Conservation of Endangered Medicinal Plants by In Vitro Propagation Methods

M. V. Lakshmi, S. Jeyaraj, and T. S. Swapna

1 Introduction

Medicinal herbs grow naturally all around us, and they have been used to treat illness and maintain health for centuries by different cultures all over the world. These easily accessible and culturally significant traditional medicines are a vital source of livelihood for indigenous and rural populations [1]. For decades, people have used a wide variety of medicinal plants for their survival; mainly, they have been used in the primary healthcare system since ancient times. Present-day medications based on traditional knowledge are developed and preserved by different indigenous communities around the globe. Medicinal plant species have received scientific and commercial attention since they have immense potential in disease prevention and curing various diseases. Explorations on plant wealth by various research teams result in the identification of potential medicinal plants having the efficacy to be used in the production of plant-based medicines. World Health Organization (WHO) stated that 80% of developing countries rely on plant-based medicines to treat various diseases. Since the usage of medicinal plants is growing rapidly with the increasing demand for herbal drugs, natural health products, and secondary metabolites [2, 3]. However, these natural resources are declining due to human interventions like habitat destruction, pesticide usage, and overexploitation.

International Union for Conservation of Nature (IUCN) is an international organization devoted to studying the impact of human interventions on nature and protecting species and habitats for their survival. The Red List of threatened species designed by IUCN has become the popular data source on the extinction risk of species worldwide. Database from IUCN indicates that 50,000–80,000 flowering plant species are widely used for medicinal purposes. About 15,000 species are already threatened [4]. Traditional practitioners mainly obtain raw drugs from

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plants, particularly IUCN's rare, endangered, and threatened (RET) category. It is believed that 25% of the estimated 250,000 species of vascular plants may become extinct within the next 50 years [5, 6].

Moreover, poor seed germination, self-incompatibility, unavailability of pollinating agents, and lack of knowledge on the wealth of plants increased the risk of extinction of medicinal plants worldwide. Such loss would be irreversible if we did not take any conservation measures at the earliest. According to Cole et al. [7], the loss of plant species is 100–1000 times higher than the expected extinction rate, and for every 2 years, we are losing at least one potential plant having medicinal importance. Henceforth, the conservation of medicinal plants that are at the edge of extinction is the prime concern since efforts are to be made to sustain plant wealth. Here, in this chapter, the importance of endangered medicinal plants and their conservation strategies were discussed in detail.

2 Medicinal Plants: A Valuable Source of Herbal Products

Medicinal plants have many advantages; according to one estimate, each new plant-derived medication is worth $94 million to pharmaceutical corporations and $449 million to society [8]. Since, in recent years, medicinal herbs that exist in nature have received more attention from the scientific community and in the commercial trade. According to WHO, 2015 [9], around 60,000 plant species are used medicinally worldwide. India is one of the mega biodiversity-rich areas, with nearly 8000 species of medicinal plants belonging to 386 families and 2200 genera of flowering plants are the primary source of raw drugs [10]. Previous studies signify that most medicinal plants are flowering plants consisting of 33% trees, followed by herbs, shrubs, climbers, and lower forms like algae, ferns, and fungi [10]. In recent times, synthetic drugs have revolutionized health care worldwide, though the majority of people still depend on herbal medicines to treat various diseases. According to WHO, traditional medicine is the knowledge, skill, and practices mainly based on beliefs, theories, and experiences of different indigenous peoples in preventing and diagnosing physical and mental illness. The most common reasons for dependence on traditional medicines are more affordable and least side effects than synthetic medicines [11]. Similarly, the usage of traditional medicines increases when other practices are ineffective in treating diseases.

3 Endangered Medicinal Plants and Their Conservation

Medicinal plants are inevitable since they have been used to prevent, diagnose, and treat various life-threatening diseases from ancient times. Pharmaceutical industries and traditional practitioners obtain raw drugs from the leaves, roots, whole plant, bark, fruits, and seeds that are vital for plant survival and regeneration. However,

deforestation, illegal trading, over-grazing, climatic disasters, and destructive harvesting contribute to the shortage of medicinal plants, ultimately affecting their existence on Earth. At the same time, rare and endangered medicinal plant species are facing regeneration failure in nature due to low germination rate, malfunctioned floral organs, and unfavorable climatic conditions.

In 2015, IUCN added 44 Indian medicinal plants in its Red List database, of which 18 plants were categorized as vulnerable, 16 under endangered, and 10 as critically endangered species (Table 1). A species classified as critically endangered faces an extremely high risk of extinction in the wild. It means that the species population has been reduced or will be reduced by 80% within three subsequent generations. For wild species, it is the highest risk category assigned by the IUCN Red List. Overexploitation of medicinal plant trees like *Hippophae rhamnoides* and *Juniperus communis* for fuelwood, medicines, and fencing is the primary reason for their reduction in population density in the entire Indian trans-Himalaya [12, 13].

Conservation of threatened medicinal plants can be attained through various sustainable utilization practices, along with the development of appropriate regeneration approaches. Similarly, the review on trade regulations and their implementation, development of cultivation packages, sustainable collection practices, habitat management, surveys, and periodic assessment become a suitable approach for conserving endangered medicinal plants [14]. Among various biotechnological approaches, micropropagation was efficient in the augmented production of valuable secondary metabolites and conservation of a wide range of medicinal plants, including endangered, rare, and threatened plant species [15]. Even though in vitro propagation has a wide range of applications in plant research, many medicinal plants lack cost-effective methods for their effective utilization. Hence, efforts must be taken to devise efficient protocols before they are commercialized.

4 In Vitro Propagation of Various Endangered Medicinal Plants with Pharmacological Properties

The environmental concern for conserving medicinal plants has become an urgent focus of discussion. It has been reported that 15,000 species of medicinal plants are globally threatened due to the loss of habitat, overexploitation, invasive species, and pollution [4]. The commercial exploitation of medicinal plants for the requirement of various herbal products is increasing drastically without its restoration. Plants are described as endangered when their exploitation exceeds 95% and are likely to become extinct in the near future. The demand for these plant products is expanding annually at the rate of 15–25% [16]. Therefore, the sustainable use and conservation of the existing medicinal plant wealth have become a matter of urgency. The in situ conservation of wild genetic plant diversity in its natural habitat is one of the practical and appropriate management strategies. Nevertheless, this onsite conservation will be difficult for the plant species that do not develop viable seeds or too minute

Table 1 Details on Indian medicinal plants listed in Red List Database [14]

Si. No.	Botanical name	Vernacular name	Endemic to	Status	Uses
1	*Aconitum chasmanthum*	Gaping monkshood	Himalayan region of India and Pakistan	CE	It is a poisonous plant with over 60 alkaloids, glycosides, sugars, and elements in its tuber. Roots are employed in a variety of ayurvedic and homeopathic compositions after they have been mitigated.
2	*Chlorophytum borivilianum*	White gold	India	CE	It is effective in alleviating carnal desires and treating diabetes, arthritis, and natal and post-natal problems
3	*Gentiana kurroo*	Himalayan Gentian	North Western Himalaya	CE	Roots and rhizomes are used to treat cough, stomach ache, and fever.
4	*Gymnocladus assamicus*	Minkling	Northeast India	CE	It is used to cure dermatological disorders and to get rid of leaches. The plant parts are used in ethno-medicine due to its anthelminthic properties
5	*Lilium polyphyllum*	White lily	Pakistan, Afghanistan, and India.	CE	Underground bulbs are used medicinally as expectorant, astringent, aphrodisiac, antipyretic, and general debility
6	*Nardostachys jatamansi*	Indian nard	Mountain range of Himalaya in Nepal, Bhutan, Myanmar, Southwest China, and India	CE	Rhizomes are used to treat epilepsy
7	*Saussurea costus*	Indian costus	Jammu Kashmir and Himachal Pradesh in India	CE	Roots used as an antiseptic and in treating bronchial asthma
8	*Tribulus rajasthanensis*		Rajasthan and Gujarat in India	CE	The whole plant is used in treating fever, sterility, and skin problems
9	*Valeriana leschenaultia*		High altitude region of southern-western Ghats	CE	Treating the diseases of eye, blood, liver, hysteria, hypochondriasis, nervous unrest, and emotional stress
10	*Commiphora wightii*	Oleo gum	Dry regions of western India and adjoining areas of Pakistan	CE	Used to decrease cholesterol synthesis in the liver

seeds. The lack of seeds, seed dormancy, non-viable seeds, and endophytic bacterial and fungal contamination of plant materials are the major limitations in the conventional propagation method of many plants [17].

Totipotency of a cell refers to its ability to develop into a whole organism. In plant tissue culture system, the totipotent cells are grown in synthetic media, along with different plant growth regulators (PGR). Previous studies demonstrated the potential of in vitro culture in raising medicinal plants. The in vitro propagation techniques are widely used for the production of disease-free plants, genetically improved varieties, superior genotypes, and plants with enhanced secondary metabolite production. Plant tissue culture techniques were established at the end of the 1960s as a probable tool for the worthwhile production of plant secondary metabolites [18]. These micropropagation techniques are highly suitable for endangered, threatened, and rare species because of their increased coefficient of multiplication and small demands on the number of initial plants and space [19]. The use of explants such as apical shoots and axillary buds is better for the conservation of rare and endangered species [20]. The contamination from different sources like bacteria and fungi should be strictly avoided for fruitful cultures of plants. The standardization of the better combinations of macro- and microelements, vitamins, amino acids, growth regulators in plant tissue culture studies is the most significant step for the regeneration of these plants. Many endangered plant species having high-quality medicinal properties were successfully conserved through different micropropagation techniques. This is preferable for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large-scale re-vegetation, and genetic manipulation studies under precisely controlled physical and chemical conditions [21]. The integrated approach of these regeneration techniques contributes effective, disease-free, and value-added herbal products for future industries.

4.1 Seed Culture

Seed culture is an in vitro method for raising sterile seedlings. So far, only a few medicinal plants have been raised through seed culture. In particular, seeds of orchids are very minute and hard to germinate in in vivo conditions; hence, seed culture was found to be an apt method for the regeneration of orchids. Ethnopharmacological studies conducted in various orchids showed that they are very significant in treating several diseases such as skin and infectious diseases, problems concerning the digestive tract, respiratory and reproductive organs, against tumors, for pain relief, and for lowering fever [22]. Successful establishment of tissue culture protocol for an endangered wild plant species *Saussurea esthonica* was achieved from seeds [23]. The optimal conditions for the seed germination and in vitro seedling growth of Bambara groundnut (*Vigna subterranea*) based on different culture media compositions were developed by Kone et al. [24]. In vitro propagation is a proper method to propagate the endangered medicinal orchid,

Dendrobium lasianthera through mature seed culture [25]. The nutrient composition of culture media for orchids is a key factor in seedling growth [26]. Hesami et al. [27] established a detailed protocol for in vitro seed germination and callus formation of *Ficus religiosa*, an important indigenous medicinal woody plant. Shatnawi et al. [28] successfully multiplied the microshoots derived from the in vitro germinated seeds of *Achillea millefolium* and *Moringa peregrina* in the different concentrations of 6-benzyl amino purine (BA). The ex vitro and in vitro plants extract of *A. millefolium* and *M. peregrina* showed antimicrobial activity against the selected microorganism.

4.2 Embryo Culture

In embryo culture, embryos from seeds and ovules are allowed to grow in a nutrient-rich medium. The plantlets develop either directly from the embryo or indirectly through callus formation. *Strychnos potatorum* is a medicinally important endangered forest tree effective in treating gonorrhea, leukorrhea, gastropathy, bronchitis, chronic diarrhea, dysentery, renal and vesicle calculi, diabetes, conjunctivitis, scleritis, and ulcers [29]. Embryo culture was found to be an efficient way to conserve this medicinally important species using zygotic embryos [30]. Similarly, the zygotic embryo was used for the in vitro culture of an endangered tree species *Givotia rottleriformis* [31] and *Kelussia odoratissima* [32]. An efficient, reproducible protocol for the regeneration of *Oroxylum indicum*, an endangered and medicinally important forest tree was also achieved through zygotic embryo culture [33]. In 2022, Kushalan et al. [34] regenerated *Curculigo orchioides* an endangered medicinal plant, through somatic embryogenesis. *C. orchioides* is considered to have aphrodisiac, immunostimulant, hepatoprotective, antioxidant, anticancer, and antidiabetic activities [35].

4.3 Callus Culture

When a differentiated tissue produces a dedifferentiated mass of callus with no regular form, it is called callus culture. Callus can be developed from any part of the plant by adding hormones, particularly auxins, at certain concentrations [36]. Callus culture is categorized into embryogenic and non-embryogenic cultures. Callus-mediated organogenesis is a widely used technique in biotechnology to conserve medicinal plants. The conservation of endangered plant species continues to be a factor in the encouragement of callus culture, and it becomes a rich source of many therapeutic compounds [37]. The in vitro regeneration of *Rauvolfia serpentina* through somatic callus culture was introduced by Mitra and Chaturvedi in 1970

[38]. An effective regeneration protocol for an endangered and potential medicinal plant, *Thymus persicus*, was achieved via in vitro callus induction and indirect organogenesis on MS medium supplemented with varying concentrations of NAA and 2,4-D, alone or in combination with BAP and KN [39]. Likewise, an in vitro propagation protocol of *Zanthoxylum armatum* through leaf explants (via callus) with high shoot multiplication (10.4 shoots/callus piece) and 100% rooting was developed by Purohit et al. in 2020 [40].

4.4 Shoot Tip/Apical Meristem Culture

The propagation through meristem culture has potent biotechnological applications for the large-scale production of plants in a relatively short span of time with reduced contamination [41]. The meristem-derived plants are virus-free and are more advantageous from a practical and pharmaceutical view [42]. The apical meristem culture studies conducted in an endangered plant species *Curculigo orchioides*, resulted in the regeneration of multiple shoots, and an average of 125 plants were obtained from a single meristem [43]. *Curculigo latifolia* is another potent medicinal herb with anticancer [44] and antidiabetic properties [45] and has the ability to inhibit the hepatitis B virus [46]. This was brought into cultivation by collecting seedlings from the wild, resulting in its population decline [43]. As a result, in vitro plantlet regeneration from shoot tip cultures was established by Babaei et al. [47] for its effective production for future cultivation. *Gentiana kurroo* is a critically endangered medicinal herb utilized as a bitter tonic, antiperiodic, expectorant, antibilious, anthelmintic, anticancer, immunomodulatory, anti-inflammatory, and analgesic agent. The rapid micropropagation of *G. kurroo* through apical meristem has been developed by Kaushal et al. [48]. Recently (2020), Pe et al. [49] established an effective in vitro propagation protocol through meristem culture to produce *Hosta* X virus-free and genetically stable plants of an endangered species, *H. capitata*, which is endemic to Korea.

4.5 Protoplast Culture

Protoplasts are cells without cell walls, widely used for the regeneration of plantlets. Protoplast cultures are established by the removal of cell walls either by mechanical or by enzymatic processes [50]. Protoplast culture and plant regeneration of medicinally important plant *Tylophora indica* were attained through callus regeneration [51]. *Gentiana straminea*, an important medicinal plant in Chinese traditional medicine, threatened due to the uncontrolled collection and poor seed germination [52]. Later, Shi et al. [53] developed an effective protocol for the regeneration of *G. straminea* from its embryonic calli protoplasts.

4.6 Bud Culture

Bud culture is a type of plant tissue culture technique widely used for raising plantlets either through stem node (single node culture) or axillary buds (axillary bud culture). Kaur et al. [54] have standardized a quick method for the multiplication and conservation of *Inula racemose,* an endangered medicinal plant that is widely used in traditional medicine in India and Tibet. In addition to this, it is commonly used in pharmaceutical industries for its antispasmodic, antiasthmatic, and digestive properties. Dohling et al. [55] established an efficient protocol (axillary bud culture) for the rapid and large-scale regeneration of *Dendrobium longicornu*, an endangered and medicinally important epiphytic orchid seriously threaten due to overexploitation and habitat destruction in Northeast India. *Nilgirianthus ciliates*, a globally endangered aromatic slender shrub of the Western Ghats with extensive applications in Ayurveda, was also regenerated through in vitro nodal culture [56]. Lately (2021), Khan et al. [57] developed a well-defined protocol for the large-scale callus induction of *Saussurea costus* using four different explants (seeds, leaf, petiole, and internodes) as well as micropropagation from auxiliary buds of *S. costus.* A novel method, namely the nylon bag culture system, is considered useful in increasing the growth and development of *Jasminanthes tuyetanhiae*, a precious medicinal herb [58]. In order to increase the population of an endangered medicinal plant *Prunus africana*, Komakech et al. [59] developed a protocol for micropropagation using nodal segment explants due to its long flowering cycle and recalcitrant seeds. *P. africana* is one of the most popular medicinal plants for treating many diseases, including prostate cancer, benign prostatic hyperplasia, erectile dysfunction, urinary tract disorders, skin lacerations, kidney disease, chest pain, stomach upset, inflammation, and as an aphrodisiac.

4.7 Somatic Embryogenesis

Somatic embryogenesis is the development of a plant or somatic embryo from a vegetative or somatic cell. There are two ways through which somatic embryogenesis may occur, such as direct and indirect embryogenesis. In vitro propagation through somatic embryogenesis has been found successful in raising many medicinal plants [60–63]. It has been used to regenerate *Centella asiatica* and *Aloe barbadensis* [64, 65]. *Phellodendron amurense* is a medicinal plant that belongs to the family Rutaceae. Though it is conventionally propagated by means of seeds, the germination rate is meager [66]. In 2009, Azad et al. [67] established a plant regeneration protocol via somatic embryogenesis from hypocotyl and internodal explants of *P. amurense.*

An effective in vitro propagation method was developed for the micropropagation of *Psoralea corylifolia* through somatic embryogenesis in cell suspension culture [68]. Rhizome explants were used for in vitro plantlet regeneration of

Podophyllum hexandrum, a critically endangered medicinal plant, through direct organogenesis [69]. In 2017, Kumar et al. [70] successfully developed a promising regeneration system for *Hypoxis hemerocallidea*, an important medicinal plant of South African traditional medicinal system. Lately (2021), *Viola canescens*, an important, threatened medicinal herb, was regenerated successfully through somatic embryogenesis using leaf-derived calli [71].

5 Conclusion

The wild plant populations from which most medicinal plants are collected are under threat since they gain more scientific and economic attention around the globe. Due to human interventions, many medicinal plant species are at the brim of extinction. Due to commercial exploitation, traditional remedies have occasionally become unavailable to indigenous peoples who have relied on them for years or millennia. The study and conservation of medicinal herbs are indispensable since they have immense potential in curing various life-threatening diseases. Approximately 15,000 medical plant species are already on the verge of extinction worldwide. Experts predict that the Earth loses at least one key therapeutic candidate every 2 years. Hence, scientists and policymakers are proposing new procedures and policies to safeguard our remaining medicinal treasures in the wild through various Governmental and non-governmental organizations' involvement.

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