Conservation of medicinal plants located in Western Ghats

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Medicinal plants play a key role in the treatment of a number of diseases and source of medicines for majority of people in the developing world. The tropical regions of the world supply the bulk of current global demand for "natural medicine," albeit with increasing threat to populations in the world and its genetic diversity. India is a major centre of origin and diversity of crop and medicinal plants, and poses out 20,000 species of higher plants, one third of it being endemic and 500 species are categorized to have medicinal values. The Western Ghats is one of the major repositories of medicinal plants that harbours around 4,000 species of higher plants of which 450 species are threatened. Currently, the number of species added to the red list category in this region is increasing, and the valuable genetic resources are being lost at a rapid rate. Demand for medicinal plants is increasing that leads to unscrupulous collection from the wild and adulteration of supplies. Providing high-quality planting material for sustainable use and thereby saving the genetic diversity of plants in the wild is important. During the last 25 years of intensive research, Tropical Botanic Garden and Research Institute has developed *in vitro* protocol for rapid regeneration and establishment of about 40 medicinally important rare and threatened plants of Western Ghats.

Key words*:* Biodiversity, Medicinal plants, Conservation, in situ conservation, ex situ conservation

1. Introduction

In developing countries, where over 80% of the population relies on herbal medicine for basic health care, there is a renewed interest in plant-based drugs due to concerns about the health hazards associated with synthetic drugs. This global inclination towards herbal medicine has led to the expansion of plant-based pharmaceutical industries, particularly in India, where only a small percentage of medicinal plants are exclusively cultivated. India, positioned along the Tropic of Cancer, serves as a prominent hub for biodiversity, boasting 8% of the world's biodiversity within a mere 2.4% of its land area. Ranking 10th globally in plant richness and 4th in Asia, the country encompasses two of the planet's 25 hotspots, the Eastern Himalayas and Western Ghats. The Western Ghats, protected by the sea to the west, Vindhya and Satpura mountain ranges to the north, and the semi-arid Deccan plateau to the east, stretch over 1,600 km through Maharashtra, Goa, Karnataka, Tamil Nadu and Kerala. This unique landscape, featuring altitudinal ranges up to 2,800 m and diverse climates and edaphic conditions, nurtures an exceptionally rich endemic flora, establishing the Western Ghats as a haven for diverse and distinctive plant species.

Medicinal plants exhibit diverse life forms, encompassing lichens, algae, ferns, herbs, shrubs, climbers and trees, spanning annuals to perennials. This botanical variety, with its intraspecific differences, represents a valuable reservoir of chemical and medicinal resources, evident in the long-standing traditions of natural drug utilization. Traditionally, the natural forests of the Western Ghats supplied nearly 500 medicinal plant species, some of which were integral to traditional and folk medicinal practices (Joy et al., 2001).

Among the plethora of species in the Western Ghats, around 50 hold significant value in folk and herbal health practices for treating various ailments (Suja, 2005). Modern medicine taps into the Western Ghat's medicinal flora, extracting phytochemicals like berberine, camptothecin, forskolin, L-dopa and reserpine from plants such as *Coscinium fenestratum*, *Nothapodytes foetida*, *Coleus forskohlii*, *Mucuna pruriens* and *Rauvolfia serpentina*. Unfortunately, comprehensive documentation and exploration of only a few higher plant species from the Western Ghats have been undertaken. Recent reports suggest that a mere 3-5% of terrestrial plants have undergone thorough investigation (Gilani, 2005).

Despite ongoing discoveries providing new drug leads, the utilization of plant-based medicines remains limited due to various factors. Challenges include the scarcity of sufficient plant material, the need for appropriate high-throughput screening bioassays, and the production of bioactive compounds in substantial quantities, as outlined by projected constraints (Ramavat et al., 2009). This underscores the imperative for extensive and intensive research on medicinal plants in general. Plant tissue culture is very useful in conserving the biodiversity of rare and endangered medicinal plant species that produce recalcitrant seeds and play a huge role mass multiplication and germplasm conservation. Also, this biotechnological approach facilitates the production of various pharmacologically valued plant products. This section is mainly focused on the micropropagation and conservation of the above mentioned highly threatened anticancer plants from the Western Ghats region of India.

2. Important medicinal plants in Western Ghats

2.1. *Acorus calamus* Linn.

A. calamus is one of the important herbs known for its immense medicinal potential. It is an aromatic herb with the creeping rhizome locally known by various names like Bachh and Sweet Flag, belongs to taxonomic family Acoraceae (Meena et al., 2010). It has a diverse range of pharmacological properties including the treatment of diseases like cancer, ulcer, hepatitis, spasm, schizophrenia, gout, arthritis, and anorexia (Singh et al., 2011; Ranjan et al., 2016). It is the source of innumerable phytoconstituents such as glycosides, tannins, alkaloids, saponins, flavonoids, phenols, and lectins. The essential oils obtained from this herb constitutes several volatile compounds such as calamene, calameone, calamenenol, α-pinene, β-pinene, p-cymene, camphene, eugenol, methyl eugenol, methyl isoeugenol, eugenyl acetate, isoeugenol, azulene, eugenol methyl ether, calamol, asaronaldehyde, dipentene, terpinolene, camphor, 1,8-cineole, caryophyllene, α-asarone, and β-asarone (Palani et al., 2009; Haghighi et al., 2017). Due to these biologically active phytocompounds, *A. calamus* is having a high demand, and thus over-exploited. The plant extracts have been documented to possess anticancer activity against various human carcinoma cells.

2.2. *Aristolochia indica* Linn.

A. indica, an endangered plant species with immense medicinal importance, belongs to Aristolochiaceae family. It is locally called by names, Garudakkodi, Eswaramooli, Iswaberusa and Ishrmul. It is a shrub with a long twining stem and is also known as “worm killer” because of its

antihelminthic properties. This plant species is listed in a red data list of South Indian medicinal herbs. It is aromatic in nature and used to treat fever, cholera, ulcer, skin diseases, leprosy, snakebite, and cancers (Dey and De, 2011; Anilkumar et al., 2014). Some of the major phytoconstituents of *A. indica* include ishwarane, aristolochen, ishwarone, aristololactam N-β- D-glucoside, 6β-hydroxy-stigmast-4-en-3-one, 3β-hydroxy-stigmast-5-en-7-one, aristolochine alkaline, isoaristolochic acid, allantoin, pinocarvone, and α-pinene. Various solvent extracts of this plant possess antiproliferative activity against MCF-7, the human breast cancer cell line (Anilkumar et al., 2014; Subramaniyan et al., 2015). Likewise, aristolochic acid isolated from the ethanolic whole plant extract has shown to prevent oral cancer in Albino rats induced by 4-nitroquinoline 1-oxide (Mariappan, 2012).

2.3. *Clerodendrum serratum* (Linn.) Moon.

C. serratum, belonging to Verbenaceae, is commonly known as Bharangi and is found in the Western Ghats of India. The plant, valued in traditional medicine like Ayurveda, Unani, and Siddha, is known for its roots and leaves, believed to have therapeutic properties. It has been historically used to address various health issues such as typhoid, syphilis, jaundice, hypertension and cancer. Some key constituents in the plant include D-mannitol, hispidulin, cleroflavone, apigenin, scutellarein, serratagenic acid and others. Additionally, it has been traditionally employed for its anti-rheumatic, anti-asthmatic, febrifuge, encephalalgia and ophthalmic properties.

2.4. *Coscinium fenestratum* (Goetgh.) Colebr.

C. fenestratum, a dioecious woody climber from Menispermaceae, is critically endangered and listed in the red list of threatened plants in India. Despite its endangered status, it is a commercially traded medicinal herb used in over 60 ayurvedic preparations. The plant addresses various health issues like skin diseases, ulcers, inflammation, eye disorders, hypertension, diabetes, jaundice and snakebites.

The stem extract of *C. fenestratum* contains berberine, an insulin-stimulating compound with hypoglycemic effects. Additionally, the leaf extract contains ecdysterone, known for anabolic, adaptogenic, antidiabetic, hepatoprotective, antitumor and immunoprotective activities. Studies have revealed that berberine isolated from *C. fenestratum* exhibits an antiproliferative effect on lung, colorectal and acute myeloid leukemia cell lines. Moreover, the plant's crude water extract demonstrates cytotoxic effects on human metastatic squamous cell carcinoma of the pharynx, linked to the modulation of signal molecules, resulting in increased cell inhibition and apoptosis.

2.5. *Curculigo orchioides* Gaertn.

The endangered plant from the Hypoxidaceae, found in the Western Ghats, is a vital component in ayurvedic preparations like Kali or Shyah-Musali, known for rejuvenation. This perennial shrub has elongated, short fleshy roots and a 1 feet pulpy rhizome. Abundant in flavone glycosides, it's tubers contain curculigosaponins, curculigosides, phenyl glycosides, orcinol glycoside, corchioside A, hentriacontanol and the alkaloid lycorine. Renowned for its immunostimulatory, aphrodisiac, hepatoprotective, antioxidant, antidiabetic, and

anticancer properties, this shrub plays a crucial role in Ayurvedic remedies for various ailments.

2.6. *Gloriosa superba* L.

G. superba, a perennial semi-woody herbaceous climber in Liliaceae, is employed in ayurvedic medicine for treating various ailments. It's medicinal properties of stem is due to alkaloids, primarily colchicine and gloriosine. The rhizomes and seeds contain colchicine, isoperlolyrine, tropolane alkaloids, β-sitosterol, glucoside, and 2-hydroxy 6-methoxy benzoic acid. It is traditionally used for conditions such as gout, arthritis, inflammation, rheumatism, ulcers, skin diseases, bleeding piles, leprosy, snakebites, and impotence. *G. superba* hydroalcoholic extract has exhibited anticancer activity against lung cancer cell lines.

2.7. *Hemidesmus indicus* R.Br. ex Schult.

H. indicus, also popularly known as "Anantmul," is a semierect shrub belonging to Asclepiadaceae. It is widely distributed throughout India and known as "God of Medicine" and used in a popular drug formulation of the ayurveda system of medicine to treat dysentery, diarrhea, skin diseases, syphilis, dyspepsia, leukoderma, diuresis, burning of body, chronic fever, and asthma and also acts as blood purifier. Pharmacological studies carried out with its extract and purified compounds indicated that this plant possesses antioxidant, hepatoprotective, antiulcer, antimicrobial, hypoglycemic, antihyperlipidemic, otoprotective, analgesic, anti-inflammatory, and immunomodulatory activities.

2.8. *Leptadenia reticulata* (Retz.) Wight & Arn.

L. reticulata, commonly known as Jivanti and belonging to Apocynaceae, is wide spread in tropical and subtropical regions. Unfortunately, over exploitation has led to its endangered status, prompting efforts for commercial cultivation in certain parts of India, driven by demand from pharmaceutical and nutraceutical industries. Traditionally used in ayurveda for conditions like hematopoiesis, tuberculosis, cough, emaciation, dyspnea, fever, dysentery, and cancer. The plant serves as a revitalizing and rejuvenating agent and rich in biologically active compounds such as α-amyrin, ferulic acid, diosmetin, β-sitosterol, luteolin, hentricontanol, stigmasterol, simiarenol, reticulin, and leptaculatin. The extracts are incorporated into various herbal preparations like speman, calshakti, envirocare, and chyawanprash. Notably, these extracts demonstrate effective activity against Dalton's ascites lymphoma in mice and inhibit various cancer cell lines in vitro.

2.9. *Ophiorrhiza mungos* L.

The endangered Mongoose plant, belonging to Rubiaceae and native to the limited regions of the Eastern and Western Ghats, is a medicinal plant of significance. Traditionally, its roots are utilized for cancer treatment and snakebite, while the root bark exhibits sedative and laxative properties. This half-woody, erect plant, reaching up to 30 cm in height, contains the cytotoxic quinoline alkaloid camptothecin (CPT), a highly valued anticancer compound.

Despite other plants like *Camptotheca acuminata* and *Nothapodytes nimmoniana* having higher CPT content, *Ophiorrhiza* species are valued for their herbaceous nature,

allowing for large-scale cultivation. The plant, containing CPT and luteolin-7-O-glucoside, demonstrates potential anticancer activity, making it noteworthy in medicinal applications.

2.10. *Rauvolfia serpentina* L. Benth. Ex Kurz.

Rauvolfia serpentina, commonly known as "Sarpagandha" and belonging to Apocynaceae, has a rich medicinal history dating back several thousand years. The dried roots of this plant contain various biologically active compounds, including reserpine, deserpidine, rescinnamine, ajamalacine, neoajmalin, ajmaline, serpentine, and α-yohimbine.

Utilized as a sedative, these roots are employed to manage conditions such as anxiety, high blood pressure, epilepsy, insomnia, and schizophrenia. Additionally, *R. serpentina* finds application in snakebites, insect stings, mental disorders, and cancer treatment. The indole alkaloid compound reserpine, derived from this plant, exhibits effective antiproliferative activity against various cancer cell lines, suggesting its potential for further use in cancer chemotherapy.

3. Conservation approaches

3.1. In situ conservation of wild medicinal plants

Medicinal Plant Conservation Areas (MPCA) were sites with known medicinal plant richness, 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". Species-focus MPCAs were established to conserve prioritized medicinal plants of high conservation concern. For example, *Saraca asoca* 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.

3.2. 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 MPCPs. 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.

3.3. Micropropagation and conservation

Micropropagation refers to the mass production of plant propagules from any part of the plant or cell. A rapid in vitro propagation method was developed *D. hamiltonii* through shoot multiplication using shoot tip explants. When calli were subcultured on MS media added with KN (1.5 mg/l) and BAP (2.5 mg/l) exhibited better shoot regeneration rate (95%) and rooted well in 1 mg/l KN contained MS media. The nodal explants showed better morphogenetic response with 95% regeneration frequency compared to leaf explants (85%). All

micropropagated plants exhibited superior growth properties in the field. In vitro rooting initiated better with the use of 1 mg/l IBA. Micropropagation and cloning of plant tissue based on different explants are commonly used to conserve different endangered plants. 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:

- 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
- Multiple shoots formation from the cultured explant
- Rooting of in vitro developed shoots
- Transplantation to the field following acclimatization

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 following.

- The ability of the biological system to minimize the growth and development in vitro
- Maintain viability of the stored material at the highest possible level along with the minimum risk of genetic stability
- Maintain full developmental and functional potential of the stored material when it is returned to physiological temperatures
- 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

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. There is direct as well as indirect somatic embryogenesis. In direct somatic embryogenesis, the plants develop directly from explants without any callus formation whereas dedifferentiation of callus to produce plants occurs in indirect somatic embryogenesis. It has great application in the rapid multiplication of endangered medicinal plants.

3.4. Long-term conservation through cryopreservation

Cryopreservation, a method for long-term preservation of plant genetic material, involves maintaining living cells at ultra-low temperatures, typically in liquid nitrogen at -196 °C. This freezing halt cellular metabolic activities, preventing genetic changes. It's valuable for conserving rare and endangered plant species by storing germplasm in suspended growth. Various tissues like seeds, pollen, embryos, and meristems can undergo cryopreservation. Notably, advantages include minimal maintenance, reduced contamination risks, and avoidance of continuous exposure to operator errors during frequent plant material manipulations. The principle behind cryopreservation is to bring the cells or tissues to a zero-metabolism stage by subjecting them to ultra-low temperature in the presence of cryoprotectants. In addition, modern techniques of Molecular Biology and Genetics can support to develop a simple and more efficient regeneration

systems and to conserve plant materials through pollen banking, seed banking, or storing in liquid nitrogen.

3.5. Traditional methods of conserving medicinal plants

The rural people who constitute the bulk of population are heavily dependent on the vegetation around them for fuel wood and for medicine. They are mainly subsistence farmers, and cannot afford alternative fuels, let alone the high prices of modern medicine. As a results vegetation is lost and environmental degradation takes place. Major steps have been taken towards conserving the medicinal plants. They include discouraging cutting down indigenous trees and encouraging the local people to plant fast growing exotic and Indigenous trees for domestic use, the inauguration of a national tree planting day and the creation of nature reserves. However, despite this intensified drive towards conservation, it is still difficult to prevent local people from destroying the plants around them. The planting of fast growing exotics is not a complete solution to the problem of environmental degradation, mainly because the locals still need indigenous plants as a source of medicine and for crafts such as carving. Local people do not approve of the planting of medicinal plants because of their belief that indigenous plants lose their curative properties when cultivated.

3.6. Conclusion

The Institute of Biodiversity Conservation (IBC) has launched a project, the Conservation and Sustainable Use of Medicinal Plants (CSMPP), aiming to address the depletion of medicinal plant biodiversity caused by both human activities and natural disasters. This initiative recognizes the potential of medicinal

plants not only for traditional healthcare but also as a source of income for farmers. The project aims to safeguard indigenous knowledge associated with the conservation and utilization of medicinal plants, which is rapidly diminishing. Conservation aims to promote sustainable development by safeguarding biological resources, preserving genetic diversity, and preventing the destruction of crucial habitats. It encompasses various activities such as collecting, propagating, characterizing, evaluating, indexing for diseases, eliminating threats, and storing for distribution. The conservation of plant genetic resources is recognized as a vital aspect of biodiversity preservation. Ex situ conservation, one of the methods employed, involves protecting plant populations outside their natural habitat. This approach is typically utilized to ensure the survival of endangered, at-risk, or deteriorating populations.

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