2015). Volatile chemical profiling of the leaves of *G. talbotii* by GC-MS analysis unveiled that the major constituents were sesquiterpene hydrocarbons β-caryophyllene, α-humulene and α-copaene (Rameshkumar et al. 2016a, b) (Figs. 10.11 and 10.12).

10.5.6 Garcinia morella *(Gaertn.) Desr.*

Garcinia morella (Gaertn.) Desr. is an endangered tree species, mainly distributed in the Indo-Malay region. Phytochemical investigation yielded the xanthones morellin and moreollin from the seeds and pericarp, while the biflavonoids dihydromorelloflavone, fukugiside and fukugetin were reported from the bark (Karanjgaokar et al. 1967; Rao 1937; Adawadkar et al. 1976). Morelloflavone was isolated from the heartwood of *G. morella* in 1967, and it is the first biflavonoid reported with a flavone and a flavonone unit (Karanjgaokar et al. 1967). *G. morella* is one of the major sources of gamboge; and several xanthones such as desoxymorellin, dihydroisomorellin, morellic acid, isomorellic acid, moreollic acid, methyl-*O*-methyl morellate, guttiferic acid, methyl morellate and dimethyl guttiferate were isolated from the resin (Bhat et al. 1964; Karanjgaokar et al. 1966; Rao et al. 2007). UHPLC-QqQLIT-MS/MS quantitative analysis of the leaves of *G. morella* showed the plant as a rich depository of the biflavonoids fukugicide, GB-1, GB-2, GB-1a, and amentoflavone (Pandey et al. 2015) (Figs. 10.13 and 10.14).

10.5.7 Garcinia wightii *T. Anderson*

Garcinia wightii T. Anderson is an endemic tree of southern Western Ghats, coming under threatened category, and the species can also be found in riparian habitats (Shameer et al. 2016). The species has not been investigated in detail, except for LC-MS and GC-MS screening. UHPLC-QqQLIT-MS/MS analysis of the leaf methanol extract of *G. wightii* identified 26 compounds; and the xanthones (cambogic acid and mangostin), benzophenone (garcinol), flavonoids (vitexin and isovitexin) and biflavonoids (GB-1 and GB-1a) were present in comparatively large quantities (Pandey et al. 2015). The sesquierpenoid bicyclogermacrene has been identified as the major compound in leaf essential oil of *G. wightii* (Rameshkumar et al. 2016a, b) (Figs. 10.15 and 10.16).

The threatened *Garcinia* species were found to be a rich source of bioactive biflavonoids, benzophenones and xanthones, in addition to volatile aroma com-

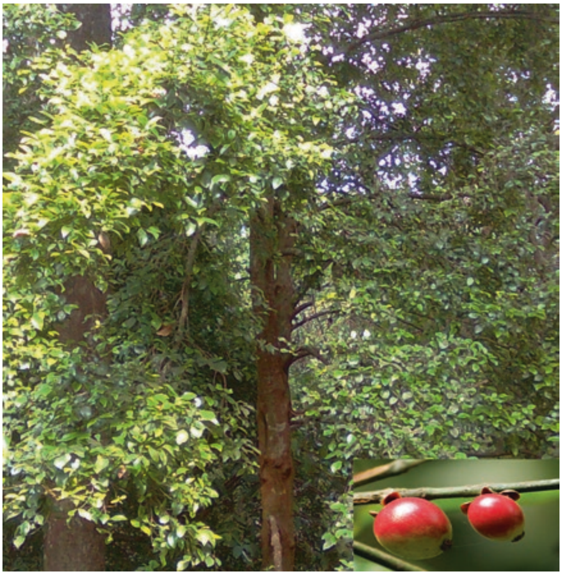


Fig. 10.13 *Garcinia morella*

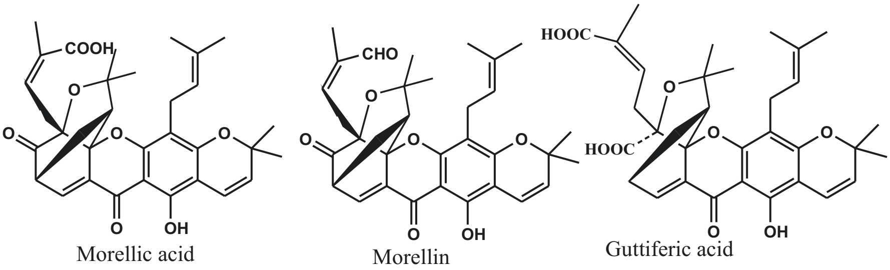


Fig. 10.14 Characteristic xanthones isolated from *Garcinia morella*

Fig. 10.15 *Garcinia wightii*

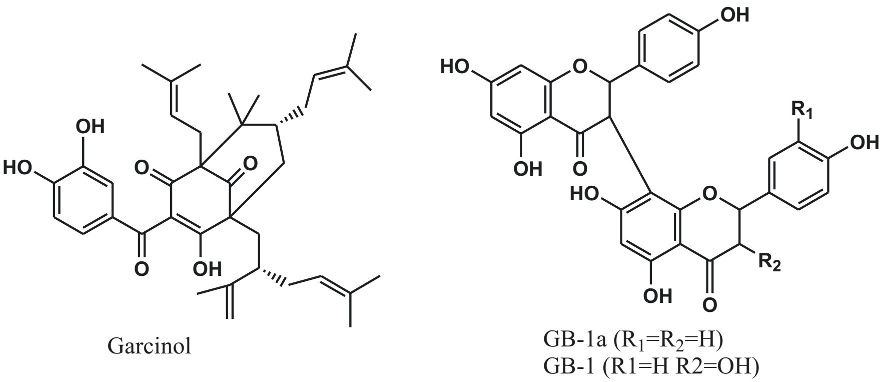


Fig. 10.16 Benzophenone and biflavonoids detected in *Garcinia wightii*

pounds. Compounds such as the biflavonoid fukugiside have been found as promising candidates for drug development, which may lead to further threat to the source plant. It is suggested to evolve conservation strategies for such species and also to find alternate synthetic derivatives for the active compounds.

10.6 *Myristica* Species

The genus *Myristica* Gronov. (Family: Myristicaceae) is an economically important group of aromatic plants with about 72 species distributed in tropical Asia, Australia and the Pacific Islands. The genus is represented by 3 species in the Western Ghats: *Myristica beddomeii* (King), *M. fatua* (Houtt.) var. *magnifica* (Bedd.) and *M. malabarica* (Lamk.); and all are endemic to the region (Sheeja et al. 2013; Nayar et al. 2014; Banik et al. 2017). In addition, *M. fragrans* is the source of the spices nutmeg and mace of high flavour, and medicinal value is now naturalised in the Western Ghats region (Nayar et al. 2014).

The seeds of *Myristica* species contain 25–40% fixed oil that is widely used in food and industrial sectors (Sarathkumara et al. 1985). *Myristica* fruits are also an excellent source of essential oils that are widely used in various food additives and cosmetic products (Ehlers et al. 1998; Choo et al. 1999). In addition to its use in food industries, nutmeg oil is widely used in the cosmetic and pharmaceutical sectors. The rind also contain up to 15% pectin and 30% fibre (Gopalakrishnan 1992). The high pectin content of the pericarp may be responsible for the antidiarrhoeal effects of *M. fragrans*, reported in Ayurvedic treatises.

Myristica species have received considerable attention from the scientific world and resulted in the isolation of several interesting structures such as acylphenols, dimeric acylphenols, diarylpropanoids, phenylpropanoid ethers, lignins, benzofuranoid neolignans and fatty acids (Cao et al. 2015; Abourashed and El-Alfy 2016; Pandey et al. 2016). The characteristic metabolites of nutmeg have demonstrated potential biological activities and that may support its use in traditional medicines.

All the species distributed in the Western Ghats: *Myristica beddomeii* (King), *M. fatua* (Houtt.) var. *magnifica* (Bedd.) and *M. malabarica* (Lamk.) are coming under threatened category, due to restricted distribution and indiscriminate harvesting of fruits (Ravikumar and Ved 2000; Santhoshkumar and Mathew 2018).

10.6.1 Myristica malabarica *(Lam.)*

Myristica malabarica Lam. is a rare species coming under threatened category and confined to the evergreen forests of the Western Ghats (Hemmila et al. 2010; Mohanan and Sivadasan 2002). The spice from *M. malabarica* is known as *rampatri* or Bombay mace and Bombay nutmeg and is used to adulterate *M. fragrans*

Fig. 10.17 *Myristica malabarica*

spices (Gamble 1967). In Ayurveda, the mace is known as *pasupasi* and is often found as a substitute for jathipathri or mace from *M. fragrans*. The species is in a threatened state because of indiscriminate harvesting of fruits for its aril (Ravikumar and Ved 2000) (Fig. 10.17).

The diarylnonanoids, malabaricones A-D, were isolated as novel compounds from *M. malabarica* fruit rinds by Purushothaman et al. (1997), and several bioactivities including antioxidant, antiulcer, anti-inflammatory and anticancer have been reported for the compounds (Banerjee et al. 2008; Maity et al. 2012). The novel compound 7,4′-dimethoxy-5-hydroxyisoflavone together with the known isoflavones, biochanin A and prunetin, 1,3-diarylpropanol and alpha-hydroxy dihydrochalcone were isolated from the heartwood of *M. malabarica* (Talukdar et al. 2000). Phytochemical investigations of the fruit rind of *M. malabarica* revealed the presence of novel diaryl nonanoids: malabaricones A-D and aryl tetradecanoid (Bauri et al. 2016). A new aryl cyclohexyl nonanoid with anti-proliferative activity against various cancer cells has been reported from the fruit rind of *M. malabarica* (Bauri et al. 2016) (Fig. 10.18).

Caryophyllene and humulene were identified as the major constituents of the leaf oil of *M. malabarica* collected from the southern Western Ghats region (Sabulal et al. 2007; Zachariah et al. 2008).

Compounds belonging to phenylpropanoid ether and their dimers, acyl phenols and their dimers, fatty acid and their ester were detected in seed, mace and pericarp of *M. malabarica* by qualitative screening through HPLC-QTOF-MS/MS and NMR analysis (Pandey et al. 2016).

M. malabarica extracts and isolated compounds were attributed with hepatoprotective (Morita et al. 2003), anti-carcinogenic (Maity et al. 2012), anti-leishmanial (Sen et al. 2007), antiulceral (Banerjee et al. 2008), antiproliferative (Manna et al. 2016), anti-inflammatory (Maity et al. 2012), anti-quorum sensing (Chong et al. 2011) and anti-thrombotic (Patro et al. 2005) activities.

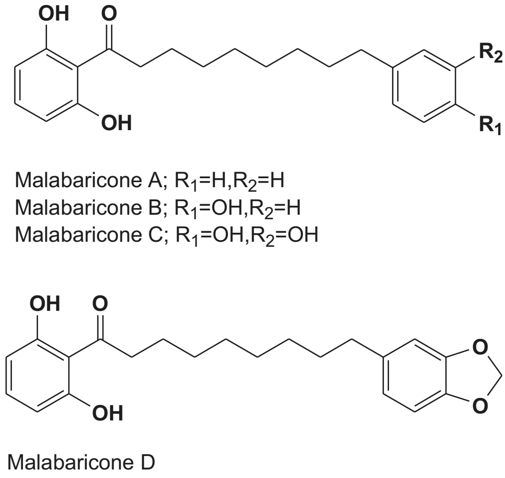


Fig. 10.18 Structures of malabaricones A-D isolated from *Myristica malabarica*

10.6.2 M. fatua *(Houtt.) var.* magnifica *(Bedd.)*

Myristica fatua (Houtt.) var. *magnifica* (Bedd.) is endemic to the Western Ghats with restricted distribution in the freshwater swamps of the Western Ghats, south India (Nayar et al. 2014). The species is adapted with stilt roots to sustain in swampy habitat (Fig. 10.19).

Recent phytochemical investigation of the stem bark of *M. fatua* (Houtt.) var. *magnifica* (Bedd.) has led to the isolation and characterisation of a new compound 3-tridecanoyl benzoic acid, along with six known acylphenols, viz, 1-(2-hydroxy-6-methoxy phenyl) tetradecan-1-one; 1-(2,6-dihydroxyphenyl) tetradecan-1-one; malabaricone A; 1-(2-hydroxy-6-methoxyphenyl)-9-(4-hydroxyphenyl)nonan-1-one; malabaricone B; and malabaricone C. All the compounds displayed moderate inhibitory activity on α-amylase and significant activity on α-glucosidase. Malabaricone B and C were identified as potent α-glucosidase inhibitors with IC_{50} values of 63.70 and 43.61 μM, respectively. Acylphenols also showed significant antiglycation property (Prabha et al. 2018) (Fig. 10.20).

LC-MS screening of different parts of the fruits of *M. fatua* (Houtt.) var. *magnifica* (Bedd.) has resulted in the identification of 16 components for the first time from, belonging to, phenylpropanoid ether and their dimers, acyl phenols and their dimers, fatty acid and their ester (Pandey et al. 2016).

M. fatua Houtt., an allied species collected from Indonesia, yielded two novel diaryl nonanoids (7S, 8R, 8'S, 7'S) 7,7′-bis(3-hydroxy-5-methoxyphenyl)-8,8′-dimethylbuthane- 7,7′-diol and 3″-hydroxydemethyldactyloidin from the leaves and the known diarylnonanoids malabaricone B and C from the stem bark (Fajriah et al. 2017; Megawati and Darmawan 2017).

Fig. 10.19 *Myristica fatua*

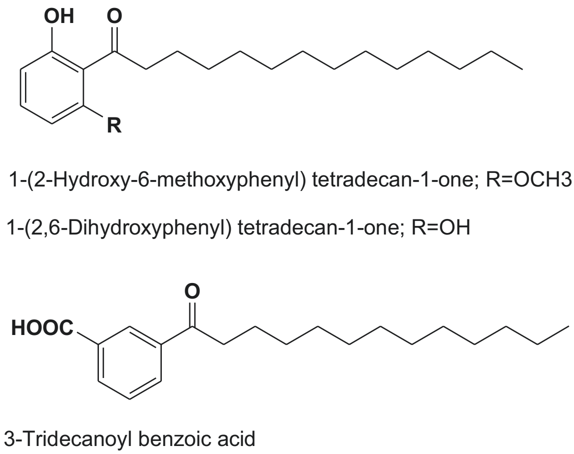


Fig. 10.20 Acylphenols isolated from *Myristica fatua* (Houtt.) var. *magnifica* (Bedd)

10.6.3 Myristica beddomei *(King)*

Myristica beddomei (King) is a wild relative of nutmeg occurring in the evergreen forests of the Western Ghats at an altitude of 1000–1500 m and coming under endangered category (Sheeja et al. 2013; IUCN 2018) (Fig. 10.21).

Methoxyeugenol, eugenol, methyl eugenol, elemicin, safrole, myristicin, malabaricone A, malabaricone B, malabaricone C, dimer of malabaricone A and C, 4-(6,7-dimethoxy-3-methyl-5-propenyl-2,3-dihydrobenzofuran-2-yl)-2-methoxyphenol, dehydro diisoeugenol (licarin A), licarin B, 1-(20,60-dihydroxyphenyl)-9-(40-hydroxy-30-methoxyphenyl)-nonan-1-one, giganteone A, giganteone C, myristic acid and trimyristin were identified in the seed, mace and pericarp of *M. beddomei* by LC-MS profiling (Pandey et al. 2016). The pericarp was found to be a rich source of nutritional compounds and

Fig. 10.21 *Myristica beddomei*

hence may be used as a functional food (Vidhya et al. 2015). The methanolic extract of pericarp showed remarkable antioxidant activities in various in vitro assays (Vidhya et al. 2015).

The essential oil constituents of leaves of *M. beddomei* were dominated by α-pinene, E-caryophyllene and β-pinene (Vidhya et al. 2015; Zachariah et al. 2008). The major volatile constituent of the pericarp has been identified as E-caryophyllene, and the total anthocyanin content was estimated as 33.064 mg/100 g (Vidhya et al. 2015).

The mace, seed and pericarp of the endemic and threatened species *M. beddomei*, *M. fatua* and *M. malabarica* are used in traditional medicines and widely consumed in south India as substituents or adulterants of true nutmeg and mace, and also as a source of fats, and are important Non-Wood Forest Produces (NWFP) of the region (Patro et al. 2005; Zachariah et al. 2008). The spice parts (mace, pericarp and seed) of the threatened *Myristica* species *M. malabarica*, *M. fatua* and *M. beddomei* showed potent in vitro antiproliferative activity by sulphorhodamine B (SRB) assay against the human cancer cell lines A549, DLD1, DU145, FaDu and MCF-7 at 100 μg/mL concentration (Pandey et al. 2016).

The genus *Myristica* has become the focus of scientific and industrial sectors because of the presence of spices, essential oils, fats, pectins and characteristic bioactive molecules such as acylphenols, dimeric acylphenols, diarylpropanoids, phenylpropanoid ethers, lignin, and benzofuranoid neolignans. Most of the wild endemic and threatened *Myristica* species are also reported to contain the characteristic compounds and remarkable level of bioactivities, supporting the use of these species in traditional medicinal practices, and the species can emerge as a potential candidate for further pharmacological evaluations.

10.7 *Rauvolfia* Species

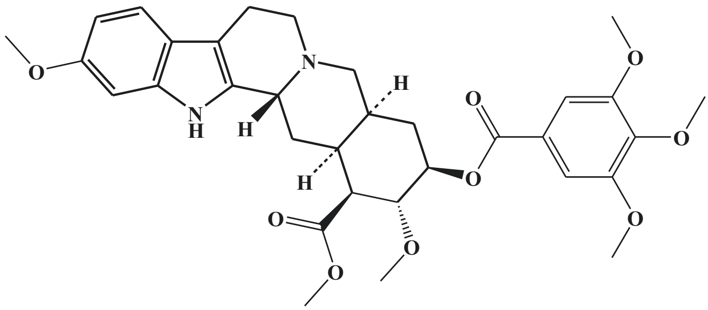
The genus *Rauvolfia* (Family: Apocynaceae), comprising about 110 species of shrubs and trees, is distributed in the tropical region. In the Western Ghats, the genus is represented by four species, viz, *R. hookeri* Srinivas *et* Chithra, *R. micrantha* Hook f., *R. serpentina* (L.) Benth. ex Kurz, and *R. verticillata* (Lour.) Baill. (Nayar et al. 2014).

The alkaloids and extracts of *Rauvolfia* species possess a wide spectrum of biological properties such as antipsychotic, antihypertensive, vasodilator and anticancer (Beljanski and Beljanski 1986). Various analytical techniques like nuclear magnetic resonance spectroscopy (NMR), high-performance thin layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GC/MS) and liquid chromatography mass spectrometry (LC/MS), direct analysis in real-time mass spectrometry (DART-MS) have been used for the phytochemical analysis of *Rauvolfia* species (Kumar et al. 2015). Reserpine, the major bioactive phytochemical isolated from the roots of *Rauvolfia* species, had been used for the control of high blood pressure, as a tranquilizer for the treatment of schizophrenia, eczema and angioplastic disorders due to peripheral vascular disorders (Rustom 1949; Sahu 1983; Barba et al. 1992; Harisaranraj et al. 2009) (Fig. 10.22).

Among the four *Rauvolfia* species in the Western Ghats, *R. hookeri*, *R. micrantha* and *R. serpentina* are coming under threatened category due to restricted distribution and destructive harvesting (Santhoshkumar and Mathew 2018).

10.7.1 Rauvolfia serpentina *(L.) Benth. ex Kurz*

Rauvolfia serpentina (L.) Benth. ex Kurz is a shrub, found in the hilly region of the Western Ghats up to an altitude of 1200 m (Alamgir and Ahamed 2005). Due to overexploitation, the plant has been listed under endangered category (Reddy and Reddy 2008; IUCN 2018) (Fig. 10.23).



Reserpine

Fig. 10.22 Reserpine, the major bioactive alkaloid isolated from *Rauvolfia* species

Fig. 10.23 *Rauvolfia serpentina*

The roots, occuring as rigid segments bearing twisted rootlets, are the drug part of the plant (Manisha et al. 2017). Traditionally, the roots of *R. serpentina* are known as 'Indian Snake root' or 'Sarpagandha' and had been used in the herbal medicinal systems in India for the treatment of hypertension and various central nervous disorders including anxiety, epilepsy, insomnia and also as an anthelmintic (Chopra et al. 1969; Madhusudanan et al. 2008). The roots and leaves of the species are being used for intestinal disorders especially dysentery, cholera and fever (Rajasree et al. 2013).

The German botanist Dr. Leonhard Rauwolf reported this plant as a potential source of therapeutic alkaloids. Indole alkaloids such as reserpine, yohimbine, ajmaline, deserpidine, rescinnamine, serpentinine and 3-oxo-rhazinilam were reported from the species (Gerasimenko et al. 2001; Itoh et al. 2005; Srivastava et al. 2006). HPLC methods were used for the separation and quantification of indole alkaloids in *Rauvolfia* species (Srivastava et al. 2006; Goel et al. 2009). Phytochemical analysis of *R. serpentina* also revealed the presence of phenols, flavonoids and tannins (Harisaranraj et al. 2009). DART-MS fingerprinting of *R. serpentina* showed the presence of monoterpenoid indole alkaloids such as demethoxy purpeline, tetraphyllicine, sarpagine, methyl sarpagine, norajmaline, ajmaline, acetyl nortetraphyllicine, sandwicolidine, methylajmaline, vomalidine, darcyriberine in roots and vellosiminol, demethoxy purpeline, tetraphyllicine, sarpagine, norajmaline and demethoxy reserpiline in leaves (Kumar et al. 2015). UHPLC-QTOF MS/MS analysis of different samples of *Rauvolfia* roots and dietary supplements containing *Rauvolfia* roots indicated that the commercial products are of variable quality with respect to the alkaloid contents (Sagi et al. 2016). Ultra high–performance liquid chromatography coupled with hybrid triple quadrupole linear ion trap mass spectrometry (UHPLC-QqQLIT-MS/MS) in multiple reaction monitoring (MRM) mode identified the content of ajmaline (52.27, 0.74 mg/g),

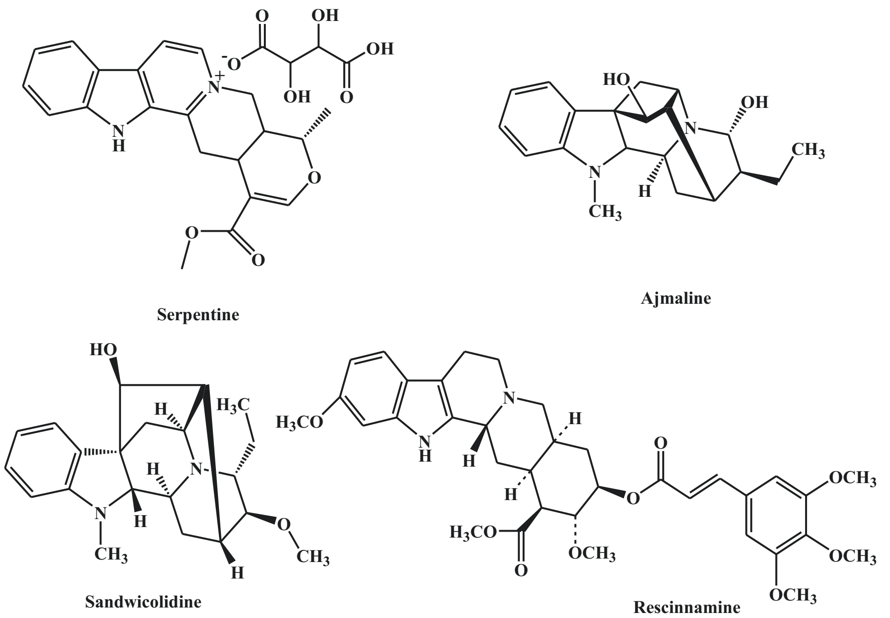


Fig. 10.24 Major alkaloids isolated from *Rauvolfia serpentina*

yohimbine (3.11, 5.86 mg/g), ajmalicine (3.14, 2.22 mg/g), serpentine (76.38, 0.52 mg/g) and reserpine (35.18, 2.40 mg/g) in the ethanolic extract of roots and leaves, respectively. Among the three threatened species, reserpine content was highest in *R. serpentina* root (Kumar et al. 2016) (Fig. 10.24).

10.7.2 Rauvolfia micrantha *Hook f.*

Rauvolfia micrantha Hook f., a woody shrub, is distributed in the evergreen forest of southern Western Ghats in Tamil Nadu and Kerala and is considered as a critically endangered species (Kulloli and Sreekala 2009; IUCN 2018). Morphologically, *R. micrantha* is allied to *R. serpentina* and *R. tetraphylla* and is used in traditional medicinal sector for nervous disorders such as insomnia and insanity (Kokate et al. 2008) (Fig. 10.25).

Root bark of *R. micrantha* contains aunamine and neosarpagine (Nair et al. 2012). Vomilenine was identified in high yield in the roots of *R. micrantha* (Kumar et al. 2015). Monoterpenoid indole alkaloids such as ajmaline, ajmalicine, reserpine, reserpiline, sarpagine and serpentine were identified from the hairy roots of *R. micrantha* (Sudha and Seeni 2006). DART-MS fingerprinting of the *R. micrantha* species showed the presence of monoterpenoid indole alkaloids such as vomalidine, ajmalicine, darcyriberine and reserpiline in roots and sarpagine, norajmaline, methyl

Fig. 10.25 *Rauvolfia micrantha*

sarpagine, vomalidine, ajmalicine, yohimbine, demethoxy reserpiline and reserpiline in leaves (Kumar et al. 2015). Ultra high–performance liquid chromatography coupled with hybrid triple quadrupole-linear ion trap mass spectrometry (UHPLC-QqQLIT-MS/MS) in multiple reaction monitoring (MRM) mode identified the content of ajmaline (1.72, 0.64 mg/g), yohimbine (2.73, 16.80 mg/g), ajmalicine (4.28, 6.27 mg/g), serpentine (6.01, 0.43 mg/g) and reserpine (32.38, 1.47 mg/g) in the ethanolic extract of root and leaves, respectively (Kumar et al. 2016). Methanolic extract of the species showed significant antioxidant property (Nair et al. 2012). The endemic species *R. micrantha* contains significant quantity of reserpine and can be considered as a source of reserpine. The species can replace *R. serpentina* and *R. tetraphylla,* which are endangered due to over exploitation, as a source of reserpine (Bindu et al. 2014) (Fig. 10.26).

10.7.3 Rauvolfia hookeri *Srinivas* et *Chithra*

Raulwolfia hookeri Srinivas *et* Chithra is a large shrub, endemic to the evergreen forests of southern Western Ghats and coming under threatened category (IUCN 2018). DART-MS fingerprinting of the *Rauvolfia hookeri* species showed the presence of monoterpenoid indole alkaloids such as sarpagine, norajmaline, methyl sarpagine, vomalidine, darcyriberine and reserpiline in roots and norajmaline, vomalidine, ajmalicine, yohimbine, darcyriberine and reserpiline in leaves (Kumar et al. 2015). Ultra high–performance liquid chromatography coupled with hybrid triple quadrupole-linear ion trap mass spectrometry (UHPLC-QqQLIT-MS/MS) in multiple reaction monitoring (MRM) mode identified the phytochemical constituents ajmaline (0.50, 0.85 mg/g), yohimbine (2.72, 11.59 mg/g), ajmalicine (0.30, 3.78 mg/g), serpentine (5.89, 0.31 mg/g) and reserpine (23.44, 4.86 mg/g) in the ethanolic extract of *R. hookeri* root and stem, respectively (Kumar et al. 2016) (Figs. 10.27 and 10.28).

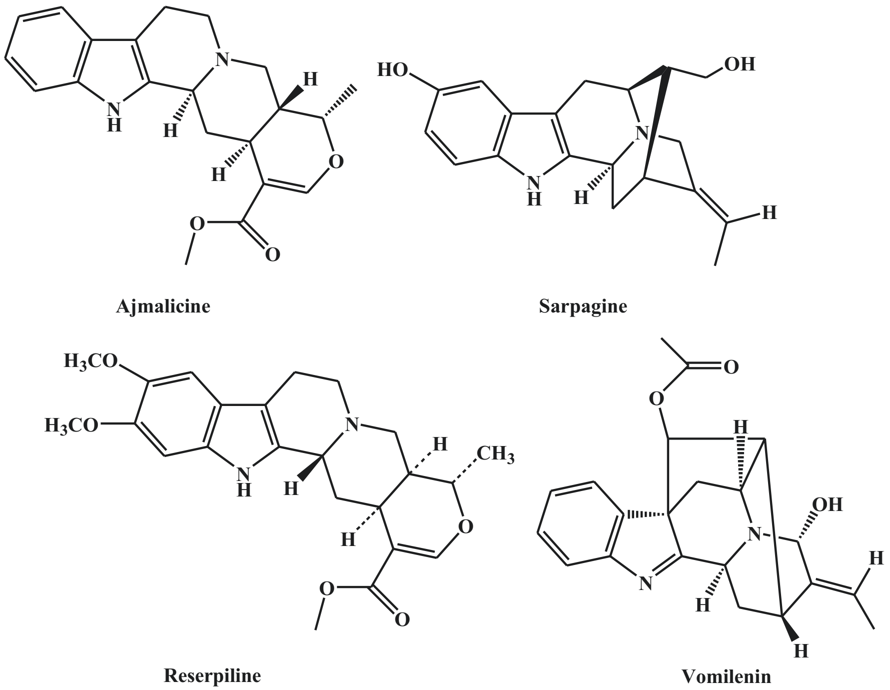


Fig. 10.26 Alkaloids isolated from *Rauvolfia micrantha*

Fig. 10.27 *Rauvolfia hookeri*

The *Rauvolfia* species are well known for bioactive constituents and potential activities and are widely used both in traditional herbal medicinal systems and in phytopharmaceutical sector, and the overexploitation has led to near extinction of the species in the wild. Phytochemical profiling of the rare species of *Rauvolfia* from the Western Ghats showed alternate sources for reserpine that may reduce the threat for already known source *R. serpentine.* Also the use of hyphenated analytical techniques for phytochemical prospecting of the threatened *Rauvolfia* species significantly help in dereplication.

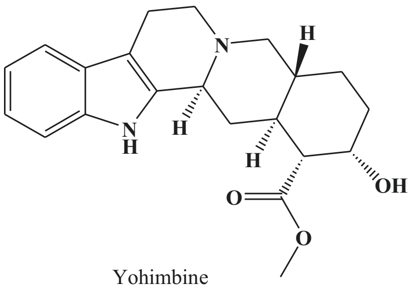


Fig. 10.28 Major alkaloid detected in *Rauvolfia hookeri*

10.8 Conclusion and Wayforward

The selected threatened medicinal plants of the Western Ghats are found to be rich sources of potential molecules that can be developed to further value-added products, and the data also highlights the importance of conservation of the threatened species. *Coscinium fenestratum,* a threatened medicinal plant extensively exploited for its root and bark from the wild, leading to near extinction, is a well-studied species; and several bioactive constituents belonging to isoquinoline alkaloids such as berberine have been reported from the species. Once the chemical compounds have been isolated and characterised through various chromatographic and spectroscopic techniques, advanced hyphenated analytical techniques such as LC-MS/MS and LC-MS/NMR can be used for further chemical profiling using minimal plant resources and the approach has been successfully applied for *C. fenestratum.* The genus *Garcinia* is an important group of plants with economical potential in medicinal, spice and food sectors. The threatened species were found to be a rich source of bioactive biflavonoids, benzophenones and xanthones, in addition to volatile aroma compounds. Compounds such as the biflavonoid fukugiside has been found as promising candidates for drug development, which may lead to further threat to the species, and it is required to evolve conservation strategies for such species and also to find alternate synthetic derivatives for the active compound. The genus *Myristica* is an economically important group of aromatic plants and a source of valued spices. The spices from the threatened species have been used as substitutes or adulterants for the original spices, and the chemical studies revealed similar profile with bioactive constituents for the threatened species as well. The extensive use may lead to extinction of the species, and urgent conservation strategies are needed for the species. Phytochemical profiling of the rare species of *Rauvolfia* showed alternate sources for reserpine that may reduce the threat for already known source *R. serpentina*.

The consumption of herbal dietary supplements and phytopharmaceuticals in the Western countries and the use of medicinal plants in traditional herbal medicines in the developing countries are expanding rapidly. However, the data related to quality control, safety and efficacy evaluation of traditional medicinal plants are far from the criteria needed to support its use in the international market. New comprehensive methodologies, utilising sophisticated and sensitive analytical techniques, that yield more reliable and accurate data using less sample within short time are needed to meet the stringent international standardisation of medicinal plants. Hitherto uninvestigated medicinal plants may provide potential lead compounds for drug discovery. However, in most of the developing countries in the tropical region where most of the floristic diversity harbours, the plant genetic resources are under threat, and it is high time to evolve strategies for systematic research on medicinal plants, especially on the threatened species, and also to evolve conservation strategies for such threatened species.

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Chapter 11
Genomics and Molecular Characterization of Threatened Medicinal Plants

M. R. Rohini

Abstract The demand for plant-based medicines is increasing day by day which has put tremendous pressure on the natural habitats, leading to dwindling of populations of many medicinally important species. Thus, the conservation of these species is of prime interest in the present scenario. The developments in genomics and molecular marker technology have played, and are playinge, a significant role in the management of threatened medicinal plants. Prior to formulating any conservation strategy, the important aspects to be evaluated include authentic taxonomic identification of the species, analyzing the amount and pattern of genetic variability present, and analyzing the population structure and phylogenetic relationships. Molecular marker technology has emerged as a rapid and efficient genomic tool to achieve these goals. The analysis of genetic diversity and population structure will enable to understand the evolution and adaptation of a species to the particular environment, and accordingly conservation measures can be implemented. Apart from this, markers have significantly contributed to assess the genetic stability of in vitro conserved endangered species which is essential to retain the original population as such even after adopting conservation strategies. Genomic tools like DNA barcoding and DNA fingerprinting have complemented for the authentic identification of medicinal plants in many herbal formulations to prevent adulteration. Thus, with the advancements in genomic technologies, the efforts to characterize and conserve the threatened medicinal plant species have become rapid and efficient.

Keywords Genomics · Molecular markers · Threatened medicinal plants · Molecular characterization

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11.1 Introduction

Plant-based medicines are gaining popularity around the world with people becoming more health conscious and have increased the medicinal plant trade exponentially in the recent years. The trade is mostly based on the material which is collected from the wild, imposing severe constraint on the availability of these natural resources. Another important issue of concern in international trade is the adulteration of important medicinal plants with useless weeds or non-medicinal species. Thus, proper cataloguing of the germplasm is very important together with formulating conservation strategies for restoring the threatened plants (Sarwat et al. 2011b). The extent of genetic diversity within a species is an important determinant of successful adaptation to adverse environmental conditions. The assessment of the extent of genetic diversity/variability is also important to monitor genetic erosion within a species. In threatened plant species, genetic diversity assessment helps in the selection of genetically diverse populations to enrich the genetically impoverished populations, thus minimizing the probability of genetic drift (Chrungoo et al. 2018). For any crop plant, characterization of the germplasm can identify novel genotypes and aid in future plant improvement programs (Sarwat et al. 2011b). But when coming to threatened medicinal plant species, apart from use in crop improvement programs, characterization and assessment of diversity is important for proper taxonomic identification and for devising long- and short-term conservation strategies. Confirming taxonomic identity of threatened species, particularly those belonging to species complexes with dispute identity, is another essential task in the conservation of threatened species which is best resolved through molecular approaches**.** Unequivocal identification is a critical step at the beginning of an extensive process of quality assurance and is of importance for the characterization of the genetic diversity, phylogeny and phylogeography as well as the protection of endangered species (Sucher and Carles 2008). Apart from this, characterization of germplasm will also help us regulate the use of germplasm as per provisions under Convention on Biological Diversity (CBD). Both morphological and molecular makers can be used for the characterization studies of plant genetic resources. The characterization aids in the identification of distinct populations or genotypes for conservation, optimum sites for germplasm collection, and gives an idea about the ongoing changes in the pattern of diversity over time (Franco et al. 2001). Morphological markers suffer from many limitations like they are dependent on environmental conditions, there are only a few marker characters available, they exhibit only dominance, less polymorphism, etc. On the other hand, molecular markers are not influenced by the external environmental factors, are numerous in number, exhibit high polymorphism percentage, are reproducible in labs, are less time-consuming, and are more reliable (Parida et al. 2017). Molecular markers not only provide a useful method for cultivar characterization but they also depict genetic relatedness, authentication of quality plant material, detection of adulteration, and protection of intellectual property right issues (Joshi et al. 1999). In this chapter, different molecular biology techniques used for management of threatened medicinal plants will be discussed.

11.2 Application of Genomic Tools in Endangered Medicinal Species

Recent advancements made in the field of genomics plays an important role in the management (characterization and conservation) of threatened medicinal plant species. The term "genome" refers to the total set of genes of an organism or refers to the complete genetic material of the organism. The term "genomics" refers to the scientific discipline dealing with the mapping, sequencing, and analysis of the genome (Xu 2012). Genomics is further divided into structural genomics and functional genomics. Structural genomics deals with the evolution, structure and organization of the genome while functional genomics deals with the expression and function of the genome. Using population genomic approaches, it is now easier to investigate the population structure and genetic variability in endangered species; at the same time genome sequences can provide hints regarding the future status for becoming endangered species. Specific biotechnological tools and techniques are used to assess the genome of threatened species to detect genetic variability and population structure. Currently, most commonly used genetic tools for the detection of genetic variations in threatened plant species include molecular markers, DNA and RNA sequence analysis, and DNA finger printing. These tools target different variables within the genome of target species, and selection of the specific tools and genome part to be analyzed is carried out based on the available information. Various genomic tools used for the detection of genetic variations in species include genome sequencing, mitochondrial SNPs, multivariate analysis, gene expression, metabolism, etc. Genomic techniques like genome-wide association studies (GWAS), development of DNA markers, marker-assisted breeding, and quantitative trait loci (QTL) analysis in endangered and threatened species can give us information about the role of natural selection at the genome level of an organism and also helps in the identification of loci that is associated with chance factors of extinction like disease susceptibility, inbreeding depression, and local adaptations (Khan et al. 2016). Genomic analysis helps in the identification of genomic regions contributing to the genetic variability of the species and also the genes coding important qualitative and quantitative traits (Goddard and Hayes 2009). Further, the use of population genetics and phylogenomics can help us in identifying conservation units for recovery, management, and protections (Steiner et al. 2013). With the progress in genomic sequencing of endangered taxa, the rescue of those taxa will become easier.

11.2.1 Molecular Markers

Molecular marker refers to a gene or a DNA sequence with a known location on a chromosome and associated with a particular character. It can be described as a variation, which may arise due to mutation or alteration of nucleotide in the genomic loci that can be observed (Srivatsava et al. 2009). A genetic marker has the potential to differentiate cells, individuals, or species based on the difference in their DNA sequence. As the DNA sequences are highly specific, they can be identified with the help of the known molecular markers which can find out a particular sequence of DNA from a group of unknown (Ganie et al. 2015). Various types of DNA-based molecular techniques are utilized to evaluate DNA polymorphism. These are hybridization-based methods, polymerase chain reaction (PCR)–based methods, and sequencing-based methods. Molecular markers provide a vast array of information related to the genetic diversity of the species, population structure, phylogenetic relationships between species, etc., which can be then utilized for devising a proper conservation strategy, management of genebank, and germplasm collections through (Sarwat et al. 2011a, b):

- Quantifying the extent of genetic diversity
- Prioritizing germplasm to be conserved
- Identifying the germplasm present in nature but missing in collections
- Identifying duplicates in collections
- Quality control tool for the authentication of germplasm
- Monitoring the genetic fidelity of in vitro propagated plants

DNA-based markers are endowed with the following properties:

- Free of environmental influence
- Independent of temporal and spatial regulation
- Heritable
- Abundant in the genome
- Polymorphic nature
- Detecting changes in both coding and non-coding regions of the genome
- Ease and efficiency of assay
- Reproducible

Molecular markers are of two types, namely, biochemical markers and nucleic acid (especially DNA-based) markers. Biochemical markers in medicinal plants include isozymes and the use of secondary metabolites. Most of the biochemical markers are expression based and are therefore dependent on the environment and are also influenced by epistatic and pleiotropic interactions, whereas DNA-based markers are free from these limitations. DNA-based markers thus have enormous advantage over biochemical markers for the cataloguing of germplasm and genetic diversity analysis.

Based on the development of these techniques in the last three decades, they are classified into three classes (Gupta et al. 2001):

(a) The first-generation molecular markers, including RFLPs, RAPDs, and their modifications
(b) The second-generation molecular markers, including SSRs, AFLPs, and their modified forms
(c) The third-generation molecular markers including ESTs and SNPs

11.2.1.1 Restriction Fragment Length Polymorphism (RFLP)

RFLPs are the first-generation molecular markers developed for genetic studies. This technique exploits variations in lengths of homologous DNA sequences which are digested by the same restriction enzyme. The digested fragments vary in size, have to be separated using Southern blot analysis and accordingly visualized by hybridization to specific probes which could be homologous or heterologous in nature. RFLPs arise in the genome due to several reasons such as point mutations, insertion, deletion, and inversion that lead to the creation, abolition, or rearrangement of restriction sites (Ganie et al. 2015). RFLP markers were used for the first time in the construction of genetic maps by Botstein et al. (1980). As they are co-dominant in nature, they are a popular tool for creating genetic maps. When the flanking regions of nucleotide sequence are known, the region meant for RFLPs could be amplified through polymerase chain reaction. In threatened medicinal plants, RFLPs were used for authentic identification against adulteration. For example, Aegle marmelos and *Oroxylum indicum* were identified through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and the regions amplified were internal transcribed spacer (ITS) with the aid of ITS1 (F) and ITS4 (R) primers (Biswas and Biswas 2013).

Recently the non-transcribed sequence (NTS) region of 5S rRNA has been employed for studying intraspecific discrimination in many medicinal and aromatic species (Mythili et al. 2012)**.** The specific function of 5S-rRNA as a component of the large ribosomal subunit in eukaryotes may be attributed due to its high level of conservation. This 5S-rRNA can be considered as a good model for organization and evolution (Cox et al. 1992). On the basis of these analysis and assumptions, variation in the NTS region has been used in a number of plant species for studying intraspecific variation, mapping 5S-rDNA arrays, genome evolution, and phylogenetic reconstruction (Negi et al. 2002; Trontin et al. 1999).

5S-rRNA-NTS gene RFLP analysis has given successful results from several endangered medicinal plants. The analysis of the 700 bp 5S-rRNA gene spacer region of three types of Acorus calamus revealed the variation in the presence of β-asarone and led to classification of two chemotypes of Acorus calamus. In Chemotype A, β-asarone is a major constituent and chemotype B is characterized mainly by sesquiterpenoids (Sugimoto et al. 1999). The limitation of RFLP in detecting diversity or for genetic mapping includes the requirement of large amounts of highly pure DNA, the use of radioactivity for detection, the need for highly skilled manpower, and the low rate of detecting polymorphism (Fig. 11.1).

11.2.1.2 Amplified Fragment Length Polymorphism (AFLP)

This technique is a combination of RFLP and PCR amplification where there is selective amplification of restriction fragments from a total digest of genomic DNA (Dorothea et al. 1996).

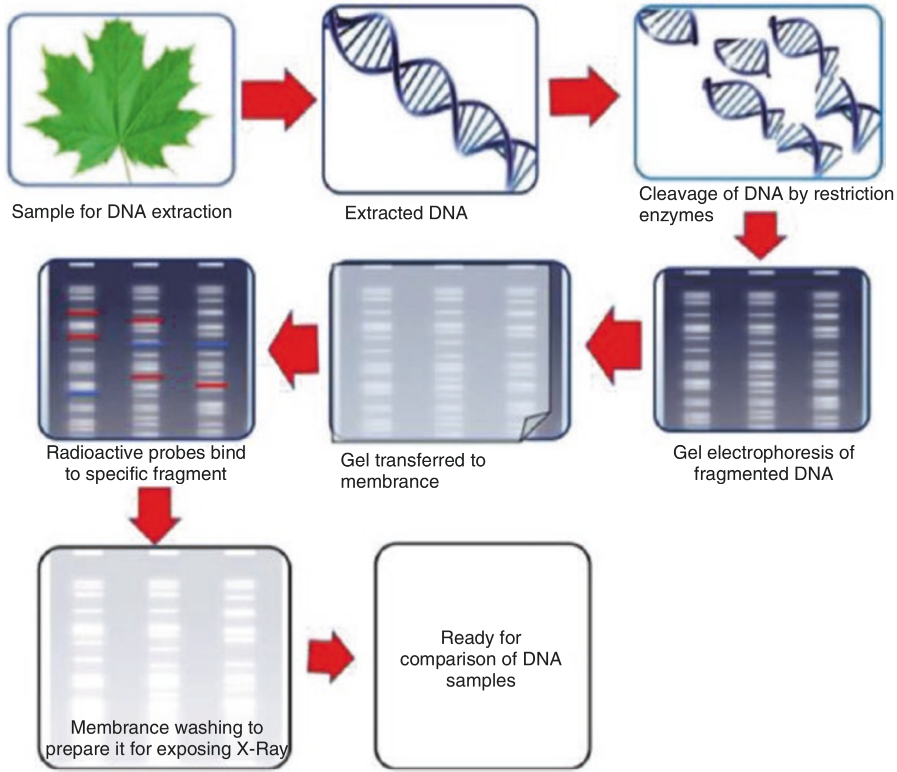

Fig. 11.1 Pictorial view methodology of RFLP. (From Ganie et al. 2015)

The technique involves three steps (Tharachand et al. 2012):

1. Digestion of total cellular DNA with one or more restriction enzymes and ligation of adaptors to all restriction fragments
2. Selective amplification of these fragments with two PCR primers that have corresponding adaptor and restriction site–specific sequences
3. Electrophoretic separation of amplicons on a gel matrix, followed by visualization of the banding pattern

One of the advantages of this technique is that prior knowledge of the sequence is not required for obtaining the fingerprint. Passinho-Soares et al. (2006) identified species-specific bands of different species of the genus *Plectranthus*. Using AFLP technique, Gowda et al. (2010) obtained species-specific banding pattern for *Embelia ribes* in the range 500–700 bp by using the P + GC/M + CTA combination. AFLPs are a dominant marker system providing multilocus and genome-wide marker profiles. These features make the AFLP technology more suitable for molecular characterization and DNA fingerprinting of any germplasm collection. AFLP not only has higher reproducibility, resolution, and sensitivity at the whole genome level compared to other techniques, but it also has the capability to amplify between 50 and 100 fragments at one time (Fig. 11.2).

11.2.1.3 Rapid Amplification of Polymorphic DNA (RAPD)

In RAPD, random oligonucleotide primers (10–12 base pairs) are used to amplify the genomic DNA through polymerase chain reaction under low annealing temperature. The amplified fragments are separated by agarose gel electrophoresis based on their size. Since the primers are short, usual annealing temperature range is 28–38 °C for RAPD primers. At this temperature range, primers anneal at various positions of the genome wherever they find complementary sequences. The amount of DNA required for RAPD markers is very less. RAPD does not require any specific knowledge of the DNA sequence of the target organism. RAPDs are the first-hand markers used for any genetic diversity study because of its easiness and efficiency. The main limitation of RAPD markers is that they are dominant; i.e., it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies) (Fig. 11.3).

11.2.1.4 Microsatellites, or Simple Sequence Repeats (SSRs)

Microsatellite or short tandem repeats or simple sequences repeats (SSR) are short stretches of 1–5 nucleotide units repeated in tandem and randomly spread in eukaryotic genomes. SSR markers are highly polymorphic due to the high mutation rate affecting the number of repeat units. SSRs arise mostly during replication due to replication slippage, and extra sets of repeated sequences are added. Polymorphism is detected based on the difference in length between the repeated sequences. Such

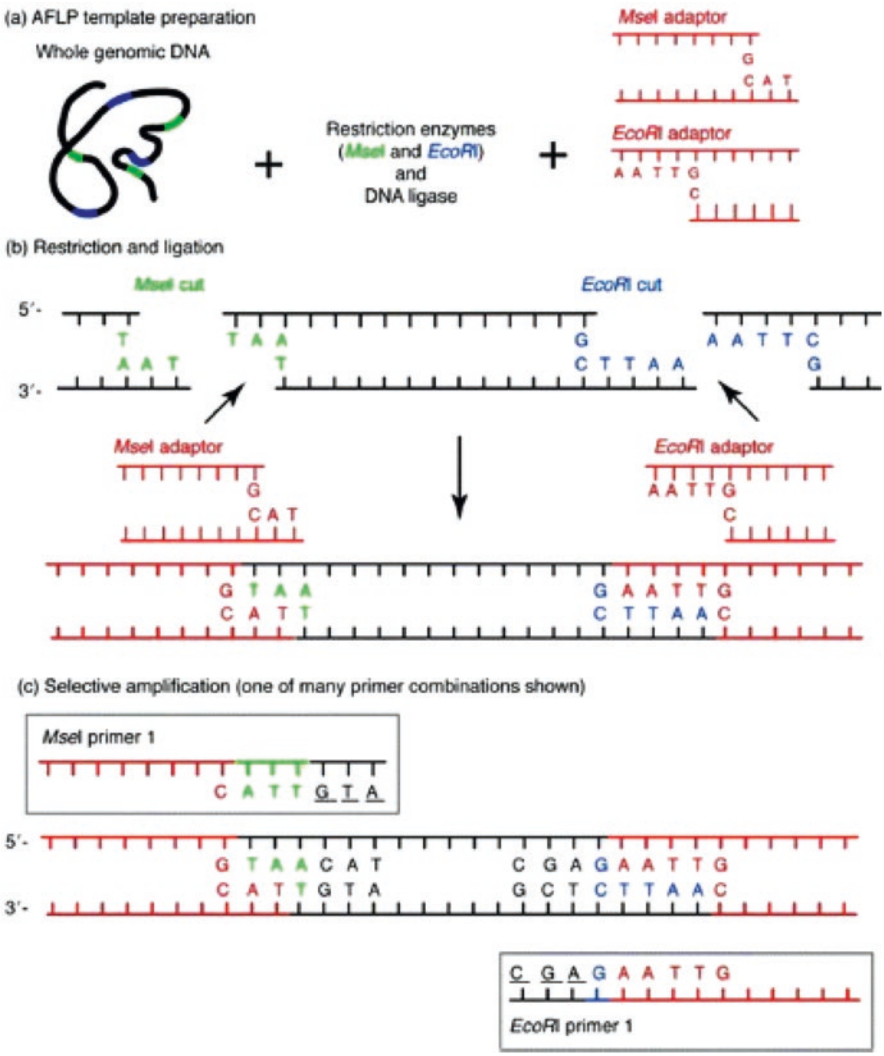

Fig. 11.2 Pictorial view methodology of AFLP. (From Ganie et al. 2015)

length polymorphisms can be easily detected on high-resolution gels (for example, sequencing gels), by running PCR amplified fragments obtained using a unique pair of primers flanking the repeat. SSR markers are widely used for fingerprinting, marker-assisted selection, kinship, breeding behavior such as selfing and outcrossing, for establishing population structure, etc.

SSRs have several advantages over other molecular markers: microsatellites allow the identification of many alleles at a single locus, they are evenly distributed all over the genome, they are co-dominant, little DNA is required, and the analysis can be semi-automated and performed without the need of radioactivity.

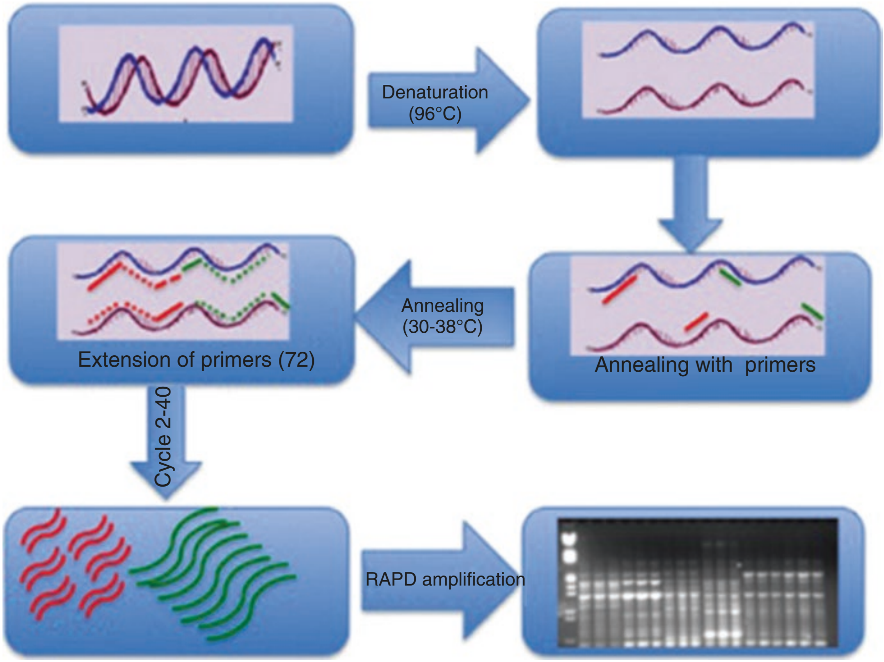

Fig. 11.3 Pictorial view methodology of RAPD. (From Ganie et al. 2015)

High level of polymorphism and their co-dominant nature have made SSRs ideal markers for studying genetic diversity in plants due to their co-dominance and ability to detect high polymorphism (Plaschke et al. 1995). Hon et al. (2003) reported the efficiency of SSR markers in genetic authentication of two *Panax* species. Katoch et al. 2013 used simple sequence repeats (SSR) and cytochrome P-450 markers to estimate genetic diversity in 25 accessions of Picrorhiza kurrooa collected from ten different eco-geographical locations. Results showed that there was a clear consistency between SSR and cytochrome P-450 trees in terms of positioning of most *Picrorhiza* accessions. Similarly, many reports are there in which SSR markers have been successfully used for genetic diversity analysis, analysis of population structure, and gene flow within populations in endangered species.

11.2.1.5 Inter-simple Sequence Repeat (ISSR)

ISSR is one of the PCR-based dominant marker system. As the name suggests, ISSR is involved in amplification of DNA segment present between two SSRs. ISSR is a general term for a genome region between microsatellite loci. The complementary sequences to two neighboring microsatellites are used as PCR primers. The variable region between them gets amplified. Microsatellites used as primers for ISSRs are di-penta nucleotide. ISSR has a few advantages because ISSR primers anneal directly to simple sequence repeat, and thus, unlike SSR markers, no prior knowledge of target sequences is required for ISSR. ISSR markers, which have longer primers, allow more stringent annealing temperatures and reveal more polymorphic fragments, can be highly variable within a species and have the advantage over RAPD markers. In addition, the cost of the analyses is relatively lower than that of some other markers such as RFLP (restricted fragment length polymorphism) and AFLP (amplified fragment length polymorphism). Therefore, ISSR has been widely used for population genetic studies of various plant species, including many medicinal plants. ISSR markers are the most used markers for genetic diversity analysis and characterization of endangered medicinal plants. ISSR markers were used for the characterization of genetic structure in Podophyllum hexandrum (Alam et al. 2008) and *Coleus forskholii*, for establishing genetic fidelity in Ceropegia spiralis, etc. Wang (2011) used ISSRs to authenticate different Rheum species with different ISSR primers. A successful attempt by Tamhankar et al. (2009) was also made for the authentication of samples of Chirayat complex (*Swertia angustifolia*, *S. chirayita*, S. cordata, *S. densifolia*, S. lurida, *S. ciliate,* S. paniculata, S. alata, S. bimaculata, Andrographis paniculata, *Enicostemma axillare, Exacum tetragonum)* (Fig. 11.4).

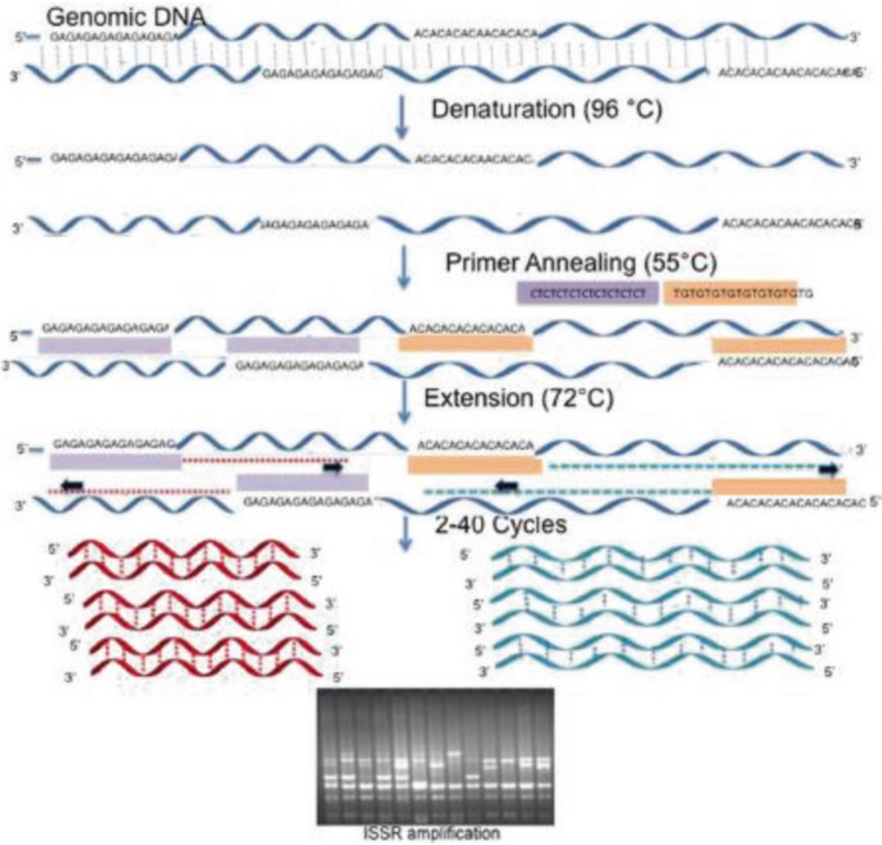

Fig. 11.4 Pictorial view methodology of ISSR. (From Ganie et al. 2015)

11.2.1.6 Selectively Amplified Microsatellite Polymorphic Loci (SAMPL)

This is a microsatellite-based modification of AFLP developed by Morgante and Vogel (1994). It involves steps similar to AFLP until pre-amplification. However, the selective amplification is slightly different as it utilizes a microsatellite-based primer in combination with an AFLP primer. The choice of microsatellite primers influences the number of amplification product that is generated. The applicability of SAMPL markers for genetic diversity assessment has been demonstrated in *Terminalia arjuna* (Sarwat et al. 2011a, b).

11.2.1.7 Single-Nucleotide Polymorphisms or SNPs

SNPs refer to variations in single nucleotide (A, T, G, and C) in the genomic sequence of individuals of a population. SNPs are the most abundant molecular markers distributed throughout the genome. SNPs are the most ideal markers for population genetic studies and candidate gene mapping studies because of their high density and mutational probability (Alves et al. 2008). SNPs can occur in both the coding and non-coding regions of the genome. SNPs which occur within a coding region will not change the protein due to degeneracy of the genetic code, but if it occurs in the non-coding region, then it may affect gene splicing and transcription factor binding or may code for a non-coding RNA (Rafalski 2002). With the advancement in genome sequencing technology, genetic variations can now be detected at the sequence level. SNP genotyping assays are based on one or two of the following molecular mechanisms: allele specific hybridization, primer extension, oligonucleotide ligation, and persistent cleavage (Sobrino et al. 2005). High-throughput genotyping methods counting DNA chips, allele-specific PCR, and primer extension approaches make SNPs especially attractive as genetic markers. In many medicinal plant species, SNP-based multiplex PCR has been used for species identification by making use of highly variable intergenic spacer and intron regions from nuclear and cytoplasmic DNA (Lee et al. 2012). SNP is able to determine genetic diversity in plants, particularly in species with limited genetic diversity (Arif et al. 2010).

11.2.1.8 Sequence Characterization of Amplified Regions (SCAR)

SCAR marker as the name suggests refers to the sequence-based mono-locus and co-dominant marker system. SCARs are the most important marker used for the authentication of medicinal plants. In SCAR, forward and reverse primers are designed from the particular region of a cloned AFLP, RAPD, and ISSR DNA fragment linked to a trait of interest (Ganie et al. 2015). The primers for SCAR marker either are located within or may be flanking the unique AFLP, RAPD, or ISSR amplified segment. SCAR markers helps in the identification of related individuals by producing single, distinct, and bright band in the desired sample (Kiran et al.

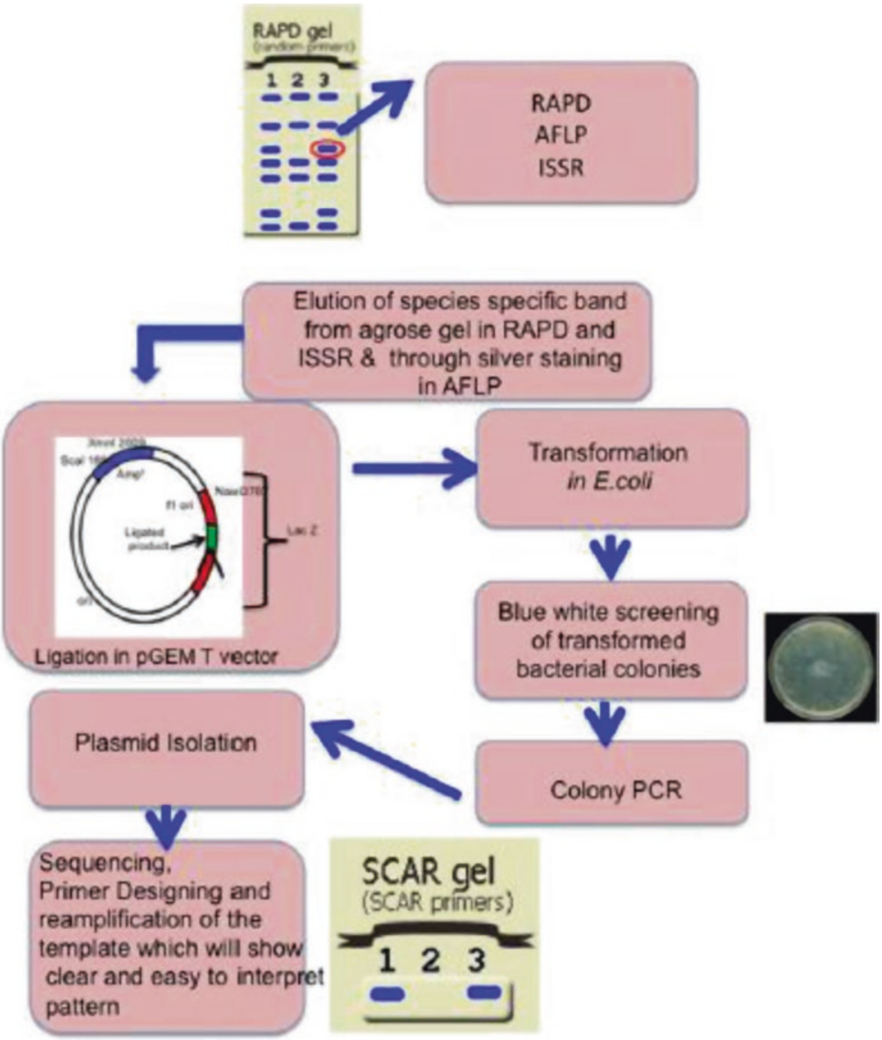

Fig. 11.5 Pictorial view methodology of SCAR. (From Ganie et al. 2015)

2010). These markers are fast, reliable, co-dominant and are highly reproducible. Seethapathy et al. (2014) authenticated Ativisha (*Aconitum heterophyllum*) and Musta (Cyperus rotundus) using nrDNA ITS sequence–based SCAR markers. When market samples of the herbal formulation were tested using SCAR system, it was found that SCAR primers could identify only tissue sample containing Musta in complex mixtures of DNA extracted from commercial herbal drugs, and Ativisha, i.e., *Aconitum heterophyllum* was not identified. This confirmed that *Aconitum heterophyllum* is not used to prepare herbal drugs despite its being labeled as one of the ingredients in formulations (Fig. 11.5).

11.2.1.9 Expressed Sequence Tags

Expressed sequence tags (ESTs) are fragments of mRNA sequences derived through single sequencing reactions performed on randomly selected clones from cDNA libraries (Parkinson and Blaxter 2009). ESTs may be used to identify gene transcripts and are instrumental in gene discovery and in gene-sequence determination. They are also useful in defining an expressed gene and also specifying the profusion of transcripts. Large-scale EST databases offer a multitude of information concerning the complexities of gene expression patterns, the functions of transcripts, and the development of SNPs (Yang et al. 2004). In plant, large-scale EST databases have been recognized, and an array of ESTs procured from different tissues, developmental stages, and stress-treated cDNA libraries have been compared with model plants and crops. In most of the medicinal plants, nevertheless, the complete genome and draft sequences are yet to be established. Consequently, EST assay represents the most rational system for the study of the genome of the plants with medicinal importance; hence, several attempts have been made in this aspect by a number of research groups. In Panax ginseng, Kim et al. (2006) found that 2896 cDNA clones represent 1576 unique sequences, consisting of 1167 singletons and 409 contig sequences. The ESTs referenced in their report were the first transcriptomes in a leaf from a half-shade ginseng plant. The majority of the identified transcripts were found to be genes related with energy, metabolism, subcellular localization, protein synthesis, and transport.

11.2.2 DNA Barcoding, Microarrays, and New Generation Sequencing

These recent versions of genomic tools have emerged as potential instruments for genetic diversity analysis and for formulating conservation strategies for threatened species. These molecular markers utilize short regions in the genome to characterize the organism to a particular species. This has the potential not only to classify the known and yet unknown species but also has a promising future to link the medicinally important plants according to their properties (Sarwat and Yamdagni 2016).

11.2.2.1 DNA Barcoding

DNA barcoding is a genomic tool that uses a part of the genome for species identification (http://www.barcoding.si.edu). The DNA segment used will be either nuclear or chloroplast or mitochondrial DNA, mostly 400–800 bp long. Authentic identification of the species is very important for formulating conservation strategies. DNA barcoding technique is highly useful in taxonomic, ecological, and evolutionary studies. This technique has immensely contributed to the authentication of many herbal drug formulations. The most important characteristic feature of a DNA barcode is its universality, specificity on variation, and easiness on employment. The genomic region used as a barcode should be suitable for a wide range of taxa, should have high variation between species but should be conserved within the species, so that the intra-specific variation will be insignificant (Kress et al. 2005, Pennisi 2007, CBOL 2009, Viayan and Tsou 2010). For plants, the universally accepted genes for DNA barcoding are of plastid origin. Several DNA barcode researches have proposed different loci and their combinations for suitable barcoding of plants. For instance, the nuclear internal transcribed spacer (ITS) region and the plastid intergenic spacer trnH-psbA region have been proposed for flowering plants (Kress et al. 2005), whereas the ITS region was suggested for land plants in general by Chase et al. (2005). The plastid rbcL gene (Ribulose-1,5 – bisphosphate carboxylase/oxygenase large subunit gene) is certainly the most sequenced locus among land plants, and therefore rbcL (Newmaster et al. 2006) and chloroplast trnL (Taberlet et al. 2007) intron are some other suggested loci for barcoding. Successful characterization of individuals to a particular species done using Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) with individual barcodes was obtained with matK (99%), followed by trnH-psbA (95%) and then rbcL (75%). The use of three-locus DNA barcode resulted in >98% correct identifications of 296 species of woody trees, shrubs, and palms (Kress et al. 2009). Recently, the two-locus combination of rbcL+matK has been recommended as the core barcode for land plants (CBOL Plant Working Group 2009) (Fig. 11.6).

El-Atroush et al. (2015) tested two endangered medicinal plants (*Cleome droserifolia* and *Iphiona scabra*) collected from Abou Galoom protectrate, South Sinai, Egypt using two DNA barcoding regions (ITS and rbcL). *Cleome droserifolia* has a long history of medicinal use especially in Sinai for the treatment of diabetes in individuals since it has a hypoglycemic effect. *C. droserifolia* extract also has antioxidant activities that protect the tissues from destructive damage of lipid peroxidation. *Iphiona scabra* is used in traditional medicine as an antispasmodic drug (Font-Quer 1990). *Its* extract has anticoagulant, anti-platelet aggregation and anti-inflammatory effects. The two selected loci for barcoding were easy to amplify and showed significant inter-specific genetic variability, making them potential DNA barcodes. Results showed that ITS region is more efficient in identification process for the two plants than rbcL. ITS region enabled the identification of plants at the species level while rbcL enabled the identification at the generic level (Fig. 11.7).

11.2.2.2 Microarrays

Microarrays are a powerful tool not only for whole genome transcript profiling but have also been suitably modified for genotyping and polymorphism detection (Sarwat et al. 2011a, b).

DNA microarray is an arrayed series of thousands of microscopic spots of DNA oligonucleotides, called features, each containing picomoles (10–12 mol) of a specific DNAsequence, known as probes (or reporters). It can be used to measure changes in expression levels, detection of SNPs, to genotype or resequence mutant

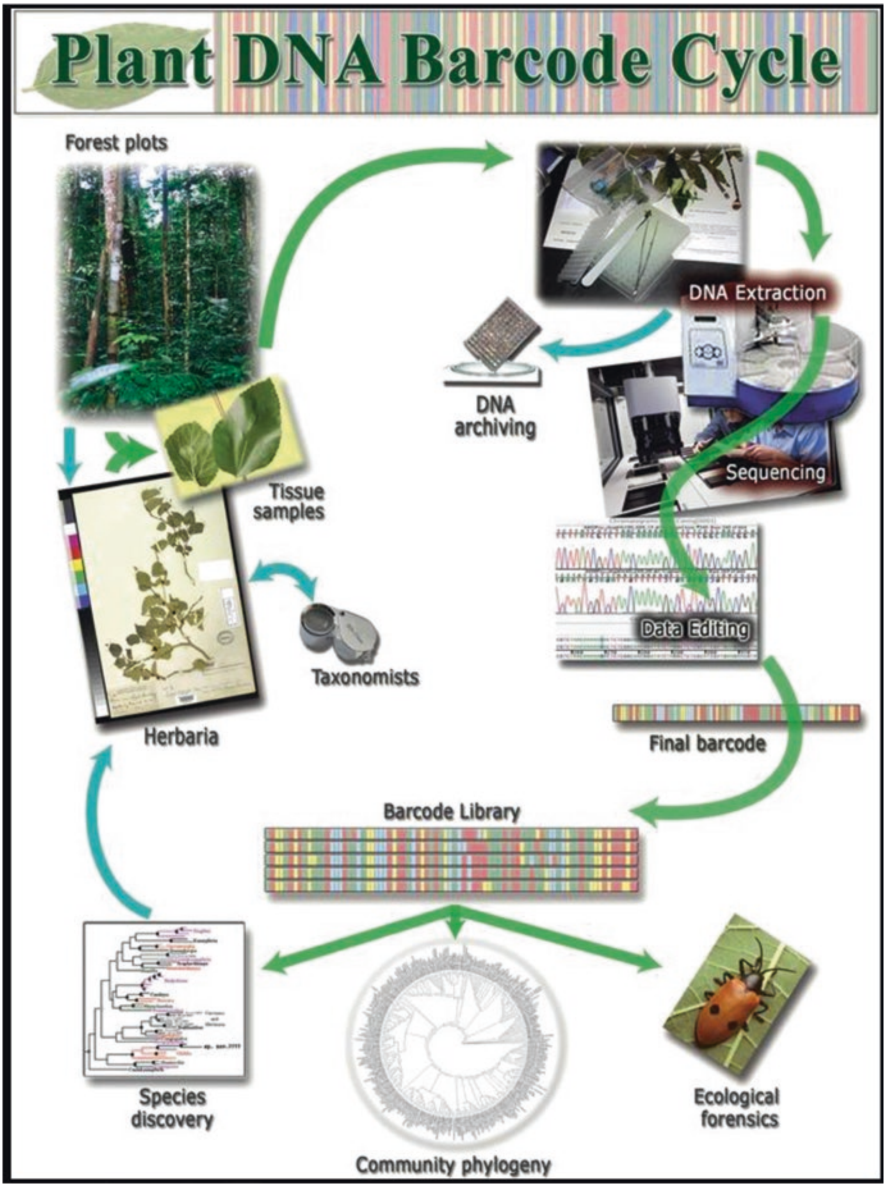

Fig. 11.6 Work flow indicating steps involved in plant DNA barcoding. (From Kress and Erickson 2012)

genomes (Hao et al. 2010). They have been widely utilized for the authentication of medicinal plants such as *Aconitum* spp., *Corton tiglium, Datura* spp. (Carles et al. 2005), molecular identification of *Dendrobium* spp. (Li et al. 2005), *Ephedra* spp. (Techen et al. 2006).

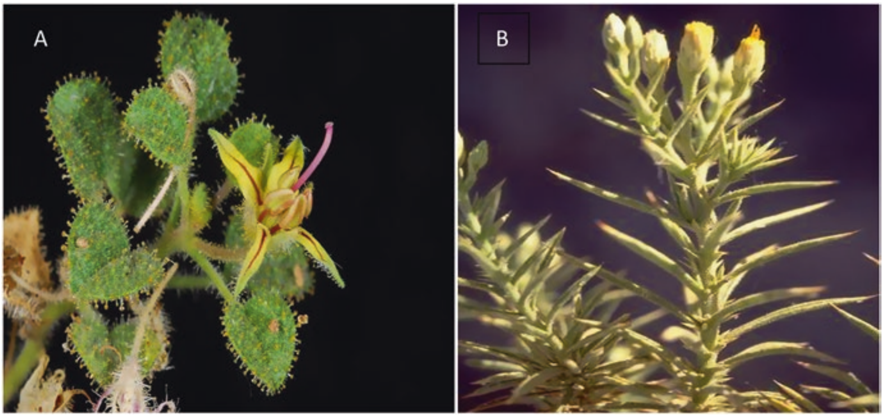

Fig. 11.7 (**a**) Plant of *Cleome droserifolia* (**b**) *Iphiona scabra*

11.2.2.3 Next Generation Sequencing

Rapid progress in genome sequences of various plant species through next-generation sequencing will further extend our understanding how genotypic variation translates into phenotypic characteristics. A comparative genomic approach is extraordinarily useful for identifying functional loci related to morphological, geographical, and physiological variation, and thus next-generation sequencing technology will enable us to better understand the process of plant evolution. Since the advent of next-generation sequencing, these techniques have been helping to uncover secondary metabolic pathways, to analyze cDNA-array-based gene expression, for genetic manipulation to improve yield of desirable secondary products and molecular marker identification. Hao et al. (2008) showed the evolutionary patterns of gene sequence divergence from the medicinal genus *Taxus* L., encoding paclitaxel biosynthetic enzymes taxadiene synthase (TS) and 10-deacetyl-baccatin III-10 beta- O-acetyl transferase (DBAT).

11.2.3 Molecular Markers for Assessing Genetic Diversity and Population Structure in Endangered Medicinal Plants

The study of genetic variability of threatened species is of prime interest to evolutionary biologists and conservation managers (Hedrick 1999). The analysis of the amount of genetic variation and genetic structure of threatened population is important in order to evaluate its impact on the amount of genetic diversity of the species. The knowledge on the genetic structure also infers about the evolutionary potential of the species, which is one of the conservation goals (Godt and Hamrick 1998).

Understanding the genetic variability and population structure of rare and isolated plant species is also important for assessing extinction risk and thereby setting up conservation plans. Information on the genetic diversity pattern of economically important, rare, and threatened plant species is a prime concern to develop strategies for sustainable harvesting of secondary metabolites and, where appropriate, reintroduction to its natural habitat (Neel and Cummings 2003). In this context, molecular markers play an important role in studying the extent and pattern of genetic diversity in threatened species and also clarifying demographic and ecological issues early in species management in order to plan long-term conservation or restoration projects (Kim et al. 2005). Due to anthropogenic activities like habitat destruction or unscrupulous harvesting from the wild, many important medicinal plants have dwindled to a smaller area with very few individuals. As per the population genetics theory, loss of genetic variation occurs due to genetic drift in species with small populations or located in narrow geographic areas. Low level of genetic variability in turn results in low fitness of the individuals (Oostermeijer et al. 1994; Fischer and Matthies 1998; Luijten et al. 2000; Hansson and Westerberg 2002), further reducing the viability or adaptability of populations in changing environments (Young et al. 1996), and in extreme cases causes the extinction of species. In threatened plant species, genetic diversity assessment helps in the selection of genetically diverse populations to enrich the genetically impoverished populations, thus minimizing the probability of genetic drift (Chrungoo et al. 2018). Genetic drift leading to reduction of variability is a more common phenomenon in self-pollinated species of plants or plants showing inbreeding. But in many clonally propagated species, a high amount of genetic diversity is found, so the amount of diversity present cannot be generalized based on the reproductive behavior of the species, and thus the use of modern molecular techniques is highly necessary to assess the extent of genetic diversity in each species and also to delineate relationships among individuals, populations, and species. Thus, before planning any conservation strategy, genetically diverse populations of the threatened species need to be identified, multiplied, and introduced in nature for avoiding possible genetic drift. Studying the patterns of genetic diversity within and among the populations of a species gives an insight into the evolutionary history and helps predict the future risk of genetic erosion (Neel and Ellstrand 2003). The amount of genetic diversity within a species is dynamic and will change with time, space, breeding behavior, ecological, and geographical factors. The assessment of such variation is particularly important in threatened species as the genetically diverse population can be used for expanding the extant population of a species, thus minimizing the probability of genetic drift thereby ensuring its conservation (Chrungoo et al. 2018).

Examples

(a) Lee et al. (2018) investigated genetic diversity and population structure of an endangered and endemic medicinal plant of Korea, *Aconitum austrokoreense*. Five nuclear microsatellites and one chloroplast marker were used in 479 individuals from seven populations throughout South Korea. Results revealed broad-scale spatial patterns of *A. austrokoreense* populations across three major mountains that were composed of seven genetically distinct subgroups. High pairwise *F*st values indicated significant differentiation between populations, but within the population, genetic variation was low. A significant correlation between geographical and genetic distance was obtained based on Mantel test indicating the pattern of isolation by distance. Study suggested that *A. austro-koreense* populations may have undergone recent population bottlenecks. Due to limited dispersal ability of the species and ongoing habitat fragmentation, population isolation may further be increased, leading to increased extinction risk.

(b) Rahimmalek et al. (2009) used ISSR markers to detect genetic polymorphism in *Thymus daenensis*, an endangered aromatic medicinal plant endemic to Iran. 15 ISSR primers in 17 *T. daenensis* accessions collected from different geographic regions in Iran showed 88.9% polymorphism. Dendrogram generated showed clear distinction between accessions collected from different regions. Principal coordinate analysis confirmed the results of clustering. The results showed that the divergence of accessions based on the Zagros Mountains is more logical in comparison with classification on the basis of provincial borders. Gene diversity and expected heterozygosity revealed that the germplasm collected from the center of the Zagros Mountains is more variable.

(c) Shukla and Sharma (2017) assessed the genetic diversity in *Shorea tumbuggaia* Roxb. population (Dipterocarpaceae family) from Tirumala Hills (Tirupati) of Andhra Pradesh using RAPD primers. As per IUCN classification, *S. tumbuggaia* is a globally endangered tree species (Ashton 1998; Reddy et al. 2003). This species has become critically endangered due to overexploitation (Savithramma and Sudarsanamma 2006), habitat degradation, and other biotic interferences. Eighteen (18) RAPD primers generated a total of 137 polymorphic bands showing 19.86% polymorphism. Genetic similarity coefficients calculated from RAPD data ranged from 0.82 to 0.95. Based on the results of this study, workers proposed two alternative conservation strategies for this species. Either the population of this species can be increased through tissue culture or vegetative propagation or proper crossing methodology may be devised after studying the floral biology in a detailed manner.

(d) Nag et al. (2015) studied genetic diversity and population structure in 24 populations of Podophyllum hexandrum, an endangered high-elevation medicinal plant species using AFLP primers. STRUCTURE analysis clustered the 24 populations of Indian Himalayan mountains into two major groups irrespective of their geographical location. This suggested that all the populations from Indian Himalayas are intermixed and are composed of two types of genetic populations. The study suggested two possibilities regarding the ancient population structure of P. hexandrum: either all the populations of Indian Himalayas emerged from once-widespread ancient population or they originated from two different types of genetic populations, which coexisted in the past but subsequently got separated. Results further showed that maximum diversity was restricted within the population level and low heterozygosity showed the existence of population bottleneck necessitating the implementation of conservation strategies.

(e) *Coleus forskohlii* has been considered as an important medicinal plant. Because of the continuous collection of roots from the wild sources, this plant has been included in the list of endangered species. This has necessitated the use of biotechnology in conservation and sustainable management of this endangered plant species. Molecular characterization will enhance our understanding in improving the optimal yields of Forskolin through breeding. Tripathi et al. (2013) used RAPD, ISSR, and AFLP marker system for molecular characterization of C. forskholii genotypes. Eleven RAPD, ten ISSRs, and eight AFLP primers produced 101, 80, and 483 fragments, respectively. Among the three-marker system used in this study, RAPD and ISSR showed 61.39 and 68.75% polymorphism, respectively, while eight AFLP primer combinations produced 70.81% polymorphism.

(f) Wang (2011) used ISSR markers to study the genetic diversity and population differentiation in 12 populations of Rheum officinale Baill., an endangered medicinal herb endemic to China. Thirteen ISSR primers were selected based on polymorphism which showed that the genetic diversity was low at the population level but high at the species level by the POPGENE analysis. The analysis of molecular variance (AMOVA) showed that the genetic variation was found mainly among populations (74.38%) and limited gene flow (Nm = 0.2766) among populations. Significant correlation was obtained between genotype and geographic region indicating the role of geographic isolation in shaping the present population genetic structure. The study implied that the conservation efforts should aim to preserve all the extant populations of this endangered species, and cultivation is proposed in this study.

(g) Thriveni et al. (2014) used ISSR markers to investigate the genetic diversity and population structure of *Coscinium fenestratum*, a critically endangered medicinal plant of Western Ghats of India. Owing to its huge requirement in drug market and industry, the species has been over-harvested, leading to rapid decline in the size of its population. Eight primer combinations produced 47.1% polymorphism. The species exhibited a moderate to low level of diversity within the population. There was only low to moderate genetic differentiation between populations and geographical distance was not significantly correlated with genetic distance, suggesting that geographically distant populations were once connected through gene flow. The results revealed that gene flow and inbreeding are likely to be the major driving force in shaping current population genetic structure of *C. fenestratum*. Thus, an understanding of the genetic diversity and population structure of *C. fenestratum* can provide insight into the conservation and management of this species.

(h) Yang et al. (2016) used eight nuclear SSR primer pairs to assess the genetic diversity and structure of 22 natural populations of Phellodendron amurense, an endangered tree with important medicinal and economic value in China. The analysis of molecular variance (AMOVA) revealed that the main variation component existed within populations (95.11%) rather than among populations (4.89%). The present genetic structure of P. amurense may be explained by geographical isolation. The decrease in genetic diversity with increasing latitude within the Northeast China group may be due to postglacial northward expansion from a single refugium. Proper conservation measures are proposed for this species based on the above results.

11.2.4 Molecular Markers to Appraise the Genetic Fidelity of In Vitro Conserved Species

Plant tissue culture is largely being used for the short- to medium-term conservation of threatened medicinal plants in the form of slow growing cultures. This method of conservation or propagation would be rewarding only if the genetic fidelity of the micropropagules are maintained over a period of time. Genetic fidelity is the maintenance of genetic constitution of a particular genotype throughout its growth span (Chatterjee and Prakash 1996). Regular monitoring of the genetic stability of in vitro conserved plants is of utmost importance for maintaining the true to type plants (Mohanty et al. 2011a). The assessment of the genetic integrity of in vitro grown regenerants at regular intervals can significantly reduce or eliminate the chance of the occurrence of somaclonal variation (Larkin and Scowcroft 1981) at early or late phase of culture. Somaclonal variation arises in the cultured plants during dedifferentiation and is uncontrollable and unpredictable. Somaclonal variations occurs due to in vitro stresses and are manifested in the form of DNA methylation, chromosome rearrangements, and point mutations (Philips et al. 1994). There are several strategies to ascertain the genetic variability or stability, each of them having merits and limitations (Alizadeh et al. 2015). Techniques based on morphophysiological, biochemical, and cytological approaches are mainly based on characters which can be affected by the in vitro manipulation, environment, and types of plant tissue; thus, the differentiation of somaclonal variation is difficult to achieve. Molecular markers play an important role in determining the uniformity and genetic stability of tissue cultured plants. Among the various DNA markers, RFLP and AFLP are the most reliable for checking the genetic stability of plants, but since they require radioactive probes, expensive enzymes and extra care are unsuitable under certain conditions. RAPD and ISSR, on the other hand, require only a small amount of DNA, do not need any radioactive labels, and are fast and cost effective. RAPDs can detect genetic stability even in closely related species. Thus, RAPD and ISSR markers are the most used ones for detecting genetic fidelity of tissue cultured plants. Highly polymorphic SSR markers are used relatively frequently. Genetic fidelity is also tested for cryopreserved germplasm after definite intervals of time to ensure its genetic stability.

Examples

(a) Lattoo et al. (2006) established an efficient micropropagation method via multiple shoot bud induction and regeneration in *Chlorophytum arundinaceum* using shoot crown explants. Genetic fidelity was tested using random amplified polymorphic DNA (RAPD), karyotype analysis, and meiotic behavior of in vitro and in vivo plants. Five primers showed same banding profile within all the in vitro plants and in vivo explant donor. The cytological and karyotype analysis also showed no genomic alterations in the regenerant plants. The results ensured the efficacy of the protocol developed for the production and conservation of this important endangered medicinal herb. One more study conducted by Samantaray and Maiti 2010 established rapid micropropagation in *Chlorophytum arundinaceum* using shoot base as explants. Here, 31 RAPD primers were used to assess the genetic stability in the micropropagated plants, and results showed that RAPD profile from micropropagated plants was genetically similar to mother plants. Thus, it can be inferred that both shoot crown and shoot base can serve as standard explants for the micropropagation of *Chlorophytum arundinaceum.*

(b) Micropropagation is reported in Ceropegia spiralis Wight (family Apocynaceae), an endangered medicinal plant of the Western Ghats of India through axillary buds, thin cell layers, and somatic embryogenesis. In this study, Chavan et al. (2013) assessed the genetic stability of micropropagated plants of C. spiralis using RAPD and ISSR markers. Study showed the same banding pattern for the in vivo plant as well as the regenerants. Thus, RAPD and ISSR markers proved to be effective tools for assessing the genetic stability in C. spiralis. These results suggested that the axillary shoot bud proliferation can be used as an efficient micropropagation tool for mass propagation of C. spiralis.

(c) Thakur et al. (2016) studied genetic stability of micropropagated plants of *Pittosporum eriocarpum* Royle, an endangered medicinal plant endemic to Uttarakhand region of Himalaya. It has become endangered due to over-collection and the loss of habitats. As seed propagation is difficult in this species, reliable protocol for micropropagation using nodal explants has been developed. For testing genetic homogeneity of the regenerants, start codon targeted (SCoT), inter-simple sequence repeats (ISSR), and random amplified polymorphic DNA (RAPD) markers were used. DNA fingerprints of in vitro regenerated plantlets displayed monomorphic bands similar to mother plant, indicating homogeneity among the micropropagated plants with donor mother plant. The dendrograms generated through UPGMA analysis revealed 97% similarity among micropropagated plants with donor mother plant, thus confirming genetic homogeneity of micropropagated clones. The protocol would be useful for the conservation and large-scale production of *P. eriocarpum* to meet the demand for medicinal formulations and also for the reintroduction of in vitro grown plants in the suitable natural habitats to restore the populations.

(d) Al-Qurainy et al. (2018) developed in vitro micropropagation protocol for *Maerua oblongifolia* (Forssk.), an important and rare medicinal plant from Saudi Arabia. Since natural regeneration of plant is very poor, in vitro micropropagation protocol is essential for its multiplication and conservation. Nodal segment explants, when cultured on MS medium supplemented with benzyl adenine (BA) and Kinetin (Kn), produced buds, eventually forming optimum multiple shoots on MS medium containing 1.0 μM BA. Inter-simple sequence repeats (ISSR) marker was used to test the genetic stability of 15 in vitro raised plants along mother plant. It resulted in monomorphic banding pattern in all the micropropagated plants as well as the mother plant. Thus, it was inferred that this propagation protocol will help to conserve the plant and also an alternative for secondary metabolite production.

(e) Preetha et al. (2015) used RAPD markers to test the genetic stability of cryopreserved samples of Kaempferia galanga, an endangered medicinal plant of Tropical Asia. In this study, no genetic variation was observed in cryopreserved and control plants of shoot tip–derived Kaempferia galanga plants. But in the case of somatic embryo–derived plants, little variation was observed in the banding pattern of control and cryopreserved samples with no phenotypic variation. The variation would have come because of the intervening callus phase. Minor genetic variations without phenotypic change in in vitro cultures are considered to be beneficial for diversity conservation. Thus, these cryopreserved samples would serve to conserve the genetic diversity of this endangered species.

(f) Al-Baba et al. (2015) developed cryopreservation protocol for long-term conservation of *Ziziphora tenuior* L., rare species with promising medicinal potential in the southern part of Jordan. Two cryopreservation techniques (encapsulation-dehydration and encapsulation-vitrification) were applied for in vitro conservation of this valuable medicinal plant, and after that the explants were tested for their genetic stability using the amplified fragment length polymorphism (AFLP) technique. The encapsulation-dehydration technique gave better results in terms of survival after cryopreservation. AFLP primers showed that there were no genetic variations between the shoot tips of *Ziziphora tenuior* L., before and after cryopreservation.

11.2.5 Molecular Markers for Establishing Taxonomic Identity

Correct taxonomic identification of threatened species is fundamental to any conservation research and action. This is particularly true for the species complexes where inter-breeding is a frequent phenomenon and phenotypic plasticity is common (Li et al. 2015). In a study conducted by Chrungoo et al. (2018), two endangered medicinal plant species, *Embelia ribes* and *Madhuca insignis*, were selected for formulating conservation studies after establishing their taxonomic identity and estimating genetic diversity. M. insignis is a riparian medicinal tree species that was classified as “extinct” by IUCN but is later rediscovered after almost 120 years from the Udupi district of Karnataka with only two surviving individuals (Bhat 2003), followed by other reports of its existence in Dakshina Kannada district, Karnataka, and the Kasaragod district of Kerala, India (Udayan 2004). In the present study, five species of the genus *Madhuca*, viz., M. insignis, *M. neriifolia*, M. latifolia, *M. longofolia*, and *M. berdollimi*, were analyzed using ITS sequences for establishing species identity. The analysis of ITS sequences of all the five different species of *Madhuca* and various ITS sequences from additional *Madhuca* taxa showed distinct clustering of different species according to their geographical region. It was observed that the Indian species like M. insignis and *M. neriifolia* were clustered together whereas *Madhuca* species from other regions like Malaysia, Indonesia, Sri Lanka, China, and Papua New Guinea clustered together. This revealed that *Madhuca* species have a Pan-Asia Pacific distribution. All the accessions of M. insignis clustered together in a separate group from the other *Madhuca* species. The present study suggests that the species within the genus *Madhuca*, particularly M. insignis, might be undergoing either extensive hybridization or incipient speciation. In this case, it can be more related to the process of incipient speciation, as the individuals of M. insignis have a limited range of distribution with scattered or fragmented populations of very small sample size (mostly one or two), which occur in isolated patches throughout the Western Ghats (Karnataka to Kerala). *Embelia ribes* is an important threatened medicinal plant species showing close similarity with other species like *Embelia tsjeriam-cottam*, *Embelia floribunda* Wall., and *Embelia subcoraceae* (Clarke) Mez. and thus poses a serious challenge in the identification of species based on morphological attributes. This genus is critically endangered as it has undergone significant genetic erosion and is at the threshold of extinction because of low seed viability, poor seed germination, and fragmentation in populations which have resulted in inbreeding in natural populations. The ITS region of different species of *Embelia* was sequenced in the present study, and sequence variations in the ITS region were analyzed to assess inter- and intraspecific relationship. The ITS region of *E. ribes* showed a highly conserved nature with 89.5% conserved sites. More specifically, ITS1 and ITS2 showed 90.1% and 81.8% conserved sites, respectively.

11.2.6 Molecular Markers for DNA Fingerprinting of Endangered Medicinal Plants

DNA fingerprinting refers to the generation of DNA profile or a banding pattern by the use of appropriate molecular markers for an individual. DNA fingerprinting of genotypes helps in the identification of closely related plant species and is one of the tools for the genetic diversity analysis and also for establishing species relationship. DNA is the most stable compound in the body of a living organism and will not vary according to the season or age or any external factor; thus the DNA pattern can easily distinguish the uniqueness of one individual from another. DNA fingerprinting is primarily used in botanicals for the protection of biodiversity, identifying markers for traits, identification of gene diversity and variation, etc. (Selvakumari et al. 2017). DNA profiling of plants can also be used in solving disputes over the identity of commercially important cultivars (Kumar et al. 2001). In endangered medicinal plant species, DNA fingerprinting can aid in the proper identification and authentication, for species differentiation, for adulteration detection, and for identification of phytoconstituents. AFLP primers were used to produce DNA fingerprints for six *Swertia* apecies including the endangered *Swertia chirayita* and *Swertia angustifolia* (Misra 2010). These AFLP fingerprints of the *Swertia* species could be used to authenticate drugs made with *Swertia* spp. Genetic inter-relationship of various *Cinnamomum* species was estimated using RAPD marker (Priya and Maridass 2008). DNA fingerprinting method for the authentication of *Taxus* species was developed (TAXUS-DNA-ID) using SNP. The technique enabled the rapid and reliable identification of species and cultivars of *Taxus* including the endangered *T. wallichiana*. The use of this method helped in the precise and timely quality controls for origin and purity of Taxus-derived raw materials (Bonardi et al. 2010) (Table 11.1).

Table 11.1 Application of genomic tools in endangered medicinal plants

Sl. No	Plant name	Genomic tool/ marker system used	Study	Reference
1	Podophyllum hexandrum	RAPD-SCAR	Molecular markers for identification and authentication of medicinal plants *Podophyllum hexandrum* Royle	Al-Shaqha et al. (2014)
2	*Aconitum heterophyllum*	AFLP	AFLP markers for the identification of *Aconitum* species	Misra et al. (2010)
3	*Cinnamomum osmophloeum*	DNA-barcoding	DNA barcoding *Cinnamomum osmophloeum* Kaneh based on the Partial Non-Coding ITS2 Region of Ribosomal Genes	Lee et al. (2010)
4	P. pseudoginseng	RAPD	Genetic and metabolomic demarcations	Mathur et al. (2003)
5	*Taxus wallichiana*	RAPD, AFLP	Assessment of genetic variation in nine natural populations from western part of the Himalayan ranges	Mohapatra et al. (2009)
6	*Trichopus zeylanicus subsp. travancoricus*	RAPD	Assessment of genetic fidelity of in vitro regenerants	Martin et al. (2011)
7	Kaempferia galanga L.	RAPD, ISSR	Molecular profiling of micropropagated plantlets	Mohanty et al. (2011a, b)
8	*Terminalia arjuna*	RAPD	Estimation of genetic diversity and evaluation of relatedness	Sarwat et al. (2008)
9	Nepenthes khasiana Hook f.	RAPD	Determination of genetic variation and gene flow estimation	Nongrum et al. (2012)
10	Vitex trifolia	RAPD	Establishment of genetic conformity of the in vitro regenerated plants	Ahmad et al. (2013)
11	*Picrorhiza kurroa* Royle ex	RAPD, ISSR	Evaluation of genetic fidelity among in vitro regenerated plants	Rawat et al. (2013)
12	*Thymus daenensis*	ISSR	Detection of genetic polymorphism using 17 accessions collected from different geographic regions in Iran	Rahimmalek et al. (2009)
13	Balanites aegyptiaca	ISSR	Evaluation of clonal integrity of micropropagated plantlets chosen from a clonal collection	Varshney and Anis (2013)

(continued)

Table 11.1 (continued)

Sl. No	Plant name	Genomic tool/ marker system used	Study	Reference
14	*Moringa oliefera*	AFLP	Determination of genetic variation	Muluvi et al. (1999)
15	Piper nigrum L.	DNA-barcoding	DNA barcoding to detect chili adulteration in traded black pepper powder	Parvathy et al. (2014)
16	*Valeriana jatamansi* Jones	AFLP	Assessment of genetic diversity and population structure in western Himalaya, India	Rajkumar et al. (2011)
17	*Oroxylum indicum*	ISSR	Assessment of genetic diversity	Rajasekharan et al. (2017)
18	*Embelia ribes Burm.F.*	SCAR	DNA fingerprinting	Devaiah and Venkatasubramanian (2008a)
19	*Taxus wallichiana*	SNP	DNA fingerprinting	Bonardi et al. (2010)
20	*Pueraria tuberose*	SCAR	DNA fingerprinting	Devaiah and Venkatasubramanian (2008a, b)
21	Cinnamomum zeylanicum	Sequencing	DNA fingerprinting	Kojoma et al. (2002)
22	*Embelia tsjeriam-cottam*	AFLP	DNA fingerprinting	Balakrishna et al. (2010)

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Chapter 12
Drugs From Threatened Medicinal Plants

Kuntal Das and P. E. Rajasekharan

Abstract Dealing with human disease problems from root level is possible only with the use of proper and authentic drugs isolated from natural sources, especially from herbal sources, because herbals are the main source of secondary metabolites, and these secondary metabolites are the main source of new drug discovery. Millions of people worldwide rely on medicinal plants for their healthcare needs; hence, herbals play a highly significant role in drug discovery and drug development. A vast number of plant sources are available in the universe; all plants have some therapeutic properties, but it is definitely impossible to make an account of the same. There are many unknown plants from which very important secondary metabolites are procured, but they are rarely known to people. Such plants become red labeled due to increased environmental damage and growing human population, which further create a threat to plant extinction. India is a rich source of plant biodiversity. The Western and Eastern Ghats are major hotspots of biodiversity in India, from where it is possible to discover drugs from threatened plants, which needs to be conserved for future research. This chapter makes an attempt to compile drug discoveries from some threatened medicinal plant species so as to give a scientific account of their future use, as well as their ability to be a source of new miracle drugs that can fight against chronic diseases. Furthermore, bioactivity-guided fractionation is recommended to identify lead compounds from these resources to be used for various activities.

Keywords Biodiversity · Drug discovery · Eastern Ghats region · Threatened plants · Phytoconstituents · Western Ghats region

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12.1 Introduction

People have faith on nature to cater to their essential needs, not the least of which are drugs for the effective treatment of a wide range of chronic diseases. Since ancient times, nature has been a source of medicinal products, and with the help of traditional knowledge about plants, many useful drugs have been developed from plant sources in recent years. As per earlier evidence (2600 BCE), the basis of sophisticated traditional medicine systems is plants, and around 1000 plant-derived substances were used in Mesopotamia (Cragg and Newman 2013). In 2900 BCE, Egyptian medicine documented over 700 plant-based drugs (Borchardt 2002), and Chinese materia medica has documented more than 1200 plant-based drugs since 1100 BCE (Huang 1999). Furthermore, around 1000 BCE, the Indian Ayurvedic system documented 341 plant-based drugs identified by Charaka and 516 plant-based drugs listed by Sushruta and Samhita (Kapoor 1990; Dev 1999). In 100 CE, a Greek physician named Dioscorides first recorded the collection, storage, and uses of medicinal herbs, and from that time onward, the Greeks and Romans have rationally developed the use of herbal drugs. Thereafter, Galen (130-200 CE.), a Roman practitioner and teacher of pharmacy and medicine, was first used his formulae in drug compounding.

Initially, the success rate of new drug discovery came from inventions of medicinal chemistry that led to the requirement for the need of development of chemical libraries through combinatorial chemistry but due to less effective in terms of success rate, an alternate source of new chemical entities for potential use as drug molecules has been focused through the natural products. From that time onward, the journey began toward herbal-related treatments and their developments. So far, herbals have been neglected due to lack of scientific evidence on their efficacy and safety on account of their complex nature and the number of phytoconstituents present in them. Furthermore, the complex nature of medicinal plants is due to greater biodiversity and more rapid environmental alteration. Some of the important drugs that have been isolated from plant sources are morphine from opium, the first drug that has been isolated in 1804 (Sertürner 1806), followed by emetine from ipecac in 1817 (Grollman and Jarkovsky 1975), strychnine from nux vomica in 1818 (Pelletier and Caventou 1818), quinine from the cinchona bark in 1820 (Dunn 1965), and thereafter nicotine (alkaloid) from the tobacco plant in 1828 (Posselt and Reimann 1828). From then on, extensive research has been carried out for the discovery of drugs with the help of modern analytical chemistry, as well as the characterization of drugs through combinatorial chemistry with the help of pharmaceutical industries.

Various analysis of new drugs that were discovered from 1981 to 2007 reveals that almost half of the drugs that have been approved since 1994 were based on natural products. From 2005 to 2007, 13 natural-product-related drugs had been approved (Harvey 2008). Thereafter, the rate of acceleration of drug discovery from natural plant sources has lessened. Medicinal plants have become threatened with extinction because of their geographical location and the cultural condition of the place. Today, medicinal plants are more prone to threats and are endangered by human activities and climate change, which greatly affects the conservation of species diversity and genetic resources and the sustainable growth of Indian traditional medicine, as well as Indian herbal industries. The World Health Organization has listed over 21,000 plant species that are used for medicinal purposes worldwide. Around 3000 plants in India are listed under the indigenous system of medicine, where there are about 450 plant entries on endangered species, of which 28 are considered extinct, 124 are endangered, 81 are rare, and 34 are unknown (Akshay et al. 2014). In India, the subject on threatened plants was first discussed in the 11th Technical Meeting of the International Union for Conservation of Nature (IUCN) in 1969, and in 1980, the Botanical Survey of India published a booklet titled "Threatened Plants of India – A State-of-the-Art Report." From then on, constant efforts have been made on the subject and valuable baseline data on nearly 1000 threatened species were gathered (World Resources Institute 1992). All data related to rare and threatened plants are compiled by the Botanical Survey of India, which published a book titled "Red Data Books." As per a report in the year 2017 by the AYUSH Ministry, the government has taken note of the information provided by Botanical Survey of India (BSI) that out of 8000 medicinal plants, about 75 species are under threatened categories (Siwach et al. 2013), such as critically endangered, endangered, and vulnerable (Fig. 12.1). About 68% of medicinal plants are found in the tropical areas of India, especially across the Western and Eastern Ghats, the Vindhyas, the Chota Nagpur plateau, the Aravalis, and the Himalayas, whereas about 30% of medicinal plants are found in temperate and alpine areas in the country (Fig. 12.2).

In Asia, India is a hub of natural herbs, but important plant species have reduced in number due to human-induced habitat loss, overcollection of these plants to supply domestic and foreign medicinal markets, rare seed germination, and deforestation. Due to overexploitation, these plants are now on the verge of extinction and have become threatened species, which can be further divided into extinct, extinct in the wild, critically endangered, endangered, vulnerable, and near threatened (Fig. 12.3). Based on scientific research, it is evident that the most threatened habitat is the tropical rainforests, and plant species that are threatened are mostly found in the tropics. Among the various plant species, gymnosperms (conifers and cycads) are the most threatened group. Recently, the Wildlife Institute of India has taken an initiative to release a special issue on "Specialized Habitats and Threatened Plants," which is contributed to by professional taxonomists and in which many threatened species, collected from the Western Himalayas, North East India, Western and Eastern Ghats, Thar Desert, Ran of Kachchh, and some semiarid regions of Deccan, and their habitats are discussed. Subsequently, state forest departments have taken responsibility for in situ conservation of biodiversity as per the policy of the Biodiversity Act (2002) and the technical support of research institutions (Fig. 12.4).

Some important drugs procured from natural plants that have became threatened in India are shown in Table 12.1.

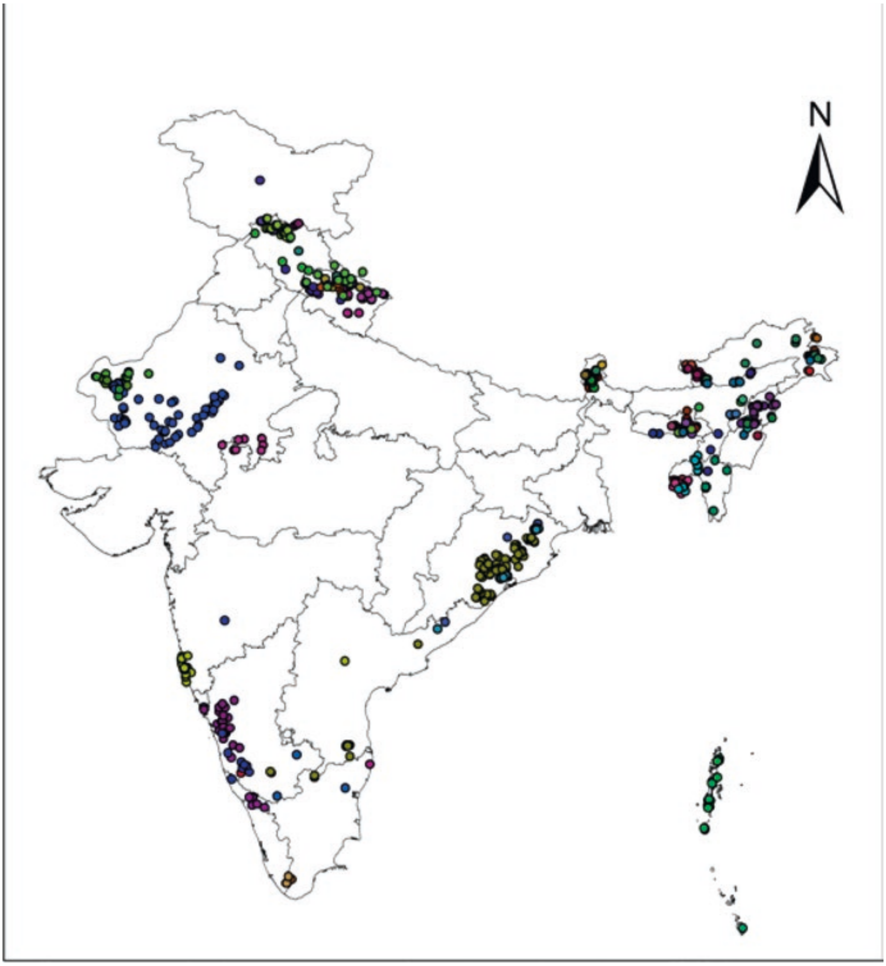


Fig. 12.1 Seventy-five threatened medicinal plant species in India. (Source: http://www.dbtindia.nic.in/dbt-conserves-100-most-threatened-species-of-india/)

12.2 Process of Drug Development

Development of herbal drug involves the collection and authentication of plant raw materials; pharmacognostic, phytochemical, and pharmacologic evaluation; and standardization. Standardization is the process of quality control of herbal drugs. Separating a single pure active constituent from the natural source is very difficult because they are present in combinations. Hence, a stepwise process is required to be followed from the identification of raw materials to characterization with the help of instrumentation methods. Multiconstituent herbals are standardized through

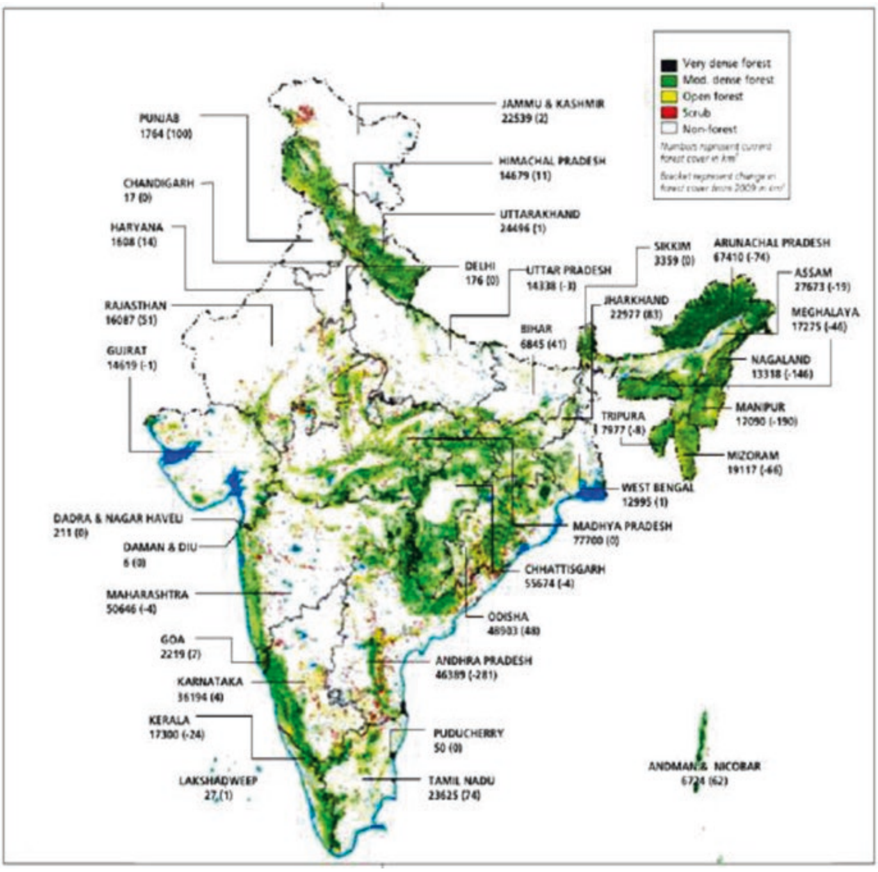


Fig. 12.2 Forest zone of India

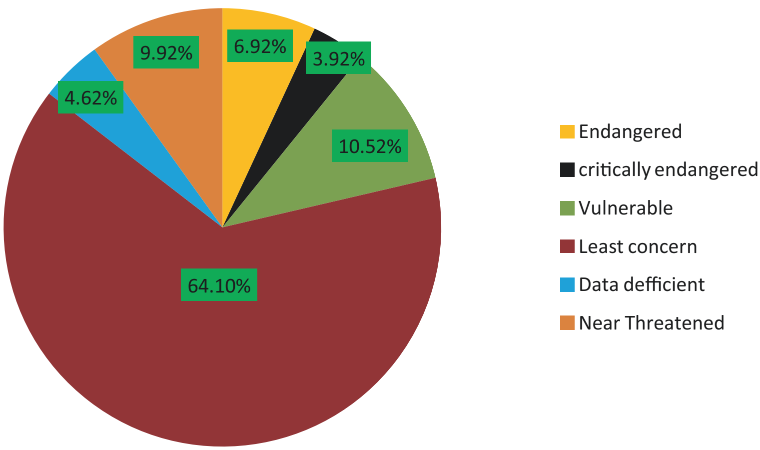


Fig. 12.3 Percentage wise classification of endangered plants

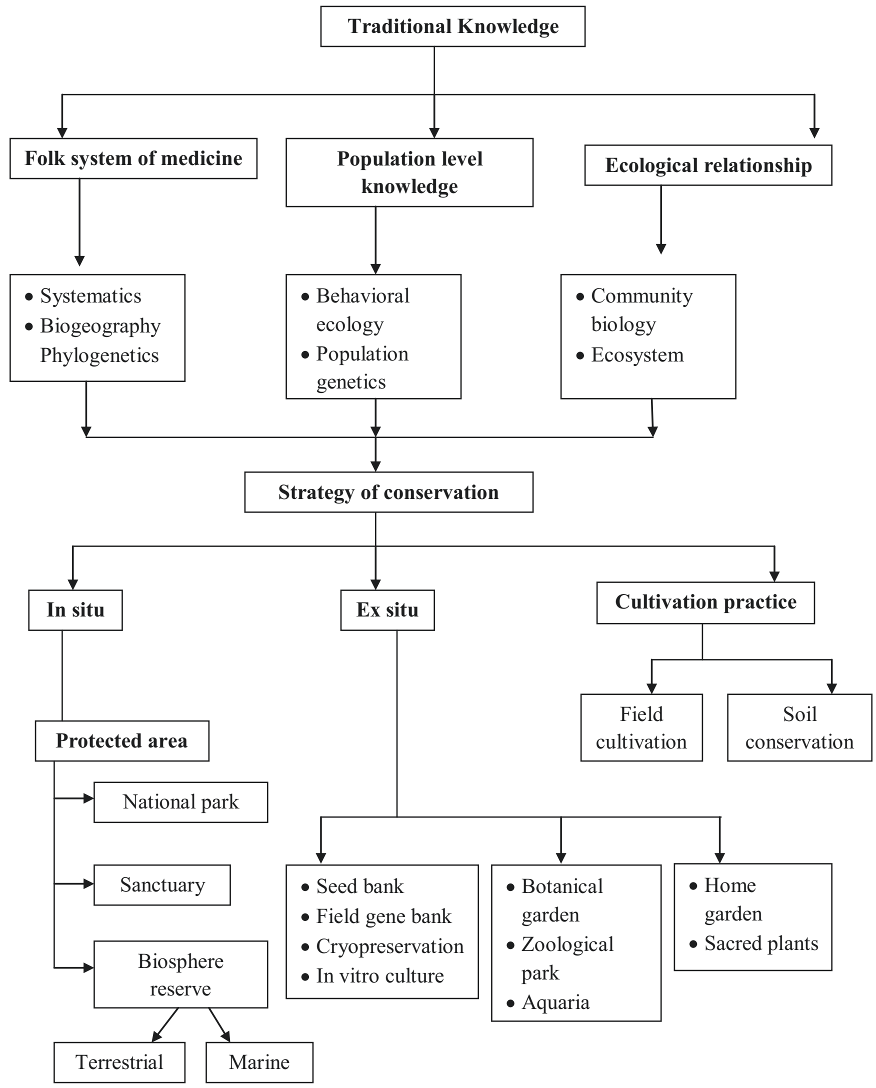
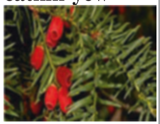


Fig. 12.4 Conservation strategies

newer techniques, such as bio-guided fractionation, DNA fingerprinting, isolation of biomarkers, high-pressure thin-layer chromatography (HPTLC), and liquid chromatography–mass spectroscopy (LCMS), followed by spectroscopic methods, such as UV, IR, NMR, MASS, HR-MS, etc. (Fig. 12.5), for structure elucidation of isolated compounds (Das 2010). Thus, for the discovery of newer drugs, a complete instrumental study is required, which is very expensive, plus it is difficult to identify and collect proper raw materials, and it is a time consuming and challenging task.

Table 12.1 List of threatened plants in India

Plants	Biological source	Family	Distribution	Drug identified	Uses
Indian mallow 	*Abutilon indicum*	Malvaceae	India	Beta-sitosterol	Demulcent, aphrodisiac, laxative
Actinodaphne 	*Actinodaphne lawsonii*	Lauraceae	India	Not identified	Not identified
Assam catkin-yew 	*Amentotaxus assamica*	Taxaceae	India	Taxol	Anticancer
Agar wood 	*Aquilaria malaccensis*	Thymelaeaceae	India, Bhutan, Indonesia, Malaysia, Nepal	Aquimavitalin, aquilanols A and B, chamaejasmone E	Anticancer, treatment for rheumatism and asthma

(continued)

Table 12.1 (continued)

Plants	Biological source	Family	Distribution	Drug identified	Uses
Brucea 	*Brucea mollis*	Simaroubaceae	India, Sri Lanka, Thailand, Nepal	Soulameanone, bruceollines C and G, bruceine B	Antimalarial, anticancer, diuretic
Colchicicum 	*Colchicum luteum*	Liliaceae	India, Afghanistan, Turkestan	Colchicine, cornigerine	Analgesic, wound healing, laxative
Dioscorea 	*Dioscorea deltoidea*	Dioscoreaceae	Afghanistan, Bhutan, Cambodia, China, India, Nepal, Pakistan, Thailand, Vietnam	Diosgenin	Contraceptive pills and sex hormones
Himalayan yew 	*Taxus wallichiana*	Taxaceae	Afghanistan, Bhutan, China, India, Indonesia, Malaysia, Myanmar, Nepal	Taxol	Anticancer, treatment for bronchitis, asthma, epilepsy
Indian sarsaparilla 	*Decalepis hamiltonii*	Apocynaceae	India, Sri Lanka, Thailand, China	2- hydroxy-4-methoxy benzaldehyde, α-atlantone	Antioxidant, antimicrobial, antipyretic, antiulcer, antidiabetic, anti-inflammatory

Ilex 	*Ilex khasiana*	Aquifoliaceae	India	Not identified	Treatment of cold, cough, tuberculosis
Jatamansi 	*Nardostachys grandiflora*	Valerianaceae	China, Bhutan, India, Nepal	Jatamansone	Nervine tonic, hypotensive, antiseptic, stomachic, carminative, tranquilizer
Kutki 	*Picrorhiza kurrooa*	Plantaginaceae	India, Pakistan	Kutkin, apocynin, kutkoside	hepatoprotective, anti-inflammatory, urinary disorder treatment
Makassar ebony 	*Diospyros celibica*	Ebenaceae	India	Macassar II, macassar quinone	Anticancer, antimicrobial
Malabar lily 	*Chlorophytum malabaricum*	Liliaceae	India, Australia, Africa	Neohecogenin	Antidiabetic, antistress, immunomodulatory

(continued)

Table 12.1 (continued)

Plants	Biological source	Family	Distribution	Drug identified	Uses
Malayuram 	*Pterospermum reticulatum*	Sterculiaceae	India	Sterculin-A	Relief of throat infection, relief of headache, antimicrobial
Red sandalwood 	*Pterocarpus santalinus*	Fabaceae	India, Sri Lanka, Taiwan, China	Pterostilbene, pterocarpol, santalins A, B	Antidiabetic, antipyretic, anti-inflammatory, anthelmintic
Snakeroot 	*Rauvolfia serpentina*	Apocynaceae	Bangladesh, Bhutan, China, Indonesia, India, Malaysia, Myanmar, Nepal, Sri Lanka, Thailand	Reserpine, serpentine	Antihypertensive
Sanchi 	*Ormosia robusta*	Fabaceae	India, Thailand, Nepal, Myanmar	Warangalone, erysenegalensein M	Antioxidant, treatment of jaundice
Spiderwort 	*Belosynapsis vivipara*	Commelinaceae	India, China, Indochina, Malaysia	Not revealed	Not revealed

Sarsaparilla 	*Smilax glabra*	Smilacaceae	China, India	Astilbin, neoastilbin, isoastilbin	Anticancer, anti-inflammatory, antiarthritic
Tree turmeric 	*Coscinium fenestratum*	Menispermaceae	India, Sri Lanka, Thailand, Vietnam, Thailand	Berberine	Useful for wounds, ulcers, jaundice, skin disease and antioxidant
Water lily 	*Nymphaea tetragona*	Nymphaeaceae	India, China, Sri Lanka, America, Africa		Treatment of dysentery, diarrhea

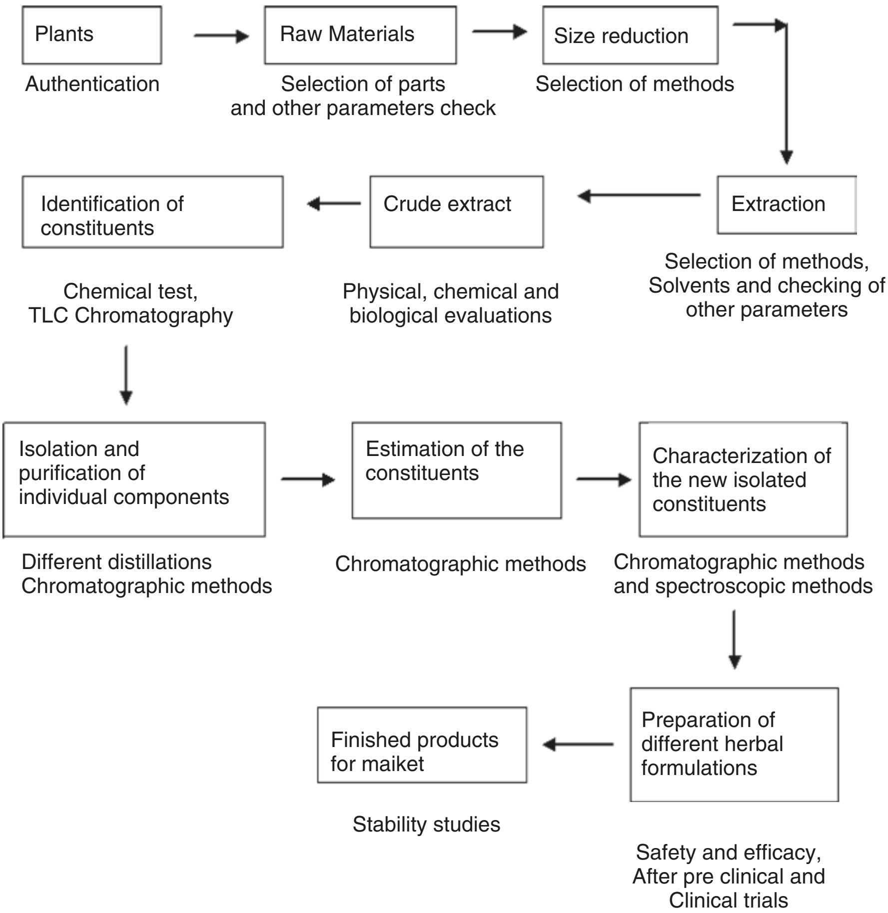


Fig. 12.5 Various steps in the standardization of herbal plants. (Ref: Das 2010)

Therefore, only very few pharmaceutical companies are involved in drug discovery screening from natural sources. Other reasons for this include the complex nature of medicinal plants due to biodiversity and improper availability of regulatory guidelines for natural products. Thus, concerns on efficacy and safety of herbals are also the main focus when it comes to isolation of new plant constituents.

Base on the figure, 12.5, the new drug discovery process is subjected to various steps. The first step is field survey and survey of related knowledge, then plant selection and authentication, processing of plants, selection of a solvent system for extraction, followed by isolation of constituents, toxicity study, and discovery of new drugs.

12.2.1 Field Survey and Survey of Related Knowledge

Surveys are commonly conducted to search for threatened, endangered, and sensitive plant species in a specific geographical area to identify particular medicinal plant species and also based on the presence or absence of key habitat requirements for the target plant species like associated native plant community types and texa, topographic and soil preferences, microhabitats (like rocky out crops, tree canopies etc.), disturbance such as fire history and type of disturbance (like canopy removal etc.). The database from the survey also provides guidance on the appropriate time to survey for individual threatened species, like flowering time, fruiting time, etc. (Cropper 1993).

12.2.2 Plant Selection and Authentication

During the field survey, if the taxon of a plant is not identified and if it is suspected to be a threatened plant, then a specimen is collected and preserved for further identification with the help of alternate resources like field guides and keys. If identification is difficult in the case of a young plant, then the plant is left in situ to grow and will be examined only when it is old enough. But even if the plant was not identified at the time it has grown, it will be submitted to the National Herbarium for inspection and formal identification. The selection of plant species are also carried out based on chemical composition uses phylogenetic or chemotaxonomic information in the search namely genera and families, for compounds from a defined chemical class with known pharmacological activity (Souza Brito 1996).

The proper and systemic authentication of raw plant materials is important to ensure that herbal medicines are safe and effective. Some of the evaluation parameters are like parts of plants collect like leaf, flower, root, stolen, regional status, family, biological source, and chemical constituents. Apart from these, other parameters like stage of collection and geographical location need also to be checked. Some of the organizations, institutes, and laboratories involved in herbal drug authentication are the Central Council for Research in Ayurveda and Siddha (CCRAS), the Central Council for Research in Unani Medicine (CCRUM), the Central Council for Research in Homoeopathy (CCRH), the Central Council for Research in Yoga and Naturopathy (CCRYN), Pharmacopoeial Laboratory for Indian Medicine (PLIM), Homoeopathy Pharmacopoeia Laboratory (HPL), the Indian Institute of Horticultural Research (IIHR), the National Medicinal Plant Board (NMPB), Central Institute of Medicinal and Aromatic Plants (CIMAP), the Regional Research Laboratory (RRL), Pharmacopoeial Laboratory for Indian Medicine (PLIM), the National Institute of Ayurveda (NIA), the National Institute of Siddha (NIS), etc.

12.2.3 Processing of Plants

Before extraction of any plant species, it is required for plants to be processed further to make them suitable for extraction. Hence, size reduction of plant materials is important. This method is the most essential method for reducing the bulkiness of plant materials and increasing the surface area for extraction, thereby providing ease of extraction. This size reduction method is also known as comminution. As per Indian Pharmacopoeia, a sieve size of 8 or 10 is used to obtain coarse particle size for the extraction of plant materials. This size reduction method is also advantageous as it improves solute–solvent contact and provides fast extraction. The main equipment used for size reduction includes equipment for grinding, crushing, mincing, milling, and dicing. As an example, leaves, flowers, and soft roots are generally powdered using a mixer grinder; hard barks and root woods are powdered through a hammer mill, knife mill, tooth mill, etc. Recently, cutting mills are being used for the preliminary grinding of soft, medium-hard, elastic, and fibrous materials, as well as heterogeneous mixes of products. Size reduction by cutting and shearing is carried out gently and quickly, which makes the mills suitable for temperature-sensitive samples; especially, woods are size reduced with the use of this method (Das 2010).

12.2.4 Selection of a Solvent System

The plant botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds and these components are extracted by using specific use of solvents with the basic principle of solubility of the components in the selected solvents and depend on the specific nature of the bioactive compound being targeted. Different solvent systems are available for the extraction of bioactive compounds from natural products. The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol, or ethyl-acetate. For the extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in the ratio of 1:1 is used. Sometimes extraction with the use of hexane and acetone solvents is done to remove chlorophyll. Ethanol is used for obtaining extracts like tincture, fluids, and soft and dry extracts. Hydroalcoholic mixture solvent is used to induce the swelling of plant particles and to increase the porosity of the cell wall, which results in more amount of constituents coming out of the solvent.

For the identification and isolation of secondary plant constituents, especially polar or semipolar constituents, methanol or ethanol, pure acetone, or acetone/water mixtures are used. For the isolation of lipophilic compounds, lipophilic solvents such as petroleum ether or hexane or chloroform or acetone is used, but they should be used with proper care because these solvents may cause health hazards; for instance, acetone is highly fire sensitive and liver toxic, chloroform creates liver toxicity, benzene is cancerous, etc. To remove chlorophyll from leaf materials, acetone is used. Recently, however, herbalists are opting for an appropriate alcohol–water mix (equal ratio) to optimize the effectiveness of the extract (Das 2010).

12.2.5 Isolation of Constituents

The isolation of the constituents from plant sample is very tedious method and hence at first method for extraction is need to select. Initially the plant extracts are qualitatively analyzed by thin-layer chromatography (TLC) and/or other chromatographic methods and screened to determine biological activity or to obtain a general evaluation of biological activities. For purification and isolation, the active plant extracts are sequentially fractionated to obtain isolated active compounds (Verpoorte 1989). A suitable method for extraction is the type that is simple, fast, and reproducible; has high herb extract ratio; and involves less consumption of solvents and time. The principle of the plant extraction is based on the solubility of the plant constituents in the solvents so that dissolved plant constituents are come out from the plant cell and further extract will form after evaporation of the solvents. The general techniques used in medicinal plant extraction include maceration; infusion; percolation; digestion; decoction; hot continuous extraction, i.e., soxhlation; aqueous-alcoholic extraction by fermentation; countercurrent extraction; microwave-assisted extraction; ultrasound extraction, i.e., sonication; supercritical fluid extraction; bio-guided fractionation; and distillation techniques (water distillation, steam distillation, phytonic extraction (with hydro fluorocarbon solvents)). For aromatic volatile oil-containing plants, hydro-distillation and steam distillation, hydrolytic maceration, followed by distillation, expression, and effleurage are commonly employed. Some of the latest extraction methods for aromatic plants are headspace trapping, solid phase micro-extraction, protoplast extraction, micro-distillation.

Based on the requirement of the plant extract, the batch size and batch volume is selected which is directly proportional to the amount of raw materials are used in the extractor. Depends on the volume of plant extraction requirement i.e. laboratory or bulk purposes, equipments are selected and proportional way volume of the solvent should added for the extraction. Due to the natural variation in the composition of a raw herbal material, the native extract ratio may vary from batch to batch. That is, herbs sourced at different times of the year or from different climactic or geographical situations may provide differing amounts of extractable herbal components. Generally, herbs along with solvents are used for the extraction at a ratio of 1:2 or 1:2.5. Extraction time and temperature are essential for extraction, which is an important step in the recovery and purification of active ingredients from plants materials. The aim of the plant extraction process is to obtain the maximum crude extract of substances and the highest quality thereof, which means that target compounds should have high concentration, which is possible only if proper extraction time and temperature are maintained throughout the process. These parameters greatly depend on the nature of the constituent and the type of extractor used for extracting. Therefore, the systemic and thorough plant extraction and extract standardization method is necessary. For example, in the case of supercritical fluid extraction method, regulation of temperature and pressure is most important because carbon dioxide is used as a solvent, which has low critical values and low chemical reactivity without used water as a solvent because of its high critical temperature and pressure. As another example, for the soxhlation method of extraction, temperature should be set at 40–50 °C for the stability of the phytoconstituents. In the case of microwave-assisted extraction, microwaves, which are nonionizing electromagnetic waves, are set at a frequency of about 300 MHz, and heating occurs in a closed system; this constant heating reduces extraction time and the use of solvent for extraction. Generally, less than 40 ml of solvent is required for this method, as compared to Soxhlet extraction, which requires 100–500 ml of solvent. This method is suitable for the isolation of polyphenol from tea, glycyrrhizin from liquorice roots, etc. In the case of pressurized liquid extraction method, the temperature is normally kept between 80° and 200 °C and pressure ranges from 10 to 20 MPa. The sample is loaded in a stainless steel extractor, into which the solvent is pumped and brought into specified temperature and pressure, and then extraction is carried out. The whole process takes about 20 minutes (Rates 2001; Das 2010).

12.2.6 Toxicity Study

Research on Pharmacologically active natural compounds from natural plant products depends on the integration of botany, chemistry, and toxicology. The plant secondary metabolites when acts as potent drugs then as per the needs of the market and public health and requirements, an adequate scientific data is necessary that provides the quality control and the efficacy and safety (Rates 2001). The toxicological, preclinical and clinical data are essential for such of the final product. The major important plant based potent drugs for therapeutic efficacy, as an investigation tool in biological research are quite productive in toxic plants. A vast number of important compounds procured from toxic plants are now used in research. Toxicity study is mandatory in order to understand the safety profile of drugs. The toxicity study of herbal drugs, as well isolated compounds, is essential for safety purposes. The toxicological study and testing helps to explore the level of harmness to all the phytochemicals, biological activity and mechanism of action at particular dose level but exposure to a specific small amount of any substance is not having any detectable effect on normal biological process and is considered safe. Substances at specific doses have beneficial effects, but exposure to higher dose level causes harmful effects, and the substance is considered toxic. Mainly four types of toxicity studies are recommended: acute, subacute, subchronic, and chronic studies. Acute toxicity testing provides information on the safety, biological activity, and mechanism of action of drugs. Information generated by the test is used in hazard identification and the risk management of drugs, as per OECD guideline 423, which restricts the use of animals for testing to only three (OECD 423, Paragraph 23) (Jonsson et al. 2013). Animals are administered drugs via oral gavage at 2000 mg/kg. Subacute toxicity study is performed for 28 days, and animals are observed for behavioral changes and general toxicity signs after dosing for the first 24 h. LD_{50} (lethal dose) is used as an indicator for acute toxicity. Acute dermal (OECD TG 402) and acute inhalation (OECD TG 403) toxicity are also observed in the case of sensitive phytoconstituents. Subacute studies are conducted in compliance with OECD guidelines No. 407 for 28 days, wherein drugs are given through oral administration. Subacute toxicity tests are performed to evaluate the toxicity of chemicals after repeated administration and also to help establish doses for longer term subchronic studies by utilizing three to four different dosages of chemicals, which are administered by mixing them in the feed (Eaton and Gallagher 2010). Subchronic toxicity is defined as the occurrence of adverse effects after repeated or continuous administration of chemicals to a test sample for up to 90 days. The rationale for the selection of a subchronic or subacute test must be based on the clinical duration of use for the medical device, the nature of exposure, and the overall testing strategy. Intravenous and/or intraperitoneal and implantation methods are used for the testing of extracts. The route of exposure is selected, based on clinical use of the device (De Jong et al. 2012). On the other hand, chronic toxicity is a condition caused by repeated or long-term exposure to low doses of toxic substances. This study generally lasts for six months to one year and is designed to determine the potential target organs of toxicity, the reversibility of the toxicities observed, and potential clinical risks in relation to anticipated clinical doses following a long-term treatment (Colerangle 2017).

12.2.7 New Drug Discovery

The development of new drugs with therapeutic efficacy from plant origin is very complicated as well as expensive. Each new drug requires an investment of huge amounts of money and a minimum of five to ten years of research work (since several steps are involved) before it is finally approved as a new drug. Of late, drug discovery by molecular modeling, combinatorial chemistry, and other synthetic chemistry methods has become a subject of interest, and natural-product-derived compounds is proving to be an invaluable source of medicine for mankind. Plant secondary metabolites are used not only as drugs but also as drug precursors, templates for synthetic modification, and pharmacological probes.

Few plant based natural products are used as drug precursors. Sometimes high efficient chemical compounds with low availability in plant body are derived by semi-synthetic approach to make more availability and cost effective. Like slow-growing Pacific yew tree, *Taxus brevifolia* Nutt., a highly potent antitumor drug paclitaxel or Taxol is originally isolated only 0.014% w/w yield from the bark of *Taxus brevifolia* (Kingston 2000) but 10-deacetylbaccatin III is isolated in relatively large amounts from the needles of other related yew species, such as *Taxus baccata* L. and is converted chemically in several steps into paclitaxel which produce more than natural one (Denis et al. 1988).

A drug prototype is the first compound discovered in a series of chemically related therapeutic agents, which is the first form of a drug or medication that is used to create alternative forms. It is also known as a lead agent. Plant based secondary metabolites are contributed more than 25% as prototype. With advances in organic chemistry, medicinal chemists have started preparing analogs from these drug prototypes to provide safer and more potent drugs. Example: podophyllotoxin is selected as drug prototypes with analogs having the same pharmacological action as the parent compound, while atropine is a drug prototype that is furnished many analogs which have additional pharmacological properties (Sneader 1996).

Sometimes secondary metabolites from plant sources are also used as pharmacological probes, which help researchers to understand the mechanism of action of intracellular signal transductions and the biological mechanisms related to human disease and helps to design better drugs. For example, phorbol esters, genistein extracted from plants, are used as pharmacological probes. Genistein, an isoflavone found naturally in soybeans (*Glycine max* Merr.), inhibits various protein tyrosine kinases (PTK). Genistein acts as probe to the interaction between PTK and cyclic nucleotide-gated (CNG) channels (Molokanova et al. 2000).

Some of the new plant-derived drugs that have been launched since 2001 are apomorphine hydrochloride, galanthamine hydrobromide, nitisinone, tiotropium bromide, and varenicline, which have been approved by the US Food and Drug Administration (FDA). Nitisinone was approved by the FDA in 2002 for the treatment of hereditary tyrosinemia type 1 (HT-1) (Butler 2004). It is a derivative of leptospermone, a new class of herbicide from the bottlebrush plant (*Callistemon citrinus* (Curtis) Skeels). Tiotropium bromide, an atropine analog, was approved by the FDA in 2005 for the treatment of bronchospasm associated with chronic obstructive pulmonary disease (Koumis and Samuel 2005). Varenicline and cytosine, which are plant-based alkaloids, were approved by the FDA in 2006 (Niaura et al. 2006).

A vast number of pharmaceutical companies in the field of natural products are tagged with small biotechnology companies, which are specialized in lead identification from natural product extracts and develop many leads from natural sources into drugs, in that many of the drugs are currently under clinical trials. These activities increase the focus toward threatened plant species with respect to drug discovery.

12.3 Conclusion and Way Forward

Medicinal plants are occupied a vital position in the healthcare system of India since ancient times and are currently a major global resource. Medicinal plants are used by various tribal and local people to cure different sickness and illnesses ranging from simple fever to injuries, wounds, diarrhea, ulcer, swelling, bone fracture, impotence, poisoning, skin diseases, night blindness, toothache, asthma, and cough and cold. But due to man-made causes and overexploitation, their natural habitat have become threatened. Hence, there is an immense need to conserve the diversity of medicinal plants for the sake of future generations through the adaptation of suitable methods of conservation.

Thereafter, the newer approaches for drug discovery process from natural sources are to utilize the application of molecular biology techniques and to develop a systematic screening method to isolate a large number of pure compounds from the plant extracts will help in identification of newer compounds and will improve the newer drug development process.

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Part IV
Case Studies on Different Threatened Medicinal Plants Distributed in Different Agroecological Regions

Chapter 13
Conservation and Utilization of High-Altitude Threatened Medicinal Plants

Ravinder Raina and Kamini Gautam

Abstract The Himalayan region is bestowed with unique flora found nowhere on the earth. The Indian Himalayas are complex and dynamic ecosystems nurturing approximately 8644 plant species. Among these, medicinal plants are predominant floral wealth of Himalayas with almost 1748 of species being utilized for curing diseases since time immemorial. These medicinal plants have enormous national and international demand as raw material leading to their illegal and unscientific harvesting at large scale from the wild in the absence of regulated cultivation practices. Almost 90% of the raw material of these herbs used in the pharmaceutical industries is collected from the wild, out of which 70% is destructively harvested. The demand for these high-value Himalayan medicinal and aromatic Plants (MAPs) is increasing every year despite of their low availability because of the multiple medicinal value of these herbs. These medicinal plants form part of Indian economy, are source of livelihood for local inhabitants, and also form part of local healthcare. Presently, most of the medicinal plants of Himalaya are in peril owing to large-scale illegal and unscientific harvesting, habitat destruction, lack of sufficient knowledge of their ecology and biology, and limited research and development initiatives. About 120 species of Himalayan medicinal plants are under various threat categories, according to International Union for Conservation of Nature (IUCN). Preventing extinction and sustainable utilization of these medicinal plants needs collaborative efforts by both government and researchers by restricting their harvest, reintroduction of species in their natural habitat, for development of in situ and ex situ conservation strategies, and developing techniques for scientific harvesting of these species. At national and international level, efforts made for conservation of Himalayan herbs by various government agencies, and nongovernmental organizations are also gearing up slowly, but immediate attention and serious efforts are

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needed to preserve these therapeutic agents for future use and for sustenance of the Himalayan ecosystem.

Keywords Himalayas · High altitude · Medicinal plants · Endangerment · Trade · Conservation · Sustainable utilization

Global use of medicinal plants in health care is increasing rapidly owing to the claims of efficacy of these plants, interest of public in herbal therapies and herbal medicines, strong belief that herbal products are superior to manufactured products, due to many side effects and high cost of modern drugs, and the growing self-medication movement (Bandaranayake 2006). It is estimated that 80% of the world's population living in the developing world depends on medicinal plants as a primary source of health care, and many people residing in the vicinity of the forests and villages use these herbs to ward off diseases traditionally (Mukherjee 2002; Bodeker et al. 2005; Bandaranayake 2006).

Nowadays developed countries are also focusing on medicinal herbs by practising complementary and alternative medicines (Calapai 2008; Braun et al. 2010; Anquez-Traxler 2011). Medicinal plants are viewed as a safe and balanced approach in healing, and people are ready to pay huge amount of money on herbal products resulting in rising demand for herbal medicines (Roberts and Tyler 1997; Blumenthal et al. 1998; WHO 2002; Kong et al. 2003; Pal and Shukla 2003; WHO 2005; Bandaranayake 2006).

As the global use of herbal medicinal products continue to grow and with the introduction of new herbal medicinal products into the market, public health issues, and concerns associated with their safety are also increasingly recognized by people. Although some of the herbal medicines have good potential in curing disease and are widely used, many herbs remain invalidated. Over 422,000 plant species worldwide possess medicinal value (Iqbal 1993) of which 52,885 species are traded globally (Schippmann et al. 2006). Wild resources serve as a main source (80–90%) of the medicinal plant species. The Indian *Ayurvedic* system alone uses around 1250–1400 medicinal plants species of which almost 80% are wild weeds (Hamilton and Radford 2007). This ever-growing global botanical market is affecting plant resources. The botanical plant market is US$ 20–40 billion worth and is increasing at an annual rate of 10–20% (Larson and Olsen 2007). A large proportion of Himalayan flora possesses medicinal value, and the region is becoming the global centre for medicinal plants (Hamilton and Radford 2007). These medicinal plants not only play an important role by directly contributing to healthcare system but also serve as primary source of income to local people (Rasul et al. 2012).

13.1 Diversity of Himalayan Medicinal Plants

The Himalayas is a rich store house of biodiversity and is known for its biodiversity hotspot in the world. The variations in its altitude, topographic, soil type, and climate factors are the reasons for sustaining such huge biodiversity (Mani 1978). The

Table 13.1 Distribution of medicinal plants

Country or region	Total number of native species in Flora	No. of medicinal plant species reported	% of medicinal plants in the region
World	297,000	52,885	10
India	17,000	7500	44
Indian Himalayas	8000	1748	22

Source: Kala (2006)

Himalayas cover 12.84% of the total geographical area of India (Negi 2009) and nearly 8000 species of flowering plants flourish there, out of which 25.30% are endemic to Indian Himalayas (Singh and Hajra 1996). Indian Himalayas being a complex and dynamic ecosystem harbours approximately 8644 plant species (Khan et al. 2012; Kumar and Maharaj 2018). The Himalayan ranges are spread into eight countries, that is, Afghanistan, Bangladesh, Bhutan, China, India, Myanmar, Nepal, and Pakistan. Indian Himalayas lie between 27°50′N to 37°06′N and 72°30′E to 97°25′E and sustain a great diversity of plant species (Singh and Singh 1992). The Indian part of Himalayas cover an area of about 5 lakh km^2 (about 16.2% of country's total geographical area) and forms the northern boundary of the country. Indian Himalayas constitute about 8000 species of angiosperms, 44 species of gymnosperms, and 600 species of pteridophytes; 1748 of these species are of medicinal value. Out of the 1748 (32.2% of India) species of medicinal plants, 1685 are angiosperms, 12 are gymnosperms, and 51 are pteridophytes. Among these medicinal plants, 1071 are herbs, 335 are shrubs, and 330 are trees (Samant et al. 1998a, b) with the maximum medicinal plants (1717 species) being reported around the 1800 m elevation range (Samant et al. 1998a, b). A total of 62 out of total 1748 species of medicinal plants are endemic to the Indian Himalayas and 208 species are near endemic (Samant et al. 1998a, b). Due to the presence of these unique miraculous medicinal plant species, the Himalayas is globally renowned as a storehouse of medicinal plants (Table 13.1).

13.2 Trade of Himalayan Medicinal Plants

Himalayan Medicinal and aromatic plants (MAPs) are much in demand due their unique therapeutic properties, endemism and small populations. During 2014–15, the export demand of Indian medicinal plants was approximately 134,500 MT with export value of US$3211 crore, whereas the domestic demand was approximately 195,000 MT (http://www.nmpb.nic.in/content/medicinal-plants-fact-sheet retrieved on seventh May 2018). India consumed 512,000 MT of herbal raw drug during the period 2014–15. According to studies, 1178 species were traded, out of which 242 species were traded in excess of 100 MT/year (http://www.nmpb.nic.in/content/medicinal-plants-fact-sheet retrieved on seventh May 2018).

A total of 18.00% of the MAP material traded in India, and out of the 960 species, 350 (mostly demanded MAPs) are demanded in pharmaceutical industries, which are Himalayan MAPs (Export-Import Bank of India, 2003). The demand for these high-value Himalayan MAPs is increasing every year despite of their low availability as upward price trend of most of the Himalayan species is an indicator of this demand. Himalayan medicinal plants have been traded since time immemorial (Jacob and Jacob 1993). However, scientific attention has been given to it recently as a potential drug to cure many rare diseases and for their role in rural livelihoods. Himalayan medicinal plants are important part of local traditional as well as national and foreign traditional system of medicine; therefore, these herbs have high local and global demand. This high demand had led to trade of huge amount of raw material from Himalayas, most of which are illegal. For almost all Himalayan MAPs data on annual quantities traded is scanty or nonexistent despite their use and trade since antiquity (Olsen 2005). Most of this trade is done locally and illegally; thus, it is a Herculean task to estimate exact amount of raw material and species traded. Moreover, the collection and trade of Himalayan medicinal herb is scattered among different Himalayan states in India and is done locally without any regulation and intervention of government, thereby is unreported. Another major reason for lack of accurate and up-to-date data on demand and supply of these Himalayan herbs is that most of the trade goes unrecorded and unclassified. Domestic as well as foreign trade is also poorly recorded. Therefore, it is impossible to assess domestic as well as global trade in Himalayan medicinal plants. Out of all the medicinal plants occurring in the Himalayas, few are in high commercial demand because of their unique property to cure rare diseases; multiple use and the active ingredients present in them have no synthetic substitute (Table 13.2).

The huge demand for raw material of the Himalayan medicinal plants is met by large-scale illegal harvesting from the wild. Almost 90% of the plant material used in the pharmaceutical industries is collected from the wild, and out of which 70% is destructively harvested (Planning Commission, Govt. of India 2000). Commercial cultivation and production of Himalayan medicinal plants is very low than actual demand for raw material. Major factor for overharvesting of these plants is easy access or absence of restriction on harvesting for medicinal plants. Another reason for overharvesting is that collectors are illiterate local people, and due to lack of knowledge of actual worth of these species, they agree to sell raw material at cheap prices to pharmaceutical companies. Therefore, pharmaceutical companies are at a win–win situation as they don't have to grow medicinal plants for raw material and they can easily get it at cheap prices, thereby they opt for harvesting from wild stock. Earlier traditional use of MAPs ensured sustainable use of medicinal plants but now forest laws has been implemented by state and central governments, but still their implementation and enforcement is weak in remote areas of the Himalayas (Planning Commission, Govt. of India 2000). A study carried out on the Himalayan MAPs indicates that 41% of the primary traders source their material through collection in wild solely and 45% from both wild collection and cultivated (Planning Commission, Govt. of India 2000). One important factor that also contributes toward overharvesting and destruction is that mostly these medicinal plants are traded for roots and rhizomes, thereby require destructive harvesting (63% of the

Table 13.2 Medicinal plant species in high trade sourced from temperate forests (Ved and Goraya 2007)

S.No	Species	Estimated annual trade (MT)	Price range of official part (Rs)
1.	*Abies spectabilis*	500–1000	30–50
2.	*Aconitum ferox*	100–200	150–250
3.	*Aconitum heterophyllum*	200–500	2000–4000
4.	*Berberis aristata*	500–1000	15–35
5.	*Bergenia ciliata*	200–500	15–20
6.	*Cedrus deodara*	500–1000	25–35
7.	*Cinnamomum tamala*	500–1000	15–35
8.	*Ephedra gerardiana*	200–500	25–35
9.	*Juniperus communis*	500–1000	35–45
10.	*Jurinea macrocephala*	1000–2000	60–150
11.	*Nardostachys grandiflora*	200–500	110–150
12.	*Onosma hispidum*	500–1000	50–60
13.	*Parmelia perlata*	1000–2000	80–90
14.	*Picrorhiza kurroa*	200–500	220–230
15.	*Pistacia integerrima*	150–200	90–110
16.	*Rheum australe*	500–1000	25–30
17.	*Rhododendron anthopogon*	100–200	15–30
18.	*Swertia chirayita*	500–1000	200–225
19.	*Taxus wallichiana*	100–200	75–90
20.	*Valeriana jatamansi*	100–200	95–100
21.	*Viola pilosa*	200–500	300–350

Source (http://www.nmpb.nic.in/sites/default/files/Projects/Chapter-10.pdf retrieved on 22/03/2018)

raw material in trade comprises roots and 5% comprise whole plants) (Planning Commission, Govt. of India 2000). Out of the total traded species, less than 20 are cultivated commercially on large scale for generating raw material for pharmaceutical companies/herbal drug companies (Planning Commission, Govt. of India 2000). A factor that discourages farmers from commercial cultivation of medicinal plants, especially in the high-altitude zones, is the long gestation period of these species (Planning Commission, Govt. of India 2000).

13.3 Issues and Challenges of Himalayan Medicinal Plants

13.3.1 Endemism and Restricted Distribution

The Himalayan medicinal plants are unique to this region and found nowhere on the earth. The unique climate of Himalayas is reason for these unique species of medicinal plants. Among various altitudinal ranges, temperate Himalayas have high endemism especially in northwest and west Himalayas compared to the entire Himalayan range (Dhar and Samant 1993; Dhar et al. 1996, 1998). Endemism is coupled with restricted distribution, as these plants require unique microclimates which lead to restriction of these medicinal species in unique microclimate including particular soil type, plant associates, and topography. For instance, *Nardostachys grandiflora* a high-altitude critically endangered Himalayan medicinal plant requires a typical habitat and grows on moist moss-laden rocky and boulder surfaces in crevices with sandy loam acidic soil consisting of residue from metamorphic crystalline rocks and high organic carbon content (7.23–8.96%) (Weberling 1975; Amatya and Sthapit 1994; Nautiyal et al. 2003; Ghimire et al. 2005). Likewise all the species of medicinal plants growing in Himalayas requires its own microclimate which has led to the restricted distribution of these species.

13.3.2 Endangerment of Himalayan Herbs

Increasing demand for medicinal plants globally has increased the annual turnover of Indian herbal medicine to 177,000 MT, which includes trade of 960 species from India (Ved and Goraya 2008). Many medicinal plants are being used in treatment of more than one disease, which has increased the demand for these species and ultimately overexploitation of the Himalayan herbs. This overexploitation resulted in endangerment of these miraculous healing herbs pushing them to the verge of extinction. Currently, about 120 species of Himalayan medicinal plants comes under the categories critically endangered, endangered, vulnerable, near threatened, and data deficient as per IUCN criteria (Samant et al. 1998a, b, 2007; Ved et al. 2003). Concerned with the loss of Himalayan biodiversity, Government of India had regulated collection of these herbs and banned endangered medicinal plants from natural habitats through Wild Life Act and Forest Conservation Act, but this ban has only promoted illegal trade as these species have long gestation period and collectors as well as pharmaceutical industries need profits in shortest time frame. Furthermore, the unscientific harvesting of these medicinal plants prevails due to lack of awareness of proper time, age, and exact growth stage for harvesting these valuable medicinal plants. Therapeutic value of these herbs is due to the presence of rare chemical content in them, and proper stage at which percentage of this content is high is the right stage to harvest for good quality raw material. Also in the Himalayas in absence of any other means of livelihood, owing to hostile climatic condition, local people depend on collection and trade of medicinal plants for livelihood (Larsen et al. 2000; Olsen and Larsen 2003; Bista and Webb 2006). This trade adds to economy of Himalayan nations (Olsen 2005), but now with this lack of alternative livelihood sources coupled with high demand by pharmaceutical companies has pushed these herbs into peril (Bista and Webb 2006). The collection of herbs traditionally for medicinal use is not a problem, until it is in harmony with the natural ecosystem (Cunningham 1993; Ghimire et al. 2005).

13.3.3 Reproductive Bottlenecks

Himalayan medicinal plants have several inherent unique features that contribute to their endangerment besides illegal trade, unscientific harvesting, and overexploitation. One among such inherent features is reproductive bottle neck. Successful reproduction is required for survival of any species, especially reproduction as it creates variability and healthy progeny that can maintain the genetic diversity of the species. These bottlenecks include self-incompatibility, pollen sterility, meiotic irregularities, low seed set, low seed viability, protracted seed germination, seed dormancy, male sterility, and exclusive dependency on pollinators. *Nardostachys grandiflora* is a critically endangered medicinal plant of the Himalayas with inherent infrequent flowering character, with only 8–10% plants in a population bearing flowers in a season impacting its multiplication (Gautam and Raina 2016). Being self-incompatible, *Aconitum heterophyllum, Sausurrea costus,* and *Inula racemosa* have to depend exclusively on pollinators or human interventions for reproduction (Nautiyal et al. 2009; Wafai et al. 2005; Wani et al. 2006). Presence of pollen sterility owing to meiotic irregularities in *Inula racemosa* makes reproduction impossible (Shabir et al. 2013). Low pollen longevity in *Gentiana kurrooa* impacts its reproduction (Raina et al. 2003). Thus, reproductive bottlenecks are other challenges faced by these herbs.

13.3.4 Lack of R&D

Research and development in Himalayan medicinal plants have not been carried out extensively although studies on their uses, cultivation practices, morphology, distribution pattern photochemistry have been carried out, but studies on their genetic system, breeding system, and reproductive biology are lacking in most of the species which hinder development of conservation plan for these species, as these studies are crucial for developing conservation protocol. The inherent features of reproduction of these species needs to be unraveled to assist successful seed set for sustaining its progeny and maximum variability in future generation. Furthermore, no empirical database on research is available regarding trade, demand, supply, and illegal trade of these medicinal plants. Because of this lacuna, a clear picture of existing situation cannot be synthesized. These studies are of utmost importance to assess the exact demand, to develop conservation programmes, and to draft policies on sustainable utilization and harvesting of these medicinal plants from wild.

13.3.5 Lack of Cultivation

Out of the total highly traded temperate medicinal plants, demand of only two or three species is met from cultivation, rest are wild harvested. Cultivation of these medicinal plants is very crucial for sustaining their supplies and conservation of endangered species. Cultivation of these medicinal plants has not received any attention despite initiatives taken by the Government of India (National Medicinal Plant Board); for example, many of these species have been prioritized for intensive cultivation and have been enlisted in negative trade practices. Package of practice on the cultivation of these medicinal plants has already been developed by researchers and scientists. In the absence of any blanket ban on the harvesting of the medicinal plants, pharmaceutical companies are exploiting wild stocks to produce herbal drugs and products, which are major cause for depletion of these natural resources.

Due to these challenges and issues of Himalayan medicinal plants, there is a need to start concerted efforts to conserve these medicinal plants for future generation as they not only act as therapeutic agents but also as integral part of our Himalayan ecosystem, and to maintain stability and integrity of any ecosystem, each and every flora and fauna (both macro and micro) needs to be protected. The need of the hour is to conserve, restore, and sustainably utilize these medicinal species without posing further threat on their survival.

13.4 Conservation of Himalayan Medicinal Plants

Conservation of Himalayan medicinal plants is of utmost importance for future generation. Conservation of these MAPs should focus on preserving the entire genetic diversity of targeted species by conserving its vast population that is adequate enough to represent all its rare and general alleles. For conservation, various conventional and nonconventional methods can be deployed along with taking concerted efforts for cultivation and reintroduction of these species into their natural habitat. Following strategies can be exploited effectively for conservation of Himalayan medicinal plants.

13.4.1 In Situ and Ex Situ Conservation

Conservation of medicinal plants in natural habitat (in situ) is a viable option as it will ensure natural regeneration; evolution of these plants, as each species is intertwined, and on-site conservation focuses on conservation of entire biodiversity/ecosystem thus maintain natural competing and evolving behavior of species. In situ conservation can be practices in national parks, biosphere reserves, wildlife sanctuaries, conservation reserves, etc. In situ conservation cannot survive on its own and must be supplemented with ex situ conservation. Ex situ conservation can be done similar to conserving these species in home gardens, sacred grooves, establishment of herbal gardens, botanical gardens, arboretum, field gene banks, etc. But in case of ex situ conservation, the species competitive ability to survive and further evolution cannot be sustained. For conservation, there must be a minimum viable population (smallest number of individuals required by any species to persist for long period, usually more than 100 years) present for the successful conservation.

13.4.2 Biotechnological Tools

Biotechnology is another important tool that can be exploited well in conservation of medicinal plants. Ex situ conservation of rare and endangered medicinal plant species through in vitro plant tissue culture techniques can be applied. Conservation of seeds, pollen, gene, and tissue culture, maintaining slow growth cultures can be done which are major biotechnological approaches for conservation of rare and endangered plant species (Paunescu 2009). These biotechnological tools allow of faster mass multiplication of endangered medicinal plant species for conservation of genotypes and can be harnessed to enhance active contents in medicinal plants (Nalawade et al. 2003).

13.4.3 Conservation by Cultivation

Conservation by cultivation of medicinal plants is also important because it will ensure production and availability of organic raw material of uniform quality without any sort of adulteration. Cultivation will also ensure continuous supply of raw material for pharmaceutical industries and high economic returns to the farmers. Moreover, cultivated products can easily be certified. It is reported that if cultivated, these species provide high potential returns to the farmer as most of these plants command high price in market. One study suggested that the cultivation of high-altitude Himalayan herbs could yield products priced anywhere between Rs. 7150 and 55,000 per hectare (Nautiyal 1994), indicating the worth of medicinal plants. Rao and Saxena (1994) reported average annual (per hectare) income of Rs. 120,000 through mixed cropping of high-altitude medicinal herbs. These medicinal can be successfully incorporated with other food crops and in any agroforestry systems of temperate regions.

National Medicinal Plant Board of India has already prioritized medicinal plants for cultivation in Himalayan states (www.nmpb.nic.in.). Although cultivation practices have been developed, but cultivation of medicinal plants in the temperate region has failed because of lack of availability of certified quality planting material, proper information on agro-techniques, exploitative market practices, minimum support price from the government and availability of package of practices in scientific languages not meant for farmers, etc.

Most of these medicinal herbs cannot be grown as a sole crop, therefore, they can be easily introduced as intercrop with other agricultural crops of the region, in agroforestry systems, fruit orchards, pastureland, and wastelands.

Moreover, this cultivation requires improved strains with the following characteristics:

- Synchronous flowering and maturity.
- Resistant to abiotic stress (drought, temperature, salinity, etc.)
- Resistant to biotic stress (disease and insect).
- Higher biomass of official part.
- High active content yield.
- Short gestation period.
- Less dependency on inorganic fertilization for higher productivity.

Understanding the reproductive biology of the species is a must for its conservation and development of improved strains. Pollination behavior (self or cross) of plant plays a significant role for the application of appropriate breeding systems. Concerted efforts have already been made by the researchers in determining the breeding system of some of the commercially important temperate/subtemperate medicinal plants (Table 13.4b). This information can be deployed for developing improved strains.

The studies conducted have been helpful in enhancing seed production in *Gloriosa superba* and developing selection of a high valepotriate yielding strain in *Valeriana jatamansi* and selection of different strains in *Hypericum perforatum* for higher biomass and active content (hyperin yield).

13.4.4 Sustainable Harvest from Wild

Escalating wild harvest is leading to overexploitation, and this practice has exposed many high-value medicinal plant species to the risk of extinction. Sustainable harvesting means that annual harvest must not exceed the annual renewal of the stock of plants.

For establishing sustainable/regulated harvesting methods, existing stocks of the medicinal species needs to be assessed. Furthermore, annual renewal rate of these medicinal species need to be studied to decide annual yield. Regulation of harvesting needs to address issues related to rights of the people over forest products, and local inhabitants should be included in regulated harvesting. There is need to generate alternate income sources for those who are entirely dependent on these resources for livelihood. If these species are sustainably harvested, they have high potential of income generation to harvesters and traders. Steps for regulating harvesting should include the following:

- Define area for regulating harvests and addressing the rights of indigenous people.

Table 13.3 List of endangered Himalayan medicinal plants

	Species	Conservation status	Trade status
1.	*Aconitum chasmanthum*	Critically endangered	Traded
2.	*Aconitum deinorrhizum*	Endangered	Traded
3.	*Aconitum ferox*	Endangered	Highly traded
4.	*Aconitum heterophyllum*	Endangered	Highly traded
5.	*Angelica glauca*	Endangered	Traded
6.	*Arnebia benthami*	Critically endangered	Traded
7.	*Arnebia euchroma*	Critically endangered	Traded
8.	*Atropa acuminate*	Critically endangered	Highly traded
9.	*Betulautilis*	Endangered	Traded
10.	*Dactylorhiza hatagirea*	Endangered	Traded
11.	*Dioscorea deltoidea*	Endangered	Traded
12.	*Epherda gerardiana*	Critically endangered	Highly traded
13.	*Ferula jaeschkeana*	Vulnerable	Traded
14.	*Fritillaria cirrhosa*	Endangered	Traded
15.	*Gentiana kurroo*	Critically endangered	Traded
16.	*Habenaria intermedia*	Endangered	Not recorded
17.	*Hyoscyamus niger*	Endangered	Traded
18.	*Jurinea dolomiaea*	Endangered	Highly traded
19.	*Lilium polyphyllum*	Critically endangered	Not recorded
20.	*Malaxis musifera*	Critically endangered	Not recorded
21.	*Meconopis aculeate*	Endangered	Not recorded
22.	*Nardostachys grandiflora*	Critically endangered	Highly traded
23.	*Panaxpseudo ginseng*	Endangered	Not recorded
24.	*Paris polyphylla*	Endangered	Traded
25.	*Picrorhizakurroa*	Endangered	Highly traded
26.	*Podophyllum hexandrum*	Endangered	Traded
27.	*Polygonatum cirrhifolium*	Endangered	Traded
28.	*Rheum emodi*	Endangered	Highly traded
29.	*Rheum moorcroftianum*	Endangered	Highly traded
30.	*Saussurea obvallata*	Critically endangered	Not recorded
31.	*Saussurea costus*	Critically endangered	Highly traded
32.	*Swertia chirayita*	Critically endangered	Highly traded
33.	*Taxus wallichiana*	Endangered	Highly traded
34.	*Zanthoxylum armatum*	Endangered	Not recorded

(*Source*: Siwach et al. 2013) high traded >100 Metric Ton/year

- Local community should be involved in management and sustainable harvesting, and thus joint forest management could be a best approach. Furthermore, local harvester must be registered legally.
- Involve nongovernmental organizations to sensitize people and implement this system along with generation of alternative income sources to people who are completely dependent on these medicinal herbs for livelihood.

Table 13.4a Medicinal plant species suitable for cultivation in temperate regions

S. No	Species	S. No	Species
1.	*Aconitum heterophyllum*	10.	*Crocus sativus*
2.	*Aconitum ferox/Aconitum balfouri*	11.	*Saussurea costus*
3.	*Hedychium spicatum*	12.	*Picrorhiza kurroa*
4.	*Swertia chirayita*	13.	*Valeriana jatamansi*
5.	*Bunium persicum*	14.	*Boerhaavia diffusa*
6.	*Berberis aristata*	15.	*Dactylozhiza hategria*
7.	*Ferula foetida*	16.	*Hippophae rhamnoides*
8.	*Nardostachys grandiflora*	17.	*Asparagus racemosus*
9.	*Podophyllum hexandrum*	18.	*Taxus wallichiana*

- Involve people in evaluation and monitoring of sustainable harvesting practices.
- Capacity building and skill development programs must be arranged for local people involved in sustainable harvesting.

Moreover, sustainable harvesting means harvesting of plant parts at proper time, that is, at maturity stage and when active content is highest; further harvesting of plant parts must be done only after seed shedding by plants to ensure natural regeneration of species. In many species, harvesting schedule have already been developed, Table 13.4c summarizes information on the harvesting schedule of some important medicinal plants. This information can be used for optimizing wild harvests, if required in accordance to biodiversity concerns.

13.4.5 Conservation by Reintroduction of Species into Nature

Conservation and reintroduction of species into wild/nature must go hand-in-hand to enhance the stock of these medicinal plants. Furthermore, bringing new species into cultivation takes lot of time as plants need to adjust to the new environment. Only those species having high commercial value can be cultivated by farmers as it is profitable. To introduce these species into the existing cropping pattern, research needs to be conducted which is a long-term effort requiring financial assistance and support.

Therefore, steps should be taken to reintroduce endangered Himalayan medicinal plants species in nature/wild for conservation and sustaining their regular supply. Thus, cultivation/reintroduction will pose minimum difficulties with minimum efforts as the environment will be natural and no problems related to establishment, growth, and yield will be encountered. Reintroduction should focus on the following steps:

- Identify endangered and prioritized medicinal plant species of Himalayan regions.

Table 13.4b Breeding system attributes of some medicinal plants

Species	Breeding system	Diploid chromosome no.	Ploidy status	Reproduction	Flower	References
Picrorhiza kurroa	Xenogamy	34	Genomic allotetraploid	Vegetative; Seeds	Bisexual	Raina et al. (2010b)
Gentiana kurroo	Xenogamy	26	Genomic allotetraploid	Vegetative; Seeds	Bisexual	Raina et al. (2003)
Swertia chirayita	Autogamy Xenogamy; Gnetenogamy	26	Genomic allotetraploid	Seeds only	Bisexual	Raina et al. (2013)
Hypericum perforatum	Autogamy	32	Genomic allotetraploid	Seeds only	Bisexual	Mustafa (2006)
Valeriana jatamansi	Autogamy Xenogamy; Gnetenogamy	32	Genomic allotetraploid	Vegetative; Seeds	Pistillate; Bisexual	Raina et al. (2010a)
Podophyllum hexandrum	Autogamy Xenogamy;	12	Diploid	Vegetative; Seeds	Bisexual; Solitary on a plant	Kamini (2016)
Nardostachys grandiflora	Xenogamy	78	Hexaploid	Vegetative; Seeds	Bisexual	Gautam and Raina (2016)
Angelica glauca	Xenogamy	22	Diploid	Vegetative; Seeds	Bisexual	Kamini (2016)

Table 13.4c Harvesting schedule of some medicinal plants based on active content

S. No	Species	Plant part to be harvested	Harvesting stage	%age content	References
1.	*Hypericum perforatum*	One-third of the plant from top	Full flowering	0.075% Hypericin	Mustafa (2006)
2.	*Swertia chirayita*	Whole plant	Full flowering	0.227% amarogentin; 0.071% amaroswerin	Raina et al. (2013)
3.	*Solanum laciniatum*	Berries	Dark green colored	≥4.0% *solasodine*	Rastogi (1990)
		Leaves	Mature	~1.0% *solasodine*	
4.	*Andrographis paniculata*	Aerial biomass	Flowering stage	~2.0% andrographolide	Bhandari (2000)
5.	*Valeriana jatamansi*	Rhizome	>Two-years old in autumn season	~4% Valepotriates;	Sharma (1993)
		Roots	>Two-years old in autumn season	~4% Valepotriates ~2.0% E. Oil	
6.	*Gloriosa superba*	Seeds	Ripened	0.70% colchicine	Gupta (1997)
		Tubers	Dormant stage	0.25% colchicine	
7.	*Mucuna sp (white seeded)*	Seeds	Ripened	5.5% L-Dopa	
	Mucuna pruriens (black seeded)	Seeds	Ripened	6.0–7.0% L-Dopa	Chandra (2001)
8.	*Picrorhiza kurroa*	Rootstock	> 3-yr old in autumn season	Picroside-I: 0.26–3.7% (rhizomes) & 0.10–1.12% (roots). Picroside-II: 2.60–7.08% (rhizomes) and 2.34–6.71% (roots)	Mehra (2006)
9.	*Podophyllum hexandrum*	Rhizome	Three-leaved plants in autumn season	4.3% podophyllotoxin	Mahajan (2004)

- Identify the best-suited forest area for reincorporation, replanting, and mass multiplication.
- Initially, selection and enclosing of a small area (say approximately 10 ha) in a reserved forest can be carried out. This area should be seeded by propagules of the selected species and focus should be given on use of superior genotypes, if available to enhance yield.
- Outside interference for at least not less than three growing cycles of the selected species should be avoided so that these introduced plants are able to produce, set, and disperse seed. Once that is achieved, then natural regeneration process will start along with its spreading to nearby areas and spread beyond the restricted areas.

- No extraction activity should be permitted till the stock of plants reach a point; it can be called as abundant.

13.5 Government's Initiative for Conservation of Medicinal Plants

At international level, organizations like IUCN (International Union for Conservation of Nature), CITES (The Convention on International Trade in Endangered Species of Wild Fauna and Flora), and Traffic (Trade Record Analysis of Flora and Fauna in Commerce) are working on the conservation of threatened plants. In India, Forest Conservation Act, 1980 and National Wildlife Act of 1972 regulates the trade and any kind of damaging activities to such plants must be punished. Various agencies like National Medicinal Plant Board of India (NMPB), Botanical Survey of India, National Biodiversity Action Plan by Ministry of Environment and Forests (MoEF) indicate that government is also concerned about our biodiversity and is taking necessary steps in this regard. All India Coordinated Research Project on Medicinal Plants and Beetle Wine (AICRP on MP&B) run by Indian Council of Agricultural Research (ICAR) in various agroclimatic zones of India including Himalayan region is also taking necessary steps for conservation of medicinal plant biodiversity. CSIR (Council for Scientific and Industrial Research) is also focusing on conservation of medicinal plants.

13.6 Way Forward

To conserve biodiversity, Himalayan medicinal plant's sustainable utilization should be targeted at various levels with particular focus on enhancing stock of these plants in wild, improving living standards of indigenous people, by changing the attitude of people to take up cultivation of these plants, development of modern technologies for cultivation, making policies on restricting illegal trade. First step would be putting restriction on harvest of raw material from wild along with commercialization of these medicinal plants by providing incentives to the farmers for growing these species. Encouraging pharmaceutical industries for taking up cultivation of these medicinal plants in association with the farmers should be the next priority. Farmers should be provided knowledge and hands-on training on cultivation, harvesting, and post-harvesting of these medicinal plants so that they reap the harvest profitably. But for cultivation, elite strains are required, therefore, researchers should take up work on developing high yielding and short gestation strains of these plants. As we know that not all species are amenable for cultivation, therefore restricted harvesting along with in situ cultivation of such species should be promoted in association with governmental and nongovernmental organizations. Various biotechnological tools and ex situ techniques should also be deployed to conserve germplasm. Most importantly, various institutes of Himalayan regions should be allotted prioritized species for tackling R&D issues and to avoid duplication of work. Moreover, state governments of the Himalayan region must develop policies to curb over exploitation and illegal trading.

13.7 Summary and Conclusion

Himalayan medicinal plants are wonderful gift of nature to us; their conservation coupled with sustainable use is the way of preserving them for our future generation. But due to various reasons, namely, illegal trade, unscientific harvest, habitat fragmentation, they are in peril and immediate attention along with well-defined steps and plan is need of the hour to save them for human beings and to maintain integrity and stability of the ecosystem. People involved in their trade and also pharmaceutical industries need to think sensibly to get involved in the sustainable, scientific harvesting of these herbs and must practice cultivation of these species for producing raw material. Concerted efforts need to be taken on research of these herbs especially to find out the exact amount of ongoing trade in these species along with finding out the number of species involved, to find out amount of raw material that can be harvested annually on sustainable basis, to promote reintroduction of these species into nature, and in developing conservation plan for these species.

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Chapter 14
Approaches Towards Threatened Species Recovery in Medicinal Plant Conservation Areas (MPCA)–Case Studies from South India

C. Kunhikannan, B. Nagarajan, V. Sivakumar, and N. Venkatasubramanian

Abstract Species recovery is an action in which reduction of a species in danger of threats at different levels is evaded or reversed. The present work explains the different steps involved in species recovery research, which further aimed at inventorization of 20 red listed medicinal plant species, their reproductive biology, seed biology, and population structure in Medicinal Plant Conservation Areas (MPCA) at Silent Valley (Kerala) and Kolli Hills (Tamil Nadu).

In Silent Valley MPCA, *Aphanamixis polystachya, Canariun strictum, Cinnamomum sulphuratum, Embelia ribes, Glycosmis macrocarpa, Hydnocarpus alpina, Nothapodytes nimmoniana* are found to be threatened in varying degrees. In Kolli Hills, populations of *Aristolochia tagala* and *Rhaphidophora pertusa* are restricted to a very few patches. In *A. tagala,* the problem is related to its breeding system. It needs specialist pollinators (very small flies), in which nothing is known about their life history cycles. In case of *Smilax zeylanica* and *Symplocos cochinchinensis var. laurina,* plenty of solitary bees are found to be potential pollinators. In *Aristolochia tagala, Symplocos cochinchinensis, var. laurina* and *E. ribes,* no bottleneck could be noticed during the pre-zygotic process or during zygote development. It is during the post-zygotic phase that most seeds are lost in the form of herbivory. Species such as *Smilax, Aristolochia, Embelia,* and *Canarium* show high rates of germination under controlled conditions; thus, establishing nursery at study sites would aid in achieving higher seed to seedling ratio. In *Embelia ribes,* fruit production is high, but very poor natural regeneration is a threat factor.

Scanty information is available for propagation and ex situ conservation of these species in seed banks. Seed-handling techniques for *Aristolochia tagala*, *Canarium strictum*, *Garcinia gummi-gutta, Persea macrantha Symplocos racemosa*, *Embelia ribes, Smilax zeylanica, and Myristica dactyloides* have been standardized. The study indicated that the seeds of *A. tagala* germinate readily and can be stored up to 18 month without serious loss of its viability. Germination of *Canarium strictum*

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seeds required some pre-treatments. The seeds can be stored at 20°C for 10 months with 42% germination. Seeds of *Persea macrantha, Symplocos racemosa, and Myristica dactyloides* were found to be sensitive to desiccation. Seed germination studies on *E. ribes* revealed the necessity of pre-treatment with GA3. Through a series of studies in different species, the status of population in wild, reproductive constraints, seed-handling techniques, and production of planting materials could be achieved. The species *Embelia ribes, Canarium strictum, Symplocos racemosa,* and *Glycosmis macrocarpa* require some in-depth study to reveal their exact status.

Keywords Medicinal plant conservation area · Species recovery · Silent Valley · Kolli Hills · Threatened medicinal plants · Seed handling · Reproductive biology

14.1 Introduction

Species recovery is a process that reduces a given species from risk of threat at different levels is evaded or reversed, so that threats to its survival are encountered and its long-term protection is guaranteed. Through this process, the species in threat could be brought to a level wherein it self-sustains its population. The primary objectives of a species recovery programme are (1) assessing the threat status, (2) understanding whether threat factor/s are biotic/abiotic, (3) quantifying population status, (4) identifying strategies that are necessary to reduce or eliminate threats, (5) applying resources available to the highest priority recovery tasks, and (6) finally reclassifying and delisting the species as appropriate to its case.

India is endowed with a spectrum of plants, animals, environmental conditions, and equally found are ethnic groups with vast knowledge of traditional medicines who exploit these resources for preparing drugs. There are more than 7000 species used in about 10,000 herbal formulations. Ninety percent of the raw material for these formulations is obtained from the wild. Due to rapid destruction of forest by various means and over exploitation of medicinal plants, most resources are becoming rare or endangered. Thus, conservation of natural forest resources including medicinal plants is inevitable. To achieve this goal, several conservation programmes have been initiated by faunal conservation, exclusive to species such as tiger, elephants, rhinos, wild asses, and several others. However, there is an exclusive conservation focusing on selected plant species such as *Rhododendrons* (Himalayas), *Nepenthes* (Meghalaya), and *Strobilanthes spp.* (Nilgiris).

Medicinal plants are one among the threatened groups due to their economic significance. It is estimated that 6000–7000 species in India have usage in folk and other system of medicine. Out of which, 960 species are traded for several medical purposes. Among these, 178 species are consumed in excess of 100 metric tons annually. Due to heavy demand, it is evident that thousands of species in our country are under various degrees of threat. Conservation of such species within its own habitats is easier and economical. However, in certain cases, individual species-level conservation measures are also important. To address the need for

Table 14.1 Species selected for the study in Silent Valley (Western Ghats) and Kolli Hills (Eastern Ghats)

Silent Valley		Kolli Hills	
Name of the species	Status	Name of the species	Status
Aphanamixis polystachya	***VU/R***	*Aristolochia tagala*	***VU/R***
Canarium strictum	***VU/R***	*Canarium strictum*	***VU/R***
Cinnamomum sulphuratum	***Vu/G***	*Celastrus paniculatus*	***VU/G***
Embelia ribes	***Lr-nt/R***	*Myristica dactyloides*	***VU/R***
Garcinia gummi-gutta	***VU/G***	*Persea macrantha*	***EN/R***
Garcinia morella	***VU/R***	*Rhaphidophora pertusa*	***VU/R***
Glycosmis macrocarpa	***Lr-nt/G***	*Santalum album*	***EN/R***
Hydnocarpus alpina	***EN/R***	*Smilax zeylanica*	***VU/R***
Nothapodytes nimmoniana	**VU/R**	*Symplocos cochinchinensis*	**Lr-nt/R**
Myristica dactyloides	**VU/R**		
Myristica malabarica	**VU/G**		
Persea macrantha	**EN/R**		
Piper mullesua	**VU/R**		
Plectranthus nilgherricus	**VU/G**		
Smilax zeylanica	**VU/R**		
Symplocos racemosa	**VU/R**		

R regional, *G* global, *VU* vulnerable, *EN* endangered, *Lr-nt* low risk near threatened
As per Ravikumar and Ved (2000)

conservation of medicinal plants, a network of in-situ (field) gene banks within forest habitats become essential as a cost-effective method to maintain the intra-specific plant diversity. These in situ (field) gene banks can also be deployed as study sites to comprehend the life history traits of the species. In order to capture the diversity of medicinal plants occurring in the forests of 12 states, a network of 108 Medicinal Plants Conservation Areas (MPCAs) have been established across different forest types and altitude zones in these 12 states of peninsular India (FRLHT 2018). Each of these sites is approximately 200 ha in size and the choice of these sites has been on the basis of criteria (Ravikumar 2010) such as the following:

1. Sites across the different forest types and altitudinal zones in the region.
2. Sites in areas that are traditionally well known for medicinal plant wealth and exploitation.
3. Sites in the areas known for high proportion of endemic species (Genetically Diverse Hot Spots, or GDHS).
4. Possibility of implementing adequate management interventions at the sites.

Later, National Medicinal Plant Board (NMPB) also started supporting the medicinal plant conservation in India through establishment of Medicinal Plants Conservation and Development Areas (MPCDAs) throughout the country. Presently, 72 MPCDAs spread over 13 states were supported by NMPB for in situ conservation of medicinal plants (Biswas et al. 2017)

The present study involves the ecological status, spatial distribution mapping, and population dynamics of the selected 20 threatened medicinal plants (Table 14.1;

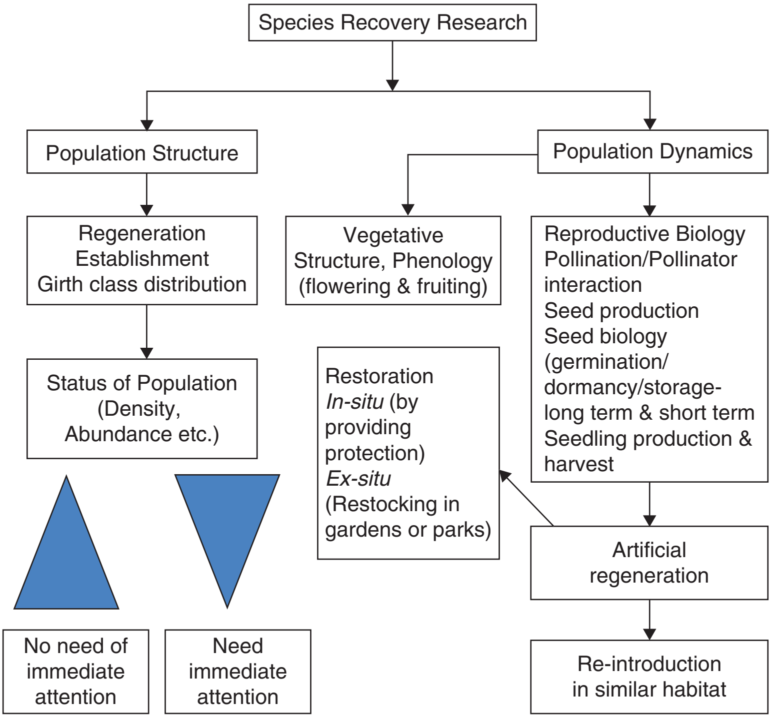


Fig. 14.1 General scheme of species recovery research

Annexure 14.1) distributed in MPCAs of Silent Valley, Western Ghats (Kerala), and Kolli Hills, Eastern Ghats (Tamil Nadu), along with reproductive biology, seed biology, and seed handling techniques so as to identify the problems in relation to these species.

14.2 Scheme of Species Recovery Research

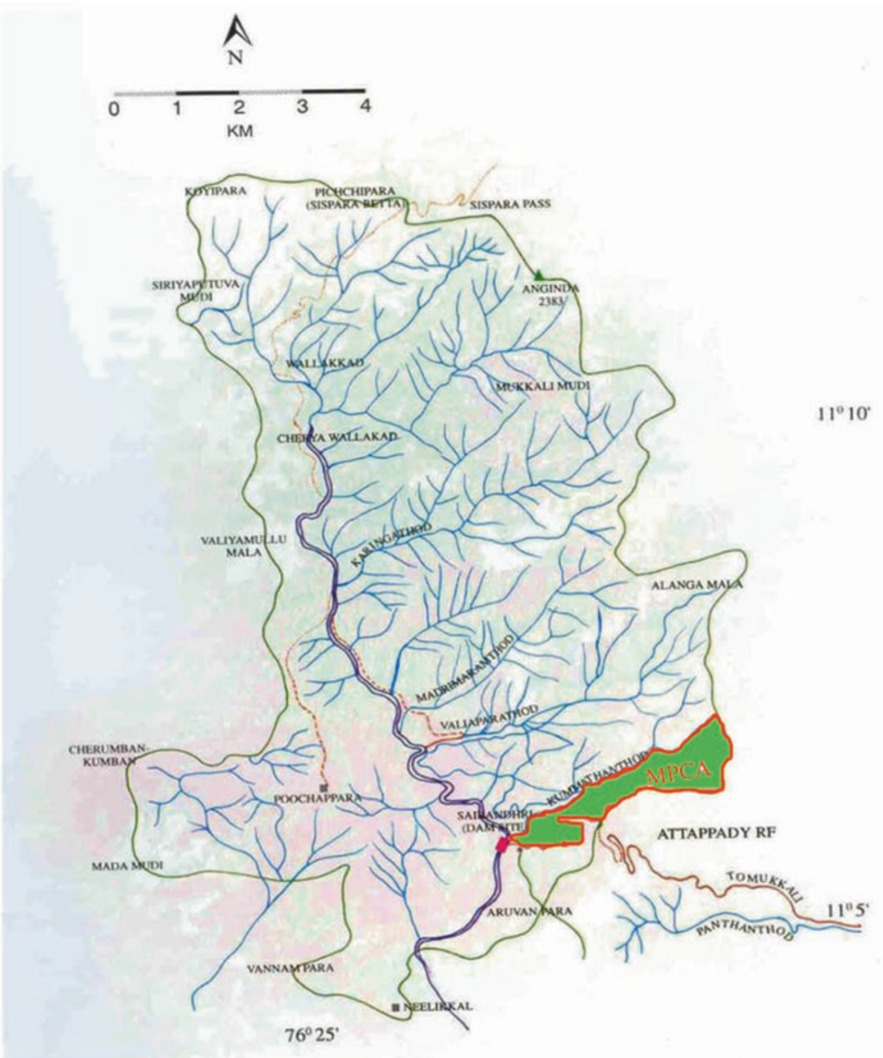
Species Recovery Research (SRR) involves population- and individual-level studies. On the basis of studies in the population in relation to the environment, phenology, and interactions, species-level studies are taken up. General Scheme of Species Recovery Research is given below (Fig. 14.1).

Plate 14.1 *Ipsea malabarica*, an orchid species rediscoverd after 130 years

14.3 Study Location

14.3.1 Silent Valley

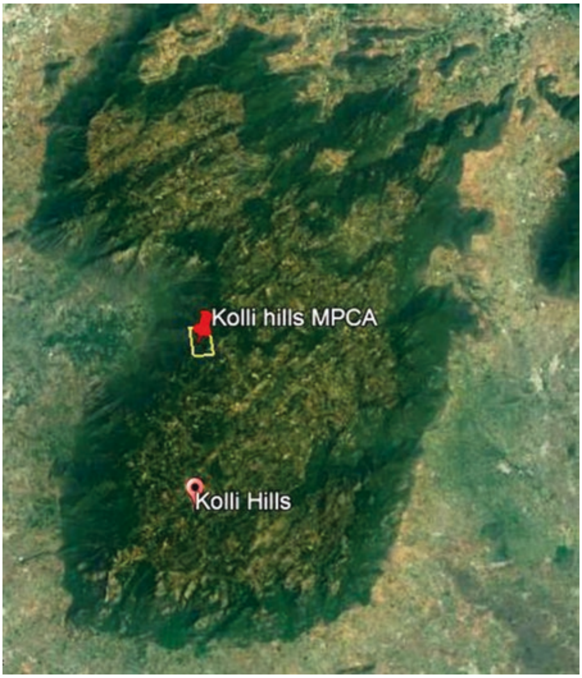
Silent Valley National Park as a part of Western Ghats (Latitude 11°03′–11°13′ N and Longitude 76°24′–76°32′ E) in Palakkad district, Kerala, is a highly protected habitat. Silent Valley forest is locally known as Sairandhrivanam. The evolutionary age of the forest is believed to be more than 50 million years. This is a cliff forest, which abruptly descends from the Nilgiri plateau to the plains of Kerala with a sudden drop in altitude from 2500 to 150 meters across a distance of 3–4 km. The biodiversity-rich forest leaped to importance in 1973 when the Kerala State Electricity Board decided to implement the Silent Valley Hydro-Electric Project (SVHEP) on a dam across the Kunthipuzha River. The resulting reservoir would have submerged 8.3 km^2 of virgin rainforest (KSSP 2009) and threatened several rare and endangered species like the lion-tailed macaque, Nilgiri langur, and the orchid *Ipsea malabarica* (Plate 14.1), rediscovered during the 1980s after its initial collection in 1852 (Manilal and Kumar 1983). The issue was brought to public attention and ultimately protected through the commitment of nature lovers, teachers, students, poets, and like-minded people after a decade-long struggle. In 1983, Smt. Indira Gandhi, the then Prime Minister of India, decided to abandon the project and the Silent Valley forest was declared as a National Park. It was formally inaugurated by Shri Rajiv Gandhi, the then Prime Minister of India on September 7, 1985, and it was designated as the core area of the Nilgiri Biosphere Reserve on September 1, 1986. Since then, a long-term conservation effort has been undertaken to preserve the Silent Valley ecosystem. The National Park extends over an area of 89 km^2 from Nilambur vested forest and part of Nilgiris in the north, vested forest of Palakkad division in the south, and the Attappadi reserve forests in the east. To



Map 14.1 Location of MPCA in Silent Valley National Park

provide better protection, an area of 147.22 km^2 of forested land was added to the Silent Valley as a buffer zone in November 2009.

The MPCA area matches the west coast tropical evergreen forest type (Basha 1987) and covers area of 200 ha all along the Kattivaramudi, down through Kummatanthodu to Kunthi River (Map 14.1). The Silent Valley National Park is perhaps the only remaining undisturbed tropical rain forest in Kerala state as well as in peninsular India. The forests show all the known characteristics of a tropical rain forest (Basha 1999). Silent Valley is unique in their vegetation, weather, flora, and



Map 14.2 Location map of MPCA in Kolli Hills (adopted from Google)

fauna. Many species of flora and fauna are yet to be discovered and described. Pulses of life can be experienced in all parts of the park either on small stone or big rock, tree trunk, river, rivulets, streams, even on tiny leaves. The plant life forms ranging from gigantic trees like *Ficus nervosa, Bischofia javanica* (Chola venga), *Acrocarpus fraxinifolius*, *Elaeocarpus tuberculatus*, woody climbers like *Ancistrocladus heyneanus, Caesalpinia cucullata, Carissa inermis, Diploclisia glaucescens, Gnetum ula, Oxyceros rugulosus, Smythea bombaiensis, Tetrastigma leucostaphylum, and Toddalia asiatica;* plants appearing only once in a year like the *Epipogium roseum* (a subterranean saprophytic orchid), *Balanophora fungosa* (a parasite on tree roots) to tiny lichens, bryophytes, fern and fern allies, as well as animals. All these life forms make this National Park a unique site.

14.3.2 Kolli Hills

Kolli Hills (Kollimalai) of Eastern Ghats, Namakkal district (11° 10′–11° 30′ N/75° 15′–75° 30′ E), Tamil Nadu, is well known for its biological diversity (Map 14.2). The area is otherwise called as Chaturagiri or square hill. It has high rising peaks and ravines. Slopes are quite steep forming several narrow and deep valleys and in some places rising abruptly from plains and generally steep near ridges, so that the

Plate 14.2 Kolli Hills: A bird eye view

edge of the plateau is sharply defined. Kolli Hills is drained by two rivers, Vasisthanadhi and Swetanadhi.

Kolli Hills falls under the categories of scrub jungle, dry deciduous, moist deciduous, semi-evergreen to evergreen, and shola vegetation (montane wet temperate forests). On the western side of the hills, patches of sholas still exist, though a great portion of the plateau has been cleared (Plate 14.2). The maximum temperature ranges between 20 and 30 °C and the minimum between 10 and 20 °C. The hills receive rain from both the south west and north east monsoons. The mean annual rainfall is 1043 mm (Lakshmi 1995).

14.4 Results and Discussion

14.4.1 Ecological Assessment

In Silent Valley, the total woody species of ≥10 cm Girth at Breast Height (GBH) available was 124, belonging to 51 families and 92 genera including 20 lianas. This includes the selected medicinal plant species (Table 14.1) for the study. Majority of the species showed contiguous distribution in both the sites. In Silent Valley MPCA, distribution of *Cinnamomum sulphuratum, Glycosmis macrocarpa, Embelia ribes,* and *Nothapodytes nimmoniana* are restricted to some patches. Most of the species in the study area showed good regeneration except *Cinnamomum sulphuratum, Hydnocarpus alpina* (seedlings were not recorded during this study), *Nothapodytes nimmoniana,* and *Garcinia gummi-gutta* (complete absence of seedlings and saplings). Among the flora, *Myristica dactyloides* showed highest frequency (96.67%) and density (142.5 stems ha^{-2}) in Silent Valley followed by *Garcinia morella* and *Persea macrantha*. The total stand density of the site was 2478.29 stems ha^{-2} and the top 10 species (Table 14.2) were accounted for occupying 44.08% of stand

Table 14.2 Phytosociological attributes of major tree species associated with the focal species of medicinal plants in Silent Valley

Species name	Density (stems/ha.)	Frequency(%)	Abundance	A/F	RD	RF	R Dom.	IVI
Cullenia exarillata	151.67	90.00	6.74	0.07	6.12	3.01	29.20	38.33
Myristica dactyloides[a]	142.50	96.67	5.90	0.06	5.75	3.23	7.92	16.90
Palaquim ellipticum	105.83	80.00	5.29	0.07	4.27	2.67	9.04	15.99
Dimocarpus longan	156.67	80.00	7.83	0.10	6.32	2.67	2.19	11.18
Syzigium laetum	125.83	73.33	6.86	0.09	5.08	2.45	0.92	8.45
Agrostistachys meeboldii	105.00	53.33	7.88	0.15	4.24	1.78	2.20	8.22
Cryptocarya bourdilloni	69.17	96.67	2.86	0.30	2.79	3.23	2.03	8.05
Persea macrantha[a]	45.00	70.00	2.57	0.04	1.82	2.34	3.75	7.91
Gomphandra tetrandra	93.33	73.33	5.09	0.07	3.77	2.45	1.40	7.62
Clerodendrum viscosum	97.50	50.00	7.80	0.15	3.94	1.67	0.50	6.11
Garcinia morella[a]	70.00	73.33	3.82	0.05	2.83	2.45	0.74	6.02
Mesua nagassarium	27.50	70.00	1.57	0.02	1.11	2.34	2.19	5.64
Oreocnide integrifolia	60.83	63.33	3.84	0.06	2.46	2.12	0.73	5.30
Aglaia anamallayana	59.17	53.33	4.44	0.08	2.39	1.78	1.10	5.27
Holigarna nigra	17.50	43.33	1.62	0.04	0.71	1.45	3.10	5.25
Turpinia malabarica	45.83	56.67	3.24	0.06	1.85	1.89	1.38	5.13
Canarium strictum[a]	22.50	53.33	1.69	0.03	0.91	1.78	2.38	5.07
Casearia wynadensis	48.33	70.00	2.76	0.04	1.95	2.34	0.52	4.80
Xanthophyllum flavescens	53.33	56.67	3.76	0.07	2.15	1.89	0.51	4.56
Aphanamixis polystachya[a]	26.67	46.67	2.29	0.05	1.08	1.56	0.95	3.58
Symplocos racemosa[a]	9.16	23.33	1.57	0.07	0.36	0.78	1.22	2.37
Hydnocarpus alpina[a]	18.33	26.67	2.75	0.10	0.74	0.89	0.20	1.83
Cinnamomum sulphuratum[a]	9.17	10.00	3.67	0.37	0.37	0.33	0.22	0.92
Garcinia gummi-gutta[a]	1.67	6.67	1.00	0.15	0.07	0.22	0.13	0.42
Nothapodytes nimmonianum[a]	0.83	3.33	1.00	0.30	0.03	0.11	0.00	0.15
Embelia ribes[a]	10.00	10.00	4.00	0.40	0.40	0.33	0.02	0.75
Piper mollusua	18.50	10.00	1.00	0.1	–	–	–	–
Glycosmis macrocarpa[a]	130.00	13.33	1.75	0.13	–	–	–	–
Smilax zeylanica[a]	315.30	43.33	2.69	0.06	–	–	–	–

[a]The focal species come across in the study plots

density and 42.95% of IVI values. Among them, *Cullenia exarillata*, *Myristica dactyloides, Palaquium ellipticum,* and *Dimocarpus longan* had the maximum share. *Cullenia exarillata* alone contributed to 6.36% of stand density and 12.78% of IVI. While *Myristica dactyloides* alone contributed 5.75% of stand density, and 5.64% of total IVI values. Contribution of 44.08% of total stand density and 42.95% of total IVI values by said 10 tree species is significant in occupying majority of spatial and nutritive resources. The focal species alone contributed 14.38% of stand density and 15.31% of IVI values. *Myristica dactyloides* contributed 39.99% of density and 36.81% of IVI of all the focal species.

Density for trees and lianas in the MPCA ranged from 0.83 to 157.67 stems ha^{-2}. Density (stems ha^{-2}) of trees and lianas among the focal species varied from 0.83 to 142.5. *Myristica dactyloides* had the maximum density (142.5) followed by *Garcinia morella* (70), *Persea macrantha* (45), and *Aphanamyxis polystachya* (26.67). *Nothapodytis nimmoniana* had the lowest density among the focal species (0.83). Chances of locating *N. nimmoniana* in the MPCA area is less, because its natural range of occurrence is in higher elevation of Silent Valley (Manilal 1988). The major associates of *Myristica dactyloides* and *Garcinia morella* are *Cullenia exarillata, Cryptocarya bourdilloni, and Palaquim ellipticum.*

In Kolli Hills, 102 woody species were enumerated, which include 79 trees and 23 lianas. All the focal species could be located in the MPCA. From the studies, it was found that highest density value was shown by *Memecylon umbellatum,* followed by *Myristica dactyloides, Diospyros ovalifolia, Symplocos cochinchinensis var. laurina,* which varied from 252 to 75 stems ha^{-2}.

Memecylon umbellatum showed highest frequency followed by *Persea macrantha, Myristica dactyloides, Scolopia crenata* that varied from 70% to 95%. Among the focal species, *Persea macrantha* showed highest frequency (80%) followed by *Smilax zeylanica* (77.5%), *Myristica dactyloides* (70%), *Canarium strictum* (60%), and *Smilax zeylanica* showed the highest density (1333/ha) followed by *Celastrus paniculata* (277 seedlings only), *Myristica dactyloides* (140), *Rhaphidophora pertusa* (110 very localized population). *Canarium strictum* showed the highest IVI value (28.24) among the entire stand (Table 14.3).

14.4.2 Regeneration and Population Structure

Relative distribution of individuals in different girth classes was used to prepare population structure. This partly indicates regeneration behaviour and future composition of the forest community. Inventorying the number of seedlings, saplings, and adult trees is an easy method to assess this aspect. Although the size of individuals may not be correlated with age, an overall trend can be obtained. The trend of population structure of different focal species in MPCA showed considerable variation. Most of the dominant species in MPCA had adequate proportion of individuals in lower size classes. On the contrary, lesser number of individuals in intermediate and very low number in larger girth classes. Relative proportions of girth classes for

Table 14.3 Phytosociological attributes of major tree species associated with the focal species of medicinal plants in Kolli Hills MPCA

Species name	Density (stems/ha.)	Frequency (%)	Abun dance	A/F	RD	RF	R Dom.	IVI
*Canarium strictum**	52.50	60.00	3.50	0.06	3.16	2.86	22.22	28.24
Memecylon umbellatum	252.50	95.00	10.63	0.11	15.21	4.52	6.29	26.02
*Myristica dactyloides**	140.00	70.00	8.00	0.11	8.43	3.33	8.62	20.39
Artocarpus heterophyllus	26.25	55.00	1.91	0.03	1.58	2.62	9.54	13.74
*Persea macrantha**	61.25	80.00	3.06	0.04	3.69	3.81	6.21	13.71
Syzygium cumini	43.75	50.00	3.50	0.07	2.64	2.38	8.32	13.33
Prunus ceylanica	15.00	45.00	1.33	0.03	0.90	2.14	8.72	11.76
Memecylon umbellatum	72.50	55.00	5.27	0.10	4.37	2.62	3.10	10.08
Diosyiros ovalifolia	95.00	55.00	6.91	0.13	5.72	2.62	0.95	9.30
Aglaia sp.	32.50	40.00	3.25	0.08	1.96	1.90	5.01	8.88
Scolopia cranata	61.25	70.00	3.50	0.05	3.69	3.33	1.62	8.64
Neolitsea scrobiculata	55.00	60.00	3.67	0.06	3.31	2.86	1.38	7.55
*Symplocos cochinchinensis var. laurina**	75.00	50.00	6.00	0.12	4.52	2.38	0.40	7.30
Pavetta indica	65.00	65.00	4.00	0.06	3.92	3.10	0.20	7.21
Canthium diococcum	50.00	55.00	3.64	0.07	3.01	2.62	0.26	5.89
Maesa indica	45.00	50.00	3.60	0.07	2.71	2.38	0.16	5.25
*Celastrus paniculata**	277.00	17.50	1.43	0.08				
*Aristolochia tagala**	83.30	5.00	1.50	0.30				
*Rhaphidophora pertusa**	110.00	2.50	4.00	1.60				
*Smilax zeylanica**	1333.99	77.50	5.26	0.07				

* Focal species

various species/ha were calculated and population structure was derived using the following girth classes (GBH in cm) for young and mature trees, that is, 10–30, 30–60, 60–90, 90–120, 120–150, 150–180, >180. Girth class-wise distribution of the focal species in Silent Valley MPCA showed that *Myristica dactyloides* only was represented in all the girth classes with higher number of individuals in lower girth classes compared to the higher ones (Fig. 14.2).

Among the selected species (Table 14.1), populations of *Aristolochia tagala* and *Rhaphidophora pertusa* were found to be restricted to just few patches. Species like *Canarium strictum, Myristica dactyloides, Persea macrantha,* and *Symplocos cochinchenensis var. laurina* showed more number of individuals in lower size classes such as 10–30 cm girth and less number in higher girth class, indicating that these species have a viable population in the site (Table 14.4). In general, *Aphanamixis polystachya, Canariun strictum, Cinnamomum sulphuratum, Embelia ribes, Glycosmis macrocarpa, Hydnocarpus alpina, Nothapodytes nimmoniana, Aristolochia tagala,* and *Rhaphidophora pertusa* were found threatened in varying degrees.

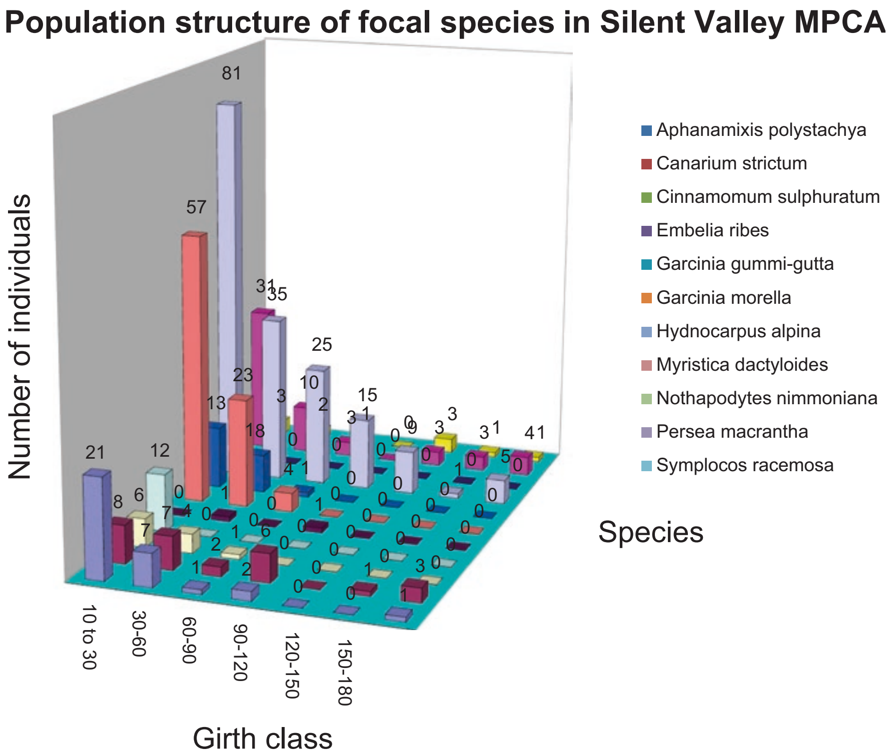


Fig. 14.2 Girth class-wise distribution of focal species of Silent Valley MPCA

In Kolli Hills, species like *Canarium strictum, Myristica dactyloides, Persea macrantha,* and *Symplocos cochinchinensis var. laurina* showed more number of individuals in lower size classes like 10–30 cm girth followed by 30–60 and so on (Fig. 14.3 and Table 14.4). The figure indicates that these species possess viable population in MPCA. *Santalum album* is represented by seedlings and sapling only. In *Smilax zeylanica*, regeneration was very good. The trend of population structure of different focal species in MPCA showed variation among species. Here, more individuals were concentrated in lower classes, comparatively lesser number of individuals in intermediate and larger girth classes. The population structure for species in forest can partly indicate its regeneration behaviour and future composition of the forest community. Regeneration is the starting stage of population, which in due course undergoes the process of sylvigenesis and builds up the stand. It leads to the increase in population number (Krebs 1972). Population structure data have also been used to interpret succession pattern and to develop succession models (Shugart 1984). Lesser number of individuals in lower girth classes shows a population on its way to local extinction while the occurrence of greater proportion of individuals in lower girth categories is indicative of frequent reproduction (Knight

Table 14.4 Regeneration status of focal species in Silent Valley and Kolli Hills

Name of species	Seedlings/ha		Saplings/ha		Mature plants/ha	
	Kolli Hills	Silent Valley	Kolli Hills	Silent Valley	Kolli Hills	Silent Valley
Aphanamixis polystachya	**–**	**111**	**–**	**0**	**–**	**27**
Aristalochia tagala	56	–	[a]	[a]	83	–
Canarium strictum	611	19	83	0	80	23
Celastrus paniculatus	194	–	83	–	0	–
Cinnamomum sulphuratum	–	0	–	0	–	10
Embelia ribes	–	56	–	0	–	10
Garcinia gummi-gutta	–	0	–	0	–	2
Garcinia morella	–	704	–	222	–	70
Glycosmis macrocarpa	–	56	–	[a]	–	130
Hydnocarpus alpina	–	0	–	56	–	18
Myristica dactyloides	1888	759	1666	352	161	143
Nothapodytes nimmoniana	–	0	–	0	–	1
Persea macrantha	583	167	333	56	72	45
Piper mullesua		185		[a]		19
Rhaphidophora pertusa	250		[a]	–	110	
Smilax zeylanica	4533	1296	[a]	[a]	1333	315
Symplocos cochinchinensis var. laurina	2972		1194		79	
Symplocos racemosa		148		0		9

[a]Shrubs/climbers

1975). Data on phytosociological studies can also be used to describe population structure of a forest where in it is difficult to measure the age of trees (Harper 1977). Adequate seed production, effective dispersal, good viability and longevity of seeds, successful establishment of seedlings, and good conversion to adult are major beneficial factors. Therefore, the population structure at each of these life stages, viz., adult trees, flower, fruit, seed, seedling, sapling, and pole, determines the structure of mature tree populations of the future. Details of changes in regeneration (species-wise) are given in Table. 14.4.

14.4.3 Reproductive Biology

Knowledge on reproductive biology is a prerequisite for both evolutionary and conservation studies (Anderson 1995). Floral morphology, phenology, and pollination studies provide insights and inferences to plant breeding systems (Nagarajan et al. 1998; Gituru et al. 2002). Adequate knowledge on breeding systems and pollination mechanisms is very essential for species conservation and recovery. Detailed field estimates on floral functions, phenology, and breeding systems shall reveal the

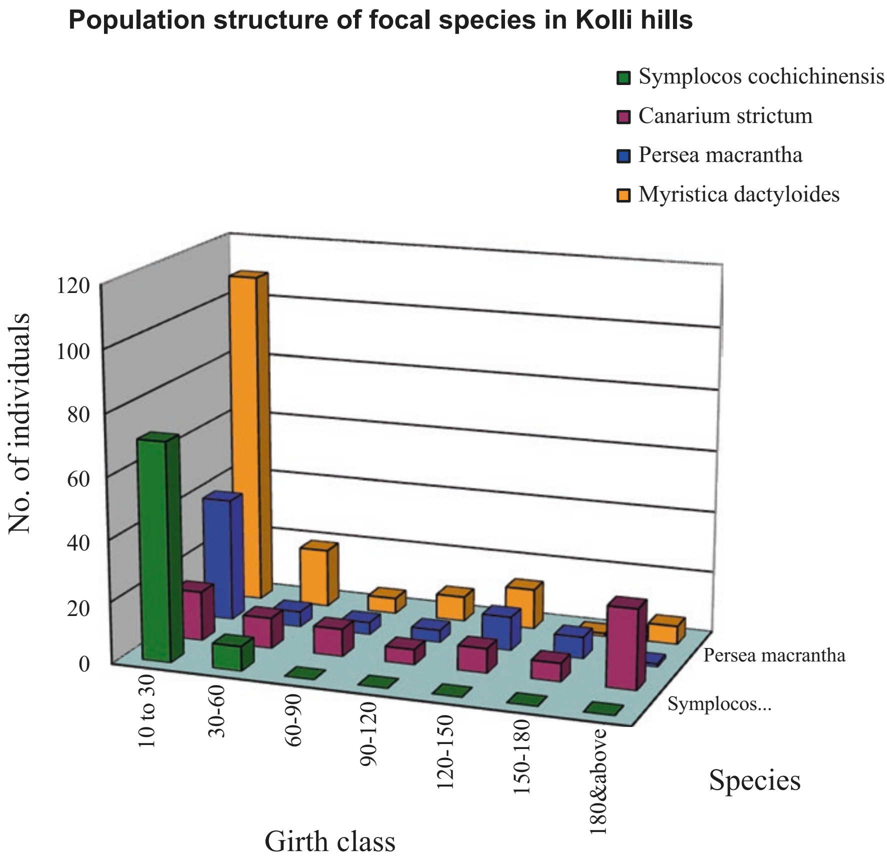


Fig. 14.3 Population structure of Focal species in Kolli hills

population structure among and within populations. Phenology, floral biology, reproductive behaviour of *Aristolochia tagala, Canarium strictum, Embelia ribes, Symplocos cochinchinensis var. laurina,* and *Smilax zeylanica* were also studied at two sites (Kunhikannan et al. 2004) to understand the reproductive bottle necks if any in that species.

14.4.4 Seed Biology and Seed Handling Techniques

Seed biology is fundamental to plant sciences. Seeds undergo discrete physiological and biochemical changes during development, maturation, storage, and germination. Very little information is available on these important developmental stages for most medicinal species in India. Information on seed biology of threatened species is vital for both in situ and ex situ conservation of the species. Information generated on seed aspects facilitates better handling of species. Problems of regeneration in relation to seed germination, dormancy, and recalcitrance leading to low population of the species, which would otherwise have gone unnoticed, could be solved. In addition, the population structure can be restored in the natural forests through reintroduction of seedlings. Research on seed collection, processing, storage, and pre-treatments for germination was carried out for eight threatened species of the MPCAs.

Conservation of germplasm in the form of seeds has many advantages as it is simple to use, easy to handle, and capable of maintaining genetic stability during storage. Successful planting and developing planting stock depend on healthy seeds of good genetic quality. Adequate quantities of seeds must be available and be able to germinate in proper time. The information available on seed handling of medicinal plants is scanty as there is large number of species with medicinal properties.

Seed handling techniques for *Aristolochia tagala*, *Canarium strictum*, *Garcinia gummi-gutta, Persea macrantha, Symplocos racemosa*, *Embelia ribes, Smilax zeylanica,* and *Myristica dactyloides* have been standardized (Kunhikannan et al. 2004). Detailed information on each species is provided below on reproductive biology, seed biology, and handling techniques.

14.5 *Aristolochia tagala* (Regionally Vulnerable: Ravikumar and Ved 2000)

14.5.1 *Phenology*

Aristolochia tagala is a moderately flowering species that colonizes on sub-canopy trees as high as 15–20 meters. It prefers to establish in well-lit open spaces and to cascade down. In Kolli, the populations flower late May to late August and in Silent Valley from late October to late November. The overall periodicity of flowering in the species ranges between 40 and 50 days. The fruit is a hanging capsule that contains six locules. Initial fruit set was high in both locations; however, proportion of matured fruits in Silent Valley was higher in comparison to Kolli (Table 14.5). Fruit maturation takes 5–6 months and contains 100–140 seeds, with a high seed to ovule ratio (Tables 14.5 and 14.6).

Table 14.5 Flower, fruit, and seed production in *A. tagala* natural population in Silent Valley and Kolli Hills

Parameters	Kolli Hills		Silent Valley	
	Mean S.E.	S.D.	Mean S.E	S.D.
Fruits/plant	4.20 ± 0.59	1.87	18.20 ± 3.28	10.39
Fruit length (cm)	4.05 ± 0.15	0.42	4.58 ± 0.03	0.09
Fruit breath (cm.)	3.03 ± 0.15	0.42	3.47 ± 0.03	0.08
Fruit set (%)	4.08 ± 0.79	2.50	15.39 ± 3.09	9.80
Seeds/capsule	83.60 ± 4.96	14.03	116.82 ± 4.14	11.72

Table 14.6 Reproductive success in *A. tagala* across Eastern and Western ghats

Location	Fr/F1 ratio	S/O ratio	PERS
Kolli Hills	0.034	0.597	0.020
Silent Valley	0.138	0.834	0.115

Fr/Fl ratio Fruit to flower ratio, *S/O ratio* Seed to ovule ratio
Values calculated from 20 individuals from each location

Plate 14.3 *Aristolochia tagala* flower showing the utricle and constricted corola tube

14.5.2 Floral Biology and Pollination

Flowers are solitary or arranged in cymes; bisexual, zygomorphic, purple to brown in colour with white patches; 6–7.5 cm in length. The 'utricle' (Plate 14.3) is the basal inflated portion of perianth, which houses the reproductive structure, gynostemium which is a specialized structure containing six stamens with reduced filaments adnate to the style. Six extra floral nectaries on the inner wall is an adaptation to feed pollinators. Nectar secretion occurs during the course of anthesis. The distal end of utricle is connected to a hollow tube, where in numerous hairs adorn the inner wall. The upper region of the tube expands into the limb. The limb is dark purplish–red in colour with creamish-white stripes in the middle to attract insect visitors. It takes 8–12 days for completing the anthesis from opening of flower. Flowers open during the daytime and stigma is receptive 2 days in advance to anther dehiscence. Anthers are large (six in number) and release large quantity of pollen. Pollen is powdery, yellowish-white in colour and highly fertile (<95%). An anther produces 3000–4000 pollens that are sticky, clumped into aggregates, and exines have no ornamentation.

Aristolochia tagala is pollinated by microdipterans through fly-trap mechanism. The pollination process is called sapromyophily. The insects get entrapped inside the utricle due to the presence of uni-seriate hairs in the throat of the tube oriented downward plane, which restrict the movement towards the opening. During their stay within the flower, insects feed on nectar and aid in cross-pollination within the first 2 days otherwise they promote obligatory self-pollination in 2–3 days. Pollen germination takes 5–8 h, in few cases, pollen germinates within anthers. This pollen tube directly penetrates the gynostemium and travel into ovaries. The breeding system is preferential out-crossing type in the protogynous phase or becomes an obligatory selfer. A very low fruit–flower ratio in Kolli is clearly indicative of pollinator limitation.

14.5.3 Reproductive Success

Reproductive success was high in Silent Valley than the Kolli population and also a lower pre-emergent reproductive success (Wiens et al. 1987) value was noted (Table 14.6).

Even though flowers produced per plant were almost equal in both locations, fruit set per plant was very low in Kolli Hills. Thus, problem persists in post-zygotic phases concerning fruit and seed set. In Silent Valley, the PERS value is high with an average of 13 fruits per plant. Seeds collected from Silent Valley (82.25%) showed a slightly higher germination in comparison to Kolli (71.25%). Silent Valley was high fecund (Table 14.6) within two populations.

14.5.4 Seed Biology and Seed Handling Techniques

Fruits were collected from both the locations. The seeds are extracted by breaking the capsule and sun drying. The seeds germinate readily without any pre-treatment. The germination percentage varied from 75% to 90%. The seeds are stored at ambient 20 °C, 10 °C, and −5 °C temperature in closed container to know the optimum storage temperatures. All temperatures found to support storage of seeds except −5 °C after 18 months of storage. Best results were obtained in 10 °C stored seeds. Complete germination took about 30 days. The result showed that the seeds can be stored at ambient temperature without loss of viability for 18 months with 80% germination.

14.5.5 Seed Dispersal and Regeneration

The seeds are gently released by septicidal dehiscence. Seeds are thick, dilated laterally and provided with a large marginal wing, and favours wind dispersal. Seeds do not fly long distances but mostly they fall within 20 metres of radius in proximity

Table. 14.7 Flower, fruit, and seed production in natural populations of *Smilax zeylanica*

	Kolli Hills (*N* = 20)		Silent Valley (*N* = 20)	
Parameters	Mean ± S.E.	S.D.	Mean ± S.E.	S.D.
Inflorescences/plant	18.11 ± 1.27	4.76	22.70 ± 1.68	5.33
Flowers/inflorescence	21.19 ± 1.47	5.50	21.86 ± 2.32	7.36
Fruits/inflorescence	15.29 ± 1.41	5.28	14.28 ± 1.47	4.65
Fruit set (%)	70.27 ± 3.15	11.80	66.66 ± 5.69	18.01
Seeds/fruit	2.07 ± 0.08	0.32	2.23 ± 0.08	0.28
Seed set (%)	66.71 ± 2.97	11.13	74.62 ± 2.96	9.37

to the mother plants. Ants and other insects were found to consume the seeds. Seed germination was as high as 90% in lab conditions. However in field, even within the proximity of high fecund individuals, only two to three seedlings could be recorded.

14.6 *Smilax zeylanica* (Regionally Vulnerable, Ravikumar and Ved 2000)

14.6.1 Phenology and Pollination

Flowering of *Smilax zeylanica* in Kolli in two distinct seasons and in Silent Valley it is once during May and June. Males flower during first and second week of June and female during the second and fourth week of June. In the second flowering season, the males flower in early August followed by females during mid to late August. Flowering in Silent Valley is restricted to only one season for 20–30 days during late December and continues until late January. Flower buds within an inflorescence open synchronously during the early part of the flowering season. Fruit set in the early phase of flowering was higher when compared to the late flowers. Flower and fruit production per inflorescence, number of inflorescences produced per plant, and fruit set and seed filling were recorded. Flower initiation in individuals in lower elevation (800–900 MSL-Mean See Level) was slightly ahead of those in higher elevations (1000–1100 MSL).

Based on flowering pattern, *S. zeylanica* is found to be strongly male-biased. In Kolli Hills, out of 130 mature individual recorded, 104 were males and 26 were females. In Silent Valley, out of 80 individuals selected, 48 were males and 22 were females. Thus, the ratio of males to female is approximately 4:1. S. zeylanica exhibits high reproductive success in both the study sites. The species shows high fruit to flower (Fr/Fl) ratio and seed to ovule (S/O) ratio and hence a high value of PERS (Tables 14.7 and 14.8).

Table. 14.8 Reproductive success in natural populations of *Smilax zeylanica* ($N = 20$)

Study sites	Fr/Fl	S/O	PERS
Kolli Hills	0.72	0.69	0.497
Silent Valley	0.65	0.74	0.485

Fr/Fl ratio Fruit to flower ratio, *S/O ratio* Seed to ovule ratio, *PERS* Pre-emergent reproductive success (Fr/F1 × S/O) (Wiens et al. (1987)

14.6.2 Seed Biology and Seed Handling Techniques

Mature fruits of *Smilax zeylanica* were collected from Kolli Hills and seeds were extracted by de-pulping, washing, and surface drying. The seeds were tested for germination with various pre-treatments and studied for the effect of storage temperature on germination. The various pre-treatments tried were cold water soaking, hot water soaking, GA_3 treatment, and sulphuric acid treatment. Cold water treatment for 3 days was found to improve the germination. Seeds were stored at different temperatures namely ambient, 20, 15, and 10 °C. Non-desiccated seeds stored at 15 °C were found to have better germination.

14.6.3 Seed Dispersal and Regeneration

Scanty regeneration could be observed in locations; however, number of regeneration in Kolli was higher than in Silent Valley. Germination study reveals asynchronous and incremental germination and observed only 20 seedlings out of 100 seeds emerging after a period of 3 months. Despite being a high PERS species (Tables 14.7 and 14.8), a long dormancy period of 2–3 months seems to be a major bottleneck in regeneration. In general, the species is a high fecund with limitation at the level of progeny to zygote (P/Z) ratio.

14.7 Embelia Ribes (Low Risk near Threatened: Ravikumar and Ved 2000)

14.7.1 Phenology

It is a polygamo-dioecious species with prolific flowering that establishes in open, well-lit areas. Often they are found cascading from trees of 11–15 meters high. Flowers are very small (3–4 mm in diameter) dull yellowish-white in colour. Each inflorescence has 50–150 flowers that bloom over a period of 4–5 weeks. Inflorescences are shorter in male plants (3–5 cm; 20–50 flowers) compared to females (6–15 cm; 50–160 flowers). Male plants bloom 2 weeks ahead of females. This is an adaptation for effective utilization of female resources. Fruits ripen by mid-November and are retained until mid-January. Birds and several insects found preferentially consuming the fruits in the following 4–5 weeks. Fruits contain single seed that do not germinate at least 10–12 weeks in untreated conditions. *E. ribes* exhibits a moderate fruit to flower and seed to fruit ratio. Flower abortion ranges from 60% to 86%. Pollinator limitation is a major cause for flower abortions; pollen–pistil interaction studies reveal absence of pollen on stigmatic surfaces. Fruit set ranges from 14% to 40%. Fruit abortiveness ranges from 1% to 27%.

14.7.2 Floral Biology and Pollination

Anthesis was studied in individuals along elevation gradient and no variations could be observed in the patterns across elevation. Opening of flower was continuous, but a greater proportion of flowers were found to open in nights indicating a tendency for nocturnal anthesis (19.00–3.00 h). Male flowers have a highly reduced pistillode and well-developed anthers. Female flowers are with pistils as well as anthers that contain no pollen (cryptic dioecy). In male flowers, an anther produces about 650–1100 pollen and each male flower may produce 3500–5000 pollen. The process of nectar presentation is quite unique in *E. ribes*. Nectar is faded golden yellow in colour and thick in consistency. During early mornings, pollinators in larger number visit male plants when compared to females due to difference in the volume and timing of nectar production. In males, nectar production was early compared to female flowers and quantity also high. Insect visitors first cover male flowers and then visit females. Due to this, by mid-day, the number of visitors to male plants recedes due to nectar depletion thus resulting in effective pollination.

Pollen is powdery, small in size 18–24 μm, cream white in colour, bi-nucleate, and and fertile up to 95%. During the morning time, insects collect pollen in clumps or aggregates, with increasing day temperature, the pollen mass becomes powdery. The exine is thick without any prominent architecture. In general, insect movement was higher in habits taller than 15 feet. Insect visit starts around 7.30–8.00 AM with bright sunshine; insect visitation fades to a few. Wasps are the most common visitors followed by social and wild solitary bees.

14.7.3 Breeding System and Reproductive Success

The species is dioecious and has a reproductive output, specifically a very high ovule to seed ratio. The levels of fecundity highly varied among individuals, most females were either moderate or low in terms of reproduction. Seven out of 13 females were highly fecund and of the 55 males, only 11 were profuse in flowering. The male–female ratio was observed to be 5:1, very low female to male ratio is of concern.

Table. 14.9 Reproductive output and success in *Embelia ribes*

Sample	Flowers	Fruits	Seeds	Fr./fl	S/O	PERS
Er1	12,432	2234	2212	0.179	0.99	0.168
Er2	1373	378	308	0.275	0.81	0.222
Er3	18,602	8465	8415	0.455	0.99	0.450
Er4	6836	976	906	0.142	0.92	0.130
Er5	14,324	5800	5180	0.404	0.89	0.359
Er6	19,782	4847	4120	0.245	0.85	0.208
Er7	9453	1345	1010	0.142	0.75	0.106
Er8	10,254	2787	2708	0.271	0.97	0.262
Er9	9644	1100	1010	0.114	0.91	0.102
Er10	13,321	4228	3120	0.317	0.73	0.231
Mean	**11602.1**	**3216.0**	**2898.9**	**0.2544**	**0.881**	**0.223**
SE	1714.58	817.25	785.27	0.0362	0.029	0.035
SD	5421.99	2584.3	2483.26	0.1145	0.094	0.111

Pollinator limitation is also noticed, which considerably influences fruit and seed set (Table 14.9). Varying fecundity observed among females is likely to influence genetic structure. The species exhibits high level of flower abortion. However, fruit to seed ratio is quite high, thus there is a high level of reproductive success.

14.7.4 Seed Biology and Seed Handling Techniques

The seeds were extracted by de-pulping and washing with water. Germination trial was conducted with pre-treatment of seeds with hot water, KNO_3 and GA_3 and found that seed coat removal and treating with GA_3 improve germination. The seeds are low temperature tolerant and can be stored in freezing temperature. The seeds can also be stored in liquid nitrogen and germinated whenever required in sand medium. The testa needs to be removed and soaked in Gibberellic acid (1000 ppm) overnight for higher germination.

14.8 *Symplocos cochinchenensis var. laurina* (Low Risk Near Threatened, Ravikumar and Ved 2000)

14.8.1 Phenology

Inflorescences consist of 10–70 white flowers. Flowering is in two distinct peaks; the first flowering is a longer period during late October and continues until mid-November. Most fecund individuals are in peak reproductive state by mid-November.

Table. 14.10 Reproductive output and success in *S. cochinchenensis* natural population

Samples	Flowers	Fruits	Seeds	Fr/fl	S/O	PERS
Sc2	356	40	32	0.112	0.8	0.089
Sc3	432	37	27	0.085	0.729	0.061
Sc4	521	62	46	0.119	0.741	0.088
Sc5	1240	110	78	0.088	0.709	0.567
Sc6	780	47	31	0.06	0.659	0.039
Sc7	230	34	23	0.147	0.657	0.096
Sc8	983	65	52	0.066	0.953	0.062
Sc9	902	103	73	0.114	0.708	0.080
Sc10	362	38	32	0.104	0.842	0.087
Sc11	438	45	30	0.102	0.666	0.067
Mean	**624.4**	**58.1**	**42.4**	**0.09**	**0.744**	**0.123**
SE	104.76	8.708	6.16	0.008	0.029	0.049
SD	331.28	27.53	19.50	0.025	0.094	0.156

The second period is a shorter one that occurs between February and March. Fruit set for the first flowering season is during December and continues till March. The second season flowers begin to fruit by April and are dispersed by late July.

14.8.2 Floral Biology and Pollination

Anthesis occurs during late nights to very early mornings. Insect movement is noticed from 9.00 to 17.00 h. A wide range of pollinators including wasps, flies, and social and solitary bees visit flowers. Flowers produce copious nectar. Flower life is restricted for a day and withers the same day or the following morning. Pollen is white and sticky, binucleate, 28–34 μm in size, and fertile up to 96%. Exine is thick without any prominent architecture. Pollinated by wasps, small insects, and rarely visited by butterflies.

14.8.3 Reproductive Success

The species is a preferential out-crosser. Under normal conditions, 8–11% of open pollination fruit set is common. Control pollination with self-pollen indicated arrest of pollen tube in stigma and style. In the presence of out-cross pollen, fruit set is very high up to 30% (Table 14.10).

14.8.4 Seed Dispersal and Regeneration

Seeds do not germinate readily. However, the species shows plenty of regeneration near the mother tree; it is also found to propagate through root suckers, thus creating a strong family structure in populations.

14.9 Canarium strictum (Regionally Vulnerable, Ravikumar and Ved 2000)

14.9.1 Phenology

In Silent Valley, *C. strictum* is found to flower between March and April while in Kolli Hills, it flowers from June to July. In Kolli Hills, male flowering could be recorded even in late August. In Kolli, the species shows strong family structure, while in Silent Valley they look very randomly distributed. Male trees flower at least 2–3 weeks ahead of females. Flowers are creamish-white in colour and have a flower of more than 3 days. Male inflorescences are shorter than female inflorescences. Asynchronous flowering is very common among individuals within population.

14.9.2 Floral Biology and Pollination

Anthesis progression was continuous; flower unwinding was not restricted to a particular period of day or night. No pollinators could be recorded during the study period. (Pollination could be through thrips or wind.) Pollen shedding happens in mornings between 10.00 and 12.00 h. Pollen is creamish-yellow in colour and are small in size (22–24 m), highly fertile (< 95%), and the exine is without any ornamentation.

14.9.3 Reproductive Success

About 14 individuals in Silent Valley and 24 in Kolli Hills were included in this study. In Kolli, observations were made in two locations, 11 trees in one patch and 13 trees in another patch. In both locations, trees exceeded 25–30 m in height. Out of the 13 individuals recorded near Chemmedu, only 5 females could be recorded, and the remaining were males. In the second patch that constituted 11 individuals, only 2 were females. In Silent Valley among the 14 individuals recorded, 3 were found to be females and 8 were males. Flowers and fruits were recorded in 100 inflorescences in each of the female trees sampled (Table 14.11).

Table. 14.11 Reproductive output and success of *C. strictum* in Kolli Hills ($N = 5$)

Samples	Flowers	Fruits	Seeds	Fr/fl	S/O	PERS
CS1	540	124	63	0.229	0.508	0.115
CS2	620	87	45	0.14	0.517	0.072
CS3	750	113	74	0.15	0.654	0.097
CS4	550	130	50	0.236	0.384	0.090
CS5	350	68	48	0.194	0.705	0.136
Mean	**562**	**104.4**	**56**	**0.189**	**0.553**	**0.102**
SE	64.91	11.70	5.44	0.019	0.057	0.010
SD	145.15	26.17	12.18	0.044	0.127	0.024

Fr/fl Fruit to flower ratio, *S/O* ratio

In comparison to the other species included in this review, *C. strictum* shows a low reproductive success. The major reasons are its low fruit to flower ratio and seed to ovule ratio. Fruits show differential seed filling. Very rarely fruits with three seed filling were recorded; most fruits were either with single or two seeds.

14.9.4 Seed Biology and Seed Handling Techniques

The germination was epigeal with trifid cotyledonary leaves. The seeds were poor in germination (10–40%). Various experiments starting from seed collection, extraction, grading, and pre-treatment were conducted. The seeds also showed poor viability. Studies were also conducted to improve the viability of seeds for long-term storage. Among the pre-treatments, the 1-day soaking in water and 1-day drying in sun light for three cycles were found to extend the viability. Drupes collected from ground that were intact with exocarp showed higher germination.

Morphological characters such as 2D surface area (cm^2), length (cm), breadth (cm), perimeter (cm), equivalent diameter (cm), roundness, aspect ratio, and fullness ratio were measured using image analyser. Diameter of the seeds was found to correlate with filling percentage (Plate 14.4), and the drupes were graded into two groups based on diameter using 20 mm sieve. Seed bulk was graded, 66% of drupes were above 20 mm and 34% of the drupes were below 20 mm. These two grades were assessed for germination percentage and seedling characteristics and found that germination and all other seedling growth characters were found to be better in above-20 mm grade drupes (Table 14.12).

Plate 14.4 Cross section of *C. strictum* fruit showing three seeds

Table 14.12 Germination of exocarp intact, damaged, and insect-affected drupes of *Canarium strictum*

Categories	Germination %	Seedlings/100 drupes (After 45 days)
Intact drupes	61	82
Exocarp, physically damaged drupes	76	108
Exocarp, insect-affected drupes	66	96

SEd: 5.594
CD $p = 0.05$: 12.65

14.9.5 *Dispersal and Regeneration*

In Kolli Hills, regeneration in proximity to mother trees was frequently noted. Some trees had up to 250 seedlings, about 70–80% in different growing stages belonging to at least 2–3 seed years. Seedlings die back over a period of 2–3 years. Only very few were found to establish as saplings over 1–2 m.

14.10 *Garcinia gummi-gutta* (Globally Vulnerable, Ravikumar and Ved 2000)

The fruits are pulpy, and a gummy substance is present over the seed. Extraction of seed needed mechanical efforts. Manual de-pulping and chemical de-pulping methods were tested. Fresh seeds were tested for germination after removing the pulp.

Table 14.13 Germination trial of fresh seeds of *Garcinia gummi-gutta*

Treatment	Germination %
Seeds with pulp (un-extracted)	14.67
Seed pulp extracted by hand	13.33
Seeds pulp extracted by hand and gummy substance removed using petroleum ether germination with seed coat	24.00
Seed pulp extracted by hand and seed coat removed	84.00

Table. 14.14 Effect of storage temperature on germination of *Persea macrantha* seeds

Temperature	20 days after storage	40 days after storage	55 days after storage
Ambient (25–28 °C)	Nil	Nil	Nil
25 °C	62.5	30.00	Nil
20 °C	73.75	71.25	38.75
15 °C	75.00	66.25	27.5

Germination was initiated after 47 days and was completed in 142 days. Germination percentage of the seeds without seed coat was 84% and with seed coat was 14.6% (Table 14.13).

Hence for immediate germination, the pulp and the seed coat need to be removed mechanically. For the purpose of storage, the pulp adhering to the seeds need to be removed by soaking in 0.1 N sodium hydroxide (NaOH) for 15 min and washed with petroleum ether to remove the gum over the testa. Seeds with moisture content above 40% need to be stored at 20 °C.

14.11 *Persea macrantha* (Regionally Endangered, Ravikumar and Ved 2000)

Persea macrantha fruits were sampled from Kolli Hills and seeds were extracted manually from the pulpy fruits. Severe insect infestation was observed in the seeds. About 28% of seeds were infested by insects. The seeds germinate readily. Seeds were high in moisture content (47.87%) and initial germination was 73%. Seedlings emerge after 30 days of sowing. The viability of the seeds was found to reduce drastically after 2-week period. Hence, studies were conducted to identify suitable storage environment. Seeds were kept in different storage temperatures like ambient, 20, 10, and 0 to −5 °C, temperature and different moisture contents, namely 45%, 40%, 35%, 30%, 25%, and 20%. Combination of 20 °C and 45% moisture content was found suitable for storing *P. macrantha* seeds compared to other temperature and moisture contents. Seeds are desiccation sensitive.

Seeds with 42.33% moisture content were stored at different temperatures namely as the details given in Table 14.14. The treatment at 20 °C was found to be optimum for storing *Persea macrantha* seeds.

It can be concluded that the seeds do not have problem in germination. The non-desiccated seeds can be stored at 20 °C.

14.12 *Myristica dactyloides* (Regionally Vulnerable, Ravikumar and Ved 2000)

14.12.1 Seed Biology and Seed Handling

Mature fruits were collected, shade dried for 2 days, de-pulped, and seeds were extracted by hand. Germination tests were conducted using roll towel method after soaking in 1000 ppm GA3 (Gibberellic acid) for 24 h. Germination initiated after 8 days of sowing and the final count was taken on the 30th day.

The actual moisture contents for the seeds were 30.5%, 19.1%, and 10.8%. Fresh seeds with 34.1% moisture content subjected to GA3 pre-treatment gave a germination of 46.67%. The seeds were found to be desiccation sensitive. The germination gradually declined to 26.67% with reduction in moisture content to 20%. From the above studies, it is evident that the seeds of *M. dactyloides* are recalcitrant and at the same time possess dormancy. Recalcitrant seeds are well known for their sensitivity to desiccation and the degree of sensitivity varies between species (Roberts 1973). Storage temperatures below 15°C were reported to be lethal for most of the tropical recalcitrant seeds (Bedi and Basra 1993). Although *M. dactyloides* seeds were recalcitrant, they did not germinate without GA3 treatment and possess dormancy. Application of plant growth regulators (especially gibberellic acid) have shown to release dormancy and enhance germination of hardwoods species (Leadem 1987). Current observation of dormancy mechanism in a tropical recalcitrant species, *M. dactyloides* paves a way for calling further studies.

Seeds were stored in a closed container at different temperatures viz. ambient (25–28°C), 20°C,15°C, 10°C, 0°C, and −5°C for a period of 2 weeks and then subjected to germination test with application of 1000 ppm GA_3 for 24 h in roll towel. Germination percentage of ambient stored seeds reduced from initial germination of 46.67% to 20%, whereas, the germination of seeds stored at 20°C increased to 76.67%. Storage of seeds at 20°C has not only prolonged the viability but also assisted in breaking the dormancy. Storage of *M. dactyloides* seeds at 20°C was found to improve the storability. The germination percentage of ambient stored seeds reduced from initial germination of 46.67% to 20%, whereas, the germination of 2 weeks stored seeds at 20 °C increased to 76.67%. After 3 months of storage, germination was good in seeds stored at 15–20°C and treated with growth regulator. *M. dactyloides* seeds are sensitive to desiccation. The non-desiccated seeds are storable at 20°C for more than 3 months. The seeds are physiologically dormant and treatment with GA3 (1000 ppm for 24 h) breaks dormancy. Low temperature storage (15–20°C) plays a supplementary role in improving the germination along with GA_3 treatment. Germination test can necessarily be conducted in germination mediums like germination paper, cotton towel, and sponge sheet.

14.13 Conclusion

Conservation of plant populations is dependent on environment, population structure, and genetic variations found within populations (Boyle 2001). Gilpin and Soulé (1986) postulated that plant populations get threatened due to four different types of extinction pathways or vortices. Of these four, two are mainly driven by environment and demographic features such as habitat fragmentation, while the remaining are genetic drift and inbreeding. Species recovery action for understanding the constraints and production of new planting materials through conventional and artificial means and reintroduction into the natural habitats for restocking are the different possible actions.

Most of the species seems to have been conditioned for flowering initiation during the onset of monsoon and develop fruits through the monsoon. Thus, fluctuating monsoon patterns could be a major factor that would implicate reproduction and regeneration severely.

In Silent Valley MPCA, *Aphanamixis polystachya, Canariun strictum, Cinnamomum sulphuratum, Embelia ribes, Glycosmis macrocarpa, Hydnocarpus alpina, Nothapodytes nimmoniana* are found to be threatened at varying levels and degrees. Population studies in 200 ha of area is far from sufficient for making any decisive conclusions; some of the species that are in very low density within MPCAs are available in plenty outside the areas (e.g., *Cinnamomum sulphuratum*). Understanding the levels of diversity in MPCAs would be more precise and informative if detailed estimates are obtained on a Species Centered Approach (SCAP).

In Kolli Hills, populations of *Aristolochia tagala* and *Rhaphidophora pertusa* are restricted to a very few patches. In *A. tagala,* the problem is related to its breeding system. The population shows preferential out-crossing and is aided through highly specialized insect vectors. A very low fruit–flower ratio in Kolli is clearly indicative of pollinator limitation as well as its preferential out-crossing nature. There is a definite need to understand the life history traits of pollinators on which no information is available.

In case of *A. tagala,* the overall reproductive success was higher in Silent Valley in comparison to Kolli Hills. Apart from high fecundity, Silent Valley population was also found to have layers of fruits with more seeds per capsule. Such variations among sites, in rates of reproductive success are critical for conservation decisions (Murren 2002). But regeneration is very poor even among the most fecund individuals. Such patterns of seed dispersal are often known to greatly influence the population size, genetic composition, and structure in tropics (Hamrick et al. 1993). In *A. tagala,* seeds were found to fall very proximal to the mother plants. This may often lead to development of a strong family structure (Shaw and Allard 1982).

Canarium strictum a tree exploited for resin extraction is low in its density in both Silent Valley and in Kolli Hills, especially in lower girth classes skewed gender ratio is of real concern. These populations have also developed strong family structure and likely to pose inbreeding problems in due course. In Kolli, the population is highly male biased with very few fecund females, even though regeneration is plenty, and most seedlings die back during the dry months. To find out the problem of establishment, a long-term monitoring of population is required. Among the dioecious breeding systems investigated in this study, *C. strictum* showed the lowest reproductive success.

Pollinator limitation seems to be a major threat as many species require specialist pollinators. In case of *Smilax zeylanica* and *S. cochinchinensis,* plenty of solitary bees are found to be the legitimate pollinators. *A. tagala* needs specialist pollinators namely mocrodipterans. Nothing is known about their life history traits. Kolli Hills, being a hub of horticulture, boasts monoculture of banana, guava, and pineapple varieties. Inadvertently, this leads to extensive usage of inorganic fertilizers and pesticides within the region. This has a direct effect on populations of pollinators and birds that aid in seed dispersal.

In *S. cochinchinensis* and *E. ribes,* no bottle neck could be noticed during the pre-zygotic or during process of zygote development. Only during the post-zygotic phase, most seeds are lost in the form of herbivory. Hence, it is important to survey and locate the most fecund individuals across altitudes for seed collections and bulk the same for nursery establishment. Species such as *Smilax, Aristolochia, Embelia,* and *Canarium* show high rates of germination under controlled conditions, thus establishing nursery at study sites would aid in achieving higher seed to seedling ratio. Thus, strategies concerning species recovery limited to the seed–progeny stage and can be overcome by methodical seed harvest, ex situ nursery development and reintroduction.

In *Embelia ribes,* fruit production is high but very poor natural regeneration is a threat factor. Seed germination studies reveal necessity of pre-treatment with GA_3. The species *Embelia ribes, Canarium strictum, Symplocos racemosa,* and *Glycosmis macrocarpa* require some in-depth study to reveal their exact status. These taxa need to be dealt exhaustively on a species base, total distribution, phenology, and dispersal.

As numerous development and conservation projects have demonstrated, support and involvement of local people is one of the basic requirements for success, whether it aims at reducing human pressure on the environment or to promote economic development (Brandon and Wells 1992; Carpenter 1998). Hence, conservation in-situ most importantly requires the cooperation and commitment of the local people and community.

Seeds of *Persea macrantha, Symplocos racemosa, and Myristica dactyloides* were found to be sensitive to desiccation. Species such as *Smilax zeylanica, Aristolochia tagala, Embelia ribes,* and *Canarium strictum* showed high rates of germination under controlled conditions, thus establishing nursery at study sites would aid in achieving higher seed to seedling ratio. Seed germination studies reveal necessity of pre-treatment with GA_3.

Populations of *Canarium strictum* have developed strong family structure and are likely to pose inbreeding problems in due course. Among the dioecious breeding systems investigated in this study, *C. strictum* showed the lowest reproductive success. In *Embelia ribes,* fruit production is high, but very poor natural regeneration, which is a threat factor. In case of *Smilax zeylanica* and *Symplocos cochinchinensis var. laurina* plenty of solitary bees were found to pollinate.

14.14 The Way Forward

India is endowed with vast resources of medicinal plants. At present, 95% of medicinal plants are still collected from the wild. The growing interest in commercialization of plant medicines leads to over exploitation of the plant resources. Current practices of harvesting are unsustainable, and many studies have highlighted depletion of resource base (Tewari 2000). About 70% is from destructive collections which include the entire plant (16.5%), reproductive parts like fruits and seeds (22.0%) or tuber, root, and stem (53.0%) (Vinay 1996). Such destructive and non-sustainable collection methods coupled with low regeneration and habitat destruction have posed serious threat to the survival and availability of various medicinal plants in the wild.

Following suggestions were made to face difficult situation in commercialization and conservation of medicinal plants.

- The recovery research will help to identify the status of population in the wild, constraints in reproduction and seed biology and handling techniques. Accordingly species-specific strategies can be made by addressing pollinator deficiency, pesticide pollution, etc.
- After studying the status of population and diversity, certain areas can be identified in different forest types and altitudinal zones, for in situ conservation and utilized for ex situ propagation of medicinal plants for cultivation.
- Some of the constraints faced in the cultivation of the medicinal plants are non-availability of the seeds and seed-handling techniques. To address this issue, all prospective farmers/entrepreneurs need to take up cultivation initially in small scale. After producing sufficient seeds/planting materials, it can be scaled up to large scale.
- Production of planting materials through conventional and artificial means by using modern tools can also be tried/adopted.

Annexure 14.1 General Information on Selected Species

1. *Aphanamixis polystachya* (Wall.) Parker (*Syn*. *Aglaia polystachya* Wall.; *Amoora rohituka* (Roxb.) Wight & Arn.; *Andersonia rohituka* Roxb.); *Family*: Meliaceae

   Vernacular name: *Hindi*: Harin-hara; *Malayalam*: Chemmaram; *Sanskrit*: Rohituka, *Tamil*: Vellai kongu, Pechambagai; *Trade name*: Amoora.

   Distribution: It is distributed in the sub-Himalayan tract; in South India, common in the Western Ghats from North Kanara downwards to Tirunelveli. Distributed in patches in Silent Valley MPCA and frequency of occurrence is less.

Description: Large evergreen tree. Leaves imparipinnate, elliptic, acute, or acuminate, entire, glabrous. Flowers polygamo-dioecious; male in panicles; female in long spike. Stamens 6, anther more or less included in the staminal tube. Ovary 3 celled. Capsule sub-globose and loculicidal.

Uses: It is used for dugouts, canoes, root structures, and light construction work. It is also used for ply boards. The seed oil is used as liniment in rheumatism. The bark is astringent and is used in cases of enlarged glands, liver, and spleen.

2. *Aristolochia tagala* Cham. (Syn.: *A. roxburghiana* Klotzch.; *A. acuminata* Roxb.); *Family*: Aristolochiaceae.

Vernacular name: Hindi: Hooka-bel; *Kannada*: Isvaberusa; *Malayalam*: Garudakkoti; *Sanskrit*: Gandhanakuli; *Tamil*: Isvaramuli; *Telugu*: Esvaraveru.

Distribution: In semi evergreen to evergreen forest of Eastern Himalayas; Deccan plateau; Konkan belt; Ceylon. Distributed in patches above 1000 m elevation in Silent Valley and Kolli Hills MPCAs and available in open patches along the border of the forest. Frequency of occurrence is very low.

Description: A quite glabrous twining shrub. Leaves cordiform, 5-nerved from base, nerves converging towards apex, base deeply cordate, sinus narrow, apex acuminate. Racemes axillary; bracts lanceolate, ciliate. Flowers purple with greenish-yellow tube. Stamens 6; anthers oblong. Ovary puberulous; stigmatic lobes 6, obtuse. Capsule pyriform or oblong with long stipes. Seed obtusely triangular, winged, tip tubercled.

Uses: Leaves used in bowel complaints and snake bites.

3. *Canarium strictum* Roxb. (Syn. *Pimela stricta* Blume) *Family*: Burseraceae.

Vernacular Names: *Tamil*: karapu kongiliam; *Malayalam*: kungilliam; *Kannada*: Halemaddu. *Trade Name*: black dammar

Distribution: Distributed across different parts of India, Myanmar, and Yunnan province of China; in South India especially in Western Ghats, parts of Konkan, Canara and Malabar, Travancore and Cochin, and some pockets in Eastern Ghats. It is available throughout the Silent Valley and in evergreen patches in Kolli Hills MPCA, excluding hilltops. The individuals observed were mostly mature trees. Frequency of occurrence is about 60%.

Description: Tree; branchlets velvety-tomentose. Leaves odd-pinnate; leaflets 3–5 pairs, opposite, oblong, thick coriaceous, sub-glabrous above, rusty-villous below, base obtuse or sub-cordate, margin serrulate or crenulate, apex acuminate; rachis tomentose; stipules obscure. Panicles axillary, interrupted; bract caducous. Flowers 3-merous, polygamous. Calyx-tube campanulate; lobes 3, triangular. Petals 3, pale yellow, oblong, concave, apiculate. Disc annular, apically pilose, intrastaminal. Stamens 6, free from disc; anthers oblong, sub-equal; pistillode short. Ovary pilose, 3-celled. Stigma capitate. Drupe oblong.

Use: Black dammer is obtained from the tree and is used for manufacturing of varnishes and bottling wax. It is also used for caulking boats. The wood has a good glue holding capacity, plywood tea boxes can be made from it. On dry distillation the resin yields 80–85% of deep blue oil.

4. *Celastrus paniculatus* Willd. (Syn. *C. rothianus* Schultes.), *Family*: Celastraceae.

Vernacular names: *Urudu*: Korsano; *Hindi*: malkahgni; *Kannada*: Kariganne; *Tamil*: Valuluvai; *Malayalam*: Cherupunna; *Sanskrit*: Jyotismati. *Telugu*: Gundumeda.

Distribution: In deciduous forests of Indo-Malaya to China, and Australia. Only seedlings were available in the sampled areas in evergreen part of Kolli Hills MPCA. Mature climbers are available below 920 m elevation. Frequency of occurrence was 17.5%.

Description: A large scandent climbing shrub; branchlets puberulous. Leaves alternate, ovate, or orbicular, thin coriaceous, base obtuse to sub-acute, margin dentate, apex acuminate to caudate. Panicles terminal, pubescent. Flowers polygamous. Calyx-tube cupular; lobes 5, sub-orbicular. Corolla 5, greenish-white, ovate to oblong, refluxed. Disc copular, lobed. Stamens 5, inserted on the margin of disc; filament short; anthers oblong; pistillode conical. Ovary inserted on the disc, globose, glabrous, 3-celled; ovules 2 per cell, erect; style short, stigma recurved; staminodes 5. Capsule ovoid.

Uses: The oil from the seed curing the pulmonary tuberculosis and berberry. It is a brain tonic for rheumatism gout and neurological disorders. Stem bark is used as an abortifacient and brain tonic. Seeds are stimulant, diaphoretic, diuretic, tonic, appetizer, anti-inflammatory, and used for abdominal disorders, leprosy, pruritus, skin diseases, paralysis asthma, leucoderma, cardiac debility, inflammation, amenorrhoea, and fever.

5. *Cinnamomum sulphuratum* Nees; *Family*: Lauraceae

Vernacular Name: *Kannada*: Pinga dalchini; *Tamil*: Sambiranimaram.

Distribution: Common in wet deciduous to evergreen forests, up to 1300 m. It is distributed mostly along the higher elevation above 1000 m on Kattivaramudi in Silent Valley MPCA.

Description: Tree; branchlets, and leaves yellow-tomentose when young. Leaves ovate-lanceolate or elliptic-oblong, rounded to acute at base, acute or obtuse at apex, prominently reticulate and glaucous beneath; petiole long. Flowers bud yellow tomentose in panicles. Berry globose; stalk short, smooth. Flower and fruit during January to May.

Uses: Bark and leaves used to cure cough, headache, and spider poison and used as a mouth refresher.

6. *Embelia ribes* Burn. (Syn. *Embelia glandulifera* Wight); *Family*: Myrsinaceae

Vernacular Name: *Sanskrit*: Vodanga; *Hindi*: Baiberang; *Telugu, Tamil & Kannada*: Vayuvilanga; *Malayalam*: Vizhal.

Distribution: It is woody climber distributed in semi-evergreen to evergreen forests (altitudinal zone of 500–2500 msl) of India, Sri Lanka, Malaysia, and China. In northeast region, it is commonly distributed in Arunachal Pradesh, Meghalaya, and Mizoram. In Western Ghats region, it is distributed in Tamil Nadu, Karnataka, and Kerala. It is frequent in Silent Valley MPCA but available mostly above the canopy of the forest and along the open borders. Flowering and fruiting season are February to April.

Description: Large climbing shrubs. Leaves elliptic-lanceolate, acute, rounded at base, prominently gland dotted on either side of midrib. Flowers pedicelled, small, polygamous. Sepals hairy. Corolla papillose, white; drupe-small, smooth.

Uses: The dried fruit is considered anthelmintic, astringent, carminative, alterative, and stimulant. The dried fruits are used in decoction for fever, piles, and for diseases of the chest and skin and also used as an ingredient of application for ringworm. The roots are used for cough and diarrhoea. Aqueous extract of fruits has anti-bacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The powder made from the dried bark of the root is a remedy for toothache.

7. *Garcinia gummi-gutta* (L.) Robson. (*Syn.*: *Cambogia gummi-gutta* L., *Garcinia cambogia* (Gaertn.) Desr., *Mangostana cambogia* (Gaertn.); *Family*: Clusiaceae (Guttiferae)

Vernacular Name: *Trade name*: Malabar Gamboge; *Kannada*: Simai hunase; *Malayalam* & *Tamil*: Kodam Puli.

Distribution: It is distributed in the evergreen forests of Western Ghats and Sri Lanka, ranging from 400 m to 900 m altitude. The species has very low frequency inside the Silent Valley MPCA and located only two individuals. It flowers in January and fruits June.

Description: Medium-sized tree with yellow latex. Leaves elliptic–oblong or obovate, acute or obtuse, cuneate, entire, glabrous. Staminate flowers yellowish-green in clusters. Stamens monodelphous forming a central globular head. Pistillate flowers solitary, larger than male. Ovary 6–12 locular, globular, grooved. Fruit berry, elliptic–oblong or sub-orbicular mamillate, grooved.

Uses: The yellow resin from the plant is used as a pigment in the manufacture of lacquer and in medicine. Citrin is extracted from the fruits used for treating obesity. The leaves, fruits, and seed oil are reported to be purgative, hydragogue, and emetic.

8. *Garcinia morella* (Gaertn.) Desr. (*Syn.*: *Mangostana morella* Gaertn.; *Garcinia gutta* L.); *Family*: Clusiaceae

Vernacular Name: *Hindi & Bengali*: Tamal; *Telugu*: Pasupuvarne; *Tamil*: Makki.

Distribution: Common in evergreen forests along banks of streams in India, Sri Lanka, and central Philippines. In India, it is mostly found in Western Ghats parts of Karnataka, Tamil Nadu, and Kerala. Distributed throughout the Silent Valley MPCA along with *Myristica dactyloides.*

Description: Moderate-sized tree, bark rusty-brown with yellow latex. Leaves oblong-lanceolate, acute, cuneate, entire. Flowers red, sub-sessile; male 2–3 together, female solitary. Sepals greenish-white, elliptic, thin. Petals rather fleshy; male flower with monodelphous stamens; anther red; female flowers solitary with globose, smooth, 4-celled ovary; stigma 4-lobed, brown-red. Fruits globose, smooth, yellowish. Flowering and fruiting occur from February to July.

Uses: The resin is used for watercolours and gold-coloured sprit varnishes for metals. In Siam, golden yellow ink is prepared from this plant resin. Several parts of the plant such as seed, pericarp, stem bark, leaves, and fruits show marked anti-bacterial activity against *Micrococcus pyogenes* var. *aureus*. It is a powerful hydragogue, cathartic causing in large doses nausea, vomiting, and griping. It is used an abortifacient and in the treatment of ulcers.

9. *Glycosmis macrocarpa* Wight: *Family*: Rutaceae

Distribution: It is understorey shrub in wet evergreen forests between 200 and 1100 m in South India and Sri Lanka; in the Western Ghats, in Wayanad, Silent Valley, and Coorg Region. It is distributed in patches in mid elevation along lower slopes of Kattivaramudi, Silent Valley MPCA.

Description: Shrub. Leaves 3–5 foliate, oblong, or elliptic-lanceolate, obtusely acuminate, cuneate. Flowers white, in terminal or axillary panicles. Fruits sub-globose, constricted at base, smooth. Flowering and fruiting occur in March to June.

Uses: Leaf juice is used for the treatment of fever, liver complaints, eczema, and other skin troubles.

10. *Hydnocarpus alpina* Wight; *Family*: Flacourtiaceae

Vernacular Name: *Tamil*: Attuchankalai; *Malayalam*: Malamaravetti; *Kannada*: Torathi.

Distribution: Western Ghats from Kerala to Kannada and also in Sri Lanka. It is distributed in Silent Valley MPCA, mid elevation from Kummattamthodu along lower slopes up to Kattivaramudi.

Description: Large tree, young shoots glabrous. Leaves oblong-lanceolate, acute or acuminate at apex, often unequal towards base, entire, glabrous, young leaves red. Flowers greenish; male one axillary fascicles; female one or two together; pedicels pubescent. Sepals ovate. Petals lanceolate, margin revolute, scale inside pubescent. Stamen shorter than petals. Ovary pubescent, stigma spreading, irregularly lobbed. Fruits globose, pointed tipped with persistent stigma, tomentose; seeds closely packed. The tree flowers during February and fruit matures in July.

Uses: The timber is good for construction purpose and is used for beams and rafters. It is also a good fuel wood. The seed contain fatty oil, which is similar to Chaulmoogra oil. This oil is used locally to cure skin diseases and leprosy.

11. *Myristica dactyloides* Gaertn. (*Syn.*: *M. laurifolia* Hook.f & Thomas, *M. beddomei* King., *M. contorta* Warb.) *Family*: Myristicaceae

Vernacular Names: *Maratti*: Jayphal; *Tamil*: Kat jathikai; *Kannada*: Jajikai; *Malayalam*: Kattujathi, Adakkapain; *Sanskrit*: Jatiphala.

Distribution: Southern India, Sri Lanka. Western Ghats from Konkan southwards and in Annamalai, Nilgiris, and Kolli Hills up to 1500 m. The species is distributed throughout the MPCA even along the river in patch near River Kunthipuzha with elevation of 810 m and at Kattivaramudi with the elevation of 1170 m. In Kolli Hills MPCA, it is distributed throughout the evergreen parts of MPCA associated with *Canarium strictum, Artocarpus heterophyllus,* etc. Frequency of occurrence is 70%.

Description: Tree to 30 m; branchlets glabrous, except for the velvety terminal bud. Leaves elliptic to broadly lanceolate, coriaceous, glabrous to glaucescent below, base rounded to acute, margin entire, apex acute. Perianth thin-fleshy, rusty-tomentose, connate in to an obovoid-globose. Staminal column produced beyond anthers; anther dome shaped. Ovary ovoid-globose, appressed pubescent, short, obtuse. Drupe broadly ovoid to elliptic, rounded below, depressed above; pericarp thick; stalk stout; seed ovoid; testa hard; aril fleshy, laciniate. Flowering and fruiting season are from October to June.

Uses: Aril with dried ginger is rubbed in cold water and given to check diarrhoea. Aril is also used in treating cough, bronchitis. Seed kernel on extraction with benzene yields 25% of light-yellow fat, which contains 89% fatty acids corresponding to 93% triglycerides. The component of fatty acids consists of palmetic acid; stearic acid 60; oleic acid; linoleic acid.

12. *Myristica malabarica* Lam. *Family*: Myristicaceae

Vernacular Name: *Tamil*: Patthiri; *Telugu*: Adavijajikaya; *Kannada*: Kanagi; *Malayalam*: Kattujattika.

Distribution: Endemic to Western Ghats distributed in Low elevation in evergreen forests of Maharashtra, Karnataka, Tamil Nadu, and Kerala. The species has not been located from the MPCA area. But it was located in Pathenthode areas in Attapadi reserve forest, the buffer zone of Silent Valley National Park.

Description: A moderate-sized tree, bark smooth. Leaves narrow-oblong, acute at both ends or obtuse, quite glabrous, glaucous beneath. Inflorescence cymose, axillary. Male flowers many, longer than the petiole. Female flowers few, globose, pubescent externally, bract very broad. Fruits cylindrical, brown tomentose; aril golden yellow, completely covering the seeds. It flowers during September–November.

Uses: The seeds yield oil used for burning and making candles. The wood is used for tea boxes in Ceylon and considered suitable for light furniture, match boxes, and splints. The seeds are used in external application for indolent ulcers, cleaning the surface, and establishing healthy action. The oil is used as an embrocation in rheumatism, sores, and pain. Aril of the seeds is used to check cough, bronchitis, fever, and burning sensation.

13. *Nothapodytes nimmoniana* (Graham) Mabb. (Syn.:*Premna nimoniana* Graham, *Stemonurus foetidus* Wight, *Mappia foetida* (Wight) Miers, *Nothapodytes foetida* (Wight) Sleumer); *Family*: Icacinaceae

Vernacular Name: *Tamil & Malayalam:* Pee-nari.

Distribution: Indo-Malaysia and Indo-China; In moist deciduous to evergreen forests of the Western Ghats of Maharashtra, Karnataka, Tamil Nadu, Kerala and parts of the Deccan peninsula, North Bengal, and Assam. Located only one individual (sapling) near the border to Attapadi Reserve Forest in Silent Valley MPCA.

Description: Small trees. Leaves ovate-oblong, slightly hairy beneath, rounded and asymmetrical at base, acute-acuminate at apex. Flowers foetid, greenish-white, in terminal corymbose cymes. Calyx toothed. Petals villous; disc copular. Stigma thick. Fruit drupe, smooth, oblong, compressed, one-seeded. Flowering and fruiting: June–October.

Uses: The wood extract of this tree contains Campothecin and is used in the treatment of cancer and tumours.

14. *Persea macrantha* (Nees) Kostern. (*Syn*.: *Machilus macrantha* Nees, *M. glaucescens.*)*; Family*: Lauraceae.

Vernacular names: *Kannada*: Kuylur mavu, Gulmao; *Malayalam*: Kulamavu; *Maratti*: Gulum; *Tamil*: Anaikkuru, Koolamavu; *Telugu*: Nara, *Trade name*: Machilus.

Distribution: Western Ghats, in most districts from S. Canara and Coorg to Nilgiris, Anamalais, Pulneys, and Hills of Travancore in evergreen forests up to 7000 ft. It is distributed throughout the Silent Valley and Kolli Hills MPCA and its frequency of occurrence was above 70%. This species is mostly seen in association with *Syzygium cumini.*

Description: Tree to 35 m; Leaves alternate, elliptic to oblong, penninerved, base acute to obliquely truncate, apex obtuse to acute. Panicles sub-terminal. Flowers bisexual. Tepals 6, equal or the outer whorl smaller, obovate, puberulous. Fertile stamens 9; filaments pubescent; anthers 4-celled; staminodes 3, stalked, arrow-shaped. Stigma simple. Berry globose. Flowering occur during December to April and fruiting during May to June.

Uses: The stem bark is used for treatment of asthma, convulsions, constipation, and rheumatism. The leaves are used as an external application for ulcers. Stem bark extensively collected for Agarbatti making.

15. *Piper mullesua* Buch. Ham.ex D.Don. (*Syn*.: *Piper brachystachyum* Wall. ex Hook., *Chavica sphaerostachya* Miq.); *Family*: Piperaceae

Distribution: Distributed in evergreen and shola forests above 840 m in Peninsular and north east India. Its distribution is patchy mostly distributed along the sloppy region and at higher elevation even above 1000 m in Silent Valley MPCA.

Description: A much-branched climber. Leaves elliptic-ovate or lanceolate, 5 × 2 cm, obtusely caudate, acuminate, acute at base, 3–5 nerved. Male spikes elongate; female globose head; rachis pubescent. Berries globose, orange red. Flowering: July – August; Fruiting: August–September.

Uses: The leaves in steam distillation gave a volatile oil with an odour reminiscent of lime oil is used for cough.

16. *Rhaphidophora pertusa* (Roxb.) Schott (*Syn*.: *Monstera pertusa* (Roxb.) Schott, *Pothos pertusa* Roxb *Scindapsus pertusus* (Roxb.) Schott); Family: Araceae

Vernacular names: *Malayalam*: Anathippali; *Tamil*: Anaipirandai; *Kannada*: Dodda tippali; *Maratti*: Ganeshkanda; *Sanskrit*: Sphotya bhujangam; *Telugu*: Enugan alleru.

Distribution: Peninsular India and Sri Lanka, Deccan peninsula, Coromandel, and Malabar coast between 500 and 1700 m altitude. Distributed on either side of stream near Nachiyamman Kovil in Kolli Hills MPCA. Frequency of occurrence is very low.

Description: Evergreen, semi-epiphytic, unarmed, root-climbers. Leaves entire, perforate or partly pinnately lobed. Inflorescence axillary; spathe deciduous; spadix sessile or sub-sessile. Flowers naked, bisexual, completely and densely covering the spadix. Stamens 4 with star-shaped filament. Ovary unilocular; ovules many. Berry many-seeded; seeds oblong to reniform, endospermous. Flowers during August to November and fruits in January to March.

Uses: Used for the treatment of snakebites and scorpion stings. Stem used for treating ulcers, pain in the colon, abdominal tumours, and also in bronchiopathy. Kani tribes in Kerala orally administer the stem juice to cure ascites inflammation of spleen and liver.

17. *Santalum album* Linn. (*Syn*.: *Sirium myrtifolium* Roxb.); *Family*: Santalaceae.

Vernacular names: *Hindi*: chandan; *Kannada*: sriganda; *Malayalam*: chandhanam; *Tamil*: sandanam.

Distribution: Peninsular India, Malaysia to Indonesia, up to 1400 m. In Kolli Hills MPCA only seedlings and saplings could be seen in deciduous forest below 980 m elevation along the slopes.

Description: An evergreen, semi-parasitic, glabrous tree. Leaves opposite, ovate, elliptic obtusely pointed, entire. Inflorescence panicle, cymose axillary and terminal. Flowers brownish-purple, fruit is a drupe, black when ripe. It flowers during October to December and fruits in February to May.

Uses: The wood is highly fragrant, so used in temples. Sandal wood paste is used to curing burnings. Essential oil distilled from pieces of heartwood, used in perfumery. The oil and wood have cooling and diaphoretic, diuretic and expectorant properties, used in gonorrhoea; reported to have antibacterial activity against *Eberthella typhosa* and *E. coli*; seed oil is used in some skin troubles.

18. *Smilax zeylanica* L. (*Syn*.: *Smilax indica* Burm.f., *Smilax elliptica* R.Br.); *Family*: Smilacaceae

Vernacular Name: *Tamil*: Kattukodi; *Hindi*: Ramdatum; *Malayalam*: Karivilanti.

Distribution: Distributed in E. Himalaya, Indo-China, Malaysia up to an altitude of 1200 m, climbing on thickets in shola border. It is common and available throughout both the MPCAs. It flowers during November–December.

Description: Armed vines, glabrous. Leaves oblong-broadly elliptic or ovate-lanceolate, 3–5 veined, coriaceous, entire, apex obtuse, subacute, somewhat notched, cuspidate; leaf-sheath narrow, not auricled. Inflorescence greenish-white, many in umbels axillary. Perianth lobes greenish, oblong; outer ones broad; inner ones narrow. Stamens 6; anthers oblong. Ovary 3-celled, 3-lobed; style 3-fid; berry globose red.

Uses: According to Nadkarni (1976), decoction of the root is given for swellings, abscesses and boils. In some parts of India, the roots are used in the treatment of venereal diseases. The Mundas of Chota Nagpur use the root in bloodless dysentry.

19. *Symplocos cochinchinensis* var. *laurina* (Retz.) Noot. (*Syn*.: *Symplocos spicata* Roxb., *Symplocos laurina* (Retz.) Wall. ex G. Don, *Symplocos spicata* Roxb. *var. laurina* (Retz.) Clarke; Family: Symplocaceae

Vernacular Name: *Sanskrit*: lodhra; *Hindi*: lodh; *Assam*: mota bhomlati; *Maratti*: mirajoli; *Tamil*: kambili vetti. *Telugu*: loddugu; *Malayalam*: pachotti. Parala, pambari.

Distribution: Shady localities between altitudes of 1200 and 1680 m. in the Himalayas, Khasi and Mikir hills, Eastern Ghats and some parts of Western Ghats. It is mostly available along the border of MPCA, associated with Syzygiun *cumini* and *Neolitsea scrobiculata* along the slopes and hill tops. Frequency of occurrence is 25%. Flowering and fruiting season are during February to November.

Description: A medium-sized, evergreen tree. Leaves elliptic-lanceolate, acuminate, crenate-serrate, and yellow when dry. Flowers white, axillary branched, fragrant, sessile, forming compound, tomentose panicles. Stamens more than 12; filament hairy; female flowers with 8 staminodes, fruits globose, green.

Uses: The wood is used for house-posts and furniture and bark is used for dyeing. Medicinally, it is used in dropsy, ulcers, arthritis, bronchitis, leprosy, asthma, diarrhoea, dysentery and skin and eye diseases.

20. *Symplocos racemosa* Roxb.; Family: Symplocaceae

Vernacular Name: *Sanskrit*: Marjana; *Hindi*: Lodh; *Tamil*: Velli-lethi; *Malayalam*: Pachotti.

Distribution: Distributed in moist deciduous to evergreen forests in India, Nepal, Bangladesh, Myanmar, Thailand, China, Vietnam. In the Western Ghats, it is found in evergreen forests of Maharashtra, Karnataka, Kerala and Tamil Nadu. It is mostly available along the border of Silent Valley MPCA and Attapadi Reserve. It is associated with *Syzygiun cumini* and *S. densiflorum* along the slopes and hilltops. Flowering and fruiting season are December to April.

Description: An evergreen tree. Leaves elliptic-lanceolate, crenate-serrate, margins reflexed. Flowers white, axillary, simple or compound racemes. Ovary hairy. Drupe purplish-black when ripe, sub-cylindric, smooth, 1–3 seeded.

Uses: The bark is used to inhibit the growth of *Microccoccus pygenes* var. *aureus*, *Escherichia coli* and enteric and dysenteric group of organisms. Bark with combination of sugar is treatment of menorrhagia and other uterine disorders. Stem bark is used to treat haemorrhage, pimples, leucorrhoea wound, and hoarseness of voice, fever, and skin disorders. The bark is used to dye red and is exported for that purpose. The red powder used during the festival of Holi and in Calico-printing and leather dyeing, it is used as an auxiliary. The wood is used for furniture.

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Chapter 15
Threatened Tree Species of the Western Ghats: Status, Diversity and Conservation

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Abstract India has been known worldwide for her rich cultural heritage, natural resources and biological diversity since ancient times. The country exhibits a variety of zones and coastlines with various ecological habitats resulting in vast richness in her floristic wealth. There are four biodiversity hotspots in our country namely the Himalayas, Indo-Burma, Sundalands and the Western Ghats. The Western Ghats has received wide conservation and research interest globally. Geologically older than the Himalayas, the Western Ghats are a chain of low mountains running 1600 km parallel to India's western coast. They have some of the finest non-equatorial tropical evergreen forests in the world with very high levels of speciation and endemism. They shelter at least 352 endemic trees of which 325 are globally threatened including 51 that are critically endangered. This irreplaceable biodiversity and ecosystem service values of the Western Ghats are threatened by a variety of human pressures. Land use changes caused by increasing population, agricultural expansion, infrastructural development along with intensive harvesting for fuel-wood, bark, leaves, fruits, exudates, etc. have contributed to the loss of biodiversity. This rich biodiversity has to be conserved, while providing adequate opportunities for livelihood security of the local people. We need to develop strategies for shielding whatever is left of the Western Ghats. There is an international effort to identify tree species that face extinction in order to make conservation efforts more efficient. Data on tree species and their distribution in the Western Ghats are increasingly available. Realizing its value, conservation efforts are on for many species. This chapter is an attempt to present threatened tree species largely found only in the Western Ghats, which form an integral part of the livelihood of the dwellers, and are fast dwindling. Different conservation strategies, which could be adopted to safeguard these treasures for posterity, are also presented.

Keywords Western Ghats · Forest trees · RET · conservation · endemics

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15.1 Introduction

One of the oldest ecosystems in the world, the Western Ghats, is a unique mountain system stretching through five States in India. Covering approximately 1,60,000 sq. km, she runs nearly 1600 km from the border of Gujarat, through the states of Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala.

The Western Ghats is home to about 300 globally threatened species and encompasses one among the last tracts of virgin tropical evergreen forest in India. Many tree species from several families of great economic importance exist in this region and provide a wide spectrum of Non-Timber Forest Products (NTFPs). Collection of forest produce such as pepper, cardamom, dammar and myrobalan has gone on for a long time in the Western Ghats. High human population density and major transformation of the landscape over the last century saw a major reduction in the natural resources emphasizing the urgency of conservation of the Ghats and sustainable use of its resources.

15.2 Need for Conservation

The Western Ghats is recognized by Conservation International as one of the world's eight hotspots for biological diversity where climatic and elevation gradients have resulted in exceptionally high speciation. The area has at least 4780 vascular plant species, of which 2180 are endemic (0.7% of the world's plants) (Myers et al. 2000). At the time of the original definition, the Western Ghats ranked seventh for endemic vascular plants per unit area. They were also eighth when comparing endemism and the remaining primary vegetation with the original extent. However, now only less than 7% of the original primary vegetation exists (Myers et al. 2000). Considering the past and predicted losses of habitat and species, the mountains are one of the 11 global hotspots in most need of conservation (Brooks et al. 2002).

The forests of the Western Ghats have been highly heterogeneous and complex having varied conditions leading to the spatial distribution of vegetation (Pascal, 1988). Reduction of forests has been from 1% to 4% annually (Laurance 1999), which may have forced many dependent species to extinction. Overexploitation, habitat destruction and unsustainable harvesting, coupled with illegal trade practices, have driven many plant species to the brink of extinction (Rajasekharan and Ganeshan 2010). Another major threat observed is collection of reproductive parts (for almost 80% of the species) which restricts/hinders natural regeneration of the species.

The awareness about the dangers of deforestation has been growing stronger in the recent years among the policy makers and the public. Over the last few decades, the liberalization of the markets and the demand for herbal medicine have led to increased pressure on the forests and its products. Since most of the extraction is from natural populations, this enormous pressure is a threat towards the plant populations and consequently also to the survival of the species. Successful long-term conservation efforts require a thorough investigation of the species distribution, genetic status and anthropological use (Shaanker et al. 2006).

15.2.1 Ex Situ Conservation

This method involves protecting an endangered species by placing it in a new location, which could either be a wild area or within the care of humans. Ex-situ conservation comprises some of the oldest and best known conservation methods, and also newer, sometimes laboratory methods. Generally, conservation takes place in facilities which support either storage or the continuity of the conditions suited to maintain the viability and genetic constitution of the genetic material or diversity. Ex situ management has been used to deliver conservation benefit for threatened species. Species extinctions have been prevented and for an increasing number of species there have been conservation restorations or introductions following periods of ex situ management (IUCN/SSC 2014).

Some possible approaches of ex situ conservation of trees are mentioned below (FAO 2012).

- Provenance trials – comparing trees grown from seed or cuttings collected in many parts of a species range.
- Seed orchards – plantations established for the production of tree seed.
- Clonal repositories – collection of clones of a species.
- Botanical gardens – plants, especially ferns, conifers and flowering plants, are grown and displayed for the purposes of research and education.
- Arboreta – trees are grown and displayed for the purposes of research and education.
- Herbal gardens – plants of known medicinal values are grown and displayed for the purposes of research and education.
- Seed and pollen banks – storing seeds as a source for planting in case seed reserves elsewhere are destroyed and pollen for controlled pollination. It facilitates germplasm exchange.
- Vegetative propagules – stored under aseptic conditions.

Ex situ conservation efforts involve the forest departments, research organizations, universities and local communities. The large number of tree species poses great challenge in ex situ conservation efforts. Many of the evergreen species seeds are recalcitrant in nature and not amenable for traditional storage methods.

S. No.	Ex situ conservation through	Species
1.	Stands	Biotechnology Research Centre (Biotrim), Andhra Pradesh
2.	Seed orchards	Eucalyptus orchards by IFGTB, Coimbatore
3.	Clone banks	Sandal clone bank by IWST, Bangalore; Casuarina germplasm bank, the largest in the world by IFGTB, Coimbatore
4.	Conservation populations	Teak at Van Sanshodhan Sanstha, Chandrapur, Maharashtra
5.	Arboreta or botanical gardens	Medicinal tree gardens supported by NMPB
6.	Seed banks	Neem at NBPGR, Delhi
7.	In vitro (including cryopreservation)	Many RET tree species at IIHR, Bangalore

15.3 The Species Selected

Ten threatened trees have been discussed in detail in this chapter. These species have been selected due to their heavy demand as NTFPs, the main source of supply being the tropical forests – the Western Ghats. Due to long gestation period, these trees do not find a place in cultivation, and hence the genetic stocks available in the forests are fast depleting. Table 15.1 details their threat status and annual demand.

Table 15.1 Demand in trade

Species	Threat status	Parts used	Source of supply	Annual demand in MT
Canarium strictum	VU(TN, KTK, KL)	Bark (resin)	Wild	2000
Garcinia gummi-gutta	NT (TN, KL, KTK, MH)	Fruit (fruit, peel)	Wild/ Cultivated	500
Hopea parviflora	EN (TN, KTK, KL)	Wood	Wild	–
Hydnocarpus alpina	VU(TN, KTK, KL)	Fruit/seed	Wild	200
Madhuca longifolia	VU (KTK)	Fruit	Wild	500
Oroxylum indicum	DD (TN); EN (KL, MH); VU (KTK)	Bark (stem, root)	Wild	1500
Perseamacarantha	EN (TN, KTK);VU (KL)	Bark	Wild	200
Santalum album	EN (TN, KTK, KL)	Heartwood	Wild/ cultivated	1000
Saraca asoca	DD (TN, KL); EN (KTK, MH)	Bark (stem)	Wild	2000
Vateria indica	VU(TN, KTK, KL)	Bark (resin)	Wild	2000

Molur et al. 1995; Molur and Walker 1997; Ganeshaiah and Uma Shaanker 2003; Ved and Goraya 2008; Goraya and Ved 2017

VU vulnerable, *NT* near threatened, *EN* endangered, *DD* data deficient, *TN* Tamil Nadu, *KTK* Karnataka, *KL* Kerala, *MH* Maharashtra

15.3.1 Canarium strictum *Roxb*

Canarium strictum Roxb. is an indigenous and endemic plant species of the Eastern and Western Ghats. A red-listed medicinal evergreen tree species, it is highly valued for its aromatic resin (Black Dammar or Dhoopa). It occurs in the tropical moist evergreen and moist mixed deciduous forests, and is commercially harvested for dammar, throughout South and South East Asia. Due to its overexploitation and the loss of habitat, it has been enlisted as an endangered species (Meena et al. 2012).

Botanical Description *C. strictum* is a large tree with a spherical crown and a clear trunk 30–35 m long**.** It is observed in both the Eastern and Western Ghats. Leaves are compound (3–9 pairs), imparipinnate, increasing in size towards apex. It is reported to be polygamous (Sasidharan 2006) with some trees having more male flowers than female. Meena et al. (2012) report the species as a poly-gamodioecious tree very rarely gregarious in flowering.

Flowers are arranged, in shortly branched axillary panicles, about 1 cm long, yellow to dull white, shortly stalked and mildly fragrant. Flowering occurs from February to April and fruits start maturing from November to January. Phenology is observed to vary with locations. Fruits are drupe, 2.5–5.0 cm long, pointed at ends, mesocarp fleshy, stone hard, aromatic and seeds trigonous, usually three celled with three seeds. The ripened fruits/drupes are collected by lopping the small branches, the fleshy mesocarp is removed with a sharp knife, and seeds are dried under proper shade. The fruits weigh about 4–6 g, while the seeds weigh about 1 g (Kunhikannan et al. 2004).

Economic Importance *C. strictum* exudates a resin called as 'Sambrani' or 'Dammar' which has medicinal as well as commercial uses. Its usage among tribal and folk people for medicinal purposes in different parts of India has been explored through ethno-botanical studies. It is also used in Siddha system of medicine. It finds its usage in incense and varnish industries and also used as a substitute for burgundy pitch in making medicinal plasters.

Resin The species is rich sources for Sambrani which is used to cure various bronchial ailments. The resin powder is given orally to cure rheumatism, fever, cough, asthma, epilepsy, chronic skin disorders, syphilis and hernia and also helps to improve complexion (Augustine and Krishnan 2006).

Timber The wood of *C. strictum* is greyish-white with a pinkish cast to the heartwood and used for making boards for ceiling, flooring and partitions from well-seasoned timber. It is also used for packing cases and for cheap utility furniture. The wood has good glue holding capacity and used for plywood tea-boxes (CSIR 1992).

Seed The seed kernel is edible and its oil is used in confectionery.

Threats and Status of the Population ENDANGERED Criteria: A2bd; B1 + 2bcde [(A2bd – Population reduction by at least 20% ({bd} due to exploitation); B1 + 2 – severely fragmented and continues to decline (bcde-area of occupancy, quality of habitat, number of subpopulations and mature individuals).

Mature individuals in all populations are >2500. The number of mature individuals declined in the past by 10–20% and is likely to decline by 10–20% in the future. Generation time is estimated at 30–35 years. The population size and numbers of the taxon are declining at the rate of <10% in the last 10 years and is predicted to decline by >50% in the next 3 generations. If the taxon were to go extinct, it may be difficult for it to recolonize.

Habitat fragmentation and landscape changes (Kolli Hills, Eastern Ghats), pollinator limitation (Kolli Hills, Eastern Ghats), seed dispersal limitation (Kolli Hills, Eastern Ghats and Silent Valley, Western Ghats) (Kunhikannan et al. 2004), exploitation for resin and wood and other human activities contributed in the declination of the population of the species.

Regeneration and Viability Regeneration or adequate protection of areas from clearance and degradation could allow it to make a fast recovery. Seeds of *C. strictum* fall close to the tree and germinate easily. Regeneration is very high; however, the recruits fail to establish. Artificially it could be propagated by directly sowing the seeds soaked for a day in water followed by accelerated ageing at 40° C. Intact drupes can rarely be collected from the forest floor. The fruits are easily susceptible to insect pests and hence physically damaged insect-affected drupes do not germinate easily. Germination is epigeal and cotyledons are trifid; it starts after 3 weeks of sowing and continues up to 120 days especially when sowing is done during winter months. Ninety-five percent germination was observed on sand substratum. Transplanting is done in the polythene bags when seedlings attain 3-leaves stage. Initial growth of the seedlings is very fast and they become ready for plantation after about 2 months of transplanting. Establishing nursery at the study site can aid in achieving high seed to seedling ratio.

Harvesting and Resin Collection The collection of the resin from the species is organized by private contractors, primary cooperative societies and is also collected by individuals throughout the year except rainy season. The harvest techniques used are making an incision on the trunk and debarking. The trade in the product is private. Collectors sell the harvested parts to private traders and primary co-operative societies. The collectors receive Rs. 30–60 per kg for the NTFP. Trade is organized and collected through Large and Multi-Purpose Cooperative Societies (LAMPS) in Wayanad. The resin of the taxon is in local, domestic, commercial and international trade. The latter result in population decline.

The harvest of black dammar is permitted for trade in Kerala, but in Tamil Nadu, as a conservation measure, harvest is permitted only for personal or home use. In Kerala, the collection intensity varies from 20 kg to 100 kg per tree annually averaging to 10,000 kg per year (Menon 2002). Information on its collection in Karnataka is lacking, though there is a mention that small quantities are collected for local consumption. The domestic share of Dammar use is very high in the incense industry – including agarbatti sticks, etc. – and is estimated to be 18,000 million tons annually (Meena et al. 2012).

Ecological Impacts Among Tapping Strategies Heavy tapping of *C. strictum* significantly increases the tree mortality and decreases the reproductive output. This could impact the pollinators and frugivores. *C. strictum* is vital to sustain Hornbills and Imperial pigeons (Kannan 1992; Ganesh and Davidar 2001). Ecological surveys reveal a decrease in the number of trees in their natural pockets which could lead to still smaller populations with time.

15.3.2 Garcinia gummi-gutta (L.) Robs

Underutilized crops have been included in worldwide plans of action after having successfully raised the interest of decision makers. One such genus *Garcinia* includes 200 species, of which 30 species are reported to be found/grown in India (Korikanthimath and Desai, 2005). Out of the 30 species, three major species evincing interest from both conservation and utilization point of view are as follows:

1. *Garcinia indica* – (Vulnerable A2cd as per IUCN) confined to India and Sri Lanka only (Patil et al. 2005).
2. *Garcinia cowa* – found only in India distributed in the eastern parts of India (Orissa, Bihar, Bengal and Assam) and in the Andaman Islands.
3. *Garcinia gummi-gutta* (L.) Robs. This species has a restricted global distribution occurring in Southern India and Sri Lanka at an altitude range of 400–900 m. It is found in semi-evergreen to evergreen forests.

Geographic Distribution *G. gummi-gutta* is commonly found in the evergreen and shola forests of the Western Ghats, Karnataka and Kerala. The tree is very much adapted to both hilltops and plain lands, but its performance is best in riverbanks and valleys. It also grows well in dry or occasionally waterlogged or flooded soils (Orwa et al. 2009). *G. gummi-gutta* species has a restricted global distribution, occurring in Southern India and Sri Lanka. Within India, it has been recorded in the Western Ghats of Maharashtra (Bombay, Konkan), Goa (Anmod, Colemrange, Sangeum), Karnataka (Chikmagalur, Dakshin and Uttar Kannada, Kodagu, Hassan, Shimoga), Kerala (Calicut, Cannanore, Palakkad, Nilambur, Thrissur and Thiruvananthapuram) and Tamil Nadu (Coimbatore, Nilgiri, Tirunelveli and Dharmapuri districts) in an altitude range of 400–900 m. It has been introduced elsewhere in the tropics.

Botanical Description *G. gummi-gutta* is an evergreen, small to medium-sized tree, 5–20 m tall, about 70 cm dbh, with dark smooth, lactiferous bark and horizontal or drooping branches. Leaves are simple, entire, opposite, petiolate (1.2–2.2 cm long), coriaceous, glossy dark green, elliptic-ovate to obovate, 1–13 long, 2–8 cm wide, shortly acuminate tip, tapered based, sub-acute and glabrous. Flowers are either androecious or bisexual, thus it is an andro-monoecious species. Male flowers occur in axillary clusters of 4–8, with long membranous sepals and concave, oblong petals which are twice as long as sepals, with monadelphous cluster of 16–18 stamens attached to a pistillode with a non-functional stigma. Hermaphrodite flowers occur solitary or in 2–3 flowered axillary or terminal clusters, with 4 greenish white, 4–6-mm long persistent sepals and 4 greenish-white, pink, or reddish, fleshy, 5–10-mm long petals, stamens 8–12 free or in 2–3 bundles, ovary globose and superior crowned by a sessile, circular papillate stigma with 8–10 tuberculate stigmatic rays. The fruit is a fleshy, globose, sub-globose to ovoid berry, 7–10 cm in diameter, green turning yellow, orangey or reddish when ripe, fluted with 5–13 longitudinal grooves, not grooved to the tip. The fruit is capped by the persistent calyx at the terminal end and a rosette of 4–5 triangular remnants of the stigma at protruding nipple-shaped mamilla. Seeds 6–8, smooth, pale brown, oval, 12 mm long, surrounded by a succulent reddish or whitish succulent aril.

Economic Importance The fruit is the economically important part of the tree. It is a poly-gamo-dioecious tree and has remained neglected from a research perspective though they are highly valued in South Indian cuisine and in traditional system of medicine (Rema and Krishnamurthy 2000). The pulp of the fruit rind, also known as 'kokum', is used in curries as a souring condiment, in preparing a refreshing drink rich in antioxidants and is known to have antiseptic properties. Dried seeds yield kokum butter, rich in protein and fat. The oil is traditionally used for treating skin diseases (Joshi et al. 2006).

The principal constituents of *G. gummi-gutta* are hydroxycitric acid, garcinol, isogarcinol and cyanidin. Different parts of the tree show anti-tumour, anti-inflammatory, anti-obesity, anti-fungal, antibacterial, hypoglycaemic, anti-oxidant and anti-ulcerogenic activity (Joshi et al. 2006).

Regeneration and Viability

Seed biology: Primates consume the fruits dispersing seeds away from parent trees, thereby increasing the probability of survival of seeds and seedlings (Rai 2003). Mature ripe fruits of *G. gummi-gutta* should be picked from the tree either by climbing the tree or using pole pruners. Collected fruits need to be spread on the floor under sun for one day to allow uniform ripening. The fully ripe yellow fruits can be depulped using a knife to extract seeds, washed in water to remove any pulp adhering to the seed coat. Shade drying seeds for 2 weeks is found suitable than sun drying. Fruits need to be collected at yellow stage. Freshly extracted and surface dried seeds have an initial moisture content and germination of 35.84% and 5% respectively. Germination is slow and initiates only after 2 months of sowing.

Pretreatments: Poor initial germination demanded presowing treatments for enhancing germination. The gummy seed pulp and coat hindered germination. Sand aberration, kerosene wash, removal of seed coat or soaking in 0.9% sodium chloride for 1 hr. are the effective pre-treatments for improving germination from the existing 5% to 45%.

Seed storage behaviour: G. gummi-gutta seeds were found to be desiccation tolerant, but temperature sensitive. The seeds possess about 35% initial moisture and shelf life is lesser than a year under ambient conditions. Desiccation did not adversely influence the viability of the seeds. Hence, it could be classified as an intermediate seed with a tendency towards recalcitrance. *G. gummi-gutta* seeds were desiccated from initial moisture content of 35% to 15%, 10% and 5% on a dessicator and were tested for germination. Though there was gradual decrease in germination, no significant variation was observed among different treatments suggesting desiccation tolerance of *G. gummi-gutta* seeds.

It was observed that when stored at different temperatures for a year, seeds could withstand ambient temperature and recorded good germination (53%) at the end of 6 months. This indicates the process of after-ripening in seeds which has been reported in many intermediate and orthodox seeds. Seeds could be stored at 20 °C for over a year with 25% germination.

Seeds of *G. gummi-gutta* have non-deep physiological dormancy (PD) and are sensitive to desiccation and low temperature. The seeds are classified as a tropical dormant recalcitrant. This has ecological significance as it helps seeds to survive dry conditions and leads to germination of seeds in the subsequent monsoon season (Joshi et al. 2017).

A major reason for decline in germination was a steady decrease in total carbohydrate content and gradual decrease in free amino acid content during storage. Reducing sugars and total protein content also diminished to very low levels at the end of 12 months. Thus, optimum levels of metabolites that can sustain viability are available in the seeds up to 6 months, beyond which reduction metabolites could affect seed vigour, and therefore germination.

The fruits of *G. gummi-gutta* are harvested intensively from the forests. 90% to 95% of fruits are removed from individual trees in the high harvest areas, where fruits along with seeds are removed. Such high seed removal rates would result in a reduction in the number of natural regenerants. Joshi et al. (2006) reported that sections of the seeds of this species develop into complete seedlings producing supernumerary plants from seed fragments. This species is one of the major food of frugivores, such as primates, civets and arboreal squirrels, who are also the main seed dispersers. Each fruit has six to seven seeds, and during frugivory, there is every probability that seeds get damaged. Seeds with damaged seed coat report higher germination than intact seeds. Defecated seeds show poor germination, indicating inhibition of germination due to the undamaged seed coat (Lieberman and Lieberman 1986). Thus damaged seeds have a higher chance of germination. However, due to high food reserve contents in the seeds, its chances of pest infestation are higher. Thus, as a means of survival, battling all pressures, the species establishes from any seed fragment that contains vasculature. With time, as the food reserves deplete, the seeds lose the ability to regenerate.

Threats and Status of the Population The species is dioecious, with separate male and female trees. Both sexes flower from February to April and fruits ripen from July to September, which coincides with the rainy season. The flowers are probably pollinated by weevils. Fruits ripen in a staggered fashion, which ensures food for the frugivores and seed dispersal (Lee et al. 1988). The major frugivores are common langur, bonnet macaque, common palm civet, and the endangered brown palm civet which move away from the trees with the fruits thereby increasing the probability of survival of seeds and seedlings. Animals discard the rind, after eating the pulp. Collection of this rind, therefore, has no explicit adverse impact on the ecology of the species as collection rate is low. Damage due to harvest of lateral limbs for easy harvest is another threat to the species. The trees are not distributed evenly. Though natural regeneration is high, the number of seedlings observed is low.

Genetic Resources: Collection, Characterization, Conservation and Documentation

Realizing the need for conservation, efforts are on to identify document and conserve 24 accessions collected from Dakshin Kannad (2 accessions), Uttar Kannad (25), Kodagu (10), Chickmagalur (8), Shimoga (1) and Belgaum (8) districts of Karnataka; Thrissur (17 accessions), Kottayam (3), Kannur (8), Alappuzha (11), Ernakulam (9), Malappuram (3), Kozhikode (3), Kollam (6), Pathanamthitta (5) and Thiruvananthapuram (1) districts of Kerala; and South Goa (4 accessions) district of Goa.

Four different forms of sexuality of flowers were noticed. They were (i) male flowers with pistillode, (ii) male flowers without pistillode or perfect pistil, (iii) female flowers with 1 to 4 bundles of staminodes and (iv) bisexual flowers. Male flower with pistillode is with perfect stamens and without perfect pistil. Male flower without pistillode is with perfect stamens only. Female flower is with perfect pistil and without perfect stamens but with staminodes. Bisexual flower is with both perfect pistil and stamens.

Two promising germplasms have been registered for their high yield. IC244100-2 (INGR No. 04061) and IC244111-1 (INGR No. 04062) (Abraham et al. 2009).

Markers have been developed to examine the diversity of the species using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers (Mohan et al. 2012; Parthasarathy et al. 2013). Ravishankar et al. (2017) isolated and characterized microsatellites from whole-genome sequence data of *G. gummi-gutta.* The NGS and mining of the *G. gummi-gutta* genome helped in the identification of thousands of SSR markers, which will support genetic studies, genotyping and conservation strategies in *G. gummi-gutta.*

The increase in the level of endemism from 50% to 65% is an important indication of the shrinking population of this species posing challenge for conservation biologists. Therefore, the management of genetic resources of *Garcinia* has to be urgently taken-up ex situ.

15.3.3 Hopea parviflora Bedd

H. parviflora is a large tree reaching a height of 30–40 m and 4–6 m girth with a clean bole height of about 20 m occurring in the Western Ghats. This species prefers deep moist soil and grows best along the side of streams and in moist valleys, but sometimes thrives even in dry hard laterite.

Geographic Distribution The species is found in moist tropical evergreen forest up to 2500 ft. It is abundant and gregarious in the interior forests of the Western Ghats from North Kanara southwards. Pure Hopea forests can be seen as large patches within the forests. It is found mainly on the hill-sides bordering on large rivers. It is essentially a semi-shola tree requiring close proximity of running water for its growth (Troup 1921).

Botanical Description *H. parviflora* trees can grow up to a height of 37 metre with maximum of 4.6 metre girth. The leaves are cordate or sub-cordate at the base with undulate margin. Leaf flushing starts from September and extends to December. Young leaves are yellowish green maturing to dark green. Though the leaf flushing is an annual phenomenon, flowering is observed only at an interval of 2–3 years. Flowers are small, bisexual, tomentose and short pedicelled. Cream in colour, they appear in February. Fruits are light with two straw-coloured wings less than 5 cm long (Sunilkumar and Sudhakara 1998). The fruits begin to mature in May during the monsoon period, and are shed off the tree during the maturation process. Hence the fallen fruits vary from green (immature) to brown (mature). Due to the presence of wings, the fruits land away from the mother tree. The seeds germinate rapidly once they reach the ground. It seeds heavily once in 2 years (Sivakumar 2004).

Economic Importance The wood is a valuable one, hard, heavy and durable, used for building and boats (Troup 1921). In addition to their timber value their bark is a good tanning material, especially for heavy leather. The bark containing 70% tannin and 22.6% non-tannin is used as an astringent (Howes 1953).

Regeneration and Viability

Seed biology: The seeds of *H. parviflora* are highly recalcitrant in nature, a characteristic feature of Dipterocarps. Mature seeds of *H. parviflora* were found to have 43.15% moisture content and 82.0% germination. Immature seeds *H. parviflora* stored better than mature seeds. Desiccating seeds up to 28.79% moisture content showed corresponding reduction in germination percentage to 53.5. Experiments carried out by Sunilkumar and Sudhakara (1998) reveal that seeds of *H. parviflora* with 29.5% moisture content stored at 10 °C in perforated polyethylene bags gave 21.0% germination after 1 week. Studies carried out by Dayal and Kaveriappa (2000) in *H. parviflora* showed a drastic reduction in the germination of seeds during desiccation below 32.0% moisture content using various drying methods namely silica gel, oven (40 °C), AC room (24 °C) and laboratory

(30 °C). Ascorbic acid and citric acid treatment prolonged the storage of *H. parviflora* up to 30 days with 45.0% and 34.0% germination. Despite testing various methods, including synthetic seeds, the viability of the seeds could not be extended beyond 30 days. Storage of *H. parviflora* seeds in wet vermiculite was found to improve the storability. After 30 days of storage, *H. parviflora* seeds showed 60–65% germination (Sivakumar 2004).

Regeneration status: Flowers start to appear in January, and flowering process completes in March. The shed fruits are severely attacked by small weevils (Coleopterans) and ants. Regeneration is very high, with all intact seeds on the forest floor germinating immediately, when left undisturbed. The seeds are highly recalcitrant; they are sensitive to drought. Shade and moisture are necessary for their survival. Artificially could be propagated by direct sowings or by transplanting the entire plant along with the ball of earth. Seeds are viable for about a fortnight and have a good germinative capacity up to 95%.

15.3.4 Hydnocarpus alpina *Wight*

There are around 40 species in the genus *Hydnocarpus*, which are indigenous to Asia's tropical rain forests. The most important are *Hydnocarpus kurzii*, *H. pentandrus*, *H. anthelmintica*, *H. macrocarpa* and *H. alpina*. *Hydnocarpus* genus is a significant component of many fragile ecosystems such as sacred groves, protected areas, national parks and other areas that deserve conservation priority. As a genus, *Hydnocarpus* supports many animals, birds, butterflies and snakes, which includes endemic and endangered species, and a part of many ecologically fragile, sensitive and fragmented ecosystems this genus can be assigned the status of a key stone species.

Geographic Distribution It is found in the borders of Shola patches though it is not a typical shola species and in evergreen forests at an altitude of about 1400 m. In the Nilgiris, Tamil Nadu it is common in the Dolphin Nose area and has been reported in semi-evergreen forests of Megamalai at Vellimalai RF, Theni, Tamil Nadu. In Kerala, the species is distributed in the districts of Palakkad, Pathanamthitta, Idukki, Thiruvananthapuram, Kollam, Kozhikkode, Thrissur, Wayanad, Ernakulam and Malappuram.

Botanical Description It is a tree growing to a height of 15 m with smooth, greyish-brown, slightly rough bark of 5–6 mm thickness. The branchlets are puberulus and young leaves are copper red in colour. Leaves are simple, alternate, drooping with lateral deciduous stipules. Petiole 5-10 mm, stout, swollen tipped, grooved above and glabrous; lamina 8–25 × 5–10 cm, ovate, elliptic-oblong or ovate-lanceolate, base oblique, round or acute, apex acute or acuminate, margin entire, glabrous, glossy, coriaceous, lateral nerves 7–10 pairs, pinnate, slender, prominent and intercostae reticulate. Flowers unisexual 22–25 mm across, yellowish-white, solitary or in stout axillary fascicles; pedicel 1.5–2 cm long, deflexed, pubescent; sepals 5,8 mm long, oblong, pubescent, imbricate; petals 5.1 cm long, narrow, glabrous, with a scale at the base, scales linear, as long as petals, sparsely hairy, stamens 5–15; filaments glabrous; connectives broad; ovary 1-celled, tomentose, stigmas 5, free, radiating, recurved. Fruit a berry, 5–7 cm across, densely tomentose, dark brown. Seeds are numerous.

Economic Importance It is used in folk medicine and in treating ailments such as leprosy, skin diseases, leucoderma, pruritus, eczema, dermatitis, phthisis, tubercular laryngitis, chronic ulcers, dyspepsia, flatulence and verminosis (Vendan et al. 2010). It has been used both as a pesticide and manure in traditional agriculture. Seed oil is used in cosmetic industry for the production of traditional cosmetic products. The wood is used to make furniture and for packing boxes.

Threats and Status of the Population The species is endemic to the Western Ghats, distributed only in South India and Sri Lanka. It is reported to be in the vulnerable status of Red listing for the states of Karnataka, Kerala and Tamil Nadu.

Regeneration and Viability Flowering and fruiting occur during February–July. Pollination is mainly by insects; self and cross-pollination occurs. Propagation is mainly through seeds and seeds are viable for about 3 months. The whole fruit is broken to extract seeds, washed and sun dried. No special seed pre-treatment is required. Soaking dried seeds in water for 24 hours before sowing registers germination of about 25% (Krishna Kumar et al. 2013).

15.3.5 Madhuca longifolia

Madhuca longifolia (L.) J. F. Macbr. var. *latifolia* (Roxb.) A. Chev. belongs to the family Sapotaceae (USDA and ARS 2009). Commonly known as Indian butter (Mahua) tree, it is cultivated in warm climates for its oil-containing seeds (Patel 1966).

Botanical Description It is a large, much branched deciduous tree usually with a short bole large rounded crown attaining a height of 12–18 m and girth up to 2.4 m in 60–80 years. Bole short, crown rounded, bark grey to black with vertical cracks, exfoliating in thin scales. Leaves oblong-shaped, rigid, clustered at the end of branches, 6–9 cm × 13–23 cm, thick and firm, exuding a milky sap when broken. Young leaves pinkish and wooly underneath. Flowers cream, corollas fleshy, juicy, clustered at the end of branches. Fruit ovoid, fleshy, greenish, 3–5 cm long, 1–4 seeded. Seed large, 3–4 cm long, elliptical, flattened on one side (World Agroforestry Centre 2004).

Geographic Distribution It grows throughout the greater part of India up to an altitude of 1200 m from North Himalayan foothills to extreme southern part of the Indian peninsula. It is a common tree found in the deciduous forest of Madhya Pradesh (Hanies 1916), Maharashtra, Gujarat (Talbot 1902), Central India, Orissa and Western Ghats from Konkan region to Southwards, usually along the banks of rivers and streams (Troup 1921). *M. longifolia* is a species of dry tropical and subtropical climate commonly found scattered in pasture and uncultivated fields all over central and southern India. In natural forest, it is seldom gregarious in nature and occurs in a wide range of temperature.

Economic Importance The main demand of Mahua is the fleshy corollas which are succulent, rich source of sugars and vitamins which are eaten raw or cooked and used in the preparation of sweets. Flowers are also used for manufacturing of country liquor, portable spirit and vinegar (Waheed Khan 1972). Its kernel contains 30–40% fatty oil called as 'Mahua oil' or 'Butter of commerce'. Oil is used by tribals in cooking, burning and also sold for the manufacture of margarine, soap, glycerine, lubricating grease, as a batching oil in jute industry, adulteration of ghee and in several chemical or industrial uses (Anon 1988; Suri and Mathur 1988). Oil cakes are profitably utilized as organic manure and biocide (insecticide and herbicide properties) in different crops. After detoxification, seed cakes can be used as a concentrate feed for cattle and fish (Banerjii and Mitra 1996). *M. longifolia* wood is hard, heavy, strong and durable but liable to split. It is used for a variety of purposes like building, furniture, turnery, sport goods, musical instruments, oil and sugar presses, ship building, agricultural implement, carving, etc.

Regeneration and Viability *Seed biology*: The germination of fresh seeds (32% moisture content) was found to be 79%. Stored seeds tested for germination at periodic intervals to study the effect of temperature and seed moisture content on storability showed that seeds stored with 32% (fresh) moisture content at 20 °C survived better than other combination treatments, thereby recording 52% germination (Sivakumar 2004). It is propagated by direct seeding, seedlings or stumps.

Genetic Resources: Collection, Characterization, Conservation and Documentation Germplasm evaluation and conservation of mahua has been carried out by State Forest Research Institute, Jabalpur. Germplasm of *Madhuca longifolia* from Maharashtra has been established as demonstration plots at Tropical Forest Research Institute, Jabalpur.

Fourteen Candidate Plus Trees (CPTs) have been identified by Narendra Dev University of Agriculture and Technology, Faizabad. S.G. College of Agriculture and Research Station, Jagdalpur identified 20 plus trees of mahua. CCS Haryana Agricultural University, Hisar characterized seed morphological variation in mahua germplasm (50 CPTs). Thirty seed sources were identified by Forest College and Research Institute, Mettupalayam (NOVOD 2014) of which five accessions have been identified as high yielders for their oil, sucrose and Total Soluble Solids content. NBPGR, Delhi identified and characterized 37 accessions of mahua for seed and biochemical traits (Yadav et al. 2011).

15.3.6 Oroxylum indicum (L.) Kurz

Oroxylum indicum is native to the Indian subcontinent, in the Western Ghats and Himalayan foothills.

Geographical Distribution *Oroxylum indicum* is native to the Indian subcontinent, in the Himalayan foothills with a part extending to Bhutan and southern China, in Indo-China and the Malaysia ecozone. It is diversely available in the forest of National Park in Assam, India, reported from Sri Lanka.

Botanical Description *O. indicum* is a small- or medium-sized deciduous tree that grows up to 12 m in height with soft light brown or greyish brown bark with corky lenticels. The leaflets are very large, 90–180-cm-long 2–3 pinnate with 5 or more pairs of primary pinnae, cylindrical, swollen at the junction of branches, leaflets 2–4 pairs ovate or elliptic, acuminate, glabrous. The large leaf stalks wither and fall off the tree and collect near the base of the trunk, appearing to look like a pile of broken limb bones. The flowers are reddish purple outside and pale, pinkish-yellow within, numerous, in large erect racemes. The flowers bloom at night and emit a strong, stinky odour which attracts bats. The tree has long fruit pods that curved downward, hang down from the branches, looking like the wings of a large bird or dangling sickles or sword in the night. Fruits are flat capsules, 40–100 cm long and 5–10 cm broad and sword shaped. When the pod bursts open the seeds flutter to the ground, often traveling some distance, looking like butterflies. The seeds are numerous, flat and winged all around like papery wings, except at the base. The plant flowers in June–July and bears fruits in November. The fresh root bark is soft and juicy; it is sweet, becoming bitter later. On drying, the bark shrinks, adhere closely to the wood and becomes faintly fissured.

Economic Importance *O. indicum* is used as one of the important ingredients in most commonly used Ayurvedic preparations such as *Dasamularistha, Syonakaputapaka, Syonaka siddaghrta, Brhatpancamulyadikvatha, Amartarista, Dantyadyarista, Narayana Taila, Dhanawantara Ghrita, Dhanawantara Tailam, Brahma Rasayana* and *Chyavanaprasa*.

Market Prices Twigs of the species are traded in India at Rs. 9/kg (about US 20 cents/kg) while its extracts on the international market fetch Rs. 500,000/kg (US$15,000/kg). Market trend (2006–07) – market price: Rs 20–30 per kg (per year stem bark) and market demand: above 600 tonnes per year.

Threats and Status of the Population The distribution of this species is identified as regionally vulnerable due to the loss of habitat and harvesting for medicine and therefore, large-scale cultivation was recommended. Further, it does not have a history of repeated introductions outside its natural range. It is rarely cultivated.

Regeneration and Viability *Flowering*: August–November; *Fruiting*: December–June. *O. indicum* produces 130–226 flower buds arranged on 1-2 m flagelliflorous inflorescences. One tree produces 1–40 simultaneously flowering inflorescences. The flowers bloom only in the night. One to four flowers per inflorescence open at a time. The flowers are pollinated by bats. The seed pods are extremely conspicuous, reaching up to 4 feet in length and about 3 inches in width. They curve downwards hanging down from the branches. The pods dehisce septicidally, and the inner septum is woody. White seeds are arranged in several rows, 100–150 in number, very thin, compressed, rounded, surrounded by a transparent broad wing. When the pods dehisce at maturity, these seeds flutter to the ground, often traveling some distance. Natural regeneration is very profuse. However, the establishment of the seedlings is very poor. As a result, natural populations of the species do not exist. Only single trees are found scattered.

Seed biology: *O. indicum* produces flowers on flagelliflorous inflorescences (1–40 simultaneously). The inflorescences are bat-pollinated. White seeds in long conspicuous pods are compressed, and surrounded by a broad papery wing. On dehiscing, they flutter away from the tree. Natural regeneration is very profuse. However, their establishment is very poor. As a result, natural populations of the species do not exist. Only single trees are found scattered.

Mature pods are collected directly from the tree and seeds extracted following the manual cracking of the pods. The average 100 seed weight is 0.60 g. One pod can yield 100–150 seeds. The moisture content of fresh seeds is 5.73%. The initial germination is 37%. Fresh seeds germinate within 5 days of sowing. Seeds do not require any pretreatments for germination. Germination is hypogeal. The germination per cent is found to improve with storage. The overall germination per cent of the seeds varies from 80–92%. The seedlings can be transplanted at 100 days (Warrier et al. 2016). Being very thin and papery in nature, collection of seeds from the ground becomes very difficult once dehiscence is complete. Mature pods are collected from the tree and seeds are extracted following manual cracking of the pods. The average 100 seed weight is 0.60 g. One pod can yield 100–150 seeds. The moisture content of fresh seeds is 5.73%.

Seed storage: The seeds are tolerant to low temperatures confirming orthodox storage behaviour. Seeds on storage show an increase in germination suggesting that there is a need for after-ripening for attaining complete physiological maturity. Seeds can be stored at 20 °C for 18 months with 75% germination. Seeds can also be stored under ambient conditions in airtight containers for the same period; however, the germination per cent of seeds reduces to 50.

Nursery practices: For raising of seedlings, it is advisable to carry out direct sowing in polybags. The soil mixture advised is 2:1:1 of Red earth, Sand and FYM. One seed per bag gives high success rate (over 95%). Dibbling of seeds at 1 cm depth facilitates better germination. Seedlings are sensitive to transplantation shock. They are highly susceptible to damping off disease; hence germination studies conducted in the laboratory give better results than in nursery. Seedlings can be hardened under 50% shade conditions to avoid damping off. Once the seedlings are hardened, they can be outplanted.

In vitro *propagation*: Seeds of *Oroxylum indicum* were soaked overnight in water and the papery wings removed. The seed free of the wings was then surface sterilized with 1% mercuric chloride followed by washing thrice in sterile distilled water. The sterilized seeds were then dried and inoculated in 1% water agar. The seeds germinated well under in vitro conditions. A very high survival rate (90–92%) was observed when plantlets were transplanted and hardened in soil:sand:farm yard manure (1:2:1).

Genetic Resources Collection, Characterization, Conservation and Documentation Three conservation stands of the species have been established in different parts of the country. Two trials of *Oroxylum* have been laid out to identify fast growing accessions. Jayaram and Prasad (2008) reported low genetic diversity in *Oroxylum* collected from eight different locations in Andhra Pradesh. A seed bank of 140 accessions has been established at IIHR, Bangalore (Rajasekharan and Ganeshan 2002). IFGTB, Coimbatore has established a Seed Production system of the species. Pollen cryobanking and further germination revealed a decline in pollen viability in the species (Rajasekharan et al. 2013).

15.3.7 Persea macrantha *(Nees) Kosterm*

Geographic Distribution *Persea macrantha* (Nees) Kosterm belongs to the family Lauraceae. It is a tree growing up to 30 m in length and is mainly distributed in Western peninsula, Ceylon and India, etc. In India, the plant is found in various states such as Karnataka, Bihar, Maharashtra and Assam up to an altitude of 2100 m. In Maharashtra, the tree is found at Kolhapur, Pune, Raigad, Ratnagiri and Sindhudurg. In Karnataka, it is distributed at Chikmagalur, Coorg, Hassan, Mysore, Shimoga, while all districts of Kerala house the tree. It has been found in forest areas of Coimbatore, Dindigul, Namakkal, Nilgiri and Theni in Tamil Nadu.

Botanical Description Evergreen trees, grow up to 30 m high, bark 20–25 mm thick, surface pale brown, mottled with dark blotches, scurfy and thinly scaly, rough, exfoliations small, brittle; blaze pinkish; branchlets glabrous. Leaves simple, alternate, clustered at the tip of branchlets, estipulate; petiole 15–40 mm long, stout, grooved above, glabrous; lamina 6.5–20 × 3.7–10 cm, oblong, elliptic-oblong or ovate-oblong, base oblique or acute, apex obtuse or obtusely acute, margin entire, glabrous, glaucous beneath, coriaceous; lateral nerves 6–12 pairs, pinnate, prominent, intercostae reticulate, obscre. Flowers bisexual, 10–12 mm across, pale yellow, in panicles from upper axils and terminal; perianth tube very short, tepals 6, subequal, in 2 series, 4–5 mm, obovate, puberulous; persistent, spreading or reflexed in fruit; stamens 9 perfect, those of first and second row opposite the perianth lobes, introrse, with long filaments, those of third row opposite the first row, extrorse with slender filaments and a pair of stipitate glands at their base, filaments pubescent 2.5 mm; anthers 4-celled; staminodes 3, in row 4 and opposite the row 2, 4 mm long, stalked, arrow shaped, pubescent; ovary half inferior, sessile, 1-celled; ovule 1, pendulous; style, slender, 2 mm; stigma discoid. Fruit a berry 15–18 mm across, globose, green with white specks, aromatic, with a basal persistent rim of perianth; lobes reflexed in young fruits, deciduous later; epicarp red when ripe; seed one, globose.

Economic Importance Sparingly seen, this well-known medicinal and NTFP tree requires much greater attention than it gets today. The bark powder is used as a binder (Jigat) in agarbathi industry. Currently, most of the Jigat barks are harvested in natural forests thereby diminishing its genetic stocks. Bark collection needs comparatively less labour in primary processing and can be stored for long periods without special arrangements. Storage does not reduce the value, unlike most NTFPs.

Threats and Status of the Population Harvesting the whole bark of the tree causes its death. The species has declined considerably in the Western Ghats and is currently being sourced from other parts of the country. In the absence of targeted programmes to augment this species these supplies will dry up while the demand is predicted to grow during the next years.

Regeneration and Viability *Seed biology:* Scant attention has been given to the species in terms of its ecology, regeneration and seed biology. The fruit of the species is a berry, globose, green, containing a globose seed (Chacko et al. 2002). It has a very thin testa. The seeds have very high moisture (47.87%) and records high initial germination (73%). Seedling emergence starts after 30 days of sowing. The viability of *P. macrantha* seeds was found to reduce drastically after 2 weeks period. The non-desiccated seeds can be stored at 20 °C for 3 months. Beyond this period, the viability of the seeds starts reducing gradually. Seeds could not tolerate low temperatures, which could be due to the formation of ice crystals within the seeds at low temperatures damaging the tissues. Combination of 20 °C and 45% moisture content was found suitable for storing *P. macrantha* seeds compared to other temperature and moisture contents for longer periods.

15.3.8 Santalum Album

Santalum album L. belongs to the family Santalaceae, and is commonly known as White or East Indian Sandalwood.

Natural Habitat *S. album* is indigenous to the tropical belt of the Indian Peninsula, eastern Indonesia and northern Australia. There is still debate as to whether *S. album* is endemic to Australia or was introduced by fishermen or birds from eastern Indonesia centuries ago. The main distribution is in the drier tropical regions of India and the Indonesian islands of Timor and Sumba. The principal sandal tracts in India are Karnataka and adjoining districts of Maharashtra, Tamil Nadu, Kerala and Andhra Pradesh. The species is mostly found in dry deciduous and scrub forests in this region. The vegetation type is a typical monsoon vine thicket growing on pure sand. It has been recorded on coastal sand dunes immediately above the normal high water mark and close to the mangroves. It also grows on low lateritic cliffs above the beach. It is a partial parasite that attaches to the roots of other trees; it needs 'nurse' species in the area of planting out. Host plants that fix nitrogen and provide light shade are preferred. It does not tolerate frost or water logging, but is drought-hardy and is a light demander in sapling and later stages. Prolonged drought and fire kill trees.

Geographic Distribution It is distributed in the dry scrub forest of Salem, Mysore, Coorg, Coimbatore and Nilgiris up to 900 m. altitude. It is also found in Andhra Pradesh, Bihar, Gujarat, Karnataka, Madhya Pradesh, Maharashtra and Tamil Nadu. It grows at an altitude of 600–1200 m, mean annual temperature: 2–38 °C, mean annual rainfall is 450–3000 mm. *S. album* grows in a wide range of soils but is most common in sandy or rocky red soil zones. The species is not found on black soil but luxuriant growth is noticeable in moist soils such as garden loam and well-drained deep alluvium. It also grows on ferruginous loam overlying metamorphic rocks, chiefly gneiss is considered the best and trees avoid calcareous situations. On shallow stony and gravely soils, growth is poor. In India, it usually grows on free draining red loams with a pH of 6.0–6.5, and occasionally on sandy soils associated with laterites.

Botanical Description *S. album* is a small evergreen, partial root parasitic tree that grows to 4 m in Australia, but in India it is much larger and can grow to a height of 20 m; girth of up to 2.4 m, with slender drooping branchlets. Bark is tight, dark brown, reddish, dark grey or nearly black, smooth in young trees, rough with deep vertical cracks in older trees, red inside. Leaves are thin, usually opposite, ovate or ovate-elliptical, 3–8 × 3-5 cm, glabrous and shining green above, glaucous and slightly paler beneath; tip rounded or pointed; stalk grooved, 5–15 cm long; venation noticeably reticulate. Flowers are purplish-brown, small, straw coloured, reddish, green or violet, about 4–6 mm long, up to 6 in small terminal or axillary clusters, unscented in axillary or terminal, paniculate cymes. Fruits are globose, fleshy drupe; red, purple to black when ripe, about 1 cm in diameter, with hard ribbed endocarp and crowned with a scar, almost stalkless, smooth, single seeded.

Economic Importance

Timber: *S. album* is mainly grown for its timber, which weighs 870 kg/cubic m, is durable and strong. Its close grained heartwood is used for ornamental and carving work. Food: Fruits are edible. Fodder: Trees are sometimes lopped for fodder; the foliage of *S. album* is palatable to grazing animals such as rabbits, sheep, goats, cattle, pigs, horses and camels.

Fuel: The wood has been used as a fuel but is generally considered too valuable for this purpose. Tannin or dyestuff: The bark contains about 12–14% tannin and has good potential in the tanning industry. Seeds yield oil that can be used in the manufacture of paint.

Essential oil: A valuable oil, 'the sandal oil', is distilled from the heartwood (yield varies from 4–10%) and is used in perfumery, soap making and medicines. The roots contain maximum quantity of oil and hence are more valuable. It is expensive and sold by weight. In 2012, the cost of 5 g of oil sold at the Karnataka Government outlet was Rs 1500 which works out to be Rs 300,000/kg (Arunkumar et al. 2012).

Other products: Powder from the heartwood is used to make incense sticks, burnt as perfumes in houses and temples, or is ground into a paste and used as a cosmetic.

Medicinal uses: Both the wood and the oil have long been employed in medicine. They are credited with cooling, diaphoretic, diuretic and expectorant properties, and sandalwood finds several applications in household remedies: a paste of the wood is applied to burns; in fevers and headache, it is applied to the forehead and upper eyelids. The oil was at one time official in many pharmacopeias. The oil from the seeds is used in skin troubles.

Threats and Status of the Population Sandalwood has been categorized as 'vulnerable' by International Union for Conservation of Nature and Natural Resources (Arunkumar et al. 2019). Mature trees are absent in the forests of Karnataka (Swaminath et al. 1998) and Tamil Nadu. Sandalwood populations of high girth class are illicitly felled resulting in genetic erosion of the species. The only natural populations of sandal left behind are at Marayoor, Kerala.

Reproductive Biology and Breeding System Plants start flowering and fruiting from the fourth year. Flowers are bisexual and the panicles appear from March to April in India, and fruits ripen in the cold season. The species is spread rapidly through seed dispersal by birds, which feed on the outer fleshy pericarp. Viable seed production occurs when the tree is 5 years old. Two flowering seasons are observed (Krishnakumar and Parthiban 2017).

Seed biology: Good seed is reported from trees over 20 years of age. Seed storage behaviour is orthodox. The seeds are viable for 2 years when stored at room temperature (seed longevity declines rapidly at room temperature). The viability is reduced from 90–15% after 3 years of storage at 7° C with 30–45% relative humidity. Seeds tolerate desiccation to 2% moisture content, and no loss in viability is observed after 16 months hermetic storage at 4 ° C with 3–10% moisture content. On an average there are 4300–6800 seeds/kg.

Most natural seedlings of sandal are found growing in the middle of thorny bushes, where the birds have dropped the seeds. Artificial propagation is easily done by directly dibbling freshly collected ripe seeds in worked up soil patches, with the onset of the monsoon, in the middle of the nurse bushes or in protected patches. Fresh seed has a dormancy period of 2 months. Manual scarification or gibberellic acid can break this. They germinate in about 8–14 days, with a germination rate of 70%. *Lantana camara*, commonly found growing in scrub forests in areas suitable for sandal, acts as a good nurse to the seedlings in the early stages. Planting of container-raised seedlings or branch cuttings is also successful; trees are raised with a host plant, for example *Cajanus cajan, Cassia siamea, Terminalia, Lagerstroemia, Anogeissus, Dalbergia, Pongamia, Albizia* and *Acacia* species. Seeds can be sown in polythene bags along with the sandal seeds and watering is once a day. Sandal seedlings attain a height of 15–20 cm by planting time and are planted out in the field along with the host plant. Seedling growth is rapid with 20–30 cm obtained at the end of the first year and 60–70 cm at the end of the second year. Root suckers are produced when roots are exposed or injured. The nursery phase to raise sturdy 30 cm plants is usually 8 months. Primary host species are grown alongside the seedlings in each pot. *S. album* has been propagated vegetatively by tissue culture, branch cuttings and cleft grafting. Direct sowing in the fields is used in some situations. Secondary host species should be well established on the planting site before planting.

Regeneration Status Natural regeneration is very high in the species. About 70% of the seeds from a mother tree germinate and reach the seedling stage. However, the sapling and pole stages are lowered due to lack of host plants, poor light conditions, browsing, pathogen infestations and site conditions.

Genetic Resources: Collection, Characterization, Conservation and Documentation Institute of Wood Science & Technology, (IWST), Bangalore has selected plus trees of *S. album* from southern states, based on the growth, heartwood and oil content (Arunkumar et al. 2011) and established Clonal Germplasm Bank at Gottipura, Bangalore. During 1982, Clonal Seed Orchard (CSO) of 25 clones was established at Nallal, Bangalore (Srinivasan et al. 1992; Srimathi et al. 1995). Seeds of the CSO are collected every year and used as a source of quality seed for improved planting stock. A Vegetative Multiplication Garden of sandal over an area of 0.8 ha and seed orchards at 4 locations is established in Tamil Nadu. Karnataka Forest Department has identified 72 plus trees and 7.39 ha of Seedling Seed Orchard have been established in the state (Arunkumar et al. 2016). They are at Gungargatti (Dharwad), Navatoor (Shimoga) and Jarakbande (Bangalore). IWST has also developed refined protocols for in vitro cloning of *S. album* through axillary shoot proliferation and somatic embryogenesis of mature trees and clones.

Genetic variation of sandalwood has been studied extensively using isozymes (Brand 1994; Suma and Balasundaran 2003; Angadi et al. 2003; Nageswara Rao et al. 2007), RAPD (Shashidhara et al. 2003; Suma and Balasundaran 2003; Azeez et al. 2009) and RFLPs (Jones 2008) and found considerable variability. Srikanta

Dani et al. (2011) in their study on genetic variation on isolated populations of *S. album* found very low to nil genetic diversity, suggesting habitat fragmentation, isolation of populations and poor vegetative reproduction as the reasons for this.

Conservation Efforts Natural sandalwood forests are found as a concentrated patch only in Kerala in Marayoor. Conservation of sandalwood trees in its natural habitat is considerably difficult. The Governments of Karnataka and Tamil Nadu have relaxed their policies with reference to Sandalwood cultivation. This has encouraged farmers and entrepreneurs to cultivate Sandalwood.

15.3.9 Saraca asoca (Roxb.) de Wilde

Saraca asoca (Roxb.) de Wilde, an evergreen tree belonging to family Caesalpinaceae, of height up to 10 m, with blackish bark and reddish-brown wood, growing along the streams or in the shades of dense evergreen patches. Their population has been dwindling over years in the country. This species is presently threatened by over-exploitation and International Union for Conservation of Nature and Natural Resources (IUCN) has red-listed this species under the threat category 'globally vulnerable' (Warrier et al. 2019).

Geographic Distribution Ashoka is a small or medium-sized tree with beautiful dense clusters of yellow and orange-red flowers. It occurs almost throughout India up to an altitude of 750 m in the Central and Eastern Himalayas; Khasi, Garo and Lushai hills. It is also found in the Andaman Islands, Dakshin Karnataka, Odisha, Kerala and lower reaches of Annamalai Hills. The tree is indigenous to India, Burma and Malaysia.

Botanical Description A small evergreen tree, 6–9 m tall, found wild along streams or in the shade of evergreen forests. The bark is dark brown to grey or almost black with warty surface. Leaves are paripinnate, 15–20 cm. long, leaflets are 6–12, oblong or oblong-lanceolate, 7.5–22.5 cm × 1.25 cm; rigidly sub-coriaceous; flowers orange or orange-yellow, eventually turning vermillion, very fragrant, in dense axillary corymbs; pods flat, leathery, 10–25 × 3.5–5 cm, seeds 4–8, ellipsoid-oblong, compressed.

Economic Importance The plant is used in dysmenorrhoea and for depression in women. The bark is reported to stimulate the uterus, making the contractions more frequent and prolonged without producing tonic contraction as in the case of pituitary ergot. It is also reported to cure biliousness, dyspepsia, dysentery, colic, piles, ulcers and pimples. Leaves possess blood purifying properties. Flowers are used in dysentery and diabetes. Ashoka is well known for its use in treating gynaecological disorders. It is especially relied upon as an astringent to treat excessive uterine bleeding from various causes (including hormone disorders and fibroids), but also for regulating the menstrual cycle and, in various complex formulae, as a tonic for women. Many Ayurvedic physicians believe that women should use this herb frequently to help avoid gynaecological and reproductive disorders. The bark, rich in tannins and cyanidins (red coloured compounds), is the primary medicinal part. The tannins provide the main astringent action for halting excessive menstrual bleeding and also for bleeding haemorrhoids, bleeding ulcers and haemorrhagic dysentery.

Threats and Status of the Population Domestic consumption of bark is quite high in pharmaceutical industries. It also has good export potential. The annual demand of Ashoka bark in the year 2004–05 was about 10724.20 tonnes and is growing at the rate of 15% per year. This increased demand of the bark has threatened this beautiful tree from the wild. This plant has been exploited unsustainably for medicinal use and therefore it has attained the status of 'Endangered' (Begum et al. 2014). Limited efforts have been taken to increase the *S. asoca* plantation. The wild populations of the species from some remote localities are being completely stripped of bark, and thus creating a greater pressure of it to become extinct species in near future. Field survey studies have shown that since this plant bears sweet kernels of seeds, which are eaten by insects and also these plants are specific to streamline of rivers, seeds are washed with water causing problems in regeneration.

Regeneration and Viability Fresh fruits of *S. asoca* collected can be distinguished as mature and immature based on the colour and hardness of the seeds. Green, soft pliable seeds are classified as immature while the hard compressed solid seeds are categorized as mature ones. Fresh seeds have an initial moisture content of 34.71%. The LSMC (Lowest Safe Moisture Content) is 45% below which seed viability reduces drastically. Following slow desiccation, it is observed that seeds stored with MC 46% in polythene bags retain higher viability when stored at lower temperatures. Due to high moisture content, the seeds show a tendency for rapid fungal attack.

Fresh seeds germinate within a week of sowing. Ground collected seeds germinate after 1 month of sowing. Germination is hypogeal. The overall germination per cent of the seeds varies from 63 to 93 with an average of 83%.

There have been reports on the poor seed set in natural populations of the species in the Western Ghats and difficulties in large-scale propagation of the species (Anjankumar et al. 2004). *S. asoca* seeds do not pose any problem in germination. No pretreatments are required for germination of the seeds. However, the high moisture content suggests that seeds could be recalcitrant in nature, losing viability within a short period due to the rapid loss of moisture and hence storability may be a problem in these seeds. The development of multiple seedlings in *S. asoca* has been reported (Singh et al. 2005). This phenomenon is believed to be apparently due to polyembryony. Similar to Garcinia, it could be explained as a means of survival for this highly recalcitrant species.

Genetic Resources: Collection, Characterization, Conservation and Documentation Forest department collects large quantities of seeds/seedlings for nursery raising, leaving less room for natural regeneration of *S. asoca* in the original habitat. TDU/FRLHT was collaboration with the Karnataka State Forest Department beginning in 1993, resulted in identifying *S. asoca* as a species of conservation concern located in Kollur, in Udupi district of Karnataka, which was demarcated as MPCA (Medicinal Plant Conservation Area) for *S. asoca* (Begum et al. 2014).

Reproductive biology studies on *S. asoca* population has also been used as one of the conservation strategies in terms of plant improvement measurement and helped in cultivation of vulnerable species (Smitha and Thondaiman 2016). Molecular marker studies on *S. asoca* to evaluate the evolutionary relationship using chloroplast mat-K gene have revealed that it is closely related to *S. palembanica, S. declinata, Endertia spectabilis* and *Lysidicerhodostegia* (Saha et al. 2015). RAPD profiling has been used for identification and characterization of *S. asoca* (Gahlaut et al. 2013).

15.3.10 Vateria indica *Linn*

Vateria indica is an endemic and economically important tree species found in the evergreen forest patches of South India especially the Western Ghats region from North Kanara to Kerala. This is planted as an avenue tree in Karnataka, and has been recommended for introducing in to the evergreen forests of Eastern Ghats. A slow-growing species, endemic and found primarily in the Southwest coast evergreen forests, up to an altitude of 750 m, and also occasionally in secondary evergreen dipterocarp forest in the states of Karnataka, Kerala and Tamil Nadu.

Botanical Description It is an evergreen tree reaching a height of 30–40 m with a clean cylindrical bole of 15 m and girth of 4–5 m. The bark of *V. indica* is smooth in young trees and rough, whitish to grey peeling off in thick round flakes in older ones. Leaves: coriaceous, ovate to oblong, entire (Leaf falls in March, new foliage appears in April–May, the second flush of foliage starts after rains, in October to December.) Flowers: White, fragrant, in terminal corymbose panicles. Fruits: capsules, ovoid, pale brown, fleshy, 8 to 11 cm long, and 3.5–6 cm in diameter. One seeded, reddish white, filled with fat.

Economic Importance The tree is a well-known species for making commercial plywood. It yields an oleo-resin called white dammar. The resins are used as tonic, carminative and possess expectorant properties against throat troubles, chronic bronchitis, piles, diarrhoea, rheumatism, tubercular glands, etc. mixed with gingelly oil; it is used against gonorrhoea, with ghee and long-pepper for the treatment of ulcers etc. (Ashton 1988). The resin comes in three forms: (1) Compact form: solid lumps and regarded as the best quality. It is very hard and bright orange to dull yellow, with a vitreous fracture, and amber like appearance. (2) Cellular form: full of air bubbles and gives a cellular structure. (3) Dark-coloured form: occurs in cavities of old and moribund trees or dead trees and of an inferior quality.

The seeds are crushed and boiled in water till the melted fat rises to the surface, which is semi-solid in consistency. The fat can also extracted by hydraulic press to obtain higher yields. It is edible after refining, used in confectionary and as an adulterant of ghee. Also used in blends with cocoa butter or as its substitute. The fat is used in making candles and Soaps.

Threats and Status of the Population *V. indica* is listed as critically endangered on the 2013 IUCN Red List based on its overexploitation for its timber and its habitat loss of more than 80% (Ashton 1988). Although the status of this species is critical, few healthy populations remain. The nuts of *V. indica* are also one of the heavily exploited NTFPs locally. This may hamper the natural recruitment of *V. indica* in natural forests (Sinu and Shivanna 2016). The timber has been overexploited, particularly for the plywood industry. An average of about 6200 tonnes of timber annually (in the year 1960) was yielded by felling the trees in the Western Ghats, which were utilized for plywood making. Loss of habitat and other human activities have also contributed to population declines.

Regeneration and Viability

Seed biology: Flowering in *V. indica* is during January–March. Fruits ripen during the monsoon (June–July). The maturation period is 10 weeks. Flowering occurs in alternate years with a mast event in every fourth year (Sinu and Shivanna 2016). Seeds collected from 8 weeks of anthesis germinate well. Fruits collected from the forest floor are usually damaged due to the seed coat being broken of mechanical injuries. Such seeds lose viability rapidly. Minor damages to the seed coat serve as an entry point for fungal attack, which is a serious cause of seed deterioration under storage conditions where fungi are active. Hence, fruits could be directly collected from the trees (Sivakumar 2004).

The initial moisture content of the seeds varies from 65% to 70%. Being highly recalcitrant in nature, the moisture sheds off rapidly, during which period the seeds are expected to germinate. The lowest safe moisture is 30% beyond which the seeds lose viability. Seeds treated with fungicides improved their storability up to 2 months.

Regeneration status: Regeneration is said to be good when left undisturbed. Adequate protection of habitats from clearance and degradation could allow it to make a recovery. Seeds fall close to the tree and germinate readily. The seeds are highly recalcitrant; they are sensitive to drought and frost. Both shade and moisture are necessary for their survival. Artificially could be propagated by direct sowings or by transplanting entire plant along with the ball of earth. Stump planting does not give satisfactory results. Seeds are viable for about a month and have a good germinative capacity up to 80%.

Conservation Efforts Some populations are found in forest reserves. Trees are being planted on a small scale and replantation efforts are being made in some degraded rainforests and barren areas. Genetic diversity, fine-scale spatial genetic structure (FSGS), inbreeding and patterns of seed and pollen dispersal in *V. indica* using microsatellite markers reveals that restoration efforts using direct seeding of *V. indica* using vigorous progenies would be successful in the species (Ismail et al. 2013, 2014). *Vateria indica* has a great potential for restoration as it grows relatively rapidly and survives well in the degraded forest (Rai 1990).

15.4 Conservation Strategies

The species described above are mainly constituent of tropical forests. Most of them are difficult to propagate, slow growing and require specific growth conditions. Further, the process of domesticating and cultivating an identified tree resource is time-consuming. Considering these issues, in addition to limitations of both species-based and ecosystem-based approaches, we need to adopt a holistic approach based on scientific techniques or approaches. This could be in situ, circa *situ*, ex situ, reintroduction, population enrichment, etc., but most suited to a particular case and circumstances (Heywood and Dulloo 2005).

Following are probable and appropriate steps in saving these highly traded and threatened trees of the Western Ghats.

- It would be necessary to carry out an intensive survey of these species which will give true picture of the distribution, abundance and regeneration status of these species.
- These species are found only in tropical evergreen forests. Identification of natural pockets is needed to initiate *in- situ* conservation measures.
- Study pollination biology and fruit setting and to know constraints in sexual reproduction and production of seeds.
- Considering the rarity of these species, steps should be taken immediately to grow or propagate them so as to conserve them as part of ex situ conservation plan. In addition, the possibilities of cultivating them must also be encouraged so as to reduce the pressure on the wild population.
- Establish field gene banks and seed production systems of the species.
- Genetic resources of these species can best be conserved through sustainable use. Circa *situ* conservation of these species would be ideal.
- Training forest officials in identification, protection and maintenance of the species both in situ and circa *situ*.
- Coordinated efforts of researchers, forest officials and people to grow, maintain and protect them.

15.4.1 Inventorization

Resource survey and inventorization of these species should be given top priority, for which training and skill development of staff, including field managers, is a vital step. A comprehensive plant resource inventory encompassing herbs, shrubs, climbers, lianas and trees will be useful to build a database on the species distribution and frequency, association, regeneration status, species interaction. Cost-effective and reliable methods need to be evolved to carry out comprehensive plant resource inventory over extensive areas within the Western Ghats. Management of natural resources cannot follow administrative boundaries. Boundaries need to be redrawn based on the ecological niches. These will form true conservation units for drawing up a scientific plan.

15.4.2 Habitat Protection

Habitat fragmentation affects the regeneration of rare tree species due to low dispersal. It also indirectly disengages ecological processes like the dependence of pollinators and seed dispersers associated with fruit bearing trees. This, in the long run, affects the total diversity within the forest fragments. Therefore, the main challenge is to maintain diversity and ensure the conservation of rare species within forest fragments. In the case of endemics, which have a narrow spatial distribution, and where populations have gone down to a critical level, special attention is required. A successful example was the identification and demarcation of dense patches of *Saracaasoca* located in Kollur, as an MPCA (Medicinal Plant Conservation Area). This strategy could be adopted for *Vateria indica* and *Hopea parviflora*, which are found as pure patches within the Western Ghats. More number of Protected Areas similar to Kurinjimala National Park, Idukki district, Kerala for *Strobilanthes*, the *Rhododendron* Sanctuary at Singba in Sikkim, the *Nepenthes* sanctuary at Jarain, National Citrus Gene Sanctuary in Meghalaya and the orchid sanctuary at Sessa in Arunachal Pradesh (FAO 2012) need to be established focusing on specific conservation-dependent species.

15.4.3 Advanced Technologies

Knowledge is lacking on many of the species which are rare, endangered or threatened. Details on its distribution, regeneration status, seed propagation, etc. have been generated more as a part of academic exercise, and lacks continuity. More than 75% of potentially valuable biodiversity resources of the country are yet to be studied (Damodaran 1992). Studies are required to generate information on aspects like species distribution, association, regeneration and interaction in a given environment. Several applications have been successfully accomplished in the science of conservation biology. Tissue culture, application of cryogenic technology for long-term seed, pollen and plant tissue cryopreservation (SCB, PCB, IVBGs), etc. should be refined for in-situ and ex-situ conservation of species of high priority. A good example is the efforts of IIHR, Bangalore where they have developed an effective ex situ conservation strategy for the establishment of Field Gene Bank (FGB), followed by in vitro conservation using tissue culture techniques for 22 species of medicinal plants distributed in south India. Propagation and re-introduction enables broadening the genetic base in species. Thus re-introduction, species rehabilitation will facilitate habitat restoration thereby balancing the ecological processes.

Defence Institute of High Altitude Research (DIHAR) (3500 msl) at Leh (Ladakh) has a National Perma Frost Based Germplasm Storage Facility at an altitude of 5360 msl (75 km from Leh). This can serve as a germplasm storage facility for the successful, cost-effective, safe and long-term conservation of valuable plant genetic resources in the form of safety duplicates (FAO 2012).

15.5 Conservation through Seed Production Systems (SPS)

An SPS is a technology which ensures continuous supply of quality seeds (due to the specific composition of the stand). It could be a natural or artificial (introduced) stand – the latter being more advantageous as it introduces maximum genetic diversity in the stand. The SPS facilitates preservation, regeneration and maintenance of resource productivity and diversity. Specific designs have to be adopted to obtain maximum benefits from the SPS (Warrier et al. 2013). Seed Production Systems have been established for ten species, with accessions from different parts of Tamil Nadu and Kerala. At the end of 10 years, these trees have attained a height of 6 m and have started flowering. Such germplasm assemblages will serve as seed stands and ensure continued supply of seeds (Warrier et al. 2011).

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Part V
Legal Aspects of Threatened Medicinal Plants

Chapter 16
Relevance of Ethnopharmacological Research Related to Threatened Medicinal Plants Associated with Traditional Knowledge

S. R. Suja, Ragesh R. Nair, S. Rajasekharan, and R. Prakashkumar

Abstract Bio-prospecting of plants based on traditional knowledge is an important area of research to develop novel process/products in terms of herbal/Ayurvedic drugs, nutraceuticals, functional foods, and other plant-based products including cosmetics. The ultimate objective of ethnopharmacological studies on plants used for food and medicine followed by its preclinical and clinical studies will lead to the development of scientifically validated process and products that can be patented and commercialized through technology transfer by ensuring the access and benefit sharing where the traditional knowledge providers/tribal knowledge holders, inventors from scientific communities, and biodiversity management committees at grassroots level in terms of conservation of bioresources would be equally benefited. In this context, the authors have made an attempt to focus on the traditional knowledge of selected threatened medicinal plants of Kerala, with an ultimate objective to develop diverse medicinal/nutraceutical products, and described few case studies on access and benefit sharing. New enterprises related to product development based on bioresources, if meticulously planned and executed, could help to generate more opportunities for employment and income in rural as well as urban sectors. To ensure the conservation of the threatened medicinal plants, new location-specific strategies should be evolved and implemented through people participatory programs at grassroots level which will help to ensure the health and economic security of the country.

Keywords Traditional knowledge · Jeevani · Kani tribe · Prior informed consent · Access and benefit sharing

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16.1 Introduction

Plants have been a major source of medicine in all cultures from ancient times. Medicinal plants have become the subjects of man's curiosity from time immemorial, and almost every civilization developed around the world has its own history of plant utilized as medication. Approximately 80% of the human population in developing countries rely on traditional medicine system for their primary health care, and about 85% of traditional medicine involves the use of plant extracts (WHO 1997). India covers 2.4% of world's area with 8% of global biodiversity and is 1 among the 19 mega diversity countries of the world. Out of the 35 hotspots recognized at global level, India has 4 major hotspots which cover Western Ghats (64.95%), Himalaya (44.37%), Indo-Burma region (5.13%), and Sundaland which includes the Nicobar group of islands (1.28%). India is also rich in medicinal plant diversity with all the three levels of biodiversity such as species diversity, genetic diversity, and ecosystem diversity.

According to an estimate of WHO, the demand for medicinal plant-based raw materials is growing at the rate of 15 to 25% annually and is likely to increase more than 5 trillion USD in 2050. One fifth of all the plants found in India are used for medicinal purpose, and medicinal plant-related trade in India is estimated to be approximately 1 billion USD per year (Kala et al. 2006). India with rich biodiversity ranks first in percent flora, which contain active medicinal ingredient (Mandal 1999). IUCN recognizes the following categories such as extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened, least concern, data deficient, and not evaluated. Species with small populations that are not at present endangered or vulnerable but are at risk are called rare (Singh et al. 2006). It has led to an estimation that about 12.5% of the world's vascular plants, totalling about 34,000 species, on a global basis are under varying degrees of threat (Phartyal et al. 2002). A total of 560 plant species of India have been included in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species, out of which 247 species are in the threatened category.

16.2 Threatened Medicinal Plants in Kerala

The state of Kerala has a wide range of distribution of several remarkable endemic medicinal plants used in various Indian traditional systems of treatments. In official assessment of the flowering plants of India, 19,048 species in 3057 genera in 266 families are recorded for the country, of which 4700 species are found to occur in Kerala, which include 250 endemics exclusively confined within the state boundaries. From the literature, around 1800 species reported from Kerala are found to be medicinal even though only 900 species are used in the classical, tribal, and folk systems of medicines. Interestingly, it is found that most of these 900 species are either rare or threatened in their natural habitats (Santhosh and Mathew 2018). Some threatened medicinal plants in the state of Kerala are listed in Tables 16.1 and 16.2.

Table 16.1 List of threatened medicinal plants associated with traditional knowledge in the state of Kerala

Sl. no.	Scientific name	Family	IUCN status
1.	*Acrotrema agastyamalayanum* E.S.S. Kumar et al.	Dilleniaceae	Endangered
2.	*Allophylus concanicus* Radlk.	Sapindaceae	Rare
3.	*Calophyllum apetalum* Willd.	Clusiaceae	Vulnerable
4.	*Cinnamomum macrocarpum* Hook. f.	Lauraceae	Vulnerable
5.	*Cinnamomum travancoricum* Gamble	Lauraceae	Endangered
6.	*Cinnamomum wightii* Meisn.	Lauraceae	Endangered
7.	*Curcuma pseudomontana* Graham	Zingiberaceae	Vulnerable
8.	*Cycas circinalis* L.	Cycadaceae	Vulnerable
9.	*Dalbergia latifolia* Roxb.	Fabaceae	Vulnerable
10.	*Diospyros montana* Roxb.	Ebenaceae	Critically endangered
11.	*Diospyros paniculata* Dalzell.	Ebenaceae	Vulnerable
12.	*Dipterocarpus bourdillonii* Brandis	Dipterocarpaceae	Critically endangered
13.	*Dipterocarpus indicus* Bedd.	Dipterocarpaceae	Endangered
14.	*Dysoxylum beddomei* Hiern	Meliaceae	Endangered
15.	*Embelia tsjeriam-cottam* (Roem. & Schult.) DC.	Myrsinaceae	Vulnerable
16.	*Garcinia imberti* Bourd.	Clusiaceae	Endangered
17.	*Garcinia travancorica* Bedd.	Clusiaceae	Vulnerable
18.	*Gardenia gummifera* L.f	Rubiaceae	Vulnerable
19.	*Glycosmis macrocarpa* Wight	Rutaceae	Vulnerable
20.	*Heracleum candolleanum* (Wight & Arn.) Gamble	Apiaceae	Endangered
21.	*Holostemma ada-kodien* Schult.	Asclepiadaceae	Vulnerable
22.	*Hopea parviflora* Bedd.	Dipterocarpaceae	Endangered
23.	*Hopea ponga* (Dennst.) Mabb.	Dipterocarpaceae	Endangered
24.	*Humboldtia decurrens* Bedd. Ex Oliver.	Caesalpiniaceae	Vulnerable
25.	*Humboldtia sanjappae* Sasidh. & Sujanapal	Caesalpiniaceae	Endangered
26.	*Humboldtia unijuga* Bedd. var. *trijuga* Joseph & Chandras	Caesalpiniaceae	Endangered
27.	*Knema attenuate* (Wall. ex Hook.f. & Thoms.) Warb.	Myristicaceae	Vulnerable
28.	*Kunstleria keralensis* Mohanan & Nair	Fabaceae	Least concern
29.	*Leea macrophylla* Roxb. ex Hornem.	Leeaceae	Least concern
30.	*Litsea quinqueflora* (Dennst.) Suresh	Lauraceae	Vulnerable
31.	*Madhuca neriifolia* (Moon) H. J. Lam	Sapotaceae	Least concern
32.	*Memecylon angustifolium* Wight.	Melastomataceae	Vulnerable
33.	*Michelia champaca* L.	Magnoliaceae	Vulnerable
34.	*Michelia nilagirica* Zenk.	Magnoliaceae	Vulnerable
35.	*Gynochthodes ridsdalei* Razafim. & B. Bremer	Rubiaceae	Endangered
36.	*Neolitsea fischeri* Gamble	Lauraceae	Vulnerable
37.	*Nervilia aragoana* Gaund.	Orchidaceae	Vulnerable

(continued)

Table 16.1 (continued)

Sl. no.	Scientific name	Family	IUCN status
38.	*Nothapodytes nimmoniana* (Graham) Mabb.	Icacinaceae	Vulnerable
39.	*Ochreinauclea missionis* (Wall. ex G. Don) Ridsdale	Rubiaceae	Vulnerable
40.	*Osbeckia aspera* (L.) Blume var. *travancorica* (Bedd. ex Gamble) Hansen	Melastomataceae	Vulnerable
41.	*Osbeckia aspera* (L.) Blume var. aspera	Melastomataceae	Least concern
42.	*Osbeckia lawsonii* Gamble	Melastomataceae	Least concern
43.	*Osbeckia travancorica* Bedd. ex Gamble	Melastomataceae	Critically
44.	*Pavetta zeylanica* (Hook. f.) Gamble	Rubiaceae	Vulnerable
45.	*Pittosporum dasycaulon* Miq.	Pittosporaceae	Vulnerable
46.	*Pterospermum reticulatum* Wight & Arn.	Sterculiaceae	Endangered
47.	*Rauvolfia hookeri* Sreenivas & Chithra	Apocynaceae	Endangered
48.	*Rauvolfia micrantha* Hook. f.	Apocynaceae	Endangered
49.	*Salacia beddomei* Gamble	Hippocrateaceae	Endangered
50.	*Salacia brunoniana* Wight & Arn.	Hippocrateaceae	Critically endangered
51.	*Salacia malabarica* Gamble	Hippocrateaceae	Endangered
52.	*Semecarpus auriculata* Bedd.	Anacardiaceae	Vulnerable
53.	*Smilax wightii* A. DC.	Smilacaceae	Vulnerable
54.	*Solena amplexicaulis* (Lam.) Gandhi	Cucurbitaceae	Least concern
55.	*Solenocarpus indicus* Wight & Arn.	Anacardiaceae	Near threatened
56.	*Strobilanthes barbatus* Nees var. *barbatus*	Acanthaceae	Endangered
57.	*Strobilanthes ciliatus* Nees	Acanthaceae	Vulnerable
58.	*Syzygium mundagam* (Bourd.) Chitra	Myrtaceae	Vulnerable
59.	*Thottea barberi* (Gamble) Ding Hou	Aristolochiaceae	Endangered
60.	*Toxocarpus beddomei* Gamble	Asclepiadaceae	Vulnerable
61.	*Utleria salicifolia* Bedd. Ex Hook. f.	Periplocaceae	Critically endangered

16.2.1 Traditional Knowledge (TK)

Traditional knowledge (TK) is considered as the blanket term which is directly linked with tradition or culture of respective countries of the world. It generally refers to the experience of long-standing tradition and practices of certain regional, indigenous, or local communities. TK also encompasses the wisdom, knowledge, teaching, and experience of these communities, and usually, it is orally transmitted from generation to generation. Since it is restricted to location-specific knowledge of common people including ethnic communities residing in a particular region/ country, the knowledge is confined to genetic and non-genetic resources available within their surroundings. The importance of TK is highlighted by the fact that more than 80% of the livelihood need of the world's poor directly or indirectly depend

Table 16.2 Traditional knowledge associated with threatened medicinal plants

Sl. no.	Scientific name and local name	Family	IUCN status	Traditional knowledge
1.	*Acorus calamus* L. (Vayabmu)	Araceae	Vulnerable	Used in combination with other ingredients to treat inflammatory pain, headaches, and migraines (Muthuraman and Singh 2011)
2.	*Acrotrema arnottianum* Wight (Nilampunna)	Dilleniaceae	Vulnerable	Medicated oil prepared from the whole plant used as a hair tonic (Saradamma et al. 1987)
3.	*Adenia hondala* (Gaertn.) W.J. de Wilde (Karimuthaku)	Passifloraceae	Rare	Used against snake bite and skin diseases, considered as health tonic to regain strength after malarial fever, and dried root powdered is given to mothers to improve milk secretion (Anonymous 2016)
4.	*Alstonia venenata* R.Br. (Analivegam)	Apocynaceae	Vulnerable	Leaves are used for relief from the rheumatic complaints and fruits are reported as a remedy for impure blood, syphilis, insanity, and epilepsy (Sutha et al. 2010)
5.	*Amorphophallus commutatus* (Schott) Engl. (Kattuchena)	Araceae	Vulnerable	Tuberous corms are reported to be used for treatment of piles, cysts, and tumors (Ravikumar et al. 2004)
6.	*Anaphyllum wightii* Schott. (Keerikkizhangu)	Araceae	Vulnerable	Used as an antidote to snake bite along with some medicinal plants (Arun et al. 2007)
7.	*Aphanamixis polystachya* (Wall.) Parker (Chemmaram)	Meliaceae	Vulnerable	Strong astringent, antimicrobial, used for the treatment of liver and spleen diseases, rheumatism, and tumors (Apu et al. 2013)
8.	*Arenga wightii* Griff. (Ayathengu)	Arecaceae	Vulnerable	Traditionally fresh toddy obtained from the young inflorescence is given internally for jaundice (Samy et al. 2008)
9.	*Aristolochia tagala* Cham. (Valia Arayan)	Aristolochiaceae	Vulnerable	Used for the treatment of snakebites and flower decoction is taken in for menstrual disorders by the Kani in Agasthiayamalai Biosphere Reserve (De Britto and Mahesh 2007)
10.	*Artocarpus hirsutus* Lam. (Aini)	Moraceae	Vulnerable	Decoction of roots and bark is supposed to cure diarrhea, venereal infections, and chronic hemorrhage, respectively (Akhil et al. 2014)
11.	*Begonia malabarica* Lam. (Kalthamara)	Begoniaceae	Endangered	Used to cure arthritis and common joint pains and leaf juice used for headache and to cure wounds (Jayanthi et al. 2012)

(continued)

Table 16.2 (continued)

Sl. no.	Scientific name and local name	Family	IUCN status	Traditional knowledge
12.	*Canarium strictum* Roxb. (Karutha Kunthirikkam)	Burseraceae	Vulnerable	Traditionally to treat rheumatism, asthma, coughs, fever, epilepsy, chronic skin diseases and hemorrhage Treat rheumatism, asthma, cough, epilepsy, etc. (Muthuswamy and Senthamarai 2014)
13.	*Cayratia pedata* (Lam.) A.Juss. ex Gagnep. var. *glabra* Gamble (Veluttachorivalli)	Vitaceae	Vulnerable	Treating uterine and other fluxes; lukewarm leaf juice is used as ear drops for fungal infections; leaves are astringent, refrigerant and also used to cure ulcers; stem paste is applied for healing bone fracture; whole plant is useful in acrid, refrigerant and beneficial in hysteria, burning of the skin and diarrhoea. Treating uterine and other fluxes, fungal infections, ulcers, healing bone fracture, burning of the skin, and diarrhea (Sharmila et al. 2018)
14.	*Celastrus paniculatus* Willd. (Kattadi nayakam)	Celastraceae	Endangered	Antibacterial, insecticidal, anti-inflammatory, sedative, anti-fatigue, analgesic, and hypolipidemic (Deodhar and Shinde 2015)
15.	*Chonemorpha fragrans* (moon) Alston (Perumkurumba)	Apocynaceae	Vulnerable	Antiamoebic, antipyretic, antidiabetic, anti parasitic, anthelmentic, anticancer, HIV disorder, skeletal muscle relaxant and gynaantiamoebic, antipyretic, antidiabetic, anti parasitic, anthelmentic, anticancer, HIV disorder, skeletal muscle relaxant and gynaecologicaldisorderecological disorder Antiamoebic, antipyretic, antidiabetic, anti-parasitic, anthelmintic, anticancer (Chandra and Rajput 2011)
16.	*Coscinium fenestratum* (Gaertn.) Coleb. (Maramanjal)	Menispermaceae	Endangered	Inflammations, wounds, ulcers, jaundice, burns, skin diseases, abdominal disorders, diabetes, fever and general debility inflammations, wounds, ulcers, jaundice, burns, skin diseases, abdominal disorders, diabetes, fever and general debility Inflammations, wounds, ulcers, jaundice, burns, skin diseases, abdominal disorders, diabetes, fever, and general debility (Tushar et al. 2008)

(continued)

Table 16.2 (continued)

Sl. no.	Scientific name and local name	Family	IUCN status	Traditional knowledge
17.	*Curcuma aromatica* Salisb. (Kasthurimanjhal)	Zingiberaceae	Endangered	Skin diseases, sprain, bruise, in snake poison, and also to enhance complexion (Sikha et al. 2015)
18.	*Decalepis arayalpathra* (J. Joseph & V. Chandras.) Venter (Amrithapaa)	Periplocaceae	Critically endangered	Remedy for peptic ulcer, as a rejuvenating tonic, and to cure for external cancers Remedy for peptic ulcer, as a rejuvenating tonic, and to cure for external cancers (Pushpangadan et al. 1990)
19.	*Decalepis hamiltonii* Wight & Arn. (Mahalikizhangu)	Periplocaceae	Endangered	Cure dysentery, cough, bronchitis, leucorrhea, uterine hemorrhage, skin disease, fever, indigestion, and vomiting, chronic rheumatism, and anemia (Vijayakumar and Pullaiah 1998)
20.	*Dysoxylum beddomei* Hiern (Vella akil)	Meliaceae	Endangered	Used as anti-inflammatory, diuretic, CNS depressant, and immunomodulatory agent (Senthil et al. 2008)
21.	*Dysoxylum malabaricum* Bedd. ex Hiern (Akil)	Meliaceae	Vulnerable	Skin diseases, anthelmintic, carminative, antibacterial, antibiotic, hypoglycemic, and antifertility properties Skin diseases, anthelmintic, carminative, antibacterial, antibiotic, hypoglycemic, and antifertility properties (Shyma and Devi 2012)
22.	*Embelia ribes* Burm.f. (Vizhalari)	Myrsinaceae	Vulnerable	Root used against toothache and sore throat and in making a soothing ointment. Anthelmintic (Bist and Prasad 2016)
23.	*Humboldtia unijuga* Bedd. var. *unijuga* (Palakan)	Caesalpiniaceae	Vulnerable	Used against snake bite, headache, and chickenpox (Arun et al. 2007)
24.	*Hydnocarpus pentandra* (Buch.-Ham.) Oken (Marotti)	Flacourtiaceae	Vulnerable	Used against leprosy, inflammation, rheumatism, sprains, bruises, sciatica, and chest affections (Sahoo et al. 2014)
25.	*Mesua ferrea* L. var. *coromandeliana* (Wight.) Singh (Nagapoo)	Clusiaceae	Vulnerable	Used as purgative, in the treatment of abscesses, inflammation, constipation, amenorrhea, and dysmenorrhea (Sahu et al. 2014)
26.	*Myristica malabarica* Lam. (Kattujathi)	Myristicaceae	Vulnerable	Used as gastroprotective, antioxidant, antifungal, nematicidal, and antiproliferative agent (Prem and Radha 2017)

(continued)

Table 16.2 (continued)

Sl. no.	Scientific name and local name	Family	IUCN status	Traditional knowledge
27.	*Operculina turpethum* (L.) Silvamanso (Thrikolppakonna)	Convolvulaceae	Endangered	Used for purgation action, balances Pitta and Kapha, wound and inflammation, anemia, liver disorders, and heart diseases (Gupta and Ved 2017)
28.	*Oroxylum indicum* (L.) Benth. ex Kurz (Pathiri)	Bignoniaceae	Endangered	Used as antimicrobial, antifungal, anti-inflammatory, and anticancer agent (Deka et al. 2013)
29.	*Persea macrantha* (Nees) Kosterm. (Kulamavu)	Lauraceae	Vulnerable	Used for the treatment of asthma and rheumatism (Prabhu et al. 2018)
30.	*Rauwolfia serpentina* (L.) Benth. ex Kurz (Sarpagandhi)	Apocynaceae	Endangered.	To treat snake bite, high blood pressure, mental agitation, epilepsy, traumas, anxiety, excitement, schizophrenia, sedative insomnia, and insanity (Reeta et al. 2013)
31.	*Salacia oblonga* Wall. ex Wight & Arn. (Eakanayakam)	Hippocrateaceae	Vulnerable	Used for the treatment of diabetes (Kushwaha et al. 2016)
32.	*Santalum album* L. (Chandanam)	Santalaceae	Vulnerable	Used against the infection, inflammation, itching, eczema, bronchitis, fever, and headache. Extract is used as a cardiac tonic (Rakesh et al. 2010)
33.	*Saraca asoca* (Roxb.) de Wilde (Asokam)	Caesalpiniaceae	Vulnerable	Used to improve skin complexion and difficulty in urinating and acts as an antidote to scorpion bite (Mohan et al. 2016)
34.	*Trichopus zeylanicus* Gaertn. subsp. travancoricus (Bedd.) Burkill (Arogyapacha)	Trichopodaceae	Endangered	Used as anti-fatigue, immune and vitality-enhancing agent (Pushpangadan et al. 1988)
35.	*Vateria indica* L. (Vellakunthirikam)	Dipterocarpaceae	Critically endangered	Used in chronic bronchitis and throat troubles and for the treatment of cough, asthma, leprosy, skin eruptions, crack infection, wounds, and ulcer (Shrijani et al. 2018)
36.	*Woodfordia fruticosa* (L.) Kurz. (Thathiripoov)	Lythraceae	Vulnerable	For the treatment of leprosy, toothache, leucorrhea, fever, dysentery, and bowel disease (Dinesh et al. 2016)

Fig. 16.1 Selected threatened medicinal plants associated with traditional knowledge

upon the use of biological resources and associate TK. TK is being eroded rapidly because of the changing lifestyle of the people; therefore, there is an urgent need to systematically document the valid information for the welfare and betterment of posterity.

In this chapter, the authors are highlighting the TK associated with threatened medicinal plants of Kerala (Figs. 16.1 and 16.2).

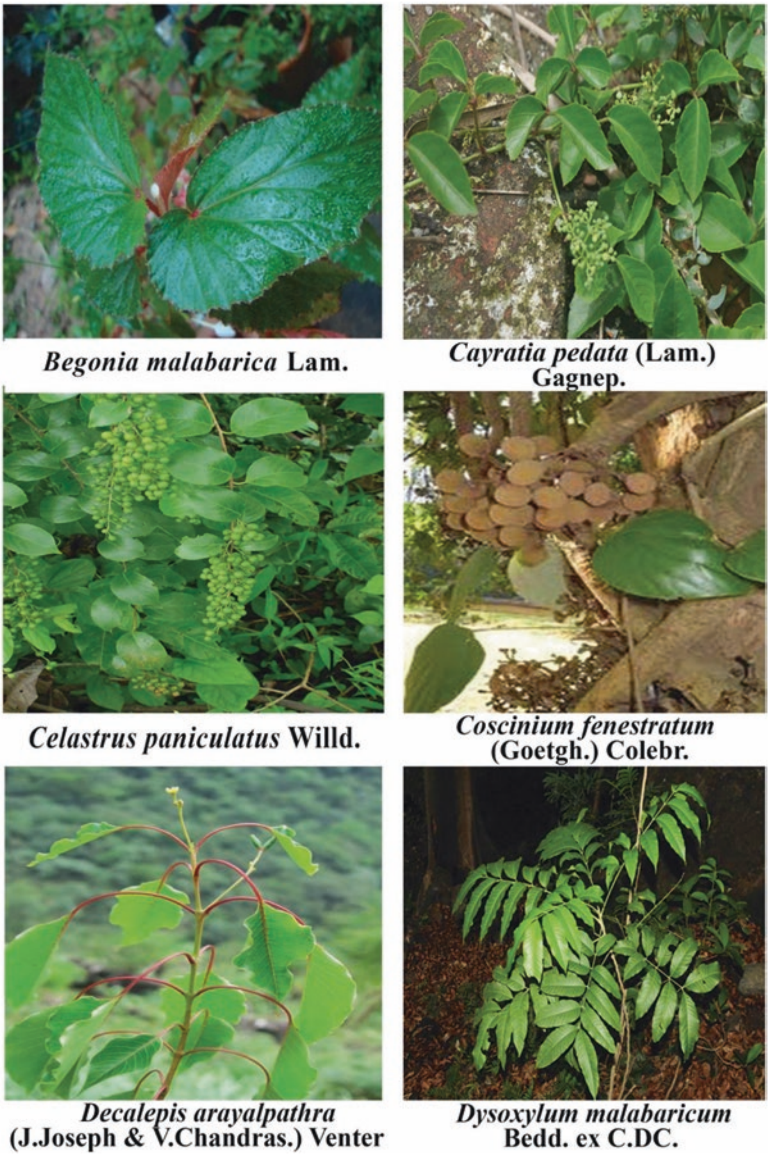

Fig. 16.1 (continued)

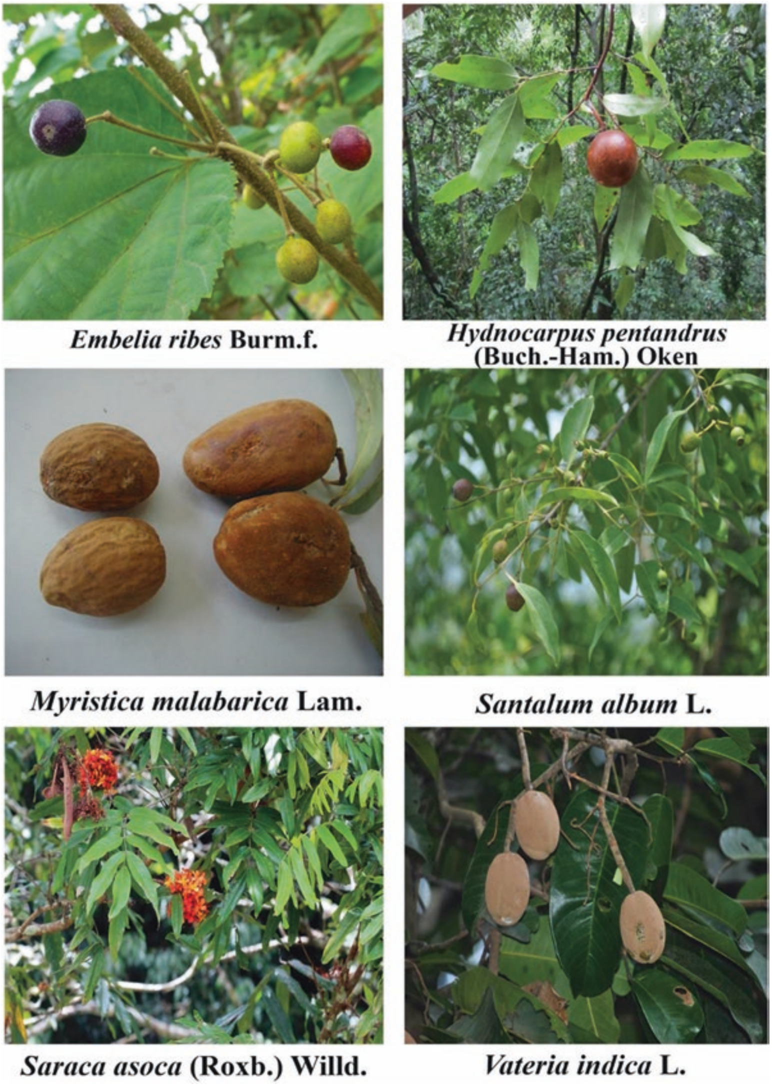

Fig. 16.1 (continued)

16.2.2 Ethnopharmacology

Ethnopharmacology as a scientific term was first introduced at an international symposium held at San Francisco in 1967 (Efron et al. 1967). Ethnopharmacology is defined as an interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man. It is one of the scientific disciplines consisting of TK holders/providers/custodians and experts from ethnomedicine,

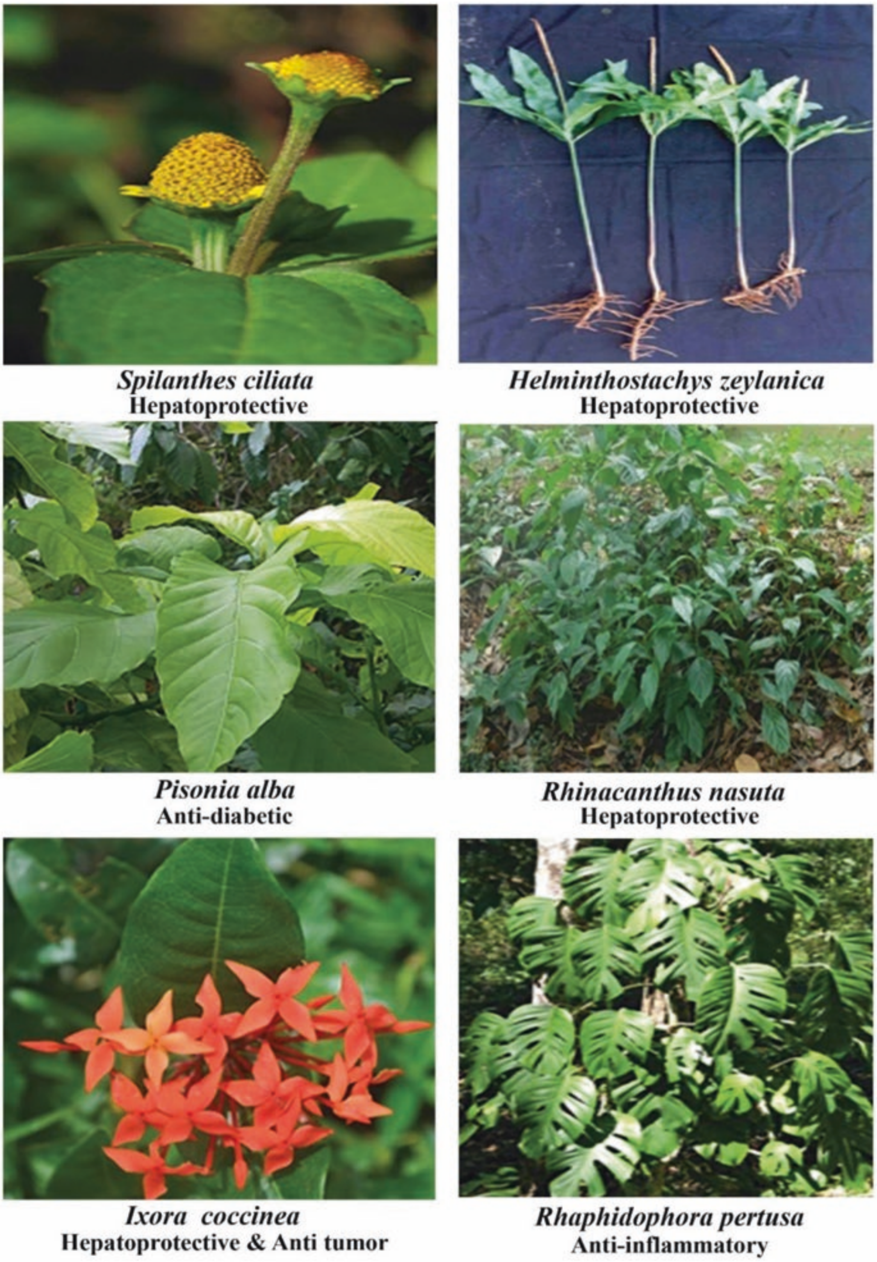

Fig. 16.2 Some important leads obtained from traditionally used medicinal plants by JNTBGRI

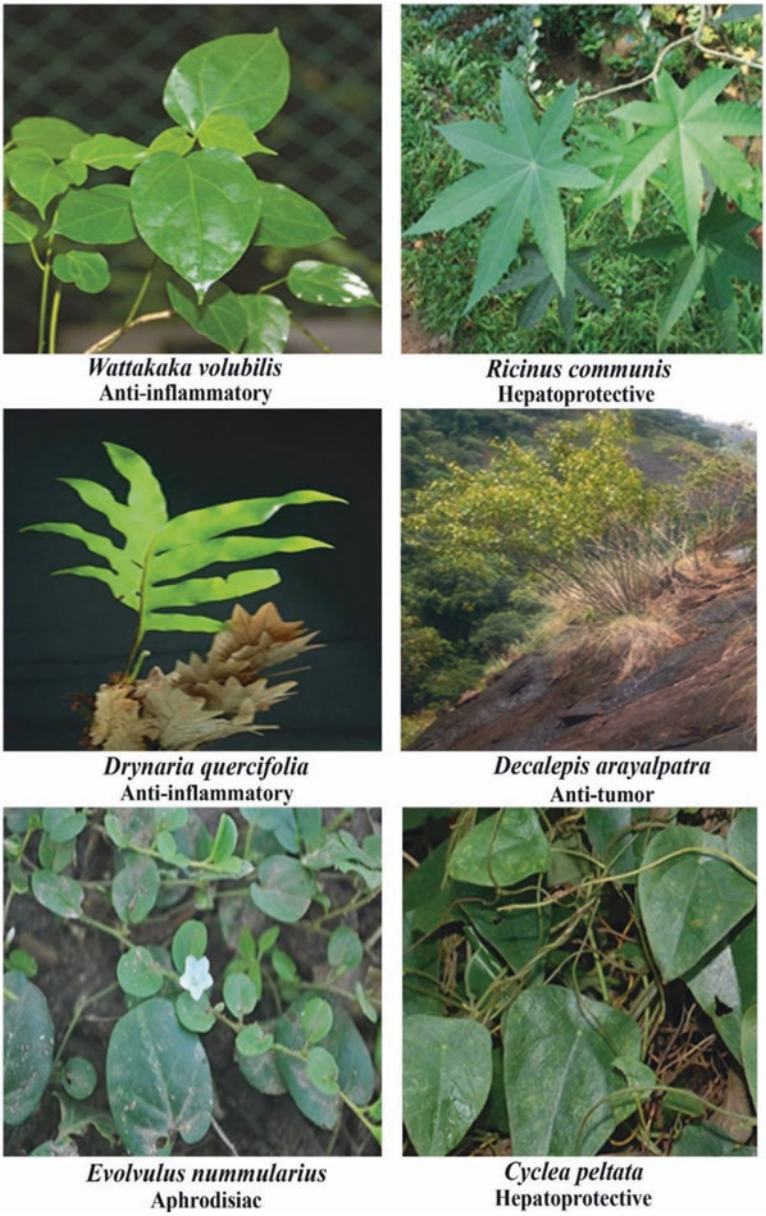

Fig. 16.2 (continued)

ethnopharmacology, Ayurveda, pharmacognosy, phytochemistry, pharmacy, and clinical pharmacology for conducting preclinical studies and clinical trials followed by drug development (herbal/Ayurvedic, modern drugs, nutraceuticals, cosmetics, and other plant-based products), patenting, technology transfer, commercialization, and benefit sharing.

A drug is broadly defined as any substance (chemical agent) that affects processes of living; therefore, briefly, the main component of ethnopharmacology can be defined as pharmacology of drugs used in ethnomedicine. The objectives of ethnopharmacology should focus on (1) the basic research aiming at giving rational explanation to how a traditional medicine works and (2) the applied research aiming at developing traditional medicine into a modern medicine (pharmacotherapy) or developing its original usage by modern methods (phytotherapy).

16.2.2.1 Significance of Ethnopharmacology

- Scientific validation of medicinal and food plants, based on TK/ethnomedical leads.
- Development of therapeutically active formulations which are commercially viable.
- Technology transfer and commercialization of the products by ensuring benefit sharing with the knowledge providers.
- Development of safer, inexpensive substitutes for expensive modern drugs or alternative drugs with fewer/no side effects.
- Utilization of ethnomedical/traditional knowledge on plants, animals, and minerals for enriching Ayurvedic/modern pharmacopoeia.

16.3 Drug Development Based on Traditional Knowledge

16.3.1 Development of an Herbal Drug 'Jeevani' from **Trichopus zeylanicus***, an Endangered Medicinal Plant with Traditional Knowledge from JNTBGRI: Case Study 1*

'Jeevani', developed from the perennial plant Arogyapacha (*Trichopus zeylanicus* Gaertn. subsp. *travancoricus* (Bedd.) Burkill) for which a national patent was filed, is an example for a potent therapeutic drug developed from an endangered traditional medicinal plant distributed in Western Ghats indicating the relevance of conservation of traditionally used medicinal plants (Fig. 16.3). *T. zeylanicus* is a small rhizomatous, perennial herb distributed in Southern India, Sri Lanka, and Malaysia. In India it is distributed at an altitude of around 1000 meters. The subspecies found in India is called *Trichopus zeylanicus* subsp. *travancoricus* and is endemic to the

Fig. 16.3 Source plant and drug development: *Jeevani*

region of the Western Ghats that falls in the Thiruvananthapuram district of the state of Kerala and the Tirunelveli district of the state of Tamil Nadu.

This case study details the benefit sharing arrangements concerning 'Jeevani', an herbal medicine developed by the scientists of the Tropical Botanic Garden and Research Institute (TBGRI) for immuno-enhancing, anti-stress, and anti-fatigue potential based on the knowledge of the Kani tribe. Scientific validation of Jeevani showed that it acts on the human system by enhancing body's natural defenses, activates delayed type hypersensitivity reactions and antibody synthesis, increases the number of polymorphonuclear granulocytes, activates the cellular immune system, exhibits hepatoprotective and choloretic activities, and has adaptogenic properties as shown by anti-peptic ulcer and anti-fatigue studies.

16.3.1.1 Discovery and Development of the Drug

In 1987, Dr. Pushpangadan stumbled upon the tribal medicinal herb while leading a team from the All India Coordinated Research Project on Ethnobiology (AICRPE) on an ethnobotanical expedition to the Western Ghats. Kani tribals, who accompanied the team as guides, were energetic even after long walks, whereas the scientists were tired. The scientist observed that the tribal guides were munching black fruits of some plants during their journey. Seeing the scientists exhausted, they offered this fruit to them during the trip, and after consuming it, they felt full of energy and vitality (Pushpangadan et al. 1988).

The therapeutic potential of *Arogyapacha* was analyzed later through a variety of chemical and pharmacological studies, and it was identified as *Trichopus zeylanicus*. Studies showed that only the species found in Western Ghats of India (*Trichopus zeylanicus* subsp. *travancoricus*) has the claimed medicinal properties, although the plant is also found in Sri Lanka and the Malay Peninsula. The analytical method for the standardization of the plant included both allopathic and Ayurvedic methods. The plant drug was evaluated on the basis of the Ayurvedic *dravyaguna* and was found to belong to the *Swathahita* (health-promoting) group. The anti-stress and immuno-stimulating properties of the plant were first discovered by the research led by Dr. P. Pushpangadan; later they also identified anti-tumor, anti-fatigue, stamina-enhancing properties, etc. The results of these open clinical trials were highly significant, and the drug developed was found to exert multi-therapeutic effect.

Three patents were filed and awarded based on the scientific validation of *T. zeylanicus*. Indian patent IN183071 dated September 1999 was awarded for the patent application entitled "A process for the isolation of a glycolipid fraction from *Trichopus zeylanicus* possessing adaptogenic activity." Indian patent IN187975 dated August 2002 was awarded for the patent application entitled "A process for preparation of novel immunoenhancing, antifatigue, antistress and hepatoprotective herbal drug." The third patent IN193609 dated 22.09.2006 was granted for a multi-drug combination containing *Trichopus zeylanicus* leaf and *Janakia arayalpathra* root, entitled "A process for preparation of a novel herbal medicinal composition for cancer treatment from *Janakia arayalpathra* root and *Trichopus zeylanicus* leaf." Utilizing modern scientific validation methods and Ayurvedic pharmacologic techniques, a new polyherbal Ayurvedic drug in granular form, named 'Jeevani', was developed later by TBGRI. The term 'Jeevani' means "elixir of life." The ingredients in 'Jeevani' were *Trichopus zeylanicus* ssp. *travancoricus* Burkill. ex Narayanan, *Evolvulus alsinoides* (Linn.) L., *Withania somnifera* (L.) Dunal., and *Piper longum* L. Clinical trial of 'Jeevani' was carried out on more than hundred subjects with different backgrounds. Apart from modern drug efficacy tests, the results were evaluated on the basis of Ayurvedic pharmacology, and subsequently, the technical knowledge for production of the drug 'Jeevani' was transferred to an Ayurvedic drug manufacturing company for a period of 7 years for a license fee of Rs.10 lakhs and 2% annual royalty on ex-factory sales price.

16.3.1.2 Access and Benefit Sharing (ABS) Model

After the technology transfer of 'Jeevani', TBGRI decided to part 50% of the license fee and royalty received from the manufacturing company to the Kani tribe who provided the lead for the development of the drug. Kani tribe registered a trust called "Kerala Kani Samudaya Kshema Trust" with the guidance from TBGRI, and 50% of the benefits received by the technology transfer and royalty were remitted to the Trust's account. This became one of earliest documented benefit sharing cases in intellectual property rights based on traditional medicinal knowledge of plants and first of its kind in India, wherein the benefits accrued from the development of a product based on an ethnobotanical lead were shared with the holders of the traditional knowledge. TBGRI, Thiruvananthapuram, obtained knowledge about the medicinal properties of *Trichopus zeylanicus* locally known as *Arogyapacha* from the Kani tribe in 1987, before the enactment of the Biological Diversity Act 2002 and the amendments to the Patents Act in 2005 that made specific provisions about traditional knowledge and biological resources. That is 50% royalty was shared by TBGRI with the Kani tribe from whom the knowledge was obtained by way of access and benefit sharing even before it became mandatory. Considering the significant outcome of this model in community empowerment, income generation, and poverty eradication of a tribal community, Dr. Pushpangadan was awarded with the UN-Equator Initiative Prize at the World Summit on sustainable development held in Johannesburg in 2002. Thereafter CBD-Bonn, WIPO guidelines, and our national legislation on biodiversity point out that TBGRI-Kani Access and Benefit Sharing case study is an ideal model of equitable benefit sharing (Pushpangadan and Pradeep 2008).

16.3.1.3 Conservation Strategies Developed for *Trichopus zeylanicus*

The plant can be propagated both by seeds and by vegetative means. Seeds usually take 6–7 months to germinate with only 10% germination rate. Sprouting is poor when planted directly. Rhizomes of 3 cm length wrapped in moist gunny sack or placed in cow dung are used for planting, and sprouting occurs within 3–5 days (Pushpangadan et al. 2016). Micropropagation of *T. zeylanicus* was achieved by shoot tip culture (0.3–0.5 cm) of 2-month-old axenic seedlings on woody plant medium (WPM) and 6-benzylaminopurine (BAP)-induced callus-free multiple shoot bud formation. These micropropagated plants were grown to maturity without defects in growth, morphological, flowering, and seed set characteristics (Krishnan et al. 1995). Martin et al. (2011) reported high-frequency in vitro propagation of *T. zeylanicus* spp. *travancoricus* using branch-petiole explants, and callus obtained from the explants was cultured on Murashige and Skoog (MS) medium. RAPD profile of the source plant and plants regenerated from callus after 4 years showed no evidence of polymorphism and the established plantlets with morpho-floral features similar to that of the source plants flowered normally and set fruits.

16.3.1.4 Current Status

Currently Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI) has a collaborative research program with Oushadhi to explore the possibilities and work out the modalities of the collaboration including signing of MOU for developing new products as well as manufacturing and marketing of Jeevani. The major players involved in this mega project were JNTBGRI, Kerala Forest and Wildlife Department, Eco-Development Committees (EDC) (Tribal) Kerala Kani Samudaya Kshema Trust, and Oushadhi. After getting the manufacturing license, JNTBGRI transferred the pre-demonstration technology of Jeevani and also renamed the product as “Jeevaniyaoushadhi,” which has been approved by the Business Management Committee. JNTBGRI alone cannot work out and implement the program as per the draft agreement prepared, and for this, we need equal support from Forest Department, Oushadhi, Kani tribes, etc. Cultivation of Arogyapacha was proposed by JNTBGRI in the forest areas of Thiruvananthapuram and Kollam districts. Based on this, the Kerala Forest and Wildlife Department decided to allow collection of seeds only from the forest areas and to build up some sustainable ex situ conservation program by local tribes with the technical support of TBGRI.

16.3.2 Studies of a Medicinal Coded Plant-222 Based on Traditional Knowledge with Special Reference to Access and Benefit Sharing: Case Study 2

This scientific study was carried out based on traditional knowledge related to a medicinal plant (Code No. 222∗) disclosed by a traditional healer after signing prior informed consent (PIC) and contractual agreement including non-disclosure agreement with Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI). The claim of the traditional healer was that he is using the particular medicinal plant species to treat diabetes, liver disorders, and jaundice and to relieve fatigue. On verification, no scientific study so far has been carried out on this plant species, and the therapeutic usage was kept as trade secret by the healer. The main objectives of the preclinical study were to conduct scientific evaluation of the claim disclosed by the traditional healer and to explore the possibilities for developing single/polyherbal formulation and its scientific validation through conducting preclinical studies. In the present study, the authors carried out plant taxonomy, pharmacognosy, phytochemistry, ethnopharmacology, and toxicity studies of the plant species (single/polyherbal formulation). The preclinical studies so far carried out by the authors show that the given coded drug (single and polyherbal formulations) possesses significant antidiabetic, hepatoprotective, and anti-fatigue properties as claimed by the traditional healer. Apart from this, the medicinal plant possesses excellent antioxidant property. The study further shows that the medicinal plant is devoid of any side effects. Based on the study, a patent application was filed with the title “A novel polyherbal formulation with multiple therapeutic effects as antidiabetic, hepatoprotective, antifatigue and antioxidant” (Application No. 2277/CHE/2011) to Regional Patent Office, Chennai. The traditional healer was also included as one of the inventors in the patent application. This is the first case study on access and benefit sharing (ABS) where a traditional healer was included as one of the inventors. The first part of the study highlights the pharmacological study on antidiabetic effect of the medicinal plant.

Preclinical Studies

1. Alloxan induces diabetes by destroying β-cells, and this model is almost comparable to type I diabetes with near-complete β-cell destruction. The blood glucose-lowering effect of the extract 222 at 125 mg/kg could possibly be due to increased peripheral glucose utilization and inhibition of tubular reabsorption mechanism for glucose in the kidney. The drug may be mimicking one or more actions of insulin at the insulin receptor level, or it may be influencing one or more post-receptor events (Krishnakumar et al. 2016a).
2. The coded plant 222 leaf ethanolic extract exhibited anti-hyperglycemic effect by significantly reducing the blood glucose levels in diabetic rats, and it also reduced the lipid profile parameters in diabetic animals. The extract prevents the free radical formation, or it may scavenge the reactive oxygen species through various antioxidant systems. The histopathological investigation along with the biochemical evaluation suggests the possibility of the regeneration of islets of diabetic pancreas by the extract treatment (Krishnakumar et al. 2016b).
3. The coded plant 222 leaf ethanolic extract exhibited hepatoprotective activity by significantly reducing the elevated serum enzyme levels in paracetamol (APAP)-induced hepatotoxic rats. The extract also showed protection against APAP-induced oxidative stress by significantly reducing the formation of reactive oxygen species (ROS) or by scavenging the free radicals by antioxidant system. The histopathological studies along with antioxidant and biochemical evaluation suggest the protective effect of coded plant 222 leaf ethanolic extract against paracetamol-induced hepatotoxicity (Krishnakumar et al. 2017a).
4. The coded plant 222 leaf ethanolic extract exhibited significant hepatoprotective effect by reducing the elevated serum enzyme levels and biochemical parameters in ethanol-induced hepatotoxic rats. The extract also showed protection against ethanol-induced lipid peroxidation and oxidative stress by significantly reducing the formation of malondialdehyde (MDA) or by scavenging the free radicals by antioxidant activity and stimulating antioxidant mechanism. Histopathological studies support the biochemical estimation of serum parameters and antioxidant enzyme status indicating hepatoprotective activity of the extract. The ability of the extract to protect the liver from ethanol-induced liver damage might be attributed to its antihepatotoxic effect or may be due to its ability to restore the activity of antioxidant enzymes (Krishnakumar et al. 2017b).
5. The coded plant 222 leaf ethanolic extract exhibited hepatoprotective activity by significantly reducing the elevated serum enzyme levels and biochemical parameters in carbon tetrachloride (CCl_4)-induced hepatotoxicity. The extract also showed protection against CCl_4-induced lipid peroxidation by significantly reducing the formation of malondialdehyde (MDA) or by scavenging the free radicals by antioxidant activity. The histopathological studies along with hepatic enzyme levels, lipid peroxidation in vivo, and biochemical evaluation suggest the protective effect of coded plant 222 leaf ethanolic extract against carbon tetrachloride induced hepatotoxicity (Krishnakumar et al. 2018).

16.3.2.1 Current Status

Preclinical studies have been completed, showing promising results. Awaiting for clinical trials and commercialization of the product.

16.3.3 The CSIR San Model of Benefit Sharing: Case Study 3

'*Hoodia gordonii*', used by the San Bushmen, was patented by the South African Council for Scientific and Industrial Research (CSIR) in 1998, for its appetite-suppressing quality. A license was granted to the British phytomedicine company Phytopharm for the development of the active ingredient in the *Hoodia* plant, p57 (glycoside), to be used as a pharmaceutical drug for dieting. Once this patent was brought to the attention of the San, a benefit sharing agreement was reached between them and the CSIR in 2003. This would award royalties to the San for the benefits of their indigenous knowledge. The San were represented by a regional organization formed under San leadership, the Working Group of Indigenous Minorities in Southern Africa (WIMSA). This benefit sharing agreement is one of the first to give royalties to the holders of TK used for drug sales. The terms of the agreement are contentious, because of their apparent lack of adherence to the Bonn Guidelines on Access to Genetic Resources and Benefit Sharing, as outlined in the Convention on Biological Diversity (CBD). The San have yet to profit from this agreement, as P57 has still not yet been legally developed and marketed (Tully 2003).

16.3.4 Benefit Sharing by Shaman Pharmaceuticals, Inc.: Case Study 4

Benefit sharing by Shaman Pharmaceuticals, Inc., a company located in South San Francisco, California, that uses ethnobotany, as well as isolation and natural product chemistry, to discover and develop novel pharmaceuticals is another example. Agreements with culture groups and countries that Shaman works with, secure benefits for the use of plant resources and TK, both during the drug discovery process and after a product is commercialized. At the beginning and during research expeditions, the company provides specific upfront compensation that responds to immediate needs of country and indigenous collaborators. Long-term compensation will be available through the Healing Forest Conservancy when a product is commercialized. When a product is marketed, Shaman will share a percentage of profits for benefit sharing through the conservancy, equally, to all collaborating countries and culture groups (UNCTAD 2019).

16.4 Conclusion and Way Forward

The plants associated with traditional knowledge, which is a potential source of discovery of lead compounds and novel therapeutics, are currently under threat due to high demand in the pharmaceutical industry. Access and benefit sharing should be implemented as and when to develop novel process or products based on the traditional knowledge as per the guidelines of the Biological Diversity Act (2002) and the Biological Diversity Rules (2004). These benefits should be shared in equitable manner in terms of monetary and non-monetary benefits with the traditional knowledge providers/holders/custodians. An important reason for the lack of progress in developing ABS regimes is mainly due to limited participation in the policy process by industries which depend on genetic resources. This may mainly because of lack of knowledge on the new policy environment, not realizing the importance of these debates for them, or having largely negative perceptions about the new paradigm. Efforts to bring industry into the ABS policy process and promote dialogue among the range of stakeholders and between the diversity of sectors remain essential. The ABS relationships have emerged as the most common model through which companies gain access to genetic resources. Under these circumstances partnerships between users and providers yield far more significant benefits than the supply of samples, or raw material, alone. Widespread frustrations are experienced by all sectors in securing prior informed consent from national competent authorities.

Appropriate ways to seek PIC, negotiate mutually agreed terms, and share benefits associated with the use of traditional knowledge remain unclear, and basic questions remain unanswered. These related questions have been raised since the CBD entered into force, but developing effective ways to address them within ABS agreements and partnerships is still in the early stages. Because of these difficulties, many companies have adopted their own approach to the use of traditional knowledge, while others have little awareness of the need to enter into ABS arrangements when using traditional knowledge. Legal certainty and clarity of rights to material is vital to promote and protect industry investment in research and development and commercialization. In this regard, the extent to which ownership and/or legal status of genetic resources is resolved at the national level plays a key role for those seeking access to genetic resources and PIC. Problems of genetic identification, combined with capacity constraints and the sheer complexity of designing a monitoring and tracking system that suits different types of genetic material and sectors, pose significant challenges for the development of a compliance system that is both cost-effective and effectual.

The relationship between intellectual property rights and benefit sharing varies considerably from sector to sector, depending on industry-specific approaches to IP protection. IPRs tend to assume greater significance in pharmaceutical, biotechnology, and seed sectors and thus play a greater role in benefit sharing in these sectors, while companies working in botanical medicine, cosmetic and personal care, fragrance and flavor, and food and beverages focus less on IPRs and more strongly on benefits linked to the supply of raw materials. In general, however, intellectual property rights are given prominence as a mechanism for benefit sharing, over and above the frequently more concrete gains of building domestic scientific and technological capacity.

Conservation of the medicinal plants should be undertaken in order to maintain the standards of the products and also maximize their potential. It is the need of the hour to rescue, recover, and rehabilitate the threatened medicinal plants through ex situ and in situ conservation strategies. According to Prof. M.S. Swaminathan, "Herbal technology and Information technology would be the two challenging enterprises which Kerala could adopt to meet its immediate economic growth and social development. The biological resources, particularly medicinal plants resources and the associated Traditional knowledge, innovations, traditions and practices are an important asset of the state, which could be judiciously exploited through appropriate scientific and technological inputs. Herbal technology alone can perhaps help Kerala to convert its biological capital to economic wealth. Such enterprises, if meticulously planned and executed, could help generate more opportunities for employment and income in rural as well as urban sectors." To ensure the conservation of the threatened medicinal plants, new location-specific strategies should be evolved and implemented through people participatory programs at grassroots level which will help to ensure the health and economic security of the country. Therefore, in the future, the scientific community has to develop a new protocol for conducting multi-sector, multidimensional, multidisciplinary collaborative research programs at national and global levels with a view to demonstrate the synergetic effect and other mechanisms of action of natural products including traditional medicines, nutraceuticals, cosmoceuticals, and other plant-based products.

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Chapter 17
Intellectual Property Rights and Threatened Medicinal Plants: The Scenario

K. Souravi and Rahul Patil

Abstract Medicinal plants are nature's gift to blossom human well-being, without any exaggeration. Research trends from decades highlight the fact that nature is at the forefront to enlighten us in finding drug leads. However, the unprecedented rate of destruction of these species is an awful dimension to this fascination. We have not explored most of the plant species, and now, most of them are threatened or extinct. There is a need for understanding and discussion on balancing resource availability, renewability, usage, and conservation of medicinal plants and products thereof. Exploration of relationships between intellectual property rights and knowledge verticals modulating biodiversity resources will attract the attention of various stakeholders in the domain.

Keywords Intellectual property rights · Threatened medicinal plants · Biopiracy · Protection

17.1 Introduction

Since the inception of drug discovery, nature is inspiring us in the exploration of drug candidates. Earliest examples like aspirin from willow bark, penicillin from fungus, and morphine from opium poppies are some of the witnesses. The surge in medicinal chemistry advancement led the transition from natural products to the development of synthetic compounds. However, the charisma developed by these medicinal plants during the evolution of millions of years in defending themselves from attacks of pathogens makes them unbeatable.

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Relentless efforts to challenge deadly cancers drove the development of treatments like trastuzumab, bevacizumab, and cetuximab. Such solutions to fight against cancers are powered by medicinal plants, fungi, and marine flora and fauna. The tree, *Taxus contorta*, a yew that grows in the Western Himalayas, is a source of the famous drug paclitaxel, a powerful chemotherapy mediation for a range of cancers like breast, lung, and ovarian. This plant species has been classified under endangered category by the International Union for Conservation of Nature (IUCN), earlier this decade (Thomas 2011). The status has drastically moved from vulnerable to the endangered category because its estimated global population reduced more than 50%, owing to varied reasons: up to 50% of the forests where this yew grows have been destroyed or heavily logged and overexploitation for fuel, fodder, and medicinal use is the reason for the declination of yew in Pakistan. On the other hand, 90% decrease in the population of yew in northwest India and western Nepal has been observed because of the exploitation for Taxol production. Similarly, *Taxus brevifolia*, Pacific yew from which paclitaxel was first discovered in the 1960s, is currently classified under the near threatened category (Thomas 2013), and it is facing threats of heavy exploitation for its bark in the recent past. Ongoing threats like logging and fires are also responsible for its reducing population. It has been estimated that the population reduction of this species is in the range of 10–30% within the last three generations, whereas its generation length is estimated to be at least 30 years with very slow growth rates. This is a similar scenario with numerous species worldwide. Efforts are being made to document their current status which will further help in designing conservation strategies.

17.2 Need for Conservation

At different levels, biodiversity assessment is being carried out – regional, national, and global. However, continuous efforts by IUCN and its partners have led to increased coverage of threatened species assessment. Such assessments of ecosystems remain helpful in tracking progress in achieving Sustainable Development Goals (SDGs). Conservation status of plant species is compiled in Fig. 17.1 (across major plant taxonomic groups) and Fig. 17.2 (across advanced and emerging economies) based on IUCN Red List Summary Statistics 2018 (IUCN 2018). The numbers of plant species in extinct (EX) and extinct in the wild (EW) are clubbed together to form the first indicator, whereas clubbing of critically endangered (CR), endangered (EN), and vulnerable (VN) formed another indicator representing threatened species.

The highest number of plants species (105340) under threatened categories is from dicotyledons (Magnoliopsida) of flowering plants, out of which 2192 species are critically endangered, 3453 species are endangered, and 4889 species are vulnerable. Figure 17.2 showcasing the country-wise study of extinct and threatened species clearly illustrates that the United States and emerging economies have a high rate of conservation risks for plant species than other enlisted advanced economies. Although IUCN Red List hosts more than 27,000 plant species, this is a small proportion of the world's existing plant population. There is a need to expand the number and coverage of taxonomic groups with conservation status assessment.

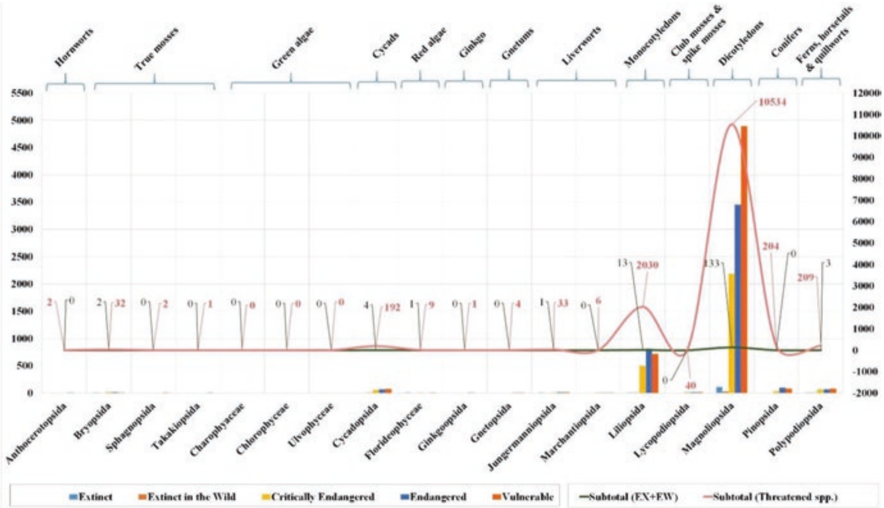

Fig. 17.1 Numbers of plant species in IUCN Red List categories by plant taxonomic groups Unable to view the figure.

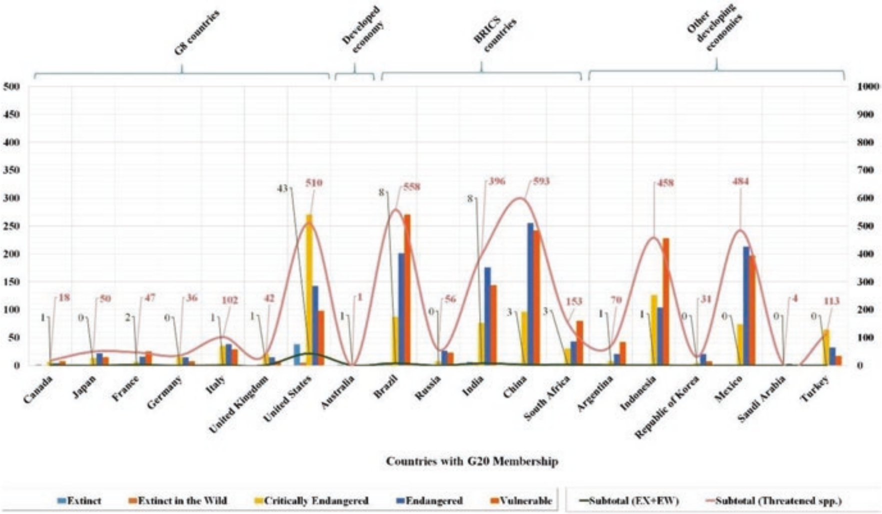

Fig. 17.2 Numbers of plant species in IUCN Red List categories by advanced and emerging economies

In 2010, the Global Checklist of Medicinal Plants (GCL-MP) recorded 21,524 taxa across countries, around the globe. Nevertheless, the numbers will increase as and when new studies report novel species and important uses of existing species. Rafael in 2001 originally reported that a total of 17.1% of flowering plant species are found worldwide, of which 72,000 plant species have been used medicinally (Schippmann et al. 2006). Based on the projections of Bramwell (2003), it is reported that 21% of the world's flora is threatened. Further, Schippmann et al. (2006) estimated that 15,000 medicinal plant species are threatened at least to some degree.

In the recent European regional study, assessment of 400 vascular medicinal plants from 90 families was reported under the initiative of Medicinal Plant Specialist Group of IUCN (David et al. 2014). Wild plant collection, general ecosystem modifications, agriculture (livestock farming, annual and perennial non-timber crops, and plantation forestry), silviculture, invasive alien species, transport, infrastructure, logging and wood harvesting, energy production and mining, dams and water abstraction, and pollution emerged as primary threats. The assessment pointed that 164 species (41%) were stable, 125 species (31%) were declining, 10 species (2.5%) were increasing, and 101 species (25%) had unknown population trends. A total ten species were classified under threatened medicinal plants at the Pan-Europe and EU-27 level, out of which seven were endangered (EN) and three were vulnerable (VU).

Further it has been reported that more than 400,000 metric tons of medicinal and aromatic plants (MAPs) are traded every year, out of which about 80% of species involved are harvested from the wild (Secretariat of the Convention on Biological Diversity 2009). This showcased the need for national/international standards/guidelines to prevent overexploitation of plants used in medicines and cosmetics. To address the issues such as maintaining wild MAPs, preventing negative environmental impacts, respecting customary rights, applying responsible management and business practices, and complying with laws, the Medicinal Plant Specialist Group of IUCN launched the International Standard for Sustainable Wild Collection of Medicinal and Aromatic Plants (ISSC-MAP) in 2007 which addresses ecological, social, and economic requirements for the sustainable wild collection of MAPs. Since 1996, the BioTrade Initiative of United Nations Conference on Trade and Development (UNCTAD) is involved in modelling transformations of MAP products, bio-resource management, value-adding processing and marketing, and promoting trade and investment in products and services originated from indigenous biodiversity (*BioTrade Initiative*, UNCTAD). Further the development of the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS) was negotiated in the Uruguay Round of negotiations, 1986–1994, under GATT (General Agreement on Tariffs and Trade) agreement which focused on the intellectual property as trade and protection instrument. The brainstorming in these negotiations led advancement in the intellectual property frameworks.

17.3 Evolution of Legal Frameworks

17.3.1 Birth and Transformations of the Multilateral Trading System, GATT

In 1946, the United Nations Economic and Social Council established the Preparatory Committee of 23 founding contracting parties, namely, Australia, Belgium, Brazil, Burma, Canada, Ceylon, Chile, China, Cuba, Czechoslovakia, France, India, Lebanon, Luxembourg, the Netherlands, New Zealand, Norway, Pakistan, Southern Rhodesia, Syria, South Africa, the United Kingdom, and the United States, to draft charter for the International Trade Organization (ITO). The ITO, International Monetary Fund (IMF), and International Bank for Reconstruction and Development (IBRD) were envisaged as the triad for integrated and harmonized world economic systems after the Second World War. Simultaneously, committee members negotiated 123 times on tariff concessions between April and October 1947. These resulted into an international agreement, General Agreement on Tariffs and Trade (GATT), which was signed by 23 founding contracting parties on October 30, 1947, at Palais des Nations in Geneva and entered into force on January 1, 1948.

The United Nations Conference on Trade and Employment (i.e., Havana Conference) was held at Havana in Cuba from November 21, 1947, to March 24, 1948. In November 1946, ITO draft was considered by 56 countries, out of which some 53 countries signed the Final Act of Havana Charter in March 1948. However, in the end, ITO was stillborn because there was no commitment from governments to ratification. Hence, GATT 1947 remained only an international instrument governing international trade until the establishment of the World Trade Organization (WTO) on January 1, 1995. GATT 1947 was applied through a Protocol of Provisional Application, whereas the Havana Charter never came into force. Therefore, Provisions of GATT 1947 were remained provisionally in force and incorporated into GATT 1994 which, further, became a component of WTO agreement. Legal instruments through which the contracting parties apply for applying GATT 1947 were terminated on January 1, 1996, after a 1-year transition period (Decision of Preparatory Committee for the WTO 1994).

During the period from the birth of GATT 1947 to the birth of WTO 1995, eight GATT rounds or multilateral trade negotiations were held. These were Geneva (1947), Annecy (1949), Torquay (1950–1951), Geneva (1956), Geneva or Dillon Round (1960–1961), the Kennedy Round (1964–1967), the Tokyo Round (1973–1979), and the Uruguay Round (1986–1994) (GATT bilateral negotiating material). In February 1987, negotiations started in the following areas: tariffs, non-tariff measures, tropical products, textiles and clothing, agriculture, subsidies, safeguards, trade-related investment measures, natural resource-based products, and trade-related aspects of intellectual property rights including trade in counterfeit goods. For the first time, exploration of trades associated with intellectual property rights was established.

17.3.2 Protection of Intellectual Property Rights

17.3.2.1 IP Protection as a Human Right

Legal aspects of intellectual property right protections vary with national territories/boundaries. National laws for intellectual property protection synchronize in granting moral and economic rights to creators for their creations and granting rights of access of these creations to the public. Article 27 of the Universal Declaration of Human Rights (The Universal Declaration of Human Rights-UDHR 1948), by the United Nations, promotes rights of participation and protection in cultural, artistic, and scientific advancement. Various international treaties bring uniformities across national legal frameworks in protecting intellectual property rights. The WIPO Convention constituted the World Intellectual Property Organization (WIPO) which was entered into force in 1970. This WIPO administers 26 treaties including the WIPO Convention. The Paris Convention, enacted in 1883, is the widest framework which considers patents, trademarks, industrial designs, utility models, service marks, trade names, geographical indications, and the repression of unfair competition.

17.3.2.2 Impact of IP Protection on Indigeneity

Most importantly, protection of intellectual property may have an adverse impact on biodiversity if there is commercial exploitation of naturally occurring biochemical or genetic material (for instance, through patent-based restriction on its future use) without paying fair compensation to the indigenous community. On the other hand, biodiversity without IP protection can also lead to adverse impacts like displacements of native and traditional crops; restricted exportation of traditional medicinal plants, negatively impacting in situ conservation; and restricted practices of saving, using, and selling farm-saved seeds by small farmers and indigenous community (Mohan 2011). IP protection mechanism should balance between defensive and positive protection where earlier protection refrains outside community from enforcing the community rights and the latter empowers the indigenous community to exercise and control their rights on traditional heritage.

Ascendancy of protection measures through the International Union for Protection of New Varieties of Plant (UPOV) Convention, Convention on Biological Diversity (CBD) 1993, International Treaty for the Protection of Plant Genetic Resources for Food and Agriculture, Intergovernmental Committee on Intellectual Property and Genetic Resources, Traditional Knowledge and Folklore, and national laws like the Protection of Plant Varieties and Farmers' Rights Act (PPVFRA), Biological Diversity Act (BD Act), and regulations for genetically modified organisms influence the biodiversity, farmer communities, and indigenous societies in developing countries. This influence is growing continuously because of the translation of agricultural trades in developing countries from the informal sector to the formal sector.

17.4 Intellectual Property Protection for Medicinal Plants

17.4.1 Patents

17.4.1.1 Patents: A State of the Art

This is the time where scientific advancement is at the peak. Researchers' tailor-made efforts are leading the world toward sustainability, and this journey is powered by breakthrough and incremental scientific inventions. The globalization of science and technology is on-trend and can be mapped through patenting activity worldwide. In 2017 only, applicants filed about 3.17 million patent applications with 5.4% average growth from 2007 to 2017 (WIPO 2018). Principally, this surge in the trend also shows the increase in awareness and necessity of patent protection across technologies. After all, granted patents provide applicants monopoly to enforce their invention rights for a limited period. This is achieved by reducing the freedom of other users of the technology. However, this is the way by which the patent system empowers and promotes the development of scientific creativity. It is a well-known fact that biotech-pharmaceutical industries invest heavily in the designing and development of drugs and biologics. Hence, these industries back the patent rights to catch returns on their investments. The biotech-pharma sector has been, consistently, profitable as compared to others. Increasing innovation and protection of inventions in the areas can be tracked in Fig. 17.3. Analysis of patenting activity (Fig. 17.4) across income groups suggests a sizable shift in filing patent applications at upper middle-income economies. It largely explains that overall interest in protecting inventions in countries like China is increasing tremendously. India has recorded one of the fastest growths (+8.3% in 2017) in the patent applications with origins which were achieved due to growth in resident applications (WIPO 2018). Coincidently, countries like China and India are rich in indigenous knowledge; also, ancient alternate systems of medicine are prominent in Asian countries like India, China, Indonesia, Bangladesh, Vietnam, Maldives, Nepal, Bhutan, Sri Lanka, and North Korea. The overall growth in innovation activity also showcases the emerging trends in harnessing the potential indigenous knowledge in these countries.

17.4.1.2 Impact of Patent Protection on Indigeneity

Basically, a patent is an exclusive right granted for an invention which confers making, using, selling, importing, and offering for sale to the patentee for a limited period. To get a patent, an applicant must disclose the invention in enough details to facilitate reproducibility without undue experimentations. The patent is granted by national patent offices to the inventions which satisfy basic patentability criteria. These requirements include subject matter eligibility, novelty, inventive step, and utility. Inventions related to medicinal plants and products may have to undergo stringent patentability assessments (TRIPS 1994, Article 27). TRIPS Article 27.3

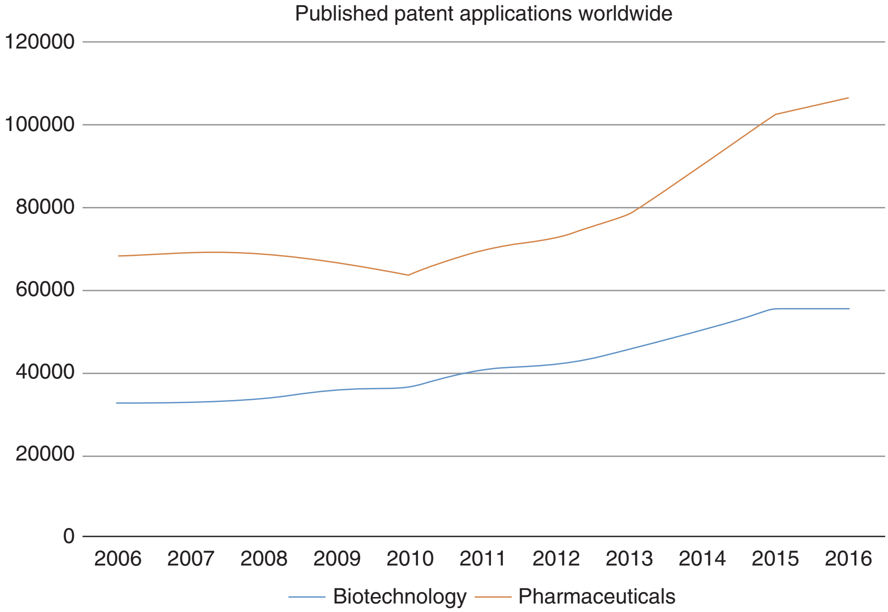

Fig. 17.3 Numbers of patent application filed worldwide in biotechnology and pharmaceutical sectors per year

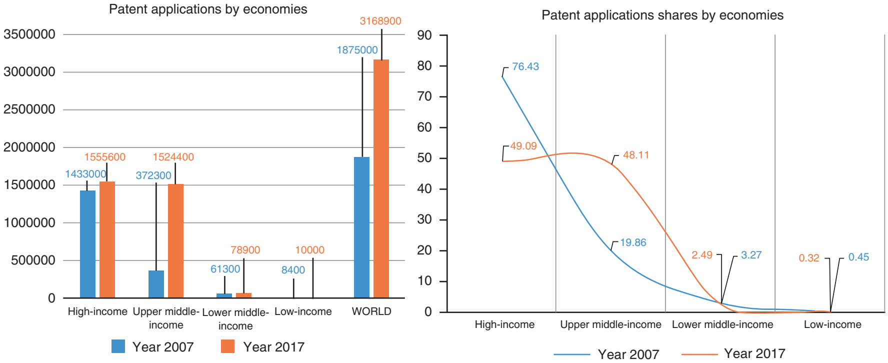

Fig. 17.4 (**a**) Comparison of patent application distribution by income groups and (**b**) analysis of patenting trend across income countries in a decade (2007–2017)

suggests that plants and animals may be excluded from patentability. However, in that case, national IP offices should provide protection provisions through either plant variety protection framework or any other sui generis mechanism. These inventions may have plant variety with medicinal properties, active ingredients obtained from plants, plant-part extracts, and new use of active ingredients or extracts from plants. Eligibility of medicinal plants as a patentable subject matter varies with the country-wise regulations. In the United States under the provisions of 35 USC 161, patents may be granted to asexually reproduced a new and distinct variety of a plant other than tuber propagated plant or a plant found in an uncultivated state, and also algae and micro-fungi are considered as plants (35 USC 161 1954). European legal framework excludes plants produced by non-technical processes such as crossing and selection whereas includes only plants made by technical methods as a patentable subject matter (Biopatent Directive 1998, Article 3.2). This is recently clarified and adopted since July 2017 by notice from the European Commission. This clarification uncurtains uncertainties of the Biopatent Directive 1998 (EPO News and Issues 2017). In India, Section 3(j) of the Patent Act 1970 excludes plants in whole and in parts including seeds, varieties, and species and essentially biological processes for the production or propagation of plants (The Patent Act 1970). On similar standards, China excluded plant varieties as a whole from patentable subject matter (SIPO Guideline for Patent Examination 2010), and the Japanese Patent Act includes patent varieties and process of development using modern plant breeding techniques (Japan: Patent Law 1959). Report by EPO suggests that most of the patent applications (about 300/year) in this category claim genetically modified (GM) plants as compared to the patent application (about 70/year) related to non-GM plants (Biotechnology patents at the EPO, EPO News & Issues).

On the other side, the world herbal market values around USD 60 billion which is expected to reach USD 150 billion in 2020 with Asia and Europe being the largest markets accounting for 39% and 34%, respectively (Alizar 2016). There are various types of herbal medicinal composition: indigenous herbal composition; herbal compositions in systems like Ayurveda, Unani, and Siddha; and modified herbal compositions with the modifications in dose, dosage form, mode of administration, herbal medicinal ingredients, method of preparations, and medical indication. The eligibility of herbal composition to overcome patentability requirements depends upon its nature. Generally, natural products are not patentable under the principle "Doctrine of Nature." If the composition is in the public domain, it cannot be patent protected by private parties. To overcome strict patentability standards, herbal compositions should have novel and synergistic combinations of plants or extracts thereof. New and inventive extraction processes of a medicinal plant have been included in the patentable subject matter. Most of the countries have excluded method of treatment, diagnosis, or surgical methods as patentable subject matter with the statuary provisions. However, the United States allows a patent to inventions claiming a method of use (Fed. Cir. Apr. 132,018). On the similar lines, USPTO granted a patent "The use of turmeric in wound healing" (Das and Cohly 1995) in March 1995, which was revoked (in November 1997) on the initiative of CSIR India by raising the objection of encroachment over traditional knowledge (Jayaraman 1997). Similarly, "Method for controlling fungi on plants by the aid of a hydrophobic extracted neem oil" (Locke et al. 2014) was granted by EPO (in September 1994). This was challenged by various nongovernmental organizations and further revoked in May 2000 (EP Board of Appeal Decisions 2005). These cases (e.g., brinjal, jamun, etc.) have a collective impact on bio-colonization and biopiracy. However, not just the cost issues but also to tackle this scenario, there remains a necessity for a strategic approach to protect the biological heritage of developing countries with international acceptance. Traditional knowledge cannot be protected under patent mechanism because it does not satisfy basic patentability requirements, namely, novelty and inventive step.

To address this situation, the Council for Scientific and Industrial Research (CSIR) India invested efforts in developing a Traditional Knowledge Digital Library (TKDL). It hosts more than 2.90 lakh medicinal formulations of Ayurveda, Unani, and Siddha which is available in five international languages, namely, English, Japanese, French, German, and Spanish. TKDL provides access of traditional knowledge to patent offices under the International Agreement (AAYUSH Press Note 2016), by regularly submitting prior art evidence in pre-grant oppositions on patent applications. In India, for the protection of traditional knowledge, various provisions are adopted under the Patent Act 1970 raising objections based on novelty [Section 2(1)(j)], inventive step [Section 2(1)(ja)], mere admixture compositions [Section 3(e)], traditional knowledge as such or aggregation of components thereof [Section 3(p)], contravention to biological material [Section (15)], pre-grant opposition [Section (25)(1)(d)/(f)/(k)], and post-grant opposition [Section (25)(2)(d)/(f)/(k)]. In addition, the Indian Patent Office had also brought out Guidelines for Processing Patent Applications relating to Traditional Knowledge and Biological Materials to facilitate due care and diligence while processing patent applications at IPO.

Since enforcement of TRIPS 1995, signatory countries have obligations to grant a patent to pharmaceutical inventions; however, earlier granting patents for such inventions were based on the decisions of the respective countries. Further, the signatories have the freedom to operate in applying their own patentability standards for allowing parallel importation. TRIPS also provides flexibilities under government use and compulsory license of patented inventions. Taking this as an opportunity, most developed countries have strong government use and compulsory licensing provision in the national laws, for instance, US Code 1498, for the government use of patented products (28 USC 1498 2012). The US government regulated the price of patented drug ciprofloxacin in 2001 considering anthrax scare in the United States, by proposing government usage as a tool. Similarly, the Directive 98/44 of 1998 of the European Parliament enables the grant of compulsory license for genetically modified plant varieties (Biopatent Directive 1998). In this way, developed countries have controlled monopolies conferring patents using patent system, government use, and compulsory licensing. An emerging middle-income country Indonesia has enabled government use of certain patented anti-retroviral drugs through the Exploitation of Patent on Anti-retroviral Drugs by the Government (Decree of the President Republic of Indonesia 76 2012) and the Procedure of Exploitation of Patent by the Government (Government Regulation 27 2004). Section 84(1) of the Indian Patent Act 1970 allows the grant of a compulsory license to an invention after the expiration of 3 years if there are objections based on availability and affordability concerns of patentable inventions to the public and local working requirement. In India, a compulsory license of Bayer's drug Nexavar (Berend et al. 2005) (granted Indian Patent No. 215758 in 2008) was assigned to Natcoin 2012 due to reduced availability of Bayer's Nexavar at an affordable price.

17.4.1.3 Patent Protection on Medicinal Species with Worrisome Conservation Status: A Case Study from Indian Medicinal Plants

Asian countries like India are rich in indigenous knowledge and ancient alternate systems of medicine. A total of 197 Red Listed medicinal plants are accessed from Foundation for Revitalisation of Local Health Traditions (FRLHT), an environmental information system (ENVIS) established by the Ministry of Environment and Forests (MoEF), Government of India, in 1982 (Ved et al. 2016). These are further refined to 84 species by the availability of complete disclosure of taxon profiles. Of the 84 species, the critically endangered are 22, endangered 17, vulnerable 27, near threatened 14, and least concerned 3, and data sufficient is 1. A patent literature search on these species resulted in 5198 INPADOC patent families across the globe. Critically endangered species *Taxus wallichiana* is the highest explored species with 1062 patent documents. Similarly CSIR India (19), Mitsui Petrochem Ind. Co. Ltd. (7), and Indena SpA (4) are the leading organizations in case of IPs related to *T. wallichiana*. *Piper nigrum* (992), *Acorus calamus* (990), and *Andrographis paniculata* (899) are further highly explored species on the same lines. Most of the inventions (about 65%) relate to plant extract from specific plants or their process of extraction. More than 50% of inventions disclose extracts with various active ingredients in combination. Angiosperm species are highly evaluated than other plants. Therapeutic activities related to anti-inflammatory, analgesic, and autonomic nervous systems are highly reported. Mainly extracts are studied for therapeutic and cosmetic applications. Extracts are normally prepared from raw materials harvested using distructive harvesting techniques from the corresponding medicinal plants leading to rapid declination in population, further aided with very slow regeneration capacity of such species. For example, a highly explored species *T. wallichiana*, East Himalayan yew, is a notable representative showing heavy exploitation for its leaves and bark which are used to produce the anti-cancer drug paclitaxel or similar chemicals like taxane alkaloids, and use of its young shoots and leaves and sometimes inner bark in various potions, tinctures, and pastes is known for a long time. The fleshy aril around the seed (only non-toxic part of yews) is consumed by local inhabitants as jams. The red dye obtained from the inner bark is often used in religious ceremonies in Nepal. Other than that, the plant is used as food for animals locally; as articles of handicrafts, jewelry, etc. locally; and as construction or structural material globally. Similar trends are also noticed for other species. This underscores the importance of harvest management planning and species management subject to ex situ conservation. Cultivation on a large scale is necessary for relation to pharmacology which will reduce the pressure on wild populations in the future (Fig. 17.5).

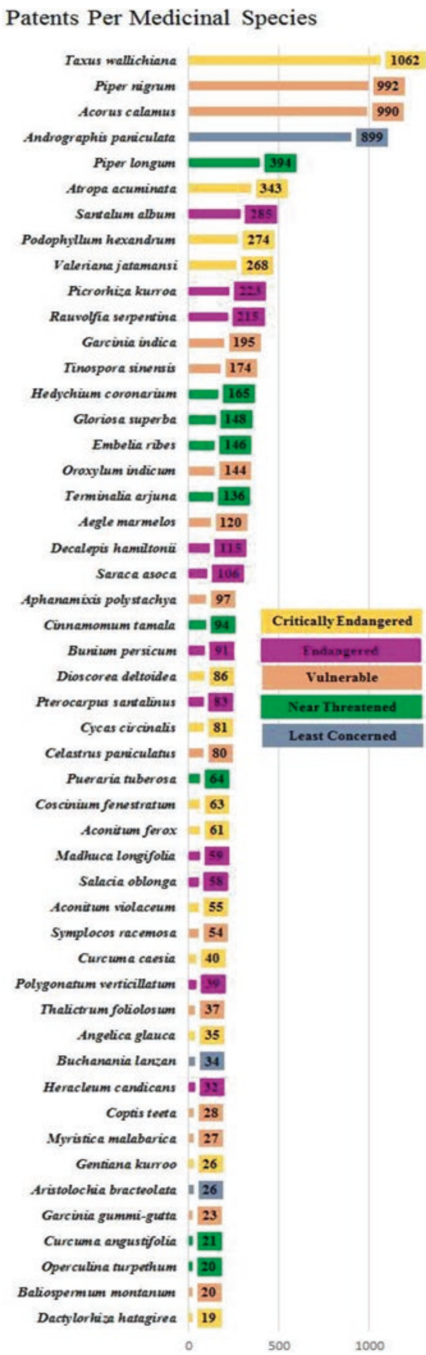

Fig. 17.5 Numbers of patent application published between 1963–2019 for inventions related to 50 Indian medicinal plants with concerned conservation status

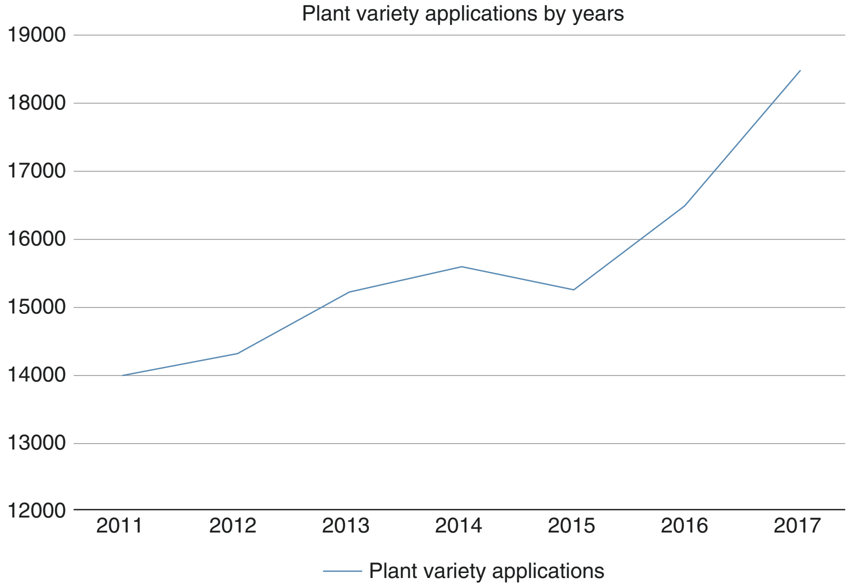

Fig. 17.6 Numbers of plant variety applications filed worldwide per year

17.4.2 Plant Variety Protection and Farmers' Rights

17.4.2.1 Plant Variety Rights: A State of the Art

Transformation in scientific knowledge and scientific society is accelerated in the past few decades. Modernization of tools and techniques led the era of genetic modifications of plants by incorporating gene of interest forming genetically modified plant variety. However, earlier, plant breeders had no such capabilities, and crossing over on the plant was the only methodology to transmit desired traits. This technique was purely non-technical and was based on random tactics. Therefore, these varieties were not protected under Patents Act. However, a different form of protection was proposed, that is, plant variety protection. Now awareness of plant variety protection is spreading like fire across the globe among farmers, breeders, researchers, and corporations. Plant variety application is growing at a rapid phase (Fig. 17.6). Significant increase in filing of plant variety applications in upper-middle and lower-middle economies in the 2007–2017 decade can be observed (Fig. 17.7). In 2017, around 18,500 applications were filed, and it was assessed as 11.7% incremental growth than the previous year. European countries and Asian countries have shares of 39.8% and 37.0%, respectively, out of worldwide filing in 2017. Assessment reveals that high-income countries have a share of 52.2% application filing worldwide. WIPO reports that by the end of 2017, 126,150 plant varieties were in force and out of these shares of active titles at CPVO and the United States are 25,914 and 25,238, respectively (WIPO 2018).

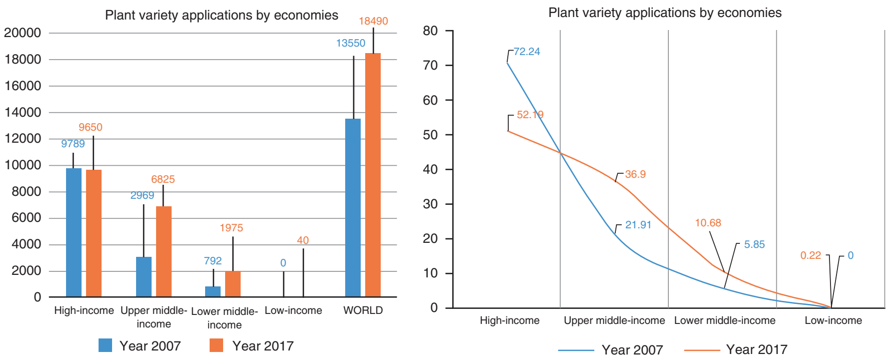

Fig. 17.7 (**a**) Comparison of plant variety application distribution by income groups and (**b**) analysis of plant variety application filing trend across income countries in a decade (2007–2017)

17.4.2.2 Impact of Plant Variety Protection on Indigeneity

Basically, a plant variety is a grouping of plants carried based on the presence of the same genome and the specifications. Plant variety as stated in Recital 30 of EC Directive on the Legal Protection of Biotechnological Inventions (Biopatent Directive 1998) is as follows: "plant variety is defined by the legislation protecting new varieties, pursuant to which a variety is defined by its whole genome and therefore possesses individuality and is clearly distinguishable from other varieties." Article 5 of the Council Regulation on Community Plant Variety Rights also defines variety as plant grouping within single botanical taxon of the lowest known rank (Council Regulation 2100/94 1994). It further illustrates that variety definition depends on the expression of characteristics that result from a genotype or their combination, variety should distinguishable from other plant groupings by the expression of at least one characteristic, and variety's suitability for propagation should remain unchanged. Simply, if the grouping of plants is based on the presence of a specific characteristic and not a whole genome, then the obtained new variety will not be considered for protection under the Plant Varieties Act. That is, to seek protection status under this category, plant varieties should have homogeneity and individuality with the same genome, and even after propagation, plant from the respective variety should have the same specifications as tolerances.

Article 4 of the Biopatent Directive suggests exclusion of plant varieties and the essentially biological processes for their production from the patentable subject matter (Biopatent Directive 1998). An essentially biological process to produce plants is excluded from patentability because it consists of natural phenomena such as crossing or selection, whereas the inventions pertaining to plants can be patentable only if the technical feasibility of that invention does not confine to a plant. On the other hand, Recital 31 of the Biopatent Directive demarks between patent protection and plant variety protection. It notifies that plant varieties with the characteristic gene and different genome cannot be covered under plant variety protection;

rather it should be covered under patent protection even if it comprises new varieties of plants (Biopatent Directive 1998).

TRIPS Agreement as per Article 27.3(b) requires a signatory state to provide protection to plant via either patent or other sui generis mechanisms (TRIPS 1994). These national acts of plant variety protection are powered by the intergovernmental organization, International Convention for the Protection of New Plant Varieties (UPOV). UPOV was adopted in 1961 at Paris and subsequently revised in 1972, 1978, and 1991. Recent revisions of UPOV in 1991 have tried to make the plant variety protection as strong as that patent protection. This revision extends protection to the whole plant from propagating plant part and importantly accompanies two exemptions where farmers can save the seeds of protected varieties for the upcoming season but cannot sell it and breeders/researchers can use protected variety as a source of variation to form and sell new varieties. Various countries have opted this sui generis mechanism of protection (refer to Table 17.1). In continuation, various other agreements have been put forward internationally: Convention on Biological Diversity (CBD 1993) (enforcement on December 29, 1993); supplementary agreement to the Cartagena Protocol on Biosafety (Cartagena Protocol 2000) (enforcement on January 29, 2000); and Nagoya Protocol on Access to Genetic Resources (Nagoya Protocol 2010) (enforcement on October 12, 2014). Another International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA 2001) was adopted on November 3, 2001, under the aegis of United Nations Food and Agriculture Organization (UNFAO; establishment in 1983).

UPOV aims to achieve an effective system for plant variety protection and encourages new variety developer farmers and breeders. CBD established pavement for the conservation of biodiversity and sustainable use of bio-resources. Other supplementary protocols enhance the effect of CBD as Cartagena Protocol seeks protection of biodiversity in the surge of modern biotechnology whereas Nagoya Protocol aims to protect genetic resources and their benefits along with the traditional knowledge associated with genetic resources. ITPGRFA works for providing access to plant genetic materials to farmers, plant breeders, and researchers. It further appreciates the contribution of farmers and promotes the return of benefits to countries of origin of genetic resources. All of these are aligned in principle with the CBD to achieve better sustainability.

17.4.2.3 Impact of Plant Variety Protection on Medicinal Plants with Worrisome Conservation Status: A Case of Indigenous Communities

In the case of medicinal plant varieties, indigenous communities can ascertain their right over the varieties by demonstrating legal requirements of respective plant varieties acts. The legal requirements are varieties should be distinct, stable, uniform, and novel. These varieties should have distinguishable characteristics from one another. Stability and uniformity focus on an exhibition of characters after propagation and homogenous genomic expression by vegetative or sexual reproduction.

Table 17.1 Examples of national laws enacted for the protection of plant varieties with respective duration of protection

Country	Legislation	Duration
United States of America	United States Plant Variety Protection Act, 7 U.S.C. 2321–2583	Tuber propagated plant variety: 20 years Tree or vine: 25 years From the date of issue of a certificate
India	Protection of Plant Varieties and Farmers' Rights Act, 2001 (PPVFRA)	Trees and vines: 18 years Extant varieties: 15 years From the date of registration
China	Protection of New Varieties of Plants	Vines, forest trees, fruit trees and ornamental plants = 20 years Others = 15 years
Canada	Plant Breeders' Rights Act (S.C. 1990, c. 20)	Trees, vines = 25 years Others = 20 years
Japan	Plant Variety Protection and Seed Act, 1998	Plant variety: 25 years Trees: 30 years
Australia	Plant Breeder's Rights Act 1994	Trees and vines = 25 years Others = 20 years
Brazil	Plant Variety Protection Law	Vines, fruits, forest, and ornamental trees = 18 years Others = 15 years
Russia	Civil Code of the Russian Federation	
South Africa	Plant Breeders' Rights Act 1976	Vines and trees = 25 years All others = 10 years
Indonesia	Plant Variety Protection (PVP) Rights in 2000	Annual plants = 25 years Seasonal plants = 20 years From the date of registration
Saudi Arabia	Law of Patents, Layout-Designs of Integrated Circuits, Plant Varieties, and Industrial Designs (promulgated by Royal Decree No. M/27 of 29/5/1425H (July 17, 2004))	Trees = 25 years Other plants = 20 years From the date of registration
Turkey	Law No. 5042 on the Protection of Plant Breeders' Rights for New Plant Varieties	Trees, vines, and potatoes = 30 years Other plants = 25 years From the date of grant

Though the protection of plant varieties is cheaper than patent protection, it shall be noted that this is active only in UPOV Convention signatories and limits the relevance of national acts. Availability of financial, legal, and scientific aid can help these indigenous breeders for protecting indigenous varieties and compete with the market players.

Plant variety protection of the medicinal species may elevate the interest of the private sector and communities as it gives incentives over their investment. This mechanism promotes the conservation of biodiversity and reduces erosion. Simultaneous efforts for the promotion of sustainable harvesting of species will balance the scenario. Hence, empowering private as well as indigenous communities will control the depletion of bio-resources. Some studies have proposed incentive-based conservation of the plant species (Gupta 2004). To maintain these reserves, high demand of the herb should be fulfilled through the cultivation of medicinal plants. Four domestic market segments are reported in a study for the utilization of medicinal herbs where crude drugs have 45% share, followed by herbal extracts (22%); essential oil, gums, and resins (19%); and condiments and food additives (14%) (Gupta 2004).

17.4.3 Geographical Indications

17.4.3.1 Geographical Indications: A State of the Art

The rise of the industrial revolution in the eighteenth century and globalization in the nineteenth century created a platform for the international trade of products from across the globe which included agricultural products, medicinal products, handicrafts, etc. To distinguish these products, products from various destinations were demarcated using various marks. These marks were the earliest forms of geographical indications (GI). Today's IP protection is evolved a quite long and facilitates protection of GIs in various forms, e.g., under sui generis mechanisms, trademark laws, international agreements, multilateral agreements, intergovernmental initiatives, etc. Now, it raises the concern about the collection of reliable data worldwide due to its variations in forms. Currently, there is lack of uniformity in global statistics of GI protections. Recently, WIPO's efforts are notable in the area of data collection and bringing uniformity in its collection with respect to geographical indications. There is a need for reliable data for enabling evidence-based researcher and policymaking. Recent data collection from 82 WIPO-affiliated countries suggested that there are approximately 59,500 protected GIs. Out of these, 4932 GIs were identified from Europe. China has the highest numbers of GI in force outside in national or regional regulators. In EU member states, Germany had largest number (14073) of GIs in force with 65% share, followed by Austria (8749), Hungary (6646), the Czech Republic (6191), and Bulgaria (6096). China, India, and Iran issued more than 100 GIs to handicrafts. In 2017, GI distribution was identified as 57.1% to wines and spirits, 28.2% to agriculture and food products, and 2.7% to handicrafts (WIPO 2018) (Fig. 17.8).

17.4.3.2 Impact of Geographical Indications on Indigeneity

Basically, GI is a sign of identification of goods differentiated based on its geographical area of origin or the characteristics assigned thereby. GI underlines quality, reputation, and characteristics of goods and its geographical origin. Article 22 of TRIPS Agreement defines geographical indications (TRIPS 1994). Recently use of

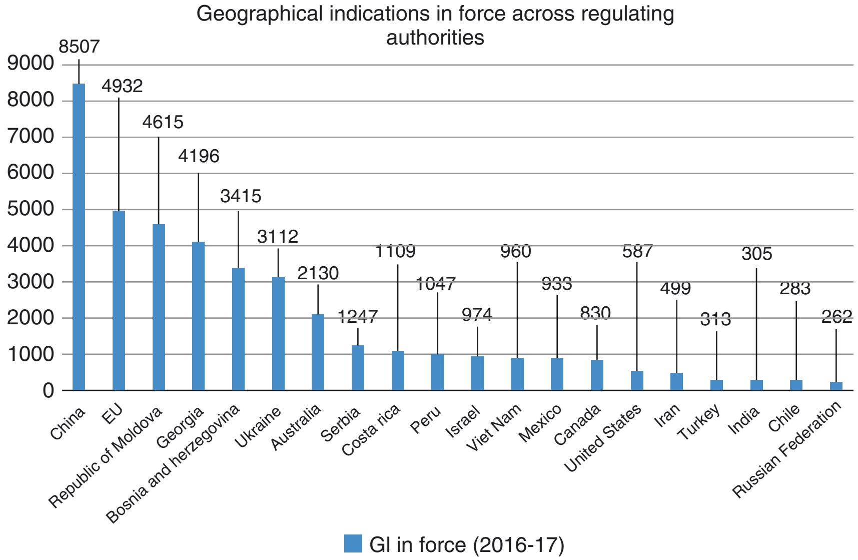

Fig. 17.8 Numbers of geographical indications in force (2016–2017)

geographic indication for the protection of knowledge of indigenous societies through protection of their genetic resources and traditional varieties is getting noticed. It's getting an immediate impression just after patent and plant variety protection. The products obtained from these societies either have exposure to superior natural geographical climate and geological environments or indigenous processing or manufacturing skills. GI may refer to the appellation of origin or indication of sources. An indication of origin is in the sense broader than appellation of origin where both refer to specific geographical origin; however, the latter restricts it to the qualities of products achieved from geographical environment modulated by natural or human factors. It can be noted that rights obtained through such protections are not transferable and should be followed through appropriate associations. Therefore, the origin of any indicated product remains associated with the community. Trading of products with false or misleading identification contrary to such identification is prohibited under Article 10(1) of the Paris Convention (Paris convention 1883). Recently 28 countries are using the Lisbon System to protect appellation of origin. By 2017, 991 appellations of origin were in force via the Lisbon System, and France remained a leading user of the Lisbon System (WIPO 2018). Tequila (Mexico), Chianti for wines (Italy), and Habanos for cigars (Cuba) are the famous examples of the appellation of origin protected under the Lisbon System. The Madrid System facilitates the protection of GI as collective and certification marks in various countries. WIPO administers the Madrid Agreement (Madrid Agreement 1981) and Madrid Protocol (Madrid Protocol 1989). Napa Valley for wine (United States) and Parmigiano Reggiano for cheese (Italy) are the famous examples of marks under the Madrid System.

Table 17.2 List of representative GI protections in force for medicinal-agricultural products in India

GI	State	GI	State
Coorg orange	Karnataka	Mysore betel leaf	Karnataka
Allahabad Surkha	Uttar Pradesh	Monsooned Malabar Arabica coffee	Karnataka
Monsooned Malabar Robusta coffee	Karnataka	Spices – Alleppey Green Cardamom	Kerala
Sikkim large cardamom	Sikkim	Mizo Chilli	Mizoram
Sangli turmeric	Maharashtra	Waigaon turmeric	Maharashtra

17.4.3.3 Impact of Geographical Indications on Medicinal Plants: A Case of Indian GI Tags

GIs are mainly used for the protection of agricultural and food products because of their close natural linkages with the origin. These GIs can have legal basis as under sui generis mechanisms, trademarks, national laws, regional laws, intergovernmental or multilateral agreements, or any others. However, they can be overlapping and not mutually exclusive. In India, GI tags are issued based on sui generis initiatives. Certain countries like India and China and countries from Europe are promoting the extension of protection of GIs as per Article 23 of TRIPS (TRIPS 1994). This will be the basis of seeking protection for products featuring expression of traditional knowledge (TRIPS: Reviews, Article 27.3(B) and Related Issues). Therapeutic properties of medicinal plants vary due to natural and climate variations in the environment. Therefore, these medicinal varieties can be protected under the Geographical Indications of Goods (Registration and Protection) Act 1999 in India (The Indian GI Act 1999). As of March 2019, India issued 130 GI tags. More than 100 of them are assigned to agricultural products (Registered GIs 2019). List of representative GI tags assigned to medicinal and agriculturally grown species in India is enlisted here (Table 17.2). Many of these are plant varieties or cultivars. Some of these applications for registration of GIs have claimed medicinal effects of the specific products. The registered variety, Navara rice from Kerala, has medicinal properties locally like treating Panchkarma and arthritis with applications in Ayurveda (James 2016). Rural communities in India have unique knowledge of traditional practices and methods to grow such species. Such tagging of GIs supplements the income farmers and breeders locally.

17.5 Conclusion and Recommendations

It is high time that steps are taken to conserve the existing valuable species in a methodological and logical manner. Any kind of development with the increasing population and need of resources will eventually lead to exploitation of these valuable resources and thus question the very foundation of environmental subsistence itself. Any single-handed approach in conservation will only have a limited result, thus falling short of its avowed objectives. There is a need to push the maximum usage of the existing IPR mechanisms effectively for conservation and make the best use of sui generis models, as well as advocating the use of the precautionary principle in all trade and other transactions involving threatened species. This can only be done effectively if there is an in-depth understanding of the relationship between IPRs and such threatened species (and their related knowledge). It is also important that such kinds of initiatives are brought into the international limelight and are more are less in line with the international obligations as well.

There is an urgent need to have a highly collaborative approach wherein laws of the land, scientific advancements, and finally participation of public and representatives of public are a must. Conservation of such populations needs to be strategically managed by public and private institutions through planned programs in association with farmers, rural communities, and indigenous people. Solutions to such complex issues will require sustainable and strategic modus operandi which may include reforestation, better administration of protected areas, and better implementation of dedicated laws and regulations.

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Chapter 18
Access and Benefit Sharing and Threatened Medicinal Plants

Atul Kumar Gupta and K. Souravi

Abstract The place of plants in medicine has always been of profound contribution; it was radically altered in the nineteenth century by the application of chemical analysis and the vast scope of synthetic biology, but nevertheless the medicinal plants have always been the guiding light for potential drugs developed and those in process, thanks to the traditional knowledge associated. It thus becomes mandatory that these valuable recourses are explored for their full potential but keeping in mind the need for conservation of these exhaustible recourses by sustainably utilizing them. This is when the interplay of bioresources such as medicinal plants, the associated traditional knowledge, and the legal mechanisms of intellectual property comes into picture. This chapter tries to focus on the various national and international legal instruments involved and the scenario in India; also diverse case studies have been discussed, both national and international that draw attention to link conservation and sustainable utilization of threatened medicinal plant species by employing legal mechanisms such as Access and Benefit Sharing, also the resulting implications on the cultural and traditional rights of the holders of traditional knowledge, the indigenous communities.

Keywords ABS · TK · Threatened · Medicinal plants · BD Act

18.1 Introduction

The sixth-century Ayurveda text *Ashtanga Hrudayam* defines "medicinal plants" as "*Jagatyevam anaoushadham na kinchit Vidyate dravyam vasatnanartha yogayoh*," which means that every plant has potential medicinal properties. However, plants are declared to be medicinal only when their properties or uses have actually been discovered by some system of medicine or healthcare, such as Ayurveda, Siddha,

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Sowa-Rigpa, Unani, Homeopathy, Allopathy, or Folk system of medicine. Medicinal plants are one of the important resource materials for many of the therapeutic agents both in developed and developing countries. *Rigveda*, one of the revered scriptures, consists of repositories of human knowledge written between 4500 and 1500 BC that mentions the use of about 67 plants for therapeutic use and *Yajurveda* enlists 81 plants, *Atharveda* written during 1200 BC describes 290 plants of medicinal values, "*Chakra Samhita*" (900 BC) describes 341 medicinal plants, and the next landmark in Ayurveda "*Sushruta Samhita*" (600 BC) mentions 395 medicinal plants. Nearly 70% of the population is dependent on traditional plant-based medicines. Over 53 million tribal people of 550 tribal communities inhabit the Indian subcontinent and are reported to use around 7500 species of plants for medicinal purposes (Medicinal plants of India 1997; The Key Role-Conceptual and Opernational Features 1999).

In India, medicinal plants have formed the most important resource base of healthcare traditions for over 2 millennia, a status that will definitely remain for centuries to come. About 6500 species of medicinal plants are reportedly used in more than 20,000 unique formulations across these healthcare systems, which is perhaps the largest use of diverse botanicals in the world. The medicinal plants are sourced from all habitats and landscapes across the country from the trans-Himalayas to the coastal regions, from arid and desert habitats to mangroves and evergreen forests (The Key Role-Conceptual and Opernational Features 1999; Task Force Report for conservation and Sustainable Use 2000).

As per the studies in 2014–2015, out of the 6500 medicinal plant species traditionally used by Indian communities, about 1622 botanicals corresponding to 1178 plant species are found to be in the trade including 242 species witnessing high volume trade at more than 100 MT/annum. Diverse parts of plants (42% herbs, 27% trees, and 31% shrubs and climbers) serve as medicinal raw drugs. The major botanical families to which these species belong to are Fabaceae, Asteraceae, Lamiaceae, Malvaceae, Euphorbiaceae, Acanthaceae, Apocyanaceae, Caesalpiniaceae, Solanaceae, Convolvulaceae, Mimosaceae, Phyllanthaceae, and Rubiaceae. Nearly 53% of the medicinal plant species are subject to destructive methods of harvest as the medicinal parts harvested include underground parts, wood, bark, and whole plant. It is observed that 85% of the traded species and 70% of the demand is met from wild sources (Goraya and Ved 2017).

A complex and diverse range of operators, viz., local communities and raw drug collectors, farmers, traders, exporters, pharmacological labs, drug manufacturers, physicians, forest resource managers, and regulatory authorities, are associated for meeting annual demand of raw material (estimated at 5,12,000 MT in 2014–2015) (Goraya and Ved 2017; Ved and Goraya 2007). The raw drug traders cater to about 8610 licensed herbal manufacturing units, of which only 3% are of large and medium scale consuming 66% of the entire quantity of raw material traded. The medicinal plants are mainly sourced from forests (wild collection), from wastelands (non-wild collection), from cultivation, and from imports to meet the demands from AYUSH Industry, Allopathy, Veterinary Drugs, Herbal Extractors, Proprietary Medicine, Traders, Exporters, Traditional Healers, and Household. The unabated trade in some of the species of medicinal plants has already pushed those under IUCN threatened category.

In spite of huge trade in medicinal plants and huge profits being earned by concerned industries, the local collectors and households barely get their dues both in terms of sustainable cultivation and harvesting and economic benefits. Moreover, the unabated extraction of medicinal plants, more than what could be sustainably harvested from the wild, is causing loss of biodiversity besides most of these species getting threatened in the wild. It is in this background that the Access and Benefit Sharing (ABS) mechanism under the Biological Diversity Act, 2002 (BDA), could be applied to meet all threefold objectives with respect to the medicinal plants, that is, conservation, sustainable use, and fair and equitable sharing of benefits accruing from the commercial use of the medicinal plants.

18.2 Access and Benefit Sharing – The Concept

(Source-ABS Mechanism- Guidance Manual)

The United Nations Conference on Human Environment (better known as Stockholm conference of 1972) for the first time focused on international environment issues and recognized that earth's resources (*biodiversity*) are finite and there is an urgent need to safeguard these resources. Twenty years later in 1992, the United Nations Convention of Rio de Janeiro (popularly known as "Earth Summit"), of which India is a signatory, recognized and declared the importance of biological diversity for evolution and for maintaining life sustaining systems of biosphere and the need for its conservation. Further, the preamble also recognizes "the close and traditional dependence of many indigenous and local communities embodying traditional lifestyles on biological resources, and the desirability of sharing equitably benefits arising from the use of traditional knowledge, innovations and practices relevant to the conservation of biological diversity and the sustainable use of its components." The objectives of this Convention are "conservation of biological diversity, sustainable use of its components, fair and equitable sharing of the benefits arising out of the utilization of genetic resources,…."

Article 8 (j) and (k) of the Rio Convention is regarding in situ conservation and calls upon the signatory parties for taking measures "Subject to its national legislation, respect, preserve and maintain knowledge, innovations and practices of indigenous and local communities embodying traditional lifestyles relevant for the conservation and sustainable use of biological diversity and promote their wider application with the approval and involvement of the holders of such knowledge, innovations and practices and encourage the equitable sharing of the benefits arising from the utilization of such knowledge, innovations and practices. Develop or maintain necessary legislation and/or other regulatory provisions for the protection of threatened species and populations." Further Article 15 [Clause (1) and (7)] of the Convention relates to Access to Genetic Resources and calls for "Each Contracting Party shall take legislative, administrative or policy measures, as appropriate, with the aim of sharing in a fair and equitable way the results of research and development and the benefits arising from the commercial and other utilization of genetic resources with the Contracting Party providing such resources. Such sharing shall be upon mutually agreed terms."

A decade later, the Johannesburg Declaration on Sustainable Development, 2002, reasserted the challenges to the conservation of biodiversity and also highlighted the important role of indigenous people and need for long-term perspective and broad-based participation in policy formulation, decision-making, and implementation at all levels.

India being a party to the United Nations Convention on Biological Diversity (Source-https://www.cbd.int/abs) enacted the Biological Diversity Act, 2002, for conservation of biological diversity, sustainable use of its components, and fair and equitable sharing of the benefits arising out of the use of biological resources and knowledge and for matters connected therewith or incidental thereto. Indigenous and local communities, who either grow “biological resources” or have a traditional knowledge of these resources, are the beneficiaries under the Act. In return for their parting with this traditional knowledge, certain benefits accrue to them as fair and equitable benefits sharing. It must be remembered here that this benefit that the “indigenous and local communities” get under the law is over and above the market price of their “biological resources.”

Another important international convention “The Nagoya Protocol” of 2010 (Source- https://en.wikipedia.org/wiki/Nagoya_Protocol) is a supplementary agreement to the 1992 Rio de Janeiro Convention on Biological Diversity that focuses on the third component, which is fair and equitable sharing of biological (genetic) resources (as per the BDA 2002, the “biological resources” means plants, animals, and microorganisms or parts thereof, their genetic material, and by-products (excluding value-added products) with actual or potential use or value, but does not include human genetic material), including the traditional knowledge associated with such resources and the benefits arising out from their use. The preamble of Nagoya Protocol, inter alia, recognized the “importance of promoting equity and fairness in negotiations and mutually agreed terms between providers and users of genetic resources.” In pursuance of the Nagoya Protocol and in exercise of the powers conferred by section 64 read with subsection (1) of section 18 and subsection (4) of section 21 of the BD Act, 2002, the National Biodiversity Authority issued “Guidelines on Access to Biological Resources and Associated Knowledge and Benefits Sharing Regulations, 2014” (Source-http://nbaindia.org/uploaded/pdf/Gazette_Notification_of_ABS_Guidlines.pdf). ABS is the only mechanism that rests on fostering fair international partnerships and explicitly encompasses not only ecological but also social and economic aspects. This underlines the relevance of ABS for achieving the SDGs, on matters as wide ranging as poverty alleviation, food security, health, economic growth, innovation, oceans, and governance.

Timeline involved in structuring of ABS Guidelines and Regulations

These ABS Guidelines give a simplistic outlook of the Act and contain the range of benefit sharing percentages that will be applicable for different activities regulated under the BD Act. The Guidelines authorize the "benefit claimers" to share the benefits from the "commercial users."

It is a fact that in most cases the "benefit claimers" are the poorest of the poor who share habitations with biological resources and the "commercial users" are the traders/commercial institutions/organizations. While the "benefit claimers," as owners and possessors of rich resources, are illiterate, poor, and unorganized and live on subsistence economy, the "commercial users" are organized, literate, resourceful, and wealthy and live on market economy. For "benefit claimers," a quick sale money on daily basis is all what they rely most on while dealing with the natural biological resources to get their legal and justified dues on a given day. Contrary to this, the commercial users have well thought out economic plans both for present and future investments for enhanced profits on each executed transaction. This hiatus in the economic gains between the "benefit claimers" and "commercial users" has always been a cause of concern and, in absence of any mechanism to set this malaise right, has been on the rise perpetually – thus pushing the "benefit claimers" more and more at the mercy of the "commercial users."

It is in this reference that the Access and Benefits Sharing (ABS) mechanism and guidelines aim at setting this disparity right by ensuring equitable sharing of the economic benefits accruing out of commercial utilization of biological resources between the deserving "benefit claimers" and resourceful "commercial users." ABS promotes biodiversity as a community asset and supports biodiversity-based businesses in an effective and sustainable manner. ABS provides for mechanisms to access biological or genetic resources and share benefits between "users" and the "providers."

18.2.1 Case Studies

Well-documented and widely discussed model for ABS is the "Jeevani-Kani tribes" model that put India as the centerpiece in the international scenario on ABS; also there have been other such models like the "Hooda-San tribes" that have acted as a reference for numerous prospective ABS models (Lewis-Lettington and Mwanyiki 2006). All of these models have showcased the potential marriage between the local communities, research organizations, and the bioresource. We have therefore tried to showcase some lesser known but diverse models of benefit sharing both national and international followed by a detailed case study on Tripura ABS model and finally a few other potential resources for ABS, in order to have a better understanding on the implementation of ABS at the local grounds and its implications.

18.2.2 Mamala-Samoa Agreement

(Pacific Case Studies)

Dr. Paul Cox from the Institute of Ethnobotany isolated an extract from the tree Mamala (*Homalanthus nutans*) based on the discussions he had with the traditional healers of Falealupo community of Samoa. Although the agreements in this case study were done even before CBD was brought into the picture, this still serves as a unique guiding model. Prior informed consent procedure was followed, and three agreements were formulated. The first agreement was the Falealupo Covenant agreement where in the debt taken for the research and commercialization was paid off and forest products were obtained for community buildings such schools, a commitment by the community to preserve the rainforest, and 33% of the income received by Dr. Cox generated through identification of newer drugs will be distributed to the community. Second is the ARA-Government of Samoa Agreement, according to which if ARA (a non-profit) partners with a company and generates revenue, then it agreed to pay one-third of the clinical trials cost and share 12.5% to government, 6.7% to Falealupo community, and 0.4% each to both of the healers who guided Dr. Cox. Last is the UC Berkeley-Government of Samoa Agreement, according to which 50% of the net revenue that arises from UC Berkeley's licensing of intellectual property will be distributed: 50% to the government, 37% to the communities, 8% to the villages who help grow Mamala, 4% to both of the guiding traditional healers, and 1% to Seacology for handling the royalty payments.

18.2.3 The PepsiCo Seaweed

(Narayankumar and Krishnan 2013)

Red algae, *Kappaphycus alvarezii*, a seaweed, is used for extraction of a gel-forming agent "carrageenan," which is widely used in pharmaceuticals, cosmetics, and pet food industries and is exported to Malaysia and Philippi. PepsiCo India Holding had initiated the cultivation of the seaweed in the Ramanathapuram district, Tamil Nadu, in 2000 after the Tamil Nadu state government declared that region as a Marine National Park and banned the harvesting of fishes and seaweeds. Around 70 families had been a part of the cultivation program's Self-Help Groups (SHGs) formed by the local community. The technology for the cultivation of the red algae and extraction of carrageenan was sourced on royalty basis from the Central Salt and Marine Chemical Research Institute (CSMCRI), which holds an international patent for the extraction methods, and initial funding was provided by State Bank of India. As per ABS Agreement, the exporter paid the NBA 5% of FoB (Free on Board) costs of the profit, and as far as the benefits to the community was concerned, they were assured buyback at pre-agreed prices and employability generation (80% women), thereby greatly improving the livelihood of the local people.

18.2.4 Cooks Islands-Koutu Nui Agreement

(Pacific Case Studies)

The main resources involved in this model were four medicinal plants, namely, *Hibiscus tiliaceus*, *Vigna marina*, *Cocos nucifera*, and *Terminalia catappa*, that were used to prepare a concoction for the treatment of bone fractures and other medicinal and therapeutic applications by local community living in Cooks Islands. In 2003, a researcher by the name Dr. Matheson developed a proposal for investigation and commercialization of these medicinal remedies and associated traditional knowledge. A prior informed consent procedure was followed. The proposal was submitted to the Koutu Nui indigenous representative body as a project. The community people handed over a Vairakau Ati prepared by Taunga Ngateina Ngapare (the local healers), but they didn't disclose the associated TK, which thereby let the researcher to formulate his own admixture and then make a benefit sharing agreement with the local community. Further a company named Cooks Islands Medical Research and Development (CIMRAD) was incorporated in the project and added Dr. Matheson and the Koutu Nui as shareholders, a vehicle through which the research and development would be commercialized.

Further on the popularity gained, an Australian company, CIMTECH, was established to take advantage of grant opportunities, for tax reasons, and also for the protection of intellectual property. This company incorporated Koutu Nui and UNSW (fund supporter) as the shareholders. CIMTECH launched a skin care product called "Te Tika." This natural product is based on Australian Scientific Research and incorporates traditional Cook Islands medicines to create a skin care range that has regenerative and anti-aging effects. As far as the benefit sharing was concerned, the Koutu Nui agreed upon allocating the monitories by themselves. The benefits provided by the CIMTECH included no royalties but in monetary benefit terms shares and dividend payments upon the sale of the products, employment of people on a part-time basis in the Cook Islands, and contributions to the local economy through laboratory, marketing, and tourism. The non-monetary benefits included research directed toward primary healthcare needs and social recognition for Cook Islands traditional medicine and most importantly recognition of the role of local community as a cultural authority involved in conservation-oriented practices like Raui.

18.2.5 Bio-India Biologicals

(Source-https://www.thehindu.com/news/national/andhra-pradesh/A-sweet-tale-of-how-neem-trees-yield-money/article12549014.ece)

The Bio-India Biologicals Corporation (BIB) sourced neem, well known for its medicinal properties, from a village Amarchintha in Mahabubnagar district of Telangana region in Andhra Pradesh, which in turn was sourced to a Japanese firm. The company thus entered into an Access and Benefit sharing Agreement with the

NBA by agreeing to provide 5% of FOB, a part of which was transferred to the concerned jurisdictional BMC for planting neem samplings and creation of awareness, thus emphasizing the idiom of conservation and sustainable utilization. The corporation also employed the local communities for collecting and drying processes instead of involving any middle man or brokers and thereby generating livelihood.

18.2.6 Prospecting Anti-malarial Medicines

(Robert and Mwanyiki 2006)

A project funded by the Department of Arts, Culture, Science and Technology (DACST), taken up by South African Medical Research Council (MRC), University of Cape Town (UCT), CSIR, NBI, University of Western Cape, and University of Pretoria (UP) aimed to develop new malarial medicines, based on indigenous medicinal plants. The project intended to create employability, multidisciplinary scientific studies, and commercialization of potential products. It was also reported that any financial benefits generated shall be shared equally as 50% by all the associated partners and 50% into a trust fund to share with the stakeholders like the indigenous communities or others who have contributed to this project.

18.2.7 ABS and Threatened Medicinal Plants: A Time-Tested Model from Tripura

Tripura, one of the northeastern states of India, is geographically advantaged with the biogeographic zone of 9B-northeast hills of eastern Himalayan region. The total land area of the state is 10,497.69 sq. km, of which 60%, i.e., 6292.681 sq. km, is of forest area. The availability of good amount of annual rainfall (2100 mm) and profound sunlight resulting in favorable temperature led to diversified floral distribution in the state. The blend of biodiversity and cultural mosaic of tribal and non-tribal population is a boon to have prevailing unique economic parley in the state of Tripura.

In Tripura, the existence of rich floral biodiversity of about 379 species of trees, 320 shrubs, 581 herbal species, 165 climbers, 35 ferns, 45 epiphytes, and 4 parasites (about 1545 species in total; Source – www.tripuraforest.nic.in) gives an opportunity for the people of Tripura to use it for varied purposes. A total of about 858 species with significant medicinal properties have been documented in Tripura (trees 206, shrubs 161, herbs 395, and climbers 96). These species, besides being used for medicinal values, are also used for various purposes, such as food supplement, local healthcare, and depict cultural significance. A total of 19 tribal groups, namely, Jamatia, Tripuri, Reang, Chakma, Kalai, Halam, Lushai, Uchai, Noatia, etc., make up for about 31% of the total population of the state. The socioeconomic and cultural diversification of the tribal people and their nature-bound lifestyle gives an opportunity to protect and utilize the forests-based produce for their subsistence and sustenance. Being a part of nature, people of the tribal communities have developed a rich knowledge on the usage of plants over a period of time, which is an undocumented wealth passed by generation to generation that makes their life adaptable to the existing situation.

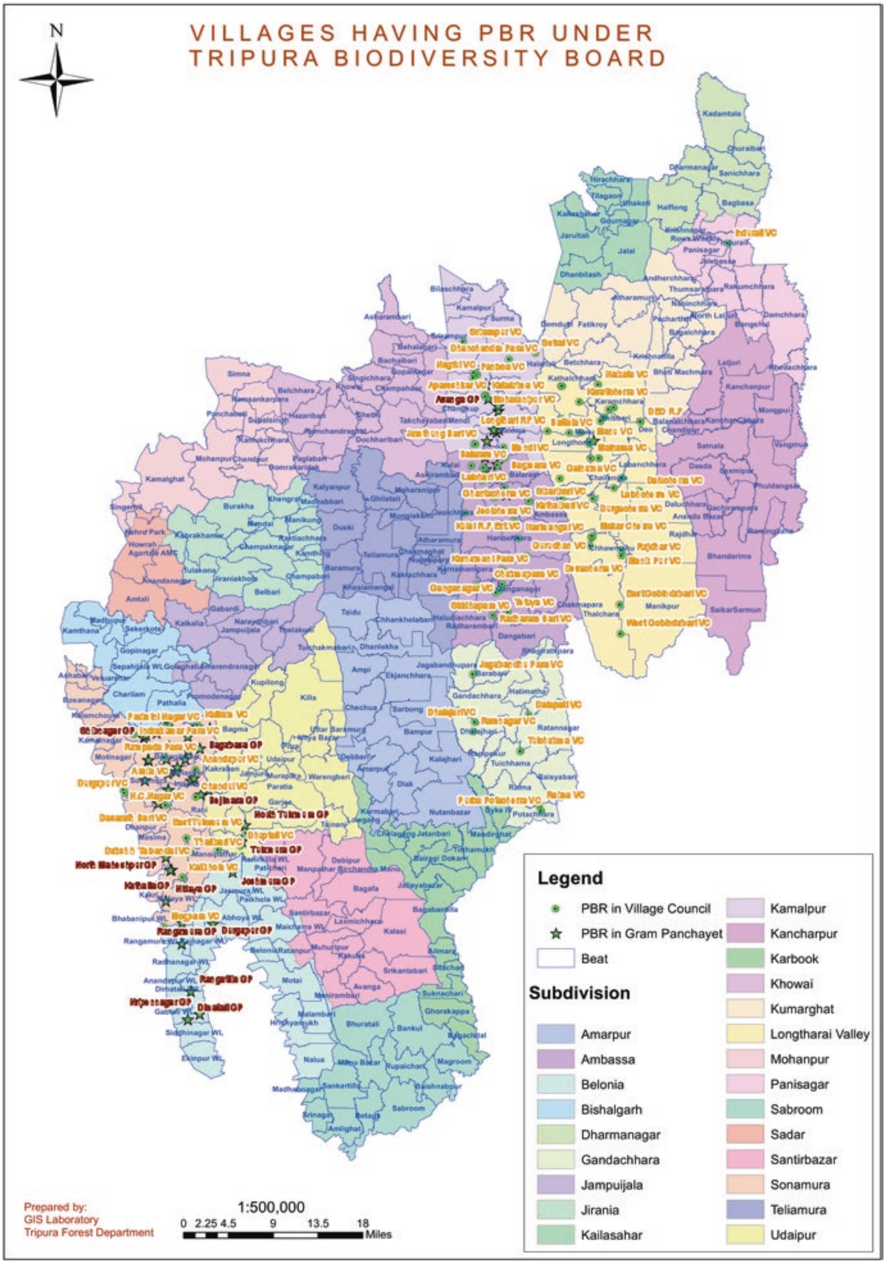
Besides usage of plants for food and fodder, people depend on various plant species for curing the diseases/health disorders in the absence of well-developed organized health system in their vicinity. The tribal people use different type of plants in their day-to-day life for various purposes, which include health issues also. The knowledge of medicinal plants lies hidden with the traditional healers known as *Kaviraj* and has not been documented in full, which include the rare, endemic, and lesser known species mainly belonging to six to nine families, viz., Fabaceae, Apocynaceae, Euphorbiaceae, Apiaceae, Asteraceae, Zingiberaceae, Lamiaceae, and Verbenaceae.

Owing to its unique geographical and topographical location, Tripura displays opulence in natural biological resources, and more than 80% of its populations are acting as "benefit claimers" as they are the possessors of the natural biological resources for meeting their day-to-day livelihood needs to sustain their lives. Practically every village has some or the other natural biological resources which is worth for its commercial utilization. However, the economic benefits out of commercial uses of those biological resources either do not reach to them or its distribution is not equitable vis-à-vis "commercial users."

Some of the most common medicinal plants which are commercially traded from Tripura (Gupta 2018) to outside states mostly in raw form are *Terminalia arjuna, Terminalia bellirica, Terminalia chebula, Aquilaria agallocha, Azadirachta indica, Gmelina arborea, Aegle marmelos, Mesua ferrea, Emblica officinalis, Syzygium aromaticum, Cinnamomum zeylanicum, Stevia* spp., *Alpinia galanga, Polygonum recumbents, Morinda citrifolia, Andrographis paniculata, Catharanthus roseus, Rauwolfia serpentina, Adhatoda vasica, Homalomena aromatica, Asparagus racemosus, Dillenia pentagyna,* gandhaki, *Aloe vera, Ocimum sanctum, Withania somnifera, Kaempferia* sps., etc. As per the present scenario in the state, the real economic benefits of these commercial natural bioresources do not equitably reach to JFMC members, tribal villagers, forest dwellers, villagers, etc. who are the main custodians/collectors of these resources and together constitute "benefit claimers." Those who utilize such resources commercially through value addition and earn the lion's share of economic profits are mostly the end-users or commercial business houses, as traders or companies within and outside Tripura. As per information gathered through studies, these biological resources of medicinal plants are transported from Tripura to places such as Assam, Shillong, West Bengal, Bihar, Uttar Pradesh, New Delhi, Uttarakhand, Madhya Pradesh, Rajasthan, Maharashtra, and Karnataka, to name only few important ones.

After the implementation of the BDA through the Tripura Biodiversity Board, there now exists a mechanism to set right this polarity of inequitable sharing of economic benefits. The TBB has facilitated constitution of village-level institutions called Biodiversity Management Committee (BMC) who are empowered under BDA and TBB Rules to enlist and record all varied kinds of natural biological resources and their status found in the given local *Panchayat* area (village committees/gram *Panchayats*) in a People's Biodiversity Register (PBR). These PBRs are documented involving the villagers, local schools, villagers having knowledge about village resources, local *vaidyas*, and traditional knowledge possessors with expert and scientific inputs from the members of the three Expert Committees under the TBB (consisting of experts in agriculture, horticulture, animal husbandry, fishery, zoology, botany, taxonomist, social scientists, traditional knowledge experts, etc.). The BMCs are empowered through the BDA to steer the process of commercial utilization of such enlisted resources in authenticated PBR. The ABS Guidelines further empower them to charge collection fee (1% for TBB and 2% for BMC of the total purchase value) and also a percentage share out of the total purchase price (1–3% if the purchaser is a trader, and 3–5% if the purchaser is a manufacturer) or out of total sale price (3–5%) profit earned by the commercial users from given natural resources. Besides, the BMC also has option to go for non-monetary benefits, such as setting up value addition facilities for the resources, imparting training to the resource owners on cultivation and sustainable harvesting, introduction of technologies, etc. The biggest advantage is that the TBB or the BMC are empowered to enter into an agreement on ABS mechanism with the commercial users, where they can decide the purchase price taking into consideration the actual market price of the given biological resources. This provision has completely undone the earlier arrangements where the purchase price was used to be dictated by the traders or manufacturers – this is a huge departure from the earlier trend and now ensuring equitable benefit sharing between the "benefit claimers" and the "commercial users." The fund received as collection fee or as percent of purchase price is deposited in the Local Biodiversity Fund of each BMC – it is a bank account being operated jointly by the chairperson and member secretary of the given BMCs.

The TBB has till March 2018 constituted 263 village-level and 40 Block-level BMCs, while 29 village BMCs are under constitution. All the BMCs have opened the Local Biodiversity Fund. A total of 222 PBRs have already been documented till March 2018 (Gupta 2017a, b). A total of more than 125 ABS agreements have been signed by the BMCs with the traders, and more and more BMCs are now willing to adopt this system that is empowering them both institutionally and financially. The local JFMCs, villagers, tribal collectors, etc. are also joining hands with the BMCs in this process as they will get their due in more organized manner and based on much better pricing system. The biggest advantage is that the BMCs can now ensure sustainable harvesting keeping in view the perennial economic gains and ecological safety net as well.


N
VILLAGES HAVING PBR UNDER
TRIPURA BIODIVERSITY BOARD
Legend
PBR in Village Council
PBR in Gram Panchayet
Beat
Subdivision
Amarpur
Ambassa
Belonia
Bishalgarh
Dharmanagar
Gandachhara
Jampuijala
Jirania
Kailasahar
Kamalpur
Kancharpur
Karbook
Khowai
Kumarghat
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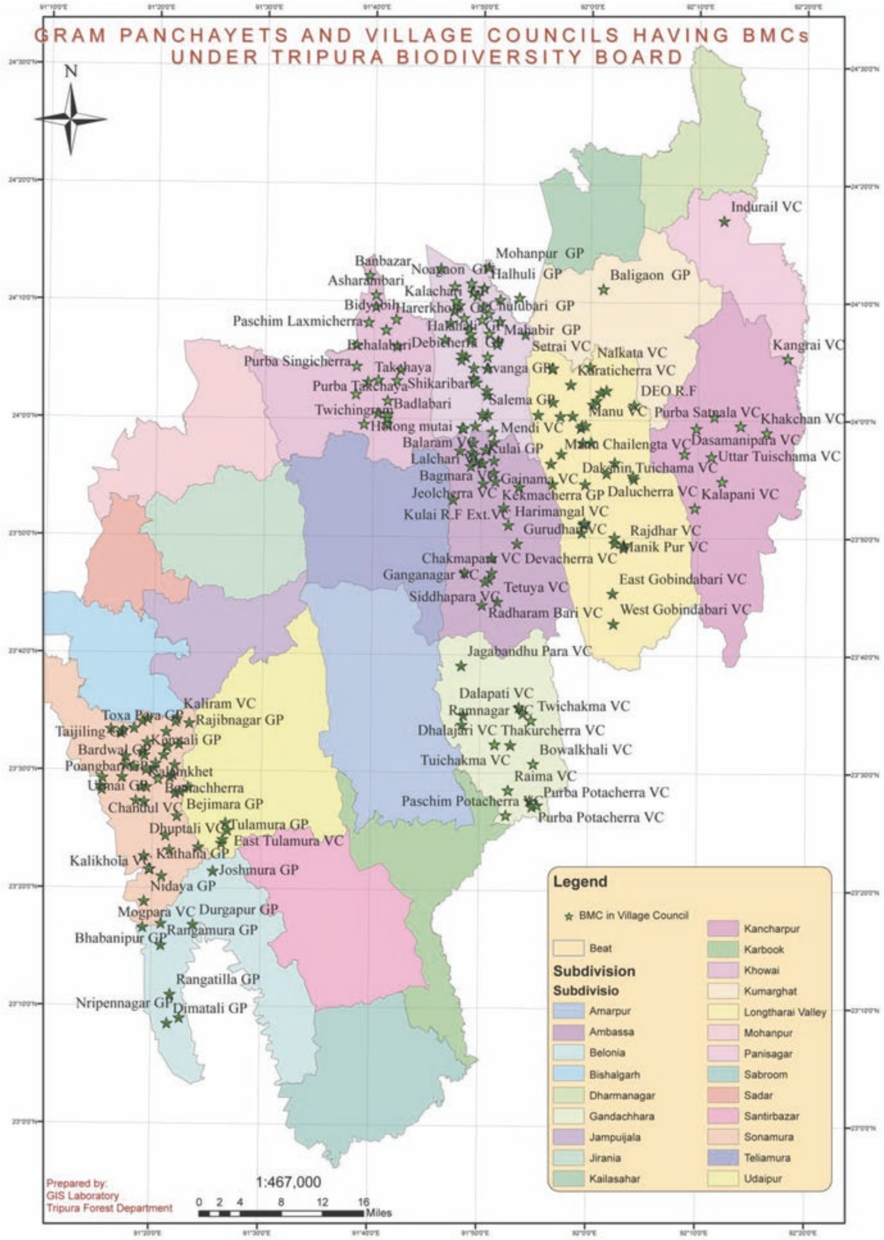
Prepared by:
GIS Laboratory
Tripura Forest Department



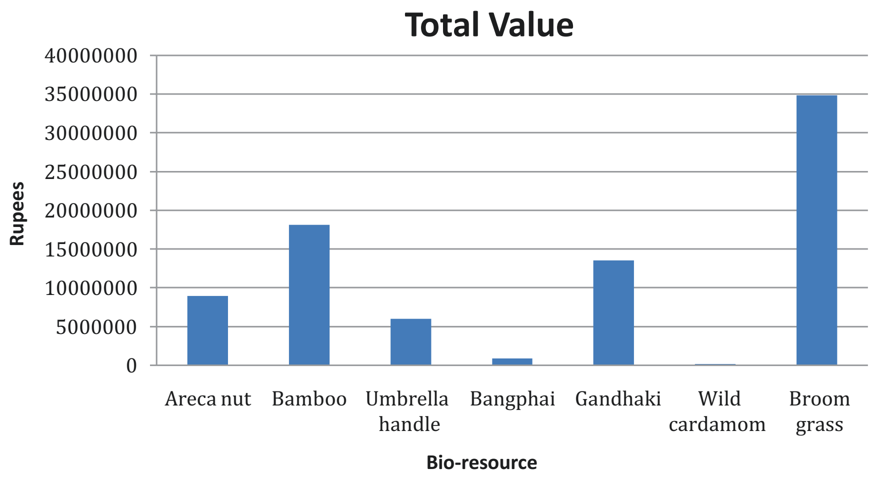
These agreements mainly cover gandhaki (*Homalomena aromatica*), haritaki (*Terminalia chebula*), wild elaichi (*Amomum aromaticum*), tokma (*Hyptis suaveolens*), mucuna (*Mucuna pruriens*), turmeric (*Curcuma amada*), bahera (*Terminalia *bellerica*), etc.; on an average four to five biological resources are traded under the agreement for each BMC. The sale price has been decided through mutually agreed terms keeping in mind the prevailing market rates, which vary for raw to semi-processed resources. On an average, each BMC has signed trade agreement involving different natural bioresources worth INR 2.00–2.15 lakhs for 1 year and that too against only four to five biological resources. This value is based on the trading being undertaken with the local traders.

List of bioresources under ABS agreement in Tripura

Sl. No.	Bioresources	Scientific name
1.	Bamboo for umbrella handle	*Bambusa affinis*
2.	Cotton	*Gossypium hirsutum*
3.	Amla	***Emblica officinalis***
4.	Areca nut	*Areca catechu*
5.	Bamboo	*Bambusoideae*
6.	Bamboo (Mrittingga-Muli)	*Bambusa tulda*
7.	Bamboo parowa	*Bambusa teres*
8.	Bangphai	***Mucuna pruriens***
9.	Boyra	***Terminalia bellirica***
10.	Broom grass	*Thysanolaena maxima*
11.	Dry chili	***Capsicum annuum***
12.	Gandhaki	***Homalomena aromatica***
13.	Ginger	***Zingiber officinale***
14.	Haritaki	***Terminalia chebula***
15.	Small bamboo stick for agarbatti	*Phyllostachys atrovaginata*
16.	Til	***Sesamum indicum***
17.	Tokma	***Hyptis suaveolens***
18.	Turmeric	***Cryphia domestica***
19.	Wild cardamom	***Amomum aromaticum***

Details of ABS agreements undertaken during a single financial year

According to the ABS agreements signed during the period between June 2016 and June 2017, it is found that broom grass is the dominant bioresource with a total value worth of Rs. 3.50 crores, followed by bamboo with a total value of about INR 1.81 crores. With a total value worth of INR 1.35 crores, gandhaki (***Homalomena aromatic)*** holds the third place which is a medicinal species. Two more species that have been revenue generators are bangphai (***Mucuna pruriens***) and wild cardamom **(*Amomum aromaticum*).**



Total value of bioresources as per the ABS agreements

Sl. No.	Bioresources	Amount (in Rupees)
1.	Broom grass	Rs. 3,48,08,810
2.	Bamboo	Rs. 1,81,42,500
3.	Gandhaki	Rs. 1,35,39,800
4.	Areca nut	Rs. 89,45,000
5.	Umbrella handle	Rs. 60,16,000
6.	Bangphai	Rs. 8,84,000
7.	Wild cardamom	Rs. 2,16,000

Although there has been substantive revenue generation, there lies vast scope to trade with the manufacturers outside the state as well to get more value for the given resources and with the companies involved in sale of finished products out of given natural biological resources to share the profits earned by them; this is in addition to the purchase values being paid by the local traders and local manufacturers to the BMCs.

18.2.8 Bioprospecting and ABS

Narayankumar and Krishnan (2013)

An Ayurveda doctor from Pune, Maharashtra, had sought permission for applying for a patent from NBA on an invention relating to an antidote for snake venom comprising of four medicinal plants, namely, *Erythrina indica*, *Eugenia jambolana*, *Mangifera indica, and Jasminum sambac.* The application was approved on mutually agreed terms that the applicant would pay 2% of gross sale revenue of the product, which can then be employed for the conservation of these medicinal plants in their natural habitats routed through the concerned BMCs, which in turn would make sure that the raw material is available in a sustainable fashion, once commercialization of the product takes off. NBA can also direct the applicant to share non-monetary benefits like transfer of technology, grant of joint ownerships, or to get into agreements with the benefit claimers. This was the first of its kind agreement made by NBA, which would set a roadmap for linking bioprospecting with ABS.

18.2.9 ABS in Sikkim: A Potential Prospect

(Pradan 2014)

Yartsa gunbu (*Ophiocordyceps sinensis*) is a fungus that has a history of usage in Tibetan and Chinese Traditional medicine and is of high demand in the international markets with roughly $20,000–$40,000/kg. Sikkim which is a store house of this valuable resource is still to formulate an effective sustainable model for tapping these resources. The local communities have been in black market trade with prices of Rs.150–200 per piece, from quite some time. In order to crib this informal trade, the Sikkim Government framed an exclusive set of state rules, namely, *Cordyceps sinensis* Collection and Selling Rules 2009 which mandated that the collection can be done only by the Joint Forest Management Committee and Eco-Development Committee with the required permissions from Forest, Environment and Wildlife Management Department. The materials are sold in government auctions, and the amount collected after deducting the expenditures are shared between the government (25%) and the above mentioned agencies (75%). However, this is restricted only to collection in national parks and wildlife sanctuaries.

In order to address the existing availability of this species from the natural habitats of Sikkim and to legalize the commercial exploitation as well as streamline trade, such that the local communities are benefited and the resource is conserved, it is important that the Biodiversity Act 2002 is fully implemented with efficient BMCs in function, as jurisdictional BMCs can help in close monitoring of access and trade of the species, create awareness and platforms for trade in the process, generate employment for the local communities, as well as help in conservation of the species.

18.3 Conclusion

As per the NBA official records, thousands of notices were issued by SBBs under Section 7 of the Act to various Indian companies including those of pharmaceuticals, ayurvedic, etc. (Source-https://www.downtoearth.org.in/news/economy/patanjali-judgement-can-have-ramifications-beyond-uttarakhand-62629). There have been numerous cases filed in courts across the country for prior intimation and deposition of ABS. One of the recent cases is the Uttarakhand HC case, filed in 2016 by Ramdev's Haridwar-based Divya Yoga Mandir Trust. Uttarakhand State Biodiversity Board (SBB) asked its pharma unit, Divya Pharmacy, to share INR 20.4 million of its INR 4.21-billion revenue in 2014–2015 with farmers as benefit sharing under the BD Act, 2002. Patanjali pleaded in the Court that it being an Indian company, ABS compliance is not applicable to it. They lost the case in 2018, which turned out to be a landmark judgment as far as the Act is concerned (Source-https://www.business-standard.com/article/current-affairs/court-rejects-ramdev-s-swadeshi-excuse-to-not-share-revenues-with-tribals-118122700608_1.html). As per NBA, only a handful of companies are sharing their profits as per ABS Guidelines, 2014, and a lot of big players are still evading (Source-https://www.downtoearth.org.in/news/economy/patanjali-judgement-can-have-ramifications-beyond-uttarakhand-62629). Hence, at present, the effectiveness of the regulatory regime on benefit sharing is not very promising in herbal sector. However, states like Tripura are a bacon of light and are paving way for implementation of the Act in its essence and would strive as an example for the other states to follow and implement the Act. The direct linking of biodiversity conservation with the economic gains at the very local level is a perfect example of "think globally and act locally." The global Convention on the Biological Diversity (CBD) is finding its execution at the local village level with the involvement of poorest of the poor families as "benefit claimers." This has helped in no uncertain terms in imparting conservation cover to the traded medicinal plant species as those are also helping them to earn their livelihoods.

18.4 Way Forward

In a larger preview, the regime for benefit sharing should also be clearer and easier to comply with by integrating or going hand in hand with other existing ABS models such as Protection of Plant Varieties and Farmers' Rights Act (PVPFRA). There needs to be more effective capacity building and awareness creation programs, as the main stakeholders are local and indigenous communities who have absolutely no knowledge of whatsoever.

To facilitate effective ABS implementation, the following are the necessary steps: 1. put in practice the ABS Guidelines with legal certainty, clarity and transparency on access of biological resources and their associated TK, 2. develop a national database 3. develop a corresponding access policy 4. effective management for listing endemic species, their associated stakeholders and TK.

Also, enhancing financial and technical resources is very important to realize ABS. These resources do not only come from the state's budget and NBA but also from the bioresources during the process of ABS implementation which presses the need for more and more sustainable ABS models.

Finally, this legal provision of ABS may be further strengthened in the local level, say, as in the discussed case study of Tripura; by linking beneficiaries who are covered under the Recognition of Forest Rights Act, 2006 (getting right over forest land for cultivation), the JFMCs, SHGs, private farmers, tribal collectors and local *hakims*, etc., with the jurisdictional BMCs. These BMCs are supported institutionally, financially, and administratively to take control of the huge natural biological resources within its legal jurisdiction, both for the ecological security of the habitats and economic well-being of the various stakeholders therein.

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https://www.tripuraforest.nic.in

Part VI
A Pathway into the Future

Chapter 19
Future of Threatened Medicinal Plants in the Era of Anthropocene and Climate Change

P. E. Rajasekharan and Shabir Hussain Wani

Abstract Medicinal plants are globally valuable sources of herbal products, and they are disappearing at a high speed. Global trends, developments, and prospects for the strategies and methodologies concerning the conservation and sustainable use of medicinal plant resources to provide a reliable reference for the conservation and sustainable use of medicinal plants are discussed in this chapter. Both conservation strategies (e.g., in situ and ex situ conservation and cultivation practices) and resource management (e.g., good agricultural practices and sustainable use solutions) should be adequately taken into account for the sustainable use of medicinal plant resources. Biotechnical approaches (e.g., tissue culture, micropropagation, synthetic seed technology, and molecular marker-based approaches) should be applied to improve yield and modify the potency of medicinal plants.

Keywords Threatend Medicinal plants · Conservation · Ex situ · In situ sustainable use

Medicinal plants are globally valuable sources of new drugs. With the increasing demand for herbal drugs, natural health products, and secondary metabolites of medicinal plants, the use of medicinal plants is growing rapidly throughout the world. Here we try to find the significance of threatened medicinal plants in the era of vast human interference in the ecosystems and climate change.

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19.1 Medicinal Plant Resource Base

Medicinal plant resource base is dwindling especially of the threatened medicinal plants all over the world mainly due to anthropogenic activities and climate change. Although these threat has been known for decades, the accelerated loss of species and habitat destruction worldwide have increased the risk of extinction of medicinal plants. To stem the rot, concerted effort on conserving these resources should be done on war footing. In situ conservation efforts need to be taken up alongside ex situ conservation in the form of gene banks in vitro and cryopreservation. Sustainable harvesting from wild practices needs to be evolved for threatened medicinal plants. Restoration of species which are threatened needs to be taken up in forest areas to muster the number of individuals in the natural habitats and to prevent the imminent danger of extinction. Modern biotechnological techniques come handy for multiplication and subsequent testing of the genetic fidelity of derived plants.

In Europe with its long tradition in the use of botanicals, about 2000 medicinal and aromatic plant species are used on a commercial basis (Lange 1998). In Germany, Lange (1996) identified not less than 1500 taxa as sources of medicinal and aromatic plant material. In Spain, it is estimated that 800 medicinal and aromatic plant species are used, of which 450 species are associated with commercial use (Blanco and Breaux 1997; Lange 1998). Leaman (1998) of the Medicinal Plants Specialist Group of the IUCN Species Survival Commission estimates the number of medicinal plants which are threatened worldwide to at least 10,000 species.

Determining Threat Status of Species

Threat status of species should be determined applying internationally accepted methods including the CAMP (Conservation Assessment and Management Plan) designed by IUCN for rapid threat assessment of species.

Issues:

- The validity of the current system of determining the threat status of medicinal plants
- Other methodologies that could be adopted to do this

19.2 Potential of Medicinal Plants in State Economy

Medicinal plant sectors in any place of the world are related to people's livelihood and primary healthcare. Collection of plants and plant materials from the wild provides livelihood for tribes and other underprivileged people. Even today, hundreds of millions of people, mostly in developing countries, derive a significant part of their subsistence needs and income from gathered plant and animal products (Iqbal 1993; Walter 2001). But most of the collections are happening in an unsustainable manner, and due to that, plants are becoming threatened and ultimately going to become extinct. 70% of the world population depends on medicinal plant for their healthcare needs. So medicinal plants play a great role in the economy of many countries which are rich in biodiversity of medicinal plants in the form of either export or import.

Table 19.1 Distribution of medicinal plants by parts used (based on analysis of 1079 South Indian species)

Parts	Percentage (%)
Roots	26.6
Leaves	5.8
Flowers	5.2
Fruits	10.3
Seeds	6.6
Stem	5.5
Wood	2.8
Whole plant	16.3
Rhizome	4.4

Diversity of medicinal plants used worldwide More than one-tenth of plant species (more than 50,000) are used in drugs and health products. However, the distribution of medicinal plants is not uniform across the world. For example, China and India have the highest numbers of medicinal plants used, with 11,146 and 7500 species, respectively, followed by Colombia, South Africa, the United States, and another 16 countries with percentages of medicinal plants ranging from 7% in Malaysia to 44% in India versus their total numbers of plant species. Certain plant families not only have higher numbers of medicinal plants but also have higher proportions of threatened species than others. Only a portion of medicinal plants that suffer from genetic erosion and resource destruction have been listed as threatened (Table 19.1).

19.3 Conservation and Utilization

The relationship between in situ and ex situ conservation of species is an interesting topic with implications for local communities, public and private land owners and managers, entire industries, and of course, wild species. Identifying the conservation benefits and costs of the different production systems for MAP should help guide policies as to whether species conservation should take place in nature or the nursery or both (Bodeker et al. 1997).

Due to the unsustainable harvesting, lack of augmentation in the wild, and cultivation, many medicinal plants are finding their way into the Red List of IUCN. At present, 90% collection of medicinal plants is from the wild, generating about 40 million man days' employment (part and full), and since 70% of plant collections involve destructive harvesting, many plants are endangered or vulnerable or threatened. If concerted efforts are not directed to stem the rot, these plants will become extinct soon. in situ conservation is the best form of conservation, due to certain issues, this form of conservation could be complemented with relevant form of ex situ conservation in the form of either seed banks (gene bank), field gene banks, in vitro gene bank, or cryobank whichever is relevant, or a combination of these will help in conservation. 34,000 species or 8% of the world's flora are threatened with extinction. If this is applied to our earlier estimate that 52,000 plant species are used medicinally, it leads us to estimate that 4160 MAP species are threatened. Medicinal plant species which are rare or endangered or threatened should be identified, and their ex situ conservation may be attempted in the established gardens, plantations, and other areas.

Of the more than 400 plants species used for the production of medicine by the Indian herbal industry, fewer than 20 species are currently under cultivation in different parts of the country (Uniyal et al. 2000). In China, about 5000 medicinal plants have been identified, and about 1000 are more commonly used, but only 100–250 species are cultivated (Xiao 1991; He and Sheng 1997). In Hungary, a country with a long tradition of MAP cultivation, only 40 species are cultivated for commercial production (Bernáth 1999; Palevitch 1991). In Europe as a whole, only 130–140 MAP species are cultivated (Pank 1998; Verlet and Leclercq 1999).

19.4 Erosion of Traditional Knowledge Base and IPR

Along with the resources, the traditional knowledge related to the medicinal plants are also fast depleting. This will prevent the people from properly using the resources. The traditional knowledge related to medicinal plants is not documented properly. It comes from generation to generation through verbal medium. The proper documentation before it gets eroded is very much required. The instruments are required to protect and commercialize these resources.

The emergence of the new intellectual property regime in the light of India joining WTO will pose important challenges in this sector. To prevent patenting of our traditional knowledge by outsiders, all the available information should be properly formatted in a digital form by using international standards for wider use at both the national and international levels. There is also a deep philosophical divide on the issue of IPR that we have to deal with. The existing IPR systems are oriented around the concept of private ownership and individual invention. They are at odds with indigenous cultures, which emphasize collective creation and ownership of knowledge. There is a concern that IPR systems encourage the appropriation of traditional knowledge for commercial use without the fair sharing of benefits or that they violate indigenous cultural percepts by encouraging the commodification of such knowledge.

19.5 Benefit Sharing

While recognizing the market-based nature of IPRs, other non-market-based rights could be useful in developing models for a right to protect traditional knowledge, innovations, and practices. Geographical indications and trademarks, or sui generis analogies, could be alternative tools for indigenous and local communities seeking to gain economic benefits from their traditional knowledge. To date, debate on IPRs and biodiversity has focused on patents and plant breeders' rights. The potential value of geographical indications and trademarks needs to be examined too. They protect and reward traditions while allowing evolution. They emphasize the relationships between human cultures and their local land and environment. They are not freely transferable from one owner to another. They can be maintained as long as the collective tradition is maintained. Models of benefit sharing are beginning to emerge in India. There is the case of a medicine that is based on the active ingredient in a plant. The plant species, *Trichopus zeylanicus*, found in the tropical forests of south-western India and collected by the Kani tribal people. Scientists at the Jawaharlal Nehru Tropical Botanic Garden and Research (JN TBGRI) Thiruvanthapuram, in Kerala, developed the tonic, which is claimed to bolster the immune system and provide additional energy, while on a jungle expedition with the Kani tribes in 1987. A few years later, they returned to collect the samples of the plant, known locally as "arogyapacha", and began laboratory studies of its potency. These scientists then isolated and tested the ingredient and incorporated it into a compound, which they christened "Jeevani" – giver of life. The tonic is now being manufactured by a major Ayurvedic drug company in Kerala. In November 1995, an agreement was struck for the institute and the tribal community to share a license fee and 2% of net profits. The process marks perhaps the first time that cash benefits have gone directly to the source of the knowledge of traditional medicines and the original innovators. We need to formalize such models.

19.6 Multiplication

The biotechnological tools are important to select, multiply, and conserve the critical genotypes of medicinal plants by adopting techniques such as micropropagation, creation of somaclonal variations, and genetic transformations. Biotechnological tools can also be harnessed for the production of secondary metabolites using plants as bioreactors.

Threat status of species should be determined applying internationally accepted methods including the CAMP (Conservation Assessment and Management Plan) designed by IUCN for rapid threat assessment of species.

19.6.1 Choosing Priorities

- It is necessary to prioritize the thrust areas to obtain the output of research efforts and other resources. Several factors help in determining the priorities. These include the distribution of flora, national or regional disease pattern, availability of modern healthcare, etc. In addition we have to keep in mind the global priorities in developing new drugs so as to get a good financial return.

- The disease pattern and the priorities have national characteristics, but there are several diseases which are common to tropical areas and in fact to most developing countries. These include protozoal and helminthic infections like malaria, filariasis, onchocerciasis, etc. Many of these diseases do not exist in developed countries, and large pharmaceutical houses, therefore, do not give high priority to develop new drugs for such conditions. There is a gross mismatch between the health needs of the developing countries and the interests of the pharmaceutical industry. These should, therefore, receive priority in national/regional plans. The above examples are only illustrative, but we have to evolve our own list of priority for communicable diseases.
- Primary healthcare usually requires comparatively milder medication, and the acceptability of herbal medicines for such conditions is also much more. The main considerations should be adequate availability or possible cultivation on required scale, lack of toxicity, and ease of formulation.
- The global thrust areas for drugs from natural sources include disease conditions whose incidence is increasing and where the modern drugs are either unavailable or unsatisfactory. Some examples of such maladies may be summarized as follows:

  - Tropical diseases: antimalarial, antifilarials, and antileishmaniasis
  - Chronic conditions: anti-arthritic agents and anti-rheumatic agents
  - Immunomodulators, immunostimulants, and adaptogens
  - Hepatoprotectors
  - Rapid wounds and ulcer-healing agents
  - Central stimulating or sedating agents
  - Alzheimer's disease: prospective agents
  - Memory enhancers
  - Analgesics
  - Sedatives

In considering the validation of the claims of ethnomedical therapies and derived preparations for introduction into the healthcare systems, the following deserve consideration:

(a) The inadequacy of animal models to serve as adequate systems to assess biological activities that can be extrapolated to the human situation. This is particularly so in some of the disease conditions for which no satisfactory modern therapy exists.
(b) The minimizing of toxicity tests needed to introduce the drug into a healthcare system. This is particularly necessary when the drug has been in long human use, toxic manifestations could be assessed by studying its long-term effect on patients already undergoing treatment in the traditional milieu, and the mode of industrial processing does not significantly vary from the ethnomedical methods. Product comparisons by modern instrumental parameters can also be made as between processed product and ethnomedical preparation.
(c) Clinical trials conducted under the supervision of competent authorities (e.g., WHO) must be a necessary prerequisite.

(d) Stimulation of traditional processing methods as well as adherence to ethnomedical regiments will be most helpful in not missing the activity present in an ethnomedical preparation. This will also stimulate examination of ethnomedical theories of disease with a view to interpretation of these, if at all possible, within modern concepts. (The idea particularly refers to long-standing and well-documented systems such as Ayurveda, Unani, and the Chinese systems.)
(e) The selection of the appropriate dosage form and mode of administration should be recently based on economic parameters as well as shelf-life potential in the situations prevailing in the developing world.
(f) There is some concrete evidence that pure compound need not necessarily be the best drugs. But on economic ground as well as on the therapeutic grounds, it will serve all interests well if the most appropriate processing methodology of a plant or combination of plants is examined in this light (this would also give rise to interesting researches on the synergistic and/or detoxificant effects of other constituents in the medicinal plants or non-medicinal plants that are found often added to polyprescriptions used in traditional systems).

19.7 New Areas in Which Research in Medicinal Plants Needed to Be Taken Up

There is an urgent need to develop new effective drugs, traditionally used medicinal plants have recently received the attention of the pharmaceutical and scientific communities. This involves the isolation and identification of the secondary metabolites produced by the plants and used as the active principles in medical preparations. Research on the scientific validation of Southern African medicinal plants used in the treatment of pain and inflammation, hypertension, and parasitic diseases including those with anthelmintic, anti-amoebic, anti-bacterial, and anti-bilharzia activity, are already taken up.

Relating to prior ethnopharmacological experiences, scientists have searched for medicinal plants that could be valued sources for endophytes yielding novel metabolites of pharmaceutical importance.

Table 19.2 List of globally significant medicinal plants (GSMPs)

S. no.	Species	Family	Parts used
1.	*Abies pindrow* Royle.	Pinaceae	Leaf
2.	*Abies spectabilis* (D. Don) Spach.	Pinaceae	Leaf
3.	*Aconitum balfouri* Stapf	Ranunculaceae	Tuber
4.	*Aconitum heterophyllum* Wall.	Ranunculaceae	Tuber
5.	*Aconitum violaceum* Jacq. ex Stapf	Ranunculaceae	Tuber
6.	*Aegle marmelos* (L.) Corr.	Rutaceae	Leaf, fruit
7.	*Allium stracheyi* Baker	Liliaceae	Whole plant
8.	*Angelica glauca* Edgew.	Apiaceae	Root

S. no.	Species	Family	Parts used
9.	*Anogeissus latifolia* (Roxb. ex DC.) Wall. Ex Guill. & Perr.	Combretaceae	Leaf, bark
10.	*Arnebia benthamii* (Wall. ex G. Don) Johnston	Boraginaceae	Root
11.	*Berberis aristata* DC.	Berberidaceae	Root, bark
12.	*Bergenia ciliata* (Haw.) Sternb.	Saxifragaceae	Leaf, root
13.	*Bergenia stracheyi* (Hk. f. & Thomson) Engl.	Saxifragaceae	Rhizome, leaf
14.	*Dactylorhiza hatagirea* (D. Don) Soo	Orchidaceae	Tubers
15.	*Dioscorea deltoidea* Wall. ex Griseb.	Dioscoreaceae	Tuber
16.	*Emblica officinalis* Gaertn.	Euphorbiaceae	Fruit
17.	*Fritillaria roylei* Hk.	Liliaceae	Bulb
18.	*Habenaria intermedia* D. Don	Orchidaceae	Tuber
19.	*Malaxis muscifera* (Lindl.) O. Kuntze	Orchidaceae	Pseudobulb
20.	*Nardostachys grandiflora* DC.	Valerianaceae	Rhizome/root
21.	*Paeonia emodi* Wall. ex Royle	Paeoniaceae	Roots and leaf
22.	*Paris polyphylla* Smith	Liliaceae	Roots
23.	*Picrorhiza kurroa* Royle ex Benth.	Scrophulariaceae	Rhizome/root
24.	*Podophyllum hexandrum* Royle	Podophyllaceae	Fruits, rhizomes
25.	*Pueraria tuberosa* (Roxb. ex Willd.) DC.	Fabaceae	Roots
26.	*Rheum emodi* D. Don	Polygonaceae	Root, rhizome
27.	*Rheum moorcroftianum* Royle	Polygonaceae	Root
28.	*Rhododendron campanulatum* D. Don	Ericaceae	Leaf and wood
29.	*Selinum candollei* DC.	Apiaceae	Root
30.	*Selinum vaginatum* (Edgew.) CB Clarke	Apiaceae	Root
31.	*Swertia chirayita* (Roxb. ex Fleming) Carsten	Gentianaceae	Whole plant
32.	*Taxus baccata* L.	Taxaceae	Leaves, bark
33.	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Fruit
34.	*Terminalia chebula* (Gaertn.) Roxb.	Combretaceae	Fruit
35.	*Tinospora cordifolia*	Menispermaceae	Tuber
36.	*Valeriana jatamansi* Wall.	Valerianaceae	Whole plant

According to Ved et al. (2008)

Table 19.3 Species totally prohibited from wild collection

S. no.	MAP species	S. no.	MAP species
1	*Picrorhiza kurroa*	11	*Swertia chirayita*
2	*Zanthoxylum armatum*	12	*Desmodium gangeticum*
3	*Acorus calamus*	13	*Uraria picta*
4	*Aconitum balfourii*	14	*Nardostachys jatamansi*
5	*Malaxis cylindrostachya*	15	*Polygonatum* spp.
6	*Dactylorhiza hatagirea*	16	*Jurinea dolomiaea*
7	*Paris polyphylla*	17	*Valeriana jatamansi*
8	*Rheum* sp.	18	*Rubia cordifolia*
9	*Taxus baccata*	19	*Tinospora cordifolia*
10	*Berberis* spp.	20	*Aconitum heterophyllum*

They can only be collected from cultivated fields when grown by registered farmers and can be exported using only permits

19.8 Factors Contributing to Rarity of Medicinal Plants

Overexploitation, indiscriminate collection, uncontrolled deforestation, and habitat destruction all affect species rarity, but all these factors are not enough to give reason for why individual species are becoming rare. Many factors contribute to extinction risk such as habitat specificity, distribution range, population size, species diversity, growth rate, and reproductive factors.

19.9 Conservation Strategies

In Situ Conservation
In situ conservation with the whole ecosystem allows to protect indigenous plants and maintain natural communities along with their intricate network of relationships. Also in situ conservation increases the volume of diversity that can be conserved and also strengthens the link between conservation and sustainable use. In situ conservation efforts worldwide are done by establishing protected areas and taking an approach that is ecosystem oriented rather than species oriented. The success of in situ conservation depends on rules, regulations, and compliance of medicinal plants within growth habitats.

Natural Reserves
As mentioned earlier degradation and destruction of habitats is a major cause of loss of genetic resources of medicinal plants. Natural reserves help to preserve and restore biodiversity. In the entire world, more than 12,700 protected areas have been established with 13.2 million km^2 of 8.88% of the land surface of the earth. There is a need to assume the ecosystem functions of individual habitats to know that medicinal plants are protected in these areas, and there is a need to establish wild nursery for species-oriented cultivation and domestication of threatened medicinal plants and protected areas. Over exploitation, habitat degradation population of many wild species are under high pressure wild nurseries will help to revive these species in the natural habitat.

Ex Situ Conservation
It is to complement to in situ conservation and not a seperate in situ conservation strategy. Especially for species which are overexploited and threatened. Medicinal plants growing slowly, low in number, ex situ conservation is relatively difficult and is better to cultivate and naturalize threatened species to ensure their continued survival. Many wild medicinal plants cannot retain high potency when grown in garden from natural habitats.

Botanic Gardens
Botanic gardens play an important role in ex situ conservation, and the survival of threatened plant species is ensured there, but in terms of genetic conservation, due to the presence of only a few individuals of each species, it is of limited use but it

contains taxonomically and ecologically diverse forms due to the presence of a wide range of plant species grown under common condition. They also play a role in medicinal plant conservation through the development of propagation and cultivation protocol and in domestication and in the breeding of rare plants.

Seed Banks

To help preserve the biological and genetic diversity of wild plant species, seed banks offer a better choice. Here it is to be mentioned the noteworthy millennium seed bank project of the Royal Botanic Garden in Britain. It allows relatively quick access to plant samples for the exploitation of the properties providing helpful information for conserving the remaining natural population, but the challenge remains in the form of how to reintroduce the plant species back to the wild and how to assist in the restoration of wild population.

Cultivation Practice

The cultivation of medicinal plants is a widely used and accepted practice although wild harvested resources of medicinal plants are considered more efficacious and it also helps to solve the problems like toxic components, pesticide contamination, low concentration of active ingredients, and wrong identification of botanical origin. If we cultivate medicinal plants under controlled growth conditions it can improve the yields of active compounds.

Sustainable Use

Unsustainable harvest results in resource exhaustion and even species extinction, there is a need to develop sustainable use of medicinal plants, and good harvesting practices must be formulated. The harvesting of roots or whole plant is destructive, e.g., in the case of herbs, shrubs or trees, then collecting their leaves and flower buds. It was found that extracts from ginseng leaf, stem or roots have similar pharmaceutical properties but leaf and stem have the advantage of being more sustainable resources.

19.9.1 In Situ *Conservation of Medicinal Plants*

The most cost-effective strategy to ensure the long-term survival of medicinal plants is to establish a network of in situ medicinal plants' conservation areas (MPCAs) [200–500 hectares size each] across all the forest types and altitudinal range in every state. At present, MPCAs have been established only in southern India. It is accepted that specific areas for the in situ conservation of medicinal plants need to be planned and that such areas should not be managed by the Forest Department (as other protected areas are today being managed), but be managed by the lowest forms of government, i.e., the Panchayati Raj Institutions. Conservation and management of such areas should be based on positive incentives.

Issues:

- The viability of the conventional approach of protected areas appropriate for medicinal plant conservation
- Suggested other strategies for conservation of medicinal plants
- Mechanisms needed in order to operationalize medicinal plant conservation
- Kinds of incentives required to make this work

19.9.2 Strategies for Sustainable Supply of Medicinal Plants

(i) Such medicinal plants wherein the parts used are fruits and flowers (e.g., *Phyllanthus emblica*, *Terminalia chebula*, *Terminalia bellirica*, *Woodfordia fruticosa*) and can therefore be harvested non-destructively and sustainably from the wild should be permitted under the NTFP collection schemes.

(ii) Poly-culture plantation models on "degraded forests" is a feasible model to encourage especially when it involves local community benefits via JFM programs.

(iii) Cultivation: Given the rapid growth of the herbal industry (annual turnover estimated to be 2000 crores), it is not feasible to encourage the presently prevailing situation, where 95% of the industry's requirement and over 700 species are harvested from the wild. This is particularly significant in the case of plants wherein wild harvest involves destructive collection, e.g., when a whole plant (e.g., *Swertia chirayita*), a bark (e.g., *Terminalia arjuna*), stem (e.g., *Coscinium fenestratum*), heart wood (e.g., *Santalum album*), root (e.g., *Picrorhiza kurroa*), or resin (e.g., *Commiphora mukul*) is made. Such harvest should be gradually stopped and the users encouraged to cultivate such species, on the desired scale. In fact almost 70% of the medicinal plant collections do involve destructive collection.

Such species, which involve destructive collection, should appear in different schedules of a negative list to be drawn up by the government from time to time. There can be at least four schedules for the negative list. The first category, i.e., negative list no. 1, can include species like *Coscinium fenestratum* whose natural populations are critically endangered on the account of population reduction exceeding 80% over a period of three generations. Since such perennial species can take 4–10 years to get established, they can be forthright banned from collection. The second negative list can include species which also involve destructive collection, but which are faster regenerating species (shrubs, herbs) or are not yet critically endangered (e.g., *Aconitum heterophyllum*), but are assessed to be in the "endangered" list based on population decline of more than 50% in 10 years. For such species users can be given a 4- to 8-year period of time during which they would need to bring the species under cultivation. There can be a third negative list that also involves species collected destructively but the species may at present only be "vulnerable," based on population reduction of 20% in 10 years (e.g., *Jurinea* *dolomiaea*). In such a case, 6–10 years' time can be provided to the users to put the species under cultivation. The fourth category would include the species which involve destructive collection and are perceived to be potentially under threat because of high current levels of consumption (e.g., *Embelia ribes*). For these species also a 6–10 years' time frame can be provided for bringing them under cultivation.

Incentives for cultivation In the initial years, going in for cultivation of medicinal plants will be resisted by farmers unless they can get assured remunerative prices and buyback guarantees. The lessons and experience of dealing with this situation in mainstream agriculture must be applied in an innovative manner to medicinal plants. One serious constraint is that reliable agro-technology and information on economics of cultivation is only available for around 70 species (most of which are spice and aromatic plants which have established markets). Industries should be given suitable financial incentives to forge backward linkages with farmers (with regard to buyback guarantees and promotion of cultivation) and also their investments in developing agro-technologies for medicinal plants.

Pricing Market price of medicinal plants collected from the wild is generally cheaper than the price of the same material derived from cultivation. This is the main reason why organized cultivation is not found to be economically feasible. Today, out of the 880 species in trade, around 70 are under commercial cultivation, and approximately 30 of these are spices, 20 are aromatic plants, and only around 20 are species used exclusively by Indian systems of medicine. For more extensive cultivation to take place, the Forest Department must immediately take policy and administrative measures to raise the "reserve price" of wild collection. Today, because of the relatively lower cost of wild material, as compared to the production cost of cultivated materials, farmers are not prepared to cultivate several of the needed medicinal plants. If the Forest Department raises the reserve price of wild harvest, then users may find it cheaper or at least equally attractive to obtain them from cultivated sources. The additional revenue thus received by the Forest Department should be invested into medicinal plants conservation programs, and the price rise should be justified in terms of meeting "conservation" costs.

Issues:

- The viability of tissue culture as an option for supplying planting material
- The viability of farmers cultivating medicinal plants
- Kinds of incentives required to encourage farmers to cultivate medicinal plants
- Kinds of incentives required for the industry to encourage buying of medicinal plants from farmers

19.9.3 Establishing a Planting Material Supply Network

A nationwide network of medicinal plant nurseries and seed banks to supply quality and certified planting materials is a very "practical" measure to immediately put in place in order to promote cultivation. This kind of infrastructure exists for agricultural crops. It is also accepted that farmers need to be given appropriate incentives for the cultivation of medicinal plants.

Issues:

- Measures to be taken to ascertain that quality planting material is provided for cultivators

19.9.4 Certification for Raw Materials

Today, many of the medicinal plants available in the marketplace are adulterated and are microbially contaminated. This is due to the absence of raw material certification requirements for the industry by the FDA and absence of suitable post-harvest technologies, especially related to "drying" of medicinal plants. It is absolutely essential that ISM (Indian System of Medicine) Department sponsors and promotes regional certification and facilit s to set "gold" standards for raw drugs. Initially however, certification need not be made compulsory. An "AGMARK" or ISO-9000 like standard for medicinal plants can be immediately promoted by the ISM Department, to encourage "quality" awareness in industry and among consumers. The ISM Department can also support consumer research and education organizations like Consumer Education and Research Center (CERC) and others to undertake consumer awareness campaign based on quality assessment of raw materials and finished products, used by the herbal industry, ways by which a nationwide network of medicinal plant nurseries and seed banks is established to supply quality and certified planting material. Valid methods of certification of medicinal plants are also suggested.

Issues:

- A valid methodology for certification of medicinal plants
- Mechanisms required to operationalize this

19.9.5 National Coordination Mechanism

A national medicinal plant council, commission, or board which can involve the Environment, Health, Science and Technology, and Agriculture Commerce Ministries, the private sector, and banks should be seriously considered as a mechanism to coordinate policy and administrative measures in this sector, which will (a) kick-start extensive cultivation, (b) promote in situ conservation of genetic resources, (c) promote gold standards for raw materials, and (d) regulate and where it proves necessary ban wild collections.

Issues:

- The role of the medicinal plant board

19.9.6 Sustainable Bio-partnerships

Commercialization of the medicinal plant sector as it stands now is unplanned, unmonitored, poorly understood, grossly inequitable, and opaque. What is needed is a process fully participated by all the major actors, downstreamed to the lowest possible level in the Production-to-Consumption and Marketing (PCM) continuum and equitable ensuring fair benefits to the local people specially the collectors. The process of globalization which has also started adversely impacting the microeconomies of the subsector has added to the additional pressure to the ongoing commercialization process which needs to be constantly monitored, evaluated, and managed.

However, increasing commercialization of medical plants does provide opportunities to the local communities to enhance their livelihoods. But in order to realize this potential, we need first to create a level playing field for all the players and stakeholders in the PCM continuum and ensure that the resources especially market information are made available to all the players. It is argued that this is possible in a framework of "bio-partnership."

Bio-partnership is a concept which aims to replace the much maligned but common concept and practice of "bio-prospecting." The basic principle of bio-partnership is development of mutually beneficial and sustainable relationships principally between the producers and users of the medical resources to satisfy both the short-term and long-term goals of the parties involved in the management of the resources. It is envisaged that traders and manufacturers, once convinced that the uninterrupted flow of quality, raw materials is possible by working in collaboration with the organized bodies at the level of local communities such as VFCs and FPCs, might be drawn into the partnership. This will call for a drastic change in the ways that trade and industry sectors are doing business today. It is argued that government agencies, NGOs, and CBOs, by working together, can change the mind-set and influence the decision-making process of the med plant-based enterprise and industry. Importance of forming a sustainable "bio-partnerships" need to be emphasized.

Issues:

In general, the demand for medicinal plants and herbal remedies and especially its Renaissance in the developed countries is driven by the following factors (Iqbal 1993; Leaman 2002):

- Increasing costs of institutional, pharmaceutical-based healthcare
- Interest of individuals, communities, and national governments in greater self-reliance in healthcare
- Interest of communities and national governments in small- and large-scale industrial development based on local/national biodiversity resources
- Increasing success in validating the safety and efficacy of herbal remedies
- Legislation improving the status of herbal medicine industry
- Renewed interest of companies in isolating useful compounds from plants
- Search for new drugs and treatments of serious and drug-resistant diseases
- Marketing strategies by the companies dealing in herbal medicine

Policy, legal, and institutional supports should be extended to the sector for adopting standards, quality control, efficacy, and effectiveness of herbal drugs.

19.10 Summary and Way Forward

The medicinal properties of plant species have made an outstanding contribution in the origin and evolution of many traditional herbal therapies. Due various anthropogenic activities and climate change, the resource base is shrinking. There is a need to conserve the same using suitable methods. The use of medicinal plants is not sustainable, and due to the same, many medicinal plant species are becoming extinct and many are in the verge of extinction. There is a need to develop integrated conservation strategies and sustainable use practices in the case of threatened medicinal plants.

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