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A Review On Threatened Medicinal Plants In Uttarakhand And Their Genetic Diversity Assessment Through Molecular Markers

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Abstract

Uttarakhand has a rich plant biodiversity, many of which have high medicinal properties. Medicinal plants have been used against insects, diseases, and herbivorous mammals through the production of chemical compounds. Various reasons like overexploitation, overgrazing, unsustainable development, etc. lead to their extinction and make them threatened species. The International Union for Conservation of Nature (IUCN) was established to prioritize species conservation, assess threat status, and develop suitable strategies due to the vast diversity of species. The genetic diversity of these threatened species can be determined using various markers. Molecular marker technology aids in analyzing genetic diversity, population structure, and species adaptation, enabling conservation measures and assessing genetic stability in conserved species. In this article, we have reviewed the genetic diversity of six threatened plants of Uttarakhand (*Picrorhiza kurroa*, *Aconitum heterophyllum*, *Nardostachys jatamansi*, *Dactylorhiza hatagirea*, *Zanthoxylum armatum*, *Saussurea costus*) in India as well as in other countries. A review highlighting the use of recent molecular markers like ISSR, SSR, RAPD, etc. to determine genetic diversity. Most of the medicinal plants show diversity less than 0.5, which indicates that the plants are at extreme risk of extinction. The diversity is calculated based on polymorphism percentage. The analysis of genetic diversity between species using ISSR, next-generation sequencing, AFLP, and microsatellite markers reveals low genetic diversity in *A. heterophyllum*, *P. kurroa*, *N. jatamansi*, *Z. armatum*, *D. hatagirea*, and *S. costus.*

Keywords: Medicinal plant, Genetic Diversity, Molecular Markers, ISSR, RAPD.

INTRODUCTION

Plants have been used for treating human diseases since ancient eras, with bioactive compounds and products serving as ethnomedicine. Rig Veda mentions medicinal plant usage in Ayurveda and Unani systems (Kuldip *et al*.,2015). WHO reports that over 80% of the world's population relies on traditional medicinal plants. In-

dia's rich heritage and diverse medicinal plants, including 7,000 species, are used to treat health issues like tuberculosis, asthma, diabetes, wound healing, heart diseases, and hypertension. Plants are facing extinction worldwide due to climate change, alien invasion, urbanization, and improper ecosystem management, with 43,000 on the IUCN Red List (Thakur *et al.,* 2021). Over 90% of medicinal plants in India face threats from excessive collection, utilization, overexploitation, and unskilled harvesting. Around 1,000 species may be under threat across different ecosystems. The IUCN Red List lists 457 species, with 73 threatened. This paper aims to review existing information and compile an exhaustive list of threatened medicinal plants in India for future generations (Gowthami *et al.,* 2021).

Uttarakhand was declared a herbal state of India in 2003 due to its diverse flora, which not only provides medicinal plants but also contributes to their income (Sharma *et al.,* 2017). 17-35%. The state's indigenous people, primarily elders and women, rely heavily on medicinal plants for health and low cost. However, over-exploitation and improper harvesting of these plants have led to the extinction of numerous species, including *Nardostachysjatamansi*, *Picrorhizakurroa,* and *Aconitum heterophyllum* (Bargali *et al.,* 2022). *Picrorhiza kurroa,* a highly traded plant in Uttarakhand, experienced reduced marketing due to declining availability. Sustainable harvesting and propagation of threatened medicinal plants are prioritized for future conservation and management (Pandey *et al.,* 2018).

Significance of markers in research and standardization of Medicinal plants

Medicinal plants produce bioactive compounds, but quantitative and qualitative analysis is limited. Specific markers, such as DNA, biomolecules, or herb parts, are used to standardize botanical preparations.

- **Chemical markers**- Such as primary and secondary metabolites, proteins, steroids, and glycosides, are found in roots, stems, or leaves and can be detected using techniques like HPTLC and TLC.
- **Biochemical markers** Isoenzyme patterns, proteins or proteins, are crucial in population genetics studies for species identification and diversity, thereby aiding in the study of crops.
- **Genetic markers –** DNA markers are small DNA fragments encoding specific genes, highly polymorphic, and used to differentiate between species due to unique genetic makeup. Ideal markers have high polymorphic nature, co-dominant inheritance, easy assay, and stability against physiological and environmental changes. Techniques used include PCR, hybridization, and sequencing. Examples include short tandem repeats, simple sequence repeats, VNTR, SNP, microsatellite polymorphism, and more (Chatterjee *et al.,*2015).

The genetic variation of some critically endangered plants is as follows-

- **Aconitum *heterophullum***

Figure 1. Pictorial representation of Plant material (Ventakasubhramanian *et al.*, 2010)

Table 1. Scientific classification of Aconitum *heterophullum*

Kingdom	Plantae
Division	Magnoliophyte
Class	Magnoliopsida
Order	Ranunculates
Family	Ranunculaceae
Genus	Aconitum

Atis, a medicinal plant in the northwestern and eastern Himalayas, is used for treating indigestion and diarrhoea as an active ingredient (Malhotra *et al.,*2014).The non- poisonous Aconitum genus root, found in India, offers medicinal benefits due to its bioactive secondary metabolites. Its roots have analgesic, anti-

arrhythmic, anti-parasitic, and anti-cancer properties, and are traditionally used for treating dyspepsia, pain, diabetes, and diarrhoea (Wani *et al*., 2021).

Chemical constituents

Anthrine, 12-secohetisan-2-ol, N-succinyl anthranilate, Atesinol 6-benzoylheterastine, N-diethyl-N-formyl-aconitine, Methyl aconitine, Aconitine (Paramanick *et al.*,2017).

Therapeutic uses of *A. heterophyllum*

- **Anti-diarrheal activity**- The root extract of *A. heterophyllum* has anti-diarrheal properties due to its stimulation of Na+ and k+ ATPase function via the nitric oxide pathway, enhancing mucosal absorption or reduced secretion.
- **Antihyperlipidemic activity and anti-obesity activity**- *A. heterophyllum* oral administration reduces cholesterol, triglycerides, and LDLs, inhibits intestinal fat absorption, and activates LCAT enzymes, exhibiting potential hypolipidemic and anti-obesity activity.
- **Anti-inflammatory activity**- The late inflammation phase of *A. heterophyllum* extract exhibits anti-inflammatory activity due to the interruption of Arachidonic acid metabolism and inhibition of prostaglandin synthesis.
- **Antibacterial and antifungal activity**- Alkaloids from *A. heterophyllum* exhibit antibacterial and antifungal properties against *Aspergillus niger* and *Alternalia solani*.
- **Antioxidant and Nephron protective activity-** The ethanolic extract of *A. heterophyllum* roots exhibited nephron protective activity, comparable to the antioxidant activity of vitamin C (Mathew *et al.*,2023).

Threat assessment

The IUCN has classified *Aconitum heterophyllum* as critically endangered species (Pal *et al.*,2015) due to its high market demand in India, distribution, population density, and anthropogenic pressure leading to its extinction (Wani *et al.*,2021).Conservation of rare species like Aconitum heterophyllum is crucial due to increasing extinction rates, but habitat fragmentation, livestock grazing, and road construction threaten its limited distribution (Mishra *et al.*,2023).

In 2023, Chauhan *et al.* examines the genetic diversity of *A. Heterophyllum* in the Rudra Prayag district of Uttarakhand using ISSR markers The cluster analysis showed wide genetic diversity within two populations, but high similarities and low diversity within each population. Improving genetic diversity is recommended to prevent species population decline.

- **Picrorhiza *kurroa***

Figure 2. Pictorial representation of Plant material (Arya *et al.*,2013)

Table 2. Scientific classification of *Picrorhiza Kurroa*

Kingdom	Plantae
Division	Dicotiledonae
Class	Asteride
Order	Scrophulariales
Family	Scrophulariaceae
Genus	*Picrorhiza*
Species	*kurroa*

Uttarakhand's Kumaun Himalayan region houses endangered species, including medicinal and aromatic plants like Picrorhiza kurroa, used for disease treatment in the Indian Himalayan region(Chandra *et al.*,2021). It is commonly called Kutki, a perennial herb found in the Himalayan regions of India, China, Pakistan, Bhutan, and Nepal. It acts as an antioxidant, anti-inflammatory, anti-allergic, hepatoprotective, choleretic, anti-asthmatic, and anti-cancerous activity because of the active iridoid glycosides presence (Debnath *et al.*,2020).

Major Phyto-constituents

Kutkoside, PicrosideI, Picroside II, Picroside III, Kutkin, Apocynin and Androsin (Almeleebia *et al.*,2022). *Picrorhiza kurroa*, traditionally used for treating malaria, snake bites, and liver disorders, is critically endangered due to overexploitation and wild collection (Masood, 2015).

Therapeutic uses of *Picrorhiza kurroa*

- **Anti-microbial- Usman *et al.*** found that *P. kurroa's* ethanolic extract has significant antimicrobial properties against various microbe strains. Sharma et al. found that the methanol extract was more potent against bacterial strains than antibacterial drugs, and its aqueous extract was more effective against fungal strains.
- **Anti-asthmatic**- The ethanolic extract from the roots shows protective actions against asthma. It is observed that *P. kurroa* has anti-asthmatic properties.
- **Anti-oxidant**- The ethanolic extract shows anti-oxidant properties because it contains flavonoid and phenolic compounds.
- **Anti-mutagenic**- The hydro alcoholic extract shows the anti-mutagenic actions of *P. kurroa.* It shows inhibition against *Salmonella typhimurium* strains (MTCC 1251 and MTCC 1252).
- **Immunomodulatory**- The alcoholic plant extract of *P. kurroa* helps in enhancing the immunostimulant activities (Chaudhary *et al.*,2021).

Threat assessment

Picrorhiza kurroa, a medicinal plant used by pharmaceutical companies, is at risk of extinction due to anthropogenic pressure, habitat fragmentation, overgrazing, climate change, lack of pollinators, and unscientific harvesting. In 2009, it was classified as a threatened species in Uttarakhand by the International Union for Conservation of Nature and Natural Resources. Conserving the species requires viable techniques like in-situ and ex-situ conservation (Bhatt *et al*,2014). Overharvesting is a significant factor in the in-situ conservation of Picrorhiza kurroa in the alpine region of Kumaun (Chandra *et al.*,2021).The characterization of an endangered species' genetic diversity is crucial for efficient resource utilization and the development of effective conservation strategies (Singh & Sharma,2020).

Kumar et al. (2021) observes the diversity using RAPD & ISSR markers in a species. RAPD showed 43.4% diversity among populations, with an average of 6.3 bands per primer, indicating 83.5% polymorphism. The study also observed ISSR diversity with 15 primers producing 88 amplification products, with an average frequency of 5.8 bands per primer. Among 91 genotypes, 71 bands or 80.6% were polymorphic. ISSR had 66.2% diversity due to variation between individuals within populations, with a low proportion of genetic variation of about 9.7%. The study found a low proportion of genetic variation of about 19.2% due to differences among populations.

- **Nardostachys *jatamansi***

Figure 3. Pictorial representation of Plant material *(Singh et al.,2020)*

Table 3. Scientific classification of *Nardostachys Jatamansi*

Kingdom	Plantae
Division	Mangnoliophyta
Class	Mangnoliophyta
Order	Dipsacales
Family	Valerinaceae
Genus	*Nardostachys*
Species	*jatamansi*

Nardostachys jatamansi, a medicinal plant found in the Himalayas from Paki-stan, India to Nepal, Tibet, and China, has been utilized in Ayurveda, Homeopathy, ethno- medicine, and modern medicines in India, Nepal, and Tibet (Chauhan et al.,2011). Nardostachys jatamansi, also known as balachara, bhytajata, Bautista, jatamansi, hanamachi, man ship, spikenard, and tapaswani, is a medicinal plant in India's Himachal Pradesh, Uttarak-hand, Sikkim, and Arunachal Pradesh states. Its essential oils are used for medicinal purposes, but over-exploitation affects its reproductive phase and germination rate.

Chemical constituents

Nardostachys jatamansi is a plant with sesquiterpenes and coumarins, with valeranone being the principal sesquiterpene. Its hydroalcoholic extract contains steroids, alkaloids, sterols, tannins, mucilage, flavonoids, carbohydrates, gums, terpenes, and glycosides, along with other compounds (Nakoti *et al.*,2017).

Threat assessment

Nardostachys jatamansi, a critically endangered medicinal plant on the IUCN red list, is being prioritized by researchers for its continuous supply and exploration of its medicinal potential (Kaur *et al.*,2020).

Some medicinal properties of *N. jatamansi*

- **Hepatoprotective activity**- *N. jatamansi*normalizes various elevated serum enzymes in response to thioacetamide-induced liver damage.
- **Antidepressant activity-** Experimentally, *N. jatamansi* has been found to have dose-dependent antidepressant activity, making it beneficial for patients suffering from sleep disturbance-related depression.
- **Antifungal and antibacterial activity**- The methanolic extract of *N. jatamansi* is proven to be effective against various microorganisms, making it a valid antimicrobial and antifungal agent.
- **Effect on Estrogen and hair growth**- The study results in the hair growth promotion activities of *N.jatamansi*.Its rhizomes can be used as immunomodulators and antiparkinsons, antidiabetic, nootropic activity, etc.(Sahu *et al.*,2016).

The genetic diversity of *Nardostachys jatamansi* is studied using various molecularmarkers in India. Some of them are as follows: -

Singh *et al.* 2013 study used the RAPD marker to analyze the genetic diversity of N. jatamansi populations in the Central Himalayan region of India and Nepal. They collected samples from different altitudes and used primers NJ-19 and NJ-45 to detect intraspecific variations. Out of 346 bands scored, 267 were polymorphic, with a polymorphism percentage ranging from 45.4% to 89.4%. The study found that the high genetic diversity may be due to reproductive isolation, habitat changes, wind dispersal of hair seeds, and small population size in different Himalayan locations. The similarity coefficient ranged from 0.38 to 1.0.

- **Zanthoxylum *armatum***

Figure 4. Pictorial representation of Plant material (Bharti,2015)

Table 4. Scientific classification of *Zanthoxylum Armatum*

Kingdom	Plantae
Division	Tracheophyta
Class	Angiosperms
Order	Sapindales
Family	Rutaceae
Genus	*Zanthoxylum*
Species	*Z. armatum*

Zanthoxylum armatum, is a medicinal plant found in Kashmir to Bhutan, North East India, China, Nepal, Malaysia, Japan, etc. It has various household, commercial, and ethno-medical applications (Bhattacharjee *et al.*,2019).

Chemical compounds

Terpenoids, Flavonoids, Alkaloids, Lignin, Sterols and Steroids, Amides, Coumarins,
Carbonyl compounds, Aliphatic compounds and Aromatic compounds (Phuyal *et al.*,2019).

Therapeutic uses

Zanthoxylum armatum is a medicinal plant used to treat various diseases like asthma, cholera, fever, fibrosis, indigestion, rheumatism, skin diseases, toothache, and varicose veins. It stimulates the lymphatic system, circulation, and mucous membranes. The bark is used to intoxicate fish, while the fruits and seeds are used to treat fever, dyspepsia, and cholera. These parts are carminative, stomachic, anthraenic, and antifungal (Paul *et al.*,2018).

Pharmacological activity

- **Anti-diabetic activity**- *Z. armatum's* anti-diabetic properties were demonstrated after 21 days of oral medication, resulting in lower blood glucose levels and reduced triglycerides, LDL, and VLDL.
- **Antidepressant activity**- The hexane extract seed both exhibit antidepressant properties.
- **Cytotoxicity**-The plant extract of the species is effective in enhancing the efficacy of chemotherapeutic drugs like camptothecin, cisplatin, and mitomycin C.
- **Anti-nociceptive and anti-convulsant activity**- Essential oil of the plant was evaluated against anti-convulsant and anti-toxicity. *Z. armatum* helps in easing neurogenic pain and inflammation.
- **Larvicidal activity**- *Z. armatum* consists of the larvacidal activities against *Aedesaegypti, Anopheles Stephens, and C. pipelines* (Verma *et al.*,2021).

The study from other country is as follows: -

In 2023, Shan *et al.* analyze genetic diversity through ISSR marker. A study analysing 132 Z. armatum individuals in China found that 73.44% showed polymorphism, with a percentage of 6.25-44.53%. The study found low genetic diversity at the species level but high diversity among populations. Gene flow was limited in *Z. armatum*, with a 45% genetic variation among the population and 55% within the population. Nei's gene diversity ranged from 0.025-0.1663 and between populations was 0.5411(Yan *et al.*, 2023).

- **Dactylorhiza *hatagirea***

Figure 5. Pictorial representation of Plant material.

Table 5. Scientific classification of *Dactylorhiza Hatagirea*

Kingdom	Plantae
Division	Angiosperms
Class	Monocots
Order	Asparagales
Family	Orchidaceae
Genus	*Dactylorhiza*
Species	*D. incarnate*

The plant, known as Panja, salam-panja, hath-panja, or hatajari in Uttarakhand, is endemic in the Indian Himalayan Region, with distribution across north east India. It thrives in open grassy slopes and alpine meadows, with an altitude range of 2500 to 5000masl. Its height is 60-70cm (Vishwakarma & Karole, 2021).

Phytochemicals

Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Tannins, Carbohydrates, Proteins. The total Flavonoid content in the species is 0.866 (Choukarya *et al.*,2019).

Pharmacological activity

- **Anti-inflammatory properties**- *D. hatagirea* roots contain hydroalcoholic extracts effective in treating anti-inflammatory diseases, while tubers exhibit significant anti-inflammatory responses against both acute and chronic inflammation.
- **Antibacterial properties**- Its rhizomatous part is highly effective against gram-positive and gram-negative bacteria, while its aerial part shows limited resistance. It can be used to treat dysentery and other stomach issues, especially against gram-positive bacteria (Dorjey *et al.*,2022).
- **Anti-cancerous activity**- *D. hatagirea* plant extracts are effective in cancerous cell lines. Root extracts have high anti- cancerous potential than shoot extracts.
- **Anti-diabetic activity**- The leaves and tubers contain various antihyperglycemic agents which show anti-diabetic properties (Wani *et al.*,2020).

Threat assessment

Heavy-bodied animals graze on D. hatagirea, causing soil erosion and habitat degradation. Local communities use it for medicinal and commercial purposes, while Himalayan mouse hare and monal pleasant feed on tuber (Chandra *et al.*,2021).*D. hatagirea* comes under the category of critically endangered species (CAMP status), and critically rare (IUCN status) in Jammu & Kashmir, Himachal Pradesh, and Uttarakhand (Chamoli & Sharan,2019).*D. hatagirea*, a medicinal plant, is under threat from overexploitation and industrial demand. Understanding genetic diversity patterns and population structure is crucial for sustainable utilization and conservation (Sharma *et al.*,2022).

In 2015, Sharma *et al.* observe the diversity using microsatellite markers. A study of 784 nucleotide sequences designed 35 primer pairs flanking microsatellite motifs, with 15 amplified unambiguous amplicons from 20 ecotypes. Out of the remaining 15 markers, 14 were polymorphic and reliable amplification, amplifying 64 alleles with a mean value of 4.2 alleles in the 130-570bp size range (Sharma *et al.*, 2015). Thakur & Kaur,in 2013 examine through RAPD analysis. The genetic diversity of *D. hatagirea* in Himachal Pradesh was analysed using RAPD markers. 343 bands were produced, with 339 polymorphic and 99% polymorphism, indicating high genetic diversity in wild accessions. This highlights the need for documentation, characterization, and conservation of this endangered plant (Thakur & Kaur, 2013).

- **Saussurea *costus***

Figure 6. Pictorial representation of Plant material (Akbar,2020)

Table 6. Scientific classification of *Saussurea Costus*

Kingdom	Plantae
Division	Tracheophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	*Saussurea*
Species	*Costus*

The Indian Himalayan region, home to 18% of endangered and rare medicinal plant species, relies on them for routine, therapeutic, and commercial purposes. Genus *Saussurea*, a high-altitude plant, has been used for over 2500 years to treat various diseases, including cough, leucorrhea, ulcers, liver, and heart problems. Its medicinal properties have been a source of traditional medicine (Nadda *et al.*,2020). Kutha, a spice from the western Himalayas, is found in Jammu and Kashmir, Himachal Pradesh, and Uttarakhand. Its roots have antimicrobial and CNS depressant properties, making it popular in India and Pakistan (Kumar & Pundir,2021).

Chemical constituents

It encompasses alkaloids, anthraquinones, and flavonoids. *S. costus* contains various terpenes, like costunolide and dihydrocostunolide, having anti-inflammatory and antitumor characteristics (Ali & Venkatesalu,2022).

Pharmacological activities

- **Antiulcerogenic activity**- The ethyl acetate of the costus roots is proven to be beneficial for gastric ulceration. UL409 was prepared from costus carrying anti-ulcer activity by improving gastric cytoprotection.
- **Anti-cancerous activity**- Dehydrocostus lactone, an essential oil compound, exhibits anti-cancerous properties, including apoptosis, decreased multi-drug resistance, cell-cycle seizing, and suppressing angiogenesis in animal models.
- **Hepatoprotective activity**-*Costus* is reported to elevate plasma liver biomarkers such as alkaline phosphatase, alanine aminotransferase, etc. This leads to a little rise in total plasma proteins.
- **Immunomodulatory activity**- The hydroalcoholic root extract of *costus* is studied for its immunomodulatory activity, but it has been found to have minimal impact on humoral immunity and the production of spleen cell-producing antibodies (Rathore *et al.*,2021).

Threat assessment

The Indian Himalayan medicinal plant, native to the region, is critically endangered due to its high harvesting load and limited distribution, as listed by the IUCN and the Red Data Book of Indian plants (Kuniyal *et al.*,2019). The genetic diversity of *S.costus.* In India, no research is done using genetic markers till time so the study done in other countries is as follows: -

In Pakistan, Idrees *et al.*, 2018 observed genetic diversity through SSR markers, a total of 14 SSR markers were used to analyze amplification and polymorphism in *S. costus.* Out of which 6 primers show successful amplification with 42.8%.

CONCLUSION

This article provides simple, updated, and ready to use information on threatened medicinal plants in Uttarakhand, India. This review paper also summarizes the use of various molecular markers for the genetic diversity assessment of these threatened plant species, along with their medicinal properties. The offline information contained in this article can be used by students, teachers, policymakers, etc. It is often needed. This will help to develop conservation and cultivation strategies to ensure these important resources are available for future generations.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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