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A review on endangered medicinal plants of India and their conservation

S. SHARMA AND R. THOKCHOM

Dept. of Floriculture, Medicinal and Aromatic Plants, Faculty of Horticulture
Uttar Banga Krishi Viswavidyalaya, Pundibari- 736165, Cooch Behar, West Bengal

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ABSTRACT

India has a very rich plant biodiversity, many of which are medicinally useful. The rich resource is disappearing at an alarming rate as a result of over-exploitation. Therefore, the management of traditional medicinal plant resources has become a matter of urgency. An ever increasing demand of uniform medicinal plants based medicines warrants their mass propagation through plant tissue culture strategy. Tissue culture technology is potent and has opened extensive areas of research for biodiversity conservation. Plant in vitro regeneration is a biotechnological tool that offers a tremendous potential solution for the propagation of endangered and superior genotypes of medicinal plants which could be released to their natural habitat or cultivated on a large scale for the pharmaceutical product of interest. Tissue culture protocols have been developed for a wide range of medicinal plants, which includes endangered, rare and threatened plant species. Some of these endangered medicinal plants are Saussaurea lappa, Picorrhiza kurroa, Ginkgo biloba, Swertia chirata, Gymnema sylvestre, Tinospora cordifolia, Salaca oblonga, Holostemma, Celastrus paniculata, Oroxylum indicum, Glycyrrhiza glabra, Tylophora indica, Bacopa monnieri, Rauwolfia serpentina. The conventional means of propagation takes a long time for multiplication and also clonal non uniform. Conventionally, there are two methods of conservation: in situ and ex situ conservation, both are complementary to each other. In situ methods allow conservation to occur with ongoing natural evolutionary processes ex situ conservation via in vitro propagation also acts as a viable alternative for increase and conservation of populations of existing bioresources in the wild and to meet the commercial requirements. A review highlighting various in vitro protocols developed for selected rare and threatened plant species of India has been done to highlight the significance of ex situ conservation in cases where regeneration through conventional methods is difficult to undertake and species are left with low population in the wild. Thus in vitro cell and tissue culture methodology is envisaged as a mean for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large scale re-vegetation and for genetic manipulation studies.

***Keywords*:** Conservation, endangered, *in vitro*, medicinal plants

Medicinal plants have been the subjects of man's curiosity since time immemorial (Constable, 1990). Almost every civilization has a history of medicinal plant use (Ensminger *et al.*, 1983). Approximately 80% of the people in the world's developing countries rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts (Vieira and Skorupa, 1993). India has 2.4% of world's area with 8% of global bio-diversity and it is one of the 12th mega diversity hot-spot countries of the world with a rich diversity of biotic resources. Out of 34 hotspots recognized, India has two major hotspots - the Eastern Himalayas and the Western Ghats. The bio-geographic 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 semiarid conditions of Peninsular India, dry desert conditions of Rajasthan and Gujarat to the tidal mangroves of the Sunderban which harbours about 47000 species of plants of which 17 000 are angiosperms (Bapat *et al.*, 2008). India is also rich in medicinal plant diversity with all the three levels of biodiversity such as species diversity, genetic diversity, and habitat diversity (Mukherjee and Wahile, 2006). Across the country, the forests are estimated to harbour 90% of India's total medicinal plants diversity. Only about 10% of the known medicinal plants of India are restricted to non-forest habitats (Wakdikar, 2004). Concerning the total number of flowering plant species, although only 18,665, the intraspecific variability found in them makes it one of the highest in the world. Out of 18,665 plants, the classic systems of medicines like Ayurveda, Siddha, and Unani make use of only about 3000 plants in various formulations (Schippmann *et al.*, 2006). Although, there is no reliable figure for the total number of medicinal plants on Earth, and numbers and percentages for countries and regions vary greatly but estimates for the numbers of species used medicinally include: 35,000-70,000 or 53,000 worldwide (Schippmann *et al.*, 2002); 10,000- 11,250 in China (He and Gu, 1997; Pei, 2002; Xiao and Yong, 1998); 7500 in India (Shiva, 1996); 2237 in Mexico (Toledo, 1995); and 2572 traditionally by North American Indians (Moerman, 1998). The World Health

Email: rockythokchomaau@gmail.com

Organization (WHO) has estimated that the present demand for medicinal plants is approximately US $14 billion per year. The demand for medicinal plant-based raw materials is growing at the rate of 15 to 25% 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 (Kala *et al.,* 2006). According to Schippmann *et al.* (1990), one fifth of all the plants found in India are used for medicinal purpose. The world average stands at 12.5% while India has 20% plant species of medicinal value and which are in use. But according to Hamilton (2003), India has about 44% of flora, which is used medicinally. Although it is difficult to estimate the total number of medicinal plants present worldwide, the fact remains true that India with rich biodiversity ranks first in per cent flora, which contain active medicinal ingredient (Mandal, 1999).

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. On a global basis, the IUCN has estimated that about 12.5% of the world's vascular plants, totalling about 34 000 species are under varying degrees of threat (Phartyal *et al.,* 2002). IUCN recognises the following categories: 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). Many of them are facing extinction. In the past few decades, there has been an ever-increasing global inclination towards herbal medicine, followed by a belated growth in international awareness about the dwindling supply of the world's medicinal plants (Bodeker, 2002). The plants used in the phyto-pharmaceutical preparations are obtained mainly from the naturally growing areas. The genetic diversity of medicinal plants in the world is getting endangered at alarming rate because of ruinous harvesting practices and over-harvesting for production of medicines, with little or no regard to the future. Also, extensive destruction of the plant-rich habitat as a result of forest degradation, agricultural encroachment, urbanization etc. is other factors, thus challenging their existence (Gupta *et al.,* 1998). In view of the tremendously growing world population, increasing anthropogenic activities, rapidly eroding natural ecosystem, etc the natural habitat for a great number of herbs and trees are dwindling and of *per capita* consumption has resulted in unsustainable exploitation of Earth's biological diversity, exacerbated by climate change, ocean acidification, and other anthropogenic environmental impacts (Rands *et al.,* 2010). A large sum of money is pumped every year to replenish the lost biodiversity and large numbers of protocols are available at present. Unfortunately, we are not witnessing any improvement in the status of these plant species in nature and the number of threatened plant species is increasing gradually (Tripathi, 2008). In order to safeguard this knowledge, it should be documented, preserved and patented (Mukherjee, 2009). Even the United Nations Conference on Environment and Development (UNCED), held recently at Rio de Janeiro, Brazil helped to place the loss of biodiversity and its conservation on the global agenda. Therefore, the management of traditional medicinal plant resources has become the matter of urgency. Hence, conservation of such a buffer is considered fundamental and provided priority in all sectors of global development (Tandon *et al.,* 2009). Although species conservation is achieved most effectively through the management of wild populations and natural habitats (*in situ* conservation) but most of the medicinal plants either do not produce seeds or seeds are too small and do not germinate in soils. Even plants raised through seeds are highly heterozygous and show great variations in growth, habit and yield and may have to be discarded because of poor quality of products for their commercial release. Likewise, majority of the plants are not amenable to vegetative propagation through cutting and grafting, thus limiting multiplication of desired cultivars. Moreover many plants propagated by vegetative means contain systemic bacteria, fungi and viruses which may affect the quality and appearance of selected items (Murch *et al.,* 2000). Thus mass multiplication of disease free planting material becomes a general problem. In order to overcome these barriers, *ex situ* techniques can be used to complement *in situ* methods and, in some instances, may be the only option for some species (Sarasan *et al.,* 2006; Negash *et al.,* 2001). Therefore, conservation of medicinal plants can be accomplished by the *ex situ*, that is, outside natural habitat by cultivating and maintaining plants through long-term preservation of plant propagules in plant tissue culture repositories (Rands *et al.,* 2010).

In vitro techniques have been increasingly applied for mass propagation and conservation of germplasm as it has superiority over conventional method of propagation and offer some distinct advantage over alternative strategies. Some of these are as follows: (1) collection may occur at anytime independent of flowering period for each species (this assumes that seed material is not required), (2) there is the potential of virus elimination from contaminated tissue through meristem culture, (3) clonal material can be produced where this is useful for the maintenance of elite genotypes, (4) rapid multiplication may occur at any time where stocks are required using micropropagation procedures, (5) germination of difficult or immature seed or embryo may be facilitated for breeding programmes, and (6) distribution across the border may be safer, in terms of germplasm health status using *in vitro* cultures. Some more general positive advantages of *in vitro* techniques include the fact that storage space requirements are vastly reduced compared with field storage. Storage facilities may be established at any geographical location and cultures are not subject to environmental disturbances such as temperature fluctuation, cyclones, insect, pests, and pathogen (Bhojwani and Dennis, 1999; Shibli *et al.,* 2006). In this regard the micro-propagation holds significant promise for true to type, rapid and mass multiplication under disease free conditions. Besides, the callus derived plants exhibit huge genetic variation that could be exploited for developing superior clones/varieties particularly in vegetatively propagated plant species. Tissue culture has emerged as a promising technique for multiplying and conserving the medicinally important species within short period and limited space, which are difficult to regenerate by conventional methods and save them from extinction. In recent years, *in-vitro* cell and tissue culture methodology is envisaged as a mean for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large-scale re-vegetation and for genetic manipulation studies under precisely controlled physical and chemical conditions. Combinations of *in vitro* propagation techniques (Fay, 1992) and cryopreservation may help in conservation of biodiversity of locally used medicinal plants (Singh *et al.,* 2006).

Important medicinal plants of India

Aegle marmelos

Aegle marmelos (L.) Corr., (Family *Rutaceae*) commonly known as "Bael Tree" is a popular vulnerable medicinal plant mostly found in tropical and subtropical regions. Almost all parts of the tree are used in preparing herbal medicine for treating diarrhea, dysentery, dyspepsia, malaria, fever, jaundice, and skin diseases such as ulcers, urticaria, and eczema.The plant is rich in alkaloids, among which aegline, marmesin, marmin, and marmelosin are the major ones (Kala, 2006).

Acorus calamus

Acorus calamus Linn. (Family *Araceae*) commonly known as "sweet flag" or "Bach" is an important endangered medicinal plant. It is a semi aquatic herb with creeping rhizomes and sword shaped long leaves. The rhizomes possess anti-spasmodic, carminative and anthelmintic properties and also used for treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent fevers and tumors (Anon, 2000).

Celastrus paniculatus

Celastrus paniculatus Willd. (Family *Celastraceae*) commonly known as Malkangni, Jyotishmati and Bitter sweet is a rare and endangered important medicinal plant believed to sharpen the memory and also used to cure a number of diseases. It is a large, woody, unarmed climbing shrub occurring naturally in hilly parts of India up to an altitude of 1200 m. This plant is widely used to cure depression, paralysis, leprosy, fever, abdominal disorders and cancerous tumors. Chemical constituents of seeds as revealed by phytochemical analysis were sesquiterpene alkaloids like celapagine, celapanigine and celapanine (Sharma *et al.,* 2001).

Commiphora mukul

Commiphora mukul (Hook. ex Stocks) Engl. (Family *Burseraceae*) popularly known as "Guggul", is an important endangered medicinal plant species. It is widely distributed in tropical regions of Africa and Asia. It grows wild in the arid, rocky tracts of north-western regions of India. The plant exudes a medicinal oleo-gum resin ('Guggul') from incisions made on the bark in cold season. Gum is bitter, acrid, aromatic, pungent, carminative and stomachic stimulating the appetite and improving digestion. It is astringent, expectorant, anthelmintic, antispasmodic, anti-inflammatory, diuretic, depurative, anodyne, vulnerary, themogenic, antiseptic, nervine tonic, aphrodisiac, stimulant, emmenagogue and diaphoretic (Sosa *et al.,* 1993). It also possesses strong purifying and rejuvenating properties and is said to be a uterine stimulant. The main constituents of guggul include phytosterols, gugulipids and the ketonic steroid compound (guggulsterones) mainly E and Z gugguisterones. These are responsible for the lipid lowering effects of guggul (Singh *et al.,* 1997).

Peganum harmala

Peganum harmala L. (Syrian Rue), a medicinally important perennial herb of family *Nitrariaceae*, distributed over semi arid areas of North-West India, North-Africa and central Asia. Medicinally the fruits and seeds of this plant are digestive, diuretic, hallucinogenic, hypnotic, antipyretic, antispasmodic, nauseant, emetic, narcotic and uterine stimulant (Chatterjee, 1997). A red dye obtained from seeds is widely used in Turkey and Iran for colouring carpets. Leaves are useful in asthma, colic, dysmenorrhea, hiccup, hysteria, neuralgia and rheumatism. The plant has also been used as antimicrobial, antitumoral, in curing malaria and has insecticidal potential (Kiritikar, 1995).

Prosopis cineraria

Prosopis cineraria (Family *Fabaceae*) is a versatile species commonly known as Jhand or Khezri. *Prosopis* species are the dominant species in Indian desert. *P. cinerarium* has a very good economic importance in arid regions and is assumed to treat snake bite and scorpion stings. Green pods of this plant are used as food. This species is highly drought tolerant and can withstand in the area having 50mm rainfall annually.

***Simmondsia chinensis* (Jojoba)**

It belongs to family *Simmondsiaceae*. It is an evergreen, dioecious desert shrub which grows wildly in Sonora desert of Arizona, northern Mexico, southern and Baja California. Jojoba is now cultivated commercially in Argentina, Australia, Chile, Egypt, India, Israel, Mexico, Peru, South Africa and the USA. Jojoba seed oil is being utilized in industrial lubricants, and in pharmaceutical and cosmetic industries. The liquid wax that makes 40–60 % of seed dry weight has properties similar to sperm whale oil. Due to the ban on the import of sperm whale products into USA and other countries, jojoba is gaining commercial importance at international level (Reddy and Chikara, 2010).

***Spilanthes acmella* Murr**

It belongs to the *Asteraceae* family and is commonly known as Akarkara or Toothache plant. This plant is widely distributed in the tropical and sub-tropical regions. The flowers and leaves of this plant have been used as traditional medicine for stammering, toothache, stomatitis and throat complaints. It has potent diuretic activity and the ability to dissolve urinary calculi. The plants have shown anti-inflammatory, antibacterial and antifungal properties. Spilanthol, the most active antiseptic alkaloid extracted from this plant, is found effective at extremely low concentrations against blood parasites, and indeed is a poison to most invertebrates while remaining harmless to warm-blooded creatures (Anon., 1989).

***Stevia rebaudiana* Bertoni**

It belongs to the *Asteraceae* family, a natural sweetener perennial herb commonly known as “Sweet Weed”, “Sweet Leaf”, “Sweet Herbs” and “Honey Leaf”. The leaves of this plant are estimated to be 300 times sweeter than sucrose and the sweetness is due to glycosides of which the most abundant is stevioside.(Dushyant *et al.*, 2014) The increasing consumption of sugar (sucrose) has resulted in several nutritional and medical problems, such as obesity. Therefore, low caloric sweeteners have been investigated to substitute sugar. The refined extracts of leaves of this plant are officially used as high potency natural-source, low calorie (non sucrose) sweetener in processed foods, artificial diets and pharmaceuticals. The sweet compounds pass through the digestive process without chemically breaking down, making it safe for diabetic and obese people (Mizutani and Tanaka, 2002).

Sapindus mukorossi

Sapindus mukorossi (Family: Sapindaceae) popularly known as ‘Ritha’ and ‘Soapnut’, is a most important deciduous tree of tropical and sub- tropical regions of Asia. The fruit of this tree contains saponins, the most active secondary metabolites extracted from this plant. It is a good substitute for washing soap and is as such used in preparation of quality shampoos, detergents etc. The fruit is of considerable importance for its medicinal value for treating a number of diseases like common cold, pimples, epilepsy, constipation, nausea etc. It is also used as expectorant and anthelmintic in small doses (Anon., 1992).

Bacopa monnieri

Bacopa monnieri belonging to the family *Scrophulariaceae* is a very popular herb in India for longevity and mental function. It is used to decrease fatigue and depression, and to stimulate the sex drive. It energizes the central nervous system, and aids the circulatory system, soothes and minimizes varicose veins and helps to minimize scarring. It is also useful in repairing skin and connective tissues and smoothing out cellulite. It is generally considered an Ayurvedic "age tonic" restoring youth and vitality. Brahmi has been used by Ayurveda in India for almost 3000 years. The Ayurvedic treatise, the Charaka Samhita (100A.D.), recommends Brahmi in formulations for a range of mental conditions including anxiety, poor cognition and lack of concentration. In India, Brahmi is currently recognized as being effective in the treatment of mental illness and epilepsy.

Glycyrrhiza glabra

It was one of the most widely known medicines in ancient history, and records of its use include Assyrian tablets of around 2000 BC and Chinese herbals of the same period. Theophrastos of Lesbos, writing in the fourth century BC wrote that 'it has the property of quenching thirst if one holds it in the mouth'. Dioscorides gave the plant its botanical name (Greek glukos = sweet, riza = root). Its 13th century English name was Lycorys, a corruption of glycyrrhiza.. Liquorice (*Glycyrrhiza glabra*) belonging to the family *Fabaceae* has long been used for both culinary and medical purposes. Used for flavoring and sweetening candies and medical remedies, licorice also has potent effects of its own, particularly for ulcers and adrenal insufficiencies. It is also used for asthmatic coughs, as an antispasmodic and ulcer remedy, and to cool 'hot' conditions.

Holostemma ada-kaodien

Holostemma ada-kodien (Synonym – *Leptadenia reticulate*, W & A) commonly known as jivanti belongs to family *Asclepiadaceae*. From the plant, stigma sterol and tocopherols are isolated, hentriacontanol amyrin, amyrin, stigmasterol and sitosterol are also isolated from the stem and leaves. From the roots – lupeol, amyrin, sitosterol, alanine, aspartic acid, glycine, serine, threonine and valine can be isolated. Jivanti is sweet in taste, sweet in the post digestive effect and has cold potency. It alleviates all the three doshas, namely, vata, pitta and kapha. It possesses light and oily attributes. It is a rejuvenative, heart (caksusya and hrdya). It is used in diseases like fever, tuberculosis, burning sensation of the body and raktapittal. Mainly the roots and the whole plant are used for medicinal purposes. Externally the paste of its leaves and roots alleviate oedema due to vitiation of pitta dosa. The herb is beneficial for external use in various skin diseases, wounds and inflammation of the skin.

Oroxylum indicum

It belongs to family *Bignoniaceae*. Syonaka is astringent and bitter in taste, pungent in the post digestive effect and has cold potency. It possesses light and dry attributes. It is used in rheumatic disorders, diarrhea, cough, diabetes and cystitis. The skin of roots of syonaka is used for medicinal purpose, both, externally as well as internally. Used externally as a paste of its skin of roots, it dries up the discharges and promotes the wound healing. The tub bath with its decoction relieves the swelling and pain in rheumatic disorders. The medicated oil of syonaka in sesame oil base instilled into ears mitigates the pain in otitis. The decoction of its sroot-skin is an effective gargle in stomatitis. The root skin is also useful in dressing the wounds in soft chancre (upadamsa). Internally, syonaka is a panacea for arthritis and rheumatism. The decoction of the roots is commonly used for arthritis. In diarrhea and dysentery the decoction combined with mocarasa (gum of samali *Bombax malabaricum*). It is given along with honey. Syonaka also stimulates appetite, improves digestion and is vermicidal.

Picrorhiza kurroa

Kutki or *Picrorhiza Kurroa* is a herbal medicinal plant from *Scrophulariaceae* family. It is also known as hellbore, katuka, kurri, Katuko, Kuru, Katukarogani. It is found in Himalayan region in India. This herbal medicine has shown effective therapeutic action in liver disorders. The crude extract of plant shows good results in liver damage caused by carbon tetrachloride, paracetamol, galactosamine and alcohol. According to Ayurveda the plant has utility as laxative, liver-stimulant, appetite and stimulant, febrifuge. The plant is also beneficial in bronchial asthma and epidemic jaundice. It is also used to ease stomachache, and is believed to promote appetite. The herb is also effective in 'Kapha'disorders, billow fever, urinary discharge, hiccup, blood troubles, burning sensations and leucoderma.

Saussurea lappa

Saussurea lappa (Family *Asteraceae*) is a tall, robust perennial Herb; leaves simple, large pubescent, heart shaped radical leaves with long petiole. The genus Saussurea has many endemic species in Hindukush, Himalayan region. The species is mainly confined to Kashmir. In the northern areas, it is confined to Astore and Minimerg forest ranges. The most important locations where these species grow wild include Kalapani, Kamari and Thanknala, Mapno and Kilshai where this species is found growing in betula forests on hill slopes at a height of 2438-3657 meters in Himalayas. In Pakistan this species is found in Kaghan and Azad Kashmir. Roots is tonic, stomachic, stimulant, carminative, used for asthama, diuretic, antiseptic, cough, cholera, aphrodisiac, anthelmintic and also used to insecticide, pesticide. The roots are highly aromatic used in perfumeries, also used for skin diseases. Locally it is used against the heart diseases of cattle and for toothache. The powdered roots are sprinkled over crops as insecticides. Externally the roots are used as an ointment or powder for the treatment of maggot-infested wound.

Swertia chirata

Swertia chirata (Family *Gentianaceae*) is found is in the temperate Himalayas at an altitude of 1,200-3,000m from Kashmir to Bhutan and in the Khasi hills in Meghalaya at a height of 1,200- 1,500 m. According to Ayurveda, this herb is a bitter tonic, stomachic. It is useful in liver disorders, eyes, heart. It is an excellent remedy for a weak stomach, especially when this gives rise to nausea, indigestion and bloating and it has also been shown to protect the liver. It is best known as the main ingredient in Mahasudarshana churna, a remedy containing more than 50 herbs. It also contains xanthones which are reputedly effective against malaria and tuberculosis, and also amarogentin, a glycoside that may protect the liver against carbon tetrachloride poisoning. The whole plant is an excellent drug for intermittent fevers, skin diseases intestinal worms, bronchial asthma, burning of the body, regulating the bowels. The root of the plant is useful in checking hiccups and vomiting. It is used in the liquor industry as a bitter ingredient.

Tinospora cordifolia

Tinospora cordifolia commonly known as Guduchi belonging to the family *Menispermaceae* is a famous plant of traditional use and also a powerful

Table 1: List of the some endangered and economically important medicinal plants of India

Sl. No.	Plant species	Family	Explants	References
1.	*Aegle marmelos*	Rutaceae	Nodal segments and shoot tip	Yadav and Singh (2011a)
2.	*Acorus calamus*	Araceae	Rhizome tip and Rhizome segments	Yadav *et al.*(2011)
3.	*Celastrus paniculatus*	Celastraceae	Seeds, nodal segments & shoot tip	Lal and Singh (2010) Lal *et al.* (2010)
4.	*Commiphora mukul*	Burseraceae	Leaf segments, apical and nodal segments	Singh *et al.* (2010b)
5.	*Peganum harmala*	Nitrariaceae	Seeds	Goel *et al.*, (2009)
6.	*Prosopis cineraria*	Fabaceae	Seeds	Kumar and Singh (2009)
7.	*Simmondsia chinensis*	Simmondsiaceae	Nodal segments	Kumar *et al.* (2010)
8.	*Spilanthes acmella*	Asteraceae	Nodal and intermodal segments	Yadav and Singh (2010) Yadav and Singh (2011b)
9.	*Stevia rebaudiana*	Asteraceae	Apical and nodal segments	Kumar and Singh (2009) Singh *et al.* (2011)
10.	*Sapindus mukorossi*	Sapindaceae	Leaf segments, apical and nodal segments	Singh *et al.* (2010a)
11.	*Bacopa monnieri*	Scrophulariaceae	Leaf explants and nodal segments	Mohapatra and Rath (2005)
12.	*Ginkgo biloba*	Ginkgoaceae	Apical and nodal segments	Tommasi & Scaramuzzi (2004)
13.	*Glycyrrhiza glabra*	Papilionaceae	Nodal segments	Vadodaria *et al.*, (2007)
14.	*Gymnema sylvestre*	Asclepiadaceae	Seeds	Komalavalli & Rao (2000)
15.	*Holostemma ada-kodien*	Asclepiadadeae	Nodal segments	Martin (2002)
16.	*Oroxylum indicum*	Bignoniaceae	Nodal segments	Dalal & Rai (2004)
17.	*Picrorhiza kurroa*	Scrophulariacae	Nodal segments	Martin *et al.* (2006)
18.	*Saussurea lappa*	Compositae	Shoot tip	Johnson *et al.* (2007)
19.	*Swertia chirata*	Gentianaceae	Shoot tip	Balaraju *et al.* (2009)
20.	*Tinospora cordifolia*	Meninspermaceae	Nodal segments	Gururaj *et al.* (2007)

rasayana mentioned in Indian ayurvedic literature. It is considered as a bitter tonic and powerful immuno modulator. Guduchi is a perennial plant of weak and fleshy stem found throughout the India. The aerial roots that arise from the stem are thread like. The leaf is heart shaped and smooth. The flowers are yellowish in colour emerges in bunch in rainy season. The fruits of guduchi are pea like which are seen in winter in India. Guduchi acts as a diuretic and found to be effective against renal obstruction like calculi and other urinary disorders. Guduchi acts as a memory booster, develops intelligence, and promotes mental clarity. It is described as one of the Medhya Rasayana (mental rejuvenative) in the Charak Samhita (The

Table 2: Medicinal plant species of conservation concern identified for West Bengal

Sl. No.	Botanical Name	Status	Sl. No.	Botanical Name	Status
1.	*Panax pseudoginseng* Wall.	Critically Endangered	23.	*Ampelocissus barbata* (Wall.) Planch.	Endangered
2.	*Persea glaucescens* (Nees) Long	Critically Endangered	24.	*Asparagus racemosus* Willd.	Endangered
3.	*Picrorhiza kurroa* Royle ex Benth.	Critically Endangered	25.	*Celastrus paniculatus* Willd.	Endangered
4.	*Podophyllum hexandrum* Royle	Critically Endangered	26.	*Cinnamomum cecidodaphne* Meissn.	Endangered
5.	*Swertia chirayita* (Roxb. ex Flem.) Karst.	Critically Endangered	27.	*Dioscorea prazeri* Prain & Burkill	Endangered
6.	*Taxus wallichiana* Zucc	Critically Endangered	28.	*Drosera burmannii* Vahl	Endangered
7.	*Aconitum bisma* (Buch.-Ham.) Rapaics	Endangered	29.	*Gynocardia odorata* R.Br.	Endangered
8.	*Aconitum ferox* Wall. ex Seringe	Endangered	30.	*Helminthostac hys zeylanica* (L.) Hook.	Endangered
9.	*Aconitum spicatum* (Bruhl) Stapf	Endangered	31.	*Lycopodiella cernua* (L.) Pichi- Sermolli	Endangered
10.	*Alpinia calcarata* Roscoe	Endangered	32.	*Mesua ferrea* L.	Endangered
11.	*Mucuna pruriens* (L.) DC.	Endangered	33.	*Gymnema sylvestre* R.Br.	Endangered
12.	*Ophioglossum reticulatum* L.	Endangered	34.	*Lumnitzera racemosa* Willd.	Vulnerable
13.	*Pterocarpus marsupium* Roxb.	Endangered	35.	*Morinda citrifolia* L.	Vulnerable
14.	*Rauvolfia serpentina* (L.) Benth. ex Kurz	Endangered	36.	*Nypa fruticans* (Thunb.) Wurmb.	Vulnerable
15.	*Sonneratia caseolaris* (L.) Engl.	Endangered	37.	*Olax nana* Wall.	Vulnerable
16.	*Aristolochia indica* L.	Endangered	38.	*Pericampylus glaucus* (Lam.) Merr.	Vulnerable
17.	*Berberis aristata* DC.	Vulnerable	39.	*Stereospermu m colais* (Dillwyn) Mabb.	Vulnerable
18.	*Cinnamomum bejolghota* (Buch.-Ham.) Sweet	Vulnerable	40.	*Thalictrum foliolosum* DC.	Vulnerable
19.	*Desmodium motorium* (Houtt.) Merr.	Vulnerable	41.	*Toona ciliata* M.J.Roem.	Vulnerable
20.	*Gloriosa superba* L.	Vulnerable	42.	*Xylocarpus granatum* Koenig	Vulnerable
21.	*Abelmoschus moschatus* Medik.	Near Threatened	43.	*Tylophora indica* (Burm.f.) Merr.	Near Threatened
22.	*Ipomoea mauritiana* Jacq.	Near Threatened			

oldest and most potent book of ayurvedic medicine). Guduchi is regarded as a liver protector. CAMP workshop was held during December 2007 at Kolkata to assess the threat status of prioritized Medicinal plants of West Bengal. During this process 43 species were assigned the Red List status of Near Threatened (NT) and above (Table 2).

A general overview of beginning of micropropagation of medicinal plants

In vitro culture is one of the key tools of plant biotechnology that exploits the totipotency nature of plant cells (Haberlandt, 1902) and unequivocally demonstrated for the first time in plants by Steward *et al.* (1964). Beyond the discovery of kinetin (Miller *et al.*, 1955), the major work on *in vitro* regeneration has been cantered around tobacco (*Nicotiana tabacum* L.) tissue culture, culminating in the first convincing demonstration of the control of differentiation of shoots or roots or both by the kinetin-auxin ratio (Skoog and Miller, 1957) followed by carrot (*Daucus carota* L.) tissue culture and birth of the concept of totipotency of plant cell with the regeneration of complete flowering plants of carrot from its phloem cells (Steward *et al.*, 1964). Thus, the micropropagation of medicinal plants remained neglected till complete plants of *Rauvolfia serpentine* (L.) Benth., were produced from its somatic callus tissue (Mitra and Chaturvedi,1970). Plant tissue culture refers to growing and multiplication of cells, tissues and organs of plants on defined solid or liquid media under aseptic and controlled environment. The commercial technology is primarily based on micropropagation, in which rapid proliferation is achieved from tiny stem cuttings, axillary buds, and to a limited extent from somatic embryos.

The process of micropropagation is usually divided into several stages *i.e.,* pre- propagation, initiation of explants, subculture of explants for proliferation, shooting and rooting, and hardening. These stages are universally applicable in large-scale multiplication of plants. The field performance of these tissue cultured plants depends on the selection of the initial material, media composition, growth regulators, cultivar and environmental factors (Chang *et al.,* 1994).

The effects of auxins and cytokinins on shoot multiplication of various medicinal plants have been reported by Skirvin *et al.* (1990). Lal and Ahuja (1996) observed a rapid proliferation rate in *Picrorhiza kurroa* using kinetin at 1.0–5.0 mg/l. Barna and Wakhlu (1998) has indicated that the production of multiple shoots is higher in *Plantago ovata* on a medium having kinetin along with NAA. Faria and Illg (1995) have also shown that the number of shoots per explant depends on concentrations of the growth regulators and the particular genotypes. The nature and condition of explants has also been shown to have a significant influence on the multiplication rate. Mao *et al.* (1995) reported that the actively growing materials were more responsive to shoot induction than dormant buds in *Clerodendrum colebrookianum*. Also BAP was proved superior to 6- purine (2ip) and TDZ for multiple shoot induction. The cultured cells and tissue can take several pathways to produce a complete plant. Among these, the pathways that lead to the production of true- to-type plants in large numbers are the popular and preferred ones for commercial multiplication (Bhojwani and Razdan, 1983; Pierik, 1989).

In clonal propagation techniques using shoot tip and nodal segments are must for mass-scale multiplication and conservation of an endangered or threatened and medicinally important species within short period and limited space. The plants produced from this method are true to type. Propagation through tissue culture provides solution for mass propagation of plants in general and threatened plants in particular. There is a need to conserve plants with medicinal values. Due to ever growing demand, the availability of medicinal plants to the pharmaceutical companies is not enough to manufacture herbal medicines. The powerful techniques of plant cell and tissue culture, recombinant DNA and bio-processing technologies have offered mankind a great opportunity to exploit the medicinal plants under *in vitro* conditions. Micropropagation: In clonal propagation, plants are multiplied using nodal segments and shoot meristems as explants. For rapid *in vitro* clonal propagation of plants, normally dormant axillary buds are induced to grow into multiple shoots by judicious use of growth regulators cytokinins and or auxin and cytokinin combinations. Shoot number increases logarithmically with each subculture to give greatly enhanced multiplication rates. As this method involves only organized meristems, hence it allows recovery of genetically stable and true to type progenies (Murashige, 1974; Hu and Wang, 1983).

Regeneration and organogenesis

For the regeneration of a whole plant from a cell or from a callus mass cyto-diffrentiation is not enough and there should be differentiation leading to organogenesis. This may occur through shoot bud differentiation (organogenesis) or through somatic embryogenesis. In the former, shoot buds (monopolar structures) are formed while in the later, somatic embryos (bipolar structures) are formed both leading to regeneration of whole plant. Callus mediated organogenesis depends on various factors. The type of callus, growth regulators used for induction of callus and also callus developed from the type of explant. The cells, although undifferentiated, contain all the genetic information present in parent plant. By suitable manipulation of growth regulators and contents of the medium, it is possible to initiate the development of roots, shoots and complete plant from callus cultures. In this pathway, groups of cells of the apical meristem in the shoot apex, axillary buds, root tips, and floral buds are stimulated to differentiate and grow into shoots and ultimately into complete plants. The explants cultured on relatively high amounts of auxin form an unorganized mass of cells, called callus. The induction of callus growth and subsequent differentiation and organogenesis is accomplished by the differential application of growth regulators and the control of conditions in the culture medium. With the stimulus of endogenous growth substances or by addition of exogenous growth regulators to the nutrient medium, cell division, cell growth and tissue differentiation are induced. There are many reports on the regeneration of various medicinal plants via callus culture. Pande *et al.* (2002) have reported the successful in-vitro regeneration of *Lepidium sativum* from various explants on MS medium supplemented with 4.0 mg/l BAP and NAA. The role of auxins and cytokinins in callus induction was also advocated by Kumar and Singh (2009) in *Stevia rebaudiana*, Goel and Singh (2009) in *Peganum harmala*, Kumar and Singh (2009) in *Prosopis cineraria*, Lal and Singh (2010) in *Celastrus paniculatus*, Yadav and Singh in *Spilanthes acmella* (2010) and *Aegle marmelos* (2011). Out of various cytokinins tested, BAP was the most effective for inducing bud break. Effectiveness of BAP was also observed in *Leucaena leucocephala* (Singh and Lal, 2007), *Prosopis cineraria* (Kumar and Singh, 2009), *Splianthes acmella* (Yadav and Singh, 2010) and *Acorus calamus* (Yadav *et al.*, 2011).

Somatic embryogenesis

Somatic embryogenesis is the process of formation of embryo like structure from somatic tissue. The somatic embryo may be produced either directly on the explant or indirectly from callus or cell suspension culture. For the first time, Haccius (1978) defined somatic embryogenesis as a non-sexual developmental process, which produces a bipolar embryo from somatic tissue. The first report of plantlet regeneration via *in vitro* somatic embryogenesis was in *Daucus carota* (Reinert, 1958; Steward *et al.*, 1958). This pathway has offered a great potential for the production of plantlets and its biotechnological manipulation. In addition to the development of somatic embryos from sporophytic cells, embryos have been induced from generative cells such as in the classic work of Guha and Maheshwari (1964) with *Datura innoxia* microspores. In this pathway, groups of somatic cells/tissues lead to the formation of somatic embryos which resemble the zygotic embryos of intact seeds and can grow into seedlings on suitable medium. The primary somatic embryos are also capable of producing more embryos through secondary somatic embryogenesis. Plant regeneration via somatic embryogenesis from single cells, that can be induced to produce an embryo and then a complete plant, has been demonstrated in many medicinal plant species (Tripathi and Tripathi, 2003). Arumugam and Bhojwani (1990) noted the development of somatic embryos from zygotic embryos of *Podophyllum hexandrum* on MS medium containing BAP and IAA. Efficient development and germination of somatic embryos are prerequisites for commercial plantlet production. Chand and Sahrawat (2002) reported the somatic embryogenesis of *Psoralea corylifolia* L. from root explants on medium supplemented with NAA and BAP. Rooting of shoots was best achieved using different concentrations of auxins. In *A.maemelos*, MS half strength medium supplemented with IAA proved better (Yadav and Singh, 2011). In P. cineraria, rooting was achieved on half strength MS medium supplemented with 3.0 mg/l IBA (Kumar and Singh, 2009), while in *L. leucocephala*, NAA resulted in better root formation (Singh and Lal, 2007).

Acclimatization and Transfer of micro-propagated plantlets to the soil

Complete regenerated plantlets with sufficient roots were taken were gradually pulled out from the medium and immersed in water to remove the remains of agar-agar particles sticking to the root system by using a fine brush. These plantlets were transferred to pots containing mixture of sterilized soil and sand (3:1). The potted plantlets were covered with a transparent polythene bag to ensure high humidity around the plants. The pots were supplied with MS (half strength) salt solution on alternate days. After about two weeks the polythene bags were removed for 3-4 hours daily to expose the plants to the conditions of natural humidity for acclimatization. These plants were shifted to bigger pots after one month of its transfer and were maintained under green house conditions. Successful acclimatization and field transfer of the *in vitro* regenerated plantlets have also been reported in *Leucaena leucocephala* (Singh and Lal, 2007), *Peganum harmala* (Goel *et al.,* 2009), *Celastrus paniculatus* (Lal and Singh, 2010).

Exvitro field evaluation of acclimated plants

These recent advances in plant tissue culture have resulted in the development of protocols for micropropagation of many important medicinal plants, but the process of transplantation and acclimatization of micropropagated plants to soil environment continues to be a major bottleneck in the micropropagation of medicinal plants. Acclimatization of a micropropagated plant to a green house or field environment is essential because anatomical and physiological characteristics of *in vitro* plantlets necessitate that they should be gradually acclimatized to the field environment (Hazarika, 2003). Successful acclimatization minimizes the percentage of dead or damaged plants, enhancing the plant growth and establishment (Sha Valli Khan, 2003). Dynamics of the process are related to the acclimatized plant species and both *in vitro* and ex vitro culture conditions (Pospisilova *et al.,* 1999). Now days, mycorrhizal technology can be applied to reduce transplantation shock during acclimatization, thus increasing plant survival and establishment rates of micropropagated medicinal plant species (Sharma *et al.,* 2008; Yadav *et al.,* 2011).

Medicinal herbs as potential source of therapeutics aids has attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as potential material for maintaining proper health. Fresh strategies of afforestation management and restoration of depleting natural resources blending with modern technologies are also required. Medicinal plants are under the threat of overexploitation and biodiversity depletion. There is urgent need of their *ex situ* conservation. Study of collection and *in vitro* conservation of important plants in the present study opens fresh avenues towards the conservation and resource management of the overexploited medicinal plants. Biotechnology is a motor of technological advancement in both the developed and developing countries though at different levels in scope and content. In recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions rather than have indifferent populations. Thus *in vitro* cell and tissue culture methodology is envisaged as a mean for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large scale re-vegetation and for genetic manipulation studies. Tissue culture protocols have been developed for several plants but there are many other species, which are over exploited in pharmaceutical industries and need conservation.

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