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A Review on Biotechnological Aspects of Conservation and Enhancements of Indian Snakeroot (*Rauwolfia serpentina*)

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Abstract

Indian snakeroot (Rauwolfia serpentina) is one of the most important medicinal plants found in the Indian subcontinent, used in the treatment of various types of diseases. Recently it has been considered as a critically endangered plant by the International Union of the Conservation of Nature and Natural Resources (IUCN) due to its heavy exploitation in the past. The plant has a high demand in the international market due to its medicinal properties and abundance of phytochemicals, which means it is necessary to save it from extinction as well as to improve the quality and quantity of the phytochemicals produced by the plant. Indian snakeroot has an abundance of an indole alkaloid–reserpine which can cure hypertension and other mental disorders, some more phytochemicals can be found in the plant but very low concentration. In-vitro micropropagation methods could be a solution for this problem, it could be effectively used in stabilizing the population of the plant as well as. The review is mainly focused on a study of pharmacological aspects of Indian snakeroot, and the biotechnological methods for conservation and enhancements of some endangered medicinal plants.

Keywords: Indian Snakeroot, Conservation, Micropropagation, Plant Tissue culture, Genetic engineering, CRISPER cas-9, Pharmaceutical activities.

Introduction

India is known to possess the oldest and most effective knowledge of medicinal plants for the remediation of several kinds of illness. According to a study, almost 80% of the world's population relies on the potential of medicinal plants owning the fact that herbal medicinal plants are highly effective, safe, cheap, and easily accessible around the world. Nevertheless, the human population is growing day by day and humans are destroying the quality of nature which is a huge threat to most of the plant species [1]. In India, there is a huge diversity of herbal medicinal plants and most of them are medicinally important for the people especially the communities of remote areas. This treasure trove of medicines is disappearing day by day due to their overexploitation, so it is crucial to saving those important medicinal plants from being extinct [2–3]. Some of the important Endangered medicinal plants include Table 1.

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India had developed many herbal drugs under alternative medicine systems known as Ayurveda, Unani, Siddha, yoga, and naturopathy, millions of Indians use the herbs as a natural remedy, supplements, and spices [10].

Methods of Conservation

There is a variety of methods available for the conservation of medicinal plants. The peoples who

are thriving in rural areas are dependent on natural resources to fulfill their food and energy requirements, consequently, they cut down trees along with herbs. This could be prevented by enforcing strict laws and restrictions on cutting the trees and planning some protocols for controlling the ecological loss. Biotechnological methods can also be applied in the conservation of medicinal plants.

Table 1. Medicinal Plants and their importance

No.	Common Name	Botanical Name	Status	Importance	Reference
1	Monk's hood	*Aconitum chasmanthum*	Critically endangered	Expectorant, Anti-inflammatory, Hepatoprotective	[4]
2	Chembilaa	*Cinnamomum wightii*	Endangered	Paralytic disorders, Gastro-protectant, Dysuria	[5]
3	Himalayan soap pod tree	*Gymnocladus assamicus*	Critically endangered	Antioxidant	[6]
4	Indian costus	*Saussurea costus*	Critically endangered	Anti-inflammatory, Anticancer, Hepatoprotective, Immunomodulator	[7]
5	Spanish chamomile	*Anacyclus pyrethrum*	Vulnerable	Antibacterial, Antioxidant Antiviral, Hepatoprotective, Anticarcinogenic, cardioprotective	[8]
6	Pimpinella tirupatiensis	*Pimpinella tirupatiensis*	Endangered	Antihyperglycemic, Nephroprotective, Antimicrobial, Hematological	[9]

In Situ Conservation

In this method of conservation, species are preserved in their natural habitat. The method is all about setting up large protected areas such as National parks, Biosphere reserves and Wildlife Sanctuaries, and wild nurseries where a large proportion of the species could be cultivated and so they could be safe from anthropogenic activities and invasive species.

Ex Situ conservation

In this method of conservation, the species are carried away from the natural habitat for their protection. The method incorporates the gathering, preserving, and maintenance of the individuals of the species in a controlled environment. Some of the critically endangered plants which are incompatible with their natural habitat can be preserved by this method. Botanical gardens, seed banks, Field gene banks, Germplasm preservation are some of the techniques which are traditionally used for the conservation of plants where either the whole plant or some part of the genome of the plant are preserved in a controlled environment [11].

Cultivation of Indian Snakeroot

Indian snakeroot is native to the Indian subcontinent allocated at the foot-hills of the Himalayan range, growing at a height of 1300–1400 m. It can be easily cultivated in the states of Uttarakhand, Jammu and Kashmir, Himachal Pradesh, and Uttar Pradesh [12].

The plant can be grown under tropical and subtropical type climates with the requirement of irrigation. Warm and shady areas are best suited for the plantation. The plant demands yearly rainfall of 300–500 mm and an average temperature between 10°C–30°C. Clay loamy soil with high proportions of humus and organic matter along with a pH of 4.7–6.5 is ideal for growth.

The propagation can be done by sowing seeds, stem cutting, and root cutting, however, the success rate is very low i.e. 40–60%. Its stem can grow up to a height of 75–100 cm and root at a depth of 50–60 cm. Land preparation is required for the efficient growth of the plant and it demands regular irrigation at an interval of 15–25 days during dry and cold weather.

Usually, this plant does not require the use of fertilizers but organic fertilizers such as cow dung and vermicompost can be used in small amounts, depending on the nature of the soil. There should be a

check on the weeds, pests, and diseases during the cultivation. Root-knot, caterpillars, and nematodes are some common pests which is a threat to the plant. Some common diseases like leaf spot and Alternaria tenuis can affect the quality of the plant.

It takes around 2–3 years to reach the maturation stage and to be ready for harvesting. Mainly the roots of the plants are processed and harvested which has the highest concentration of useful phytochemicals [13].

The main disadvantages of using the traditional methods of cultivation of the Indian snakeroot are

1. Lower viability of seed germination.
2. Take a very long time (2–3 Years) to become fully mature and ready for harvest.
3. Requires intense irrigation and labor until maturation.

Phytochemical Composition of Indian Snakeroot

Indian snakeroot has been there for thousands of years, used in the treatment of a variety of disorders which is possible due to the presence of numerous active compounds present in it. The Chemical compounds synthesized by the plants are classified as primary metabolites and secondary metabolites. Secondary metabolites carry significant pharmaceutical activities.

Some of the most important secondary metabolites include Alkaloids, Terpenoids, phenolics, glycosides, and flavonoids. These secondary metabolites influence the activities of the body by directly or indirectly interacting with the receptors or tissues [14]. The Table 2 describes the secondary metabolites present in the Indian snakeroot.

Table 2. Secondary Metabolite Present in Indian snakeroot.

No.	Active compounds	Class	Part	Function	Reference
1.	Reserpine	Alkaloid	Root	Hypertension, neuroprotective, cardioprotective.	[15]
2.	Ajmalicine	Alkaloid	Root	Lowers blood pressure, preventing strokes.	
3.	Ajmaline	Alkaloid	Root	Diagnosis of Brugada syndrome	
4.	Serpentine	Alkaloid	Vacuoles	Antipsychotic	
5.	Rescinnamine	Alkaloid	Root	Antihypertensive, Decreasing vasopressor	
6.	Deserpidine	Ester Alkaloid	Root	Antipsychotic, antihypertensive.	
7.	Yohimbine	Alkaloid	Stem	Alpha-blocker in the blood vessel, treatment of erectile dysfunction	
8.	Phenols	-	Whole plant	Expectorant, hypolipidemic, antidiabetic	
9.	Tannins	-	Whole plant	Stringent, healing wounds	
10.	Flavonoids	-	Whole plant	Antioxidant, Anti-inflammatory, Cardioprotective.	
11.	Saponins	Glycoside	Whole plant	Hemolytic activity, cholesterol-binding properties, coagulation of blood, healing wounds.	

Biotechnological Aspects of Cultivation and Enhancement of Indian Snakeroot

Biotechnology is a field of study which includes the use of various tool that could be employed to improve the cultivation of any medicinal plant or food crops by overcoming the disadvantages of the traditional methods, secondary metabolite enhancement, hence preventing it from being extinct.

Next in this review, two of the plant biotechnology methods are described that could be employed in the purpose of cultivation, secondary metabolite enhancement, as well as conservation of the Indian snakeroot. Additionally, different methods of phytochemical extraction has also being discussed

Micropropagation of Indian snakeroot

Plant tissue culture/Micropropagation is the *in vitro* generation and multiplication of plant cells and tissues. It is an important tool, employed to study the plant tissues, hormones and chemical compositions and some operations to enhance the quality of the plant. Explant, tissue or a single cell can be used to regenerate entire plants *in vitro* by growing them on a nutritional media under sterile conditions, this property is known as *totipotency*. By the help of this method clones of some elite plant can be produced, endangered plants and their germplasm can be conserved, quality and quantity of secondary metabolites can be enhanced.

A successful micropropagation of a plant lies on certain growth factors such as the concentration of hormones, composition of nutrient medium, and certain environmental conditions such as pH, Temperature, humidity and light. Nutrient media consist of certain important vitamins and minerals which is necessary for the growth of the plant. Some of the important minerals includes manganese, zinc, boron, copper, iron, molybdenum. Vitamins such as thiamine, nicotinic acid, pyridoxine, are included in the media, additionally folic acid, tocopherol, ascorbic acid and biotin are included depending on the requirement of certain plants. Growth regulators and hormones is essential for manipulation and development of tissues, i.e. for proliferation of callus, and production of roots and shoots. The important growth regulators are auxin, cytokinin and gibberellins, these are essential for the cell division and shoot growth [16].

Advantages of micropropagation include:

1. High multiplication rate.
2. Controlled environment can be provided according to the needs of the plant.
3. Production of clones with some specialized characteristics.
4. Enhancement of phytochemical production.
5. Production of genetically modified crops could be easy.
6. Conservation of endangered plant species.
7. Genome preservation could be easier.

Explant

Explant is the prime retirement for initiation of plant tissue culture. The success of micropropagation is dependent on the quality, age and spot of explant. Mostly the explants are chosen from leaves, shoot tips, root and nodal buds [17].

Sterilization

Pathogen contamination is the major problem that can destroy the process completely. Common bacterial species such as *Staphylococcus, Bacillus, Lactobacillus and Pseudomonas* cause major threat to the micropropagation process [17]. These contaminants can grow on the culture media very quickly and release some harmful chemicals that alters the quality of the environment and plant material. The explants are cleaned with deionized water and sterilized by ethyl alcohol, bleach, and mercuric chloride. The sterilization of utensils and instruments is done by backing, flame treatment, fumigation, autoclaving and alcohol washing.

Tissue Culture Media

The culture media is vital for the growing living organisms or cell in an in-vitro conditions. It contains all the necessary nutrients and vitamins essential for a successful growth of pants. The prime composition of a growth media are Vitamins, hormones, macro and micro nutrients, carbon source. Most commonly used culture media is Murashige and Skoog (MS) media, Linsmaier-Skoog (LS) media, Woody Plant media (WPM), Nitsch and Nitsch media and Schenk and Hilderbrant (SH) media. Agar is an essential gelling agent, the pH between 5.0–6.0 is favorable for the growth of plant.

Growth Hormones/Regulators

Plant growth regulators (PGRs) or phytohormones govern a variety of physiological and morphological processes in plants. These regulators are naturally synthesized in the plants, so most of

the plant species does not require any supplement. Hormones are vital promoting faster growth of the plant as well as enhancing the phytochemicals produced by the plant [17] Table 3.

Table 3. Requirements for the growth of Phytochemicals produced by palnts

Category	Name	Referance
Growth media	Murashige and Skoog(MS) media containing vitamins, macro and micro salts, and agar powder	[18]
Hormones	6-benzyl amino purine (BAP), α-Naphthalene acetic acid (NAA), indole-3 aceticacid, kinetin (KIN), gibberellic acid (GA3)	[19]
Cleaning and sterilizing agents	Tween 20, Distilled water, Bavistin, ethanol, mercuric chloride, sodium hypochlorite.	[19]

Tissue Culture Techniques

Callus Culture

A callus is an undifferentiated mass of cells that develops on explants within a few weeks after being placed in growth media with the appropriate hormones. Dedifferentiation or redifferentiation, a well-known process of cell differentiation, results in the development of calluses [17].

Suspension Culture

Suspension cultures are created in vitro by growing friable calli on liquid medium in a suitable container and agitating it continually to maintain a suspension of free cells. Because of its enormous surface area, conical flasks are utilized to maintain a liquid medium to allow continuous gas exchange. Batch and continuous suspension cultures are the two forms of suspension cultures. A part of the original cell suspension is removed and sub-cultured on fresh medium at regular intervals in batch cultures. Fresh medium is supplied to existing cultures in continuous cultures, and unwanted cell suspensions are discarded periodically [17].

Somatic Embryogenesis

Somatic embryogenesis is the production of a non-zygotic embryo from plant tissue or cell that can grow into a new plant. Somatic embryos are formed in two steps: first, calluses are grown on auxin-rich media (2,4-D is usually employed), resulting in embryogenic clumps, and then these clumps are moved to auxin-free medium, resulting in mature embryos. Auxins and nitrogen levels in the medium influence the growth and development of mature embryos.

Protoplast Culture

Plant cells that have had their cell walls destroyed by enzyme digestion or mechanical means are known as protoplasts. Plant tissue is dipped in hypertonic solution to cause the plasma membrane to shrink away from the cell wall, resulting in protoplast isolation. Enzymatic digestion (pectinase and cellulose) or mechanical methods can now be used to remove the cell wall [17].

Enhancement of Secondary Metabolite Production

Plant cells that are grown *in vitro*, are in the form of callus either undifferentiated or semi-differentiated form. The production of secondary metabolites is initiated at this stage, in some cases, the synthesis of important secondary metabolites can be initiated in the cell lines of selected herbal plants, for example, Sanguinarine and Shikonin synthesis in *Papaver sominifera* and *Lithospermum erythrorhizon* can be initiated in a cell suspension state [20].

The following rules and steps were developed 30 years ago which aimed at enhancing the phytochemicals production using plant tissue culture:

1. Selection of the plant material (organs or tissues) with the highest concentration of secondary metabolites.

2. Identifying the appropriate growth media which is highly favorable for the production of secondary metabolites. The components such as micro and macronutrients, vitamins, carbon and nitrogen sources, and growth regulators, along with the growing conditions such as light, temp, pH, oxygen levels, do affect the rate of growth of the plant and the number of secondary metabolites produced. To enhance the synthesis of secondary metabolites, it is important to do experimentation by changing the ratios of all the conditions and select the best method. For example, changing the ratio of auxins and cytosine concentration determines whether the cell will remain completely undifferentiated or it will tend to differentiate into leaves or root. The production of secondary metabolites would be affected by light, temperature, and oxygen levels [20].
3. The addition of cultures containing certain types of precursors, intermediates, and inhibitors can influence the metabolic pathways of the plant leading to the enhancement in the production of secondary metabolites. For example, using Phenylalanine as a precursor of phenolic compounds to the suspension cultures of *Coleus blumei* and *Salvia officinalis* can enhance the production of rosmarinic acid, and to the suspension culture of *Taxus cuspidata* which enhance the synthesis of N-benzoliphenylisoserine in the respective plants, this method could be used for enhancing the secondary metabolite production in other plants such as *Rauwolfia sepantina* by the use of if the precursors and inhibitors are known.
4. Also, elicitors can be assigned to the plant material which can enhance the secondary metabolite production significantly. Elicitors are certain foreign signaling molecules produced by pathogens which when interacting with plant cells, and as a response, the plant cells trigger the defense mechanism, hence synthesizing respective phytochemicals. For example, when *Botrytis* homogenate is added to the cell suspension of *Papaver somniferum,* which enhances the Sanguinarine synthesis significantly [20].
5. Scale-up strategy can also be a method that can be used to enhance biomass and secondary metabolite production. Using appropriate type of bioreactors, scale-up can be achieved, some of the additional factors such as the dimensions of the bioreactor, size of the inoculum, aeration, and agitation can influence the production of secondary metabolites and scale-up success [20].

Genetic Engineering Techniques for Enhancing the Synthesis of Phytochemicals

Genetic engineering is an in vitro method of inducing a foreign DNA into an organism to modify its genetic makeup to obtain enhanced traits from the organism. This method has opened a new possibilities to modify the genome of a medicinal plant and enhance the production of secondary metabolites from it. The practice of genetically modification of plant dates back over three decades, allowing plant genetic engineering methods to be used enabling the modification of agronomic and qualitative characteristics, resulting in the cultivation of transgenic crops in several countries.

Modern uses of *Rauwolfia serpentina*

Rauwolfia serpentina is a treasure trove of active compounds which has been used for thousands of years. Many tribes of India especially in the southern parts, have used as per ethnomedicinal knowledge for treating fever, wounds, snakebites, boils and stomach ache and much more. In the states of Jammu and Kashmir, Orrisa, Karnataka, Tamil Nadu and Madhya Pradesh, there are reported of many native tribes using the extracts of this *Rauwolfia serpentina* for the treatment of snakebites and scorpion sting [21]. Besides this, scientists are engaged in developing drugs, metabolites and other synthetic compounds by studying the potential of this plant. Some of the modern uses are described below:

Treatment of Hypertension

Reserpine is an indole alkaloid contained in the plant, is used in the treatment of hypertension. It is found in roots and leaves at a higher concentration. A drug was developed in 1952 named as Serpsail, it was obtained by isolation of reserpine from the *Rauwolfia serpentina.* Reserpine mainly act on the various organs of the body such as brain, liver kidney, spleen and adipose tissue. The molecule targets vasicular monoamine transporter. found in the membranes of specialized secretory vesicles of presynaptic neurons and acts as a transporter of catecholamines neurotransmitters such as serotonin,

norepinephrine, dopamine, histamine and epinephrine molecule reach the membranes and inhibits these catecholamine neurotransmitter from binding with VMAT protein which results in depletion of neurotransmitters in the cytoplasm and leads to nerve depolarization this causes lower heart rate and arterial blood pressure hence curing hypertension [22].

Synthesis of Silver Nanoparticles

Silver nanoparticles can be synthesized by eco-friendly, non-toxic and inexpensive method from the plant extracts. Silver nanoparticles is synthesized in a one-step process of treatment of silver nitrate by the action of the pant extract. These nanoparticles possess antibacterial antilarval and antifungal properties. Besides this, the nanoparticles carry certain cytotoxic properties which are harmful of the human body [23].

Fungicidal Properties

The Plant carry fungicidal properties which means it could be useful in manufacturing fungicides for agricultural purposes. Fungus such as the Pathogenic fungi is a prime cause of fungal infection in plants. Aspergillus flavus, Alternariaspp., and Rhizopus stolonife are the most common fungus that cause post-harvest illnesses. Use of chemical fungicides is expensive as well as it is harmful for human health and ecology. Plants extracts derived from *Rauwolfia serpentina* carry antifungal properties, in some studies it was found that root extracts derived from this plants are highly effective against a fungal infection, furthermore it is highly biodegradable which means it would be safe for humans and environment [24].

Neutralization of Snake Venom

There is huge diversity of venomous snake species in the Indian subcontinent. Snake bite is a serious concern for most of the tribal people and communities living in rural areas. Due to the lack of health facilities in the area, most of the people die due to snake bite. Snake venom mainly consist of enzymes such as proteinases, cholinesterase, hyaluronidase, metalloproteinase and phospholipase. The snake venom is rich in alpha-bungarotoxin and cobratoxin. Venom binds to Acetylcholine receptors in specific, preventing Acetylcholine from interacting with receptors on the post synaptic membrane, resulting in neuromuscular blockade. The only treatment for this condition is anti-venom which is expensive and inaccessible to the communities of rural and remote areas, also antivenom can cause some harmful side effects. Root extracts of *Rauwolfia serpentina* has shown promising resultsfor neutralization of snake venom by inhibiting the protease activities, ATPase activities and acetyl cholinesterase activities [25].

Genetic Transformation of Sarpagandha

At present, growing demands of the pharmaceutical industry has rendered listing the species as ‘‘endangered’’ by the International Union for Conservation of Nature and Natural Resources (IUCN) [26, 27]. Thus various in vitro techniques have been developed to safeguard the existence of this immensely important species, among which genetically transformed root cultures established by transformation with Agrobacterium rhizogenes is an attractive alternative. These roots are characterized by rapid growth on hormone-free media, genetic stability and high productivity of secondary metabolite characteristic to the parent plants [27–29].

CONCLUSION

This review primarily focuses on a research of Indian snakeroot's pharmacological properties as well as biotechnological techniques for the preservation and improvement of some medicinal plants that are in risk of extinction.

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