A MEDICINAL PLANT THAT IS IN RISK OF EXTINCTION, HAS SEVERAL THERAPEUTIC USES: MECONOPSIS ACULEATA Vikas Kumar

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Abstract:

Meconopsis aculeata, also referred to as Queen of Himalaya, is a member of the Papaveraceae family. This herb holds significant importance in traditional medicine practises within Indian, Pakistani, Tibetan, and Nepali cultures, dating back to ancient times. It has been utilised as a remedy for a wide range of diseases and ailments. Globally, there are an estimated 49 species within the genus Meconopsis, which have been recognised for their medicinal properties to this day. Due to the unique habitat of the Himalayas, coupled with adverse environmental conditions and excessive exploitation, this species is currently classified as critically endangered. The primary chemical constituents of M. aculeata consist of a group of naturally occurring compounds, including alkaloids, phenolics, tannins, cardiac glycosides, flavonoids, saponins, steroids, triterpenoids, carbohydrates, proteins, amino acids, phytosterols, and phlobatannins. There is a limited availability of scientific literature pertaining to the antioxidant, antifungal, anti-inflammatory, anticancer, and antimicrobial properties of it. Hence, the purpose of this review is to provide a comprehensive analysis of the ecological, pharmacological, and phytochemical aspects of M. aculeata, based solely on evidence-based information. Several conservation strategies have been proposed for this valuable gem of the Himalayas. Due to its numerous therapeutic potentials, this particular plant species has garnered significant attention from the modern scientific community.

Key Words: Meconopsis Aculeate, Papaveraceae, ConservationStrategies

1. Introduction:

India is ranked as the seventh largest country in the world, with a total area of 3,287,263 square kilometres. Its geographical coordinates span from 8.4 to 37.61 degrees north latitude and 68.7 to 97.251 degrees east longitude. Furthermore, India holds the sixth position globally in terms of mega biodiversity centres. It is estimated that around 6000-7000 species of flowering plants, out of a total of 17,000-18,000, have been assessed for their potential medicinal properties as documented in traditional knowledge. According to the literature on traditional medical systems, Ayurveda utilises the highest number of plant species, followed by Siddha, Unani, and Tibetan practises. The indigenous communities residing in the Himalayan region of India, Pakistan, China, Nepal, and Tibet have gained recognition for their extensive knowledge of the ethnobotanical resources, particularly the medicinal plant species [23, 10, 21].

The Himalaya region is widely recognised globally for its abundant collection of medicinal, aromatic, and endangered plant species. Meconopsis aculeata Royle has garnered significant attention due to its status as an endangered medicinal plant. It is listed in the Red Data Book of Indian plants [32]. This is a genus of flowering plants that are considered ornamental and decorative. They belong to the family Papaveraceae and are typically found at higher altitudes, specifically around 4000m above mean sea level. In India, it is estimated that there are approximately 8000 species of medicinal plants. It is worth noting that over 90% of the medicinal plants used by the herbal drug industries are sourced from natural habitats. This reliance on natural habitats has led to significant exploitation of these plants [1]. Among the 49 species of the Meconopsis genus found globally, approximately 38 species are found in the western regions of China, specifically in Qinghai, Sichuan, Yunnan provinces, and the Tibet region [12, 47].

The initial description of this species can be attributed to the French botanist Viguier in 1814 [1]. Subsequently, the species was formally described by Royle in 1833 based on specimens that were collected. The natural prominent habitats of this species are located in the Tungnath region of Western Garhwal Himalaya, Uttarakhand (Coordinates: 30°29'23.50"N -79°13'00.64"E), as well as in Gurez, Harmukh, Thajwas, Nagbaren, and Munwarsar in the Northwest Himalayan range of Kashmir valley (330 20 to 340 54 N latitudes and 750 55 to 750 35 longitudes), India.

According to literature, M. aculeata has been extensively studied in various regions around the world. These include locations such as Pakistan (specifically below Makra top, above Nila, above Tambu Naka, Danda Baik, Shingan, Pithoragarh, Bageshwar, Gauri Kund, Suru, and Zanskar Valley), the Western Himalaya spanning from Kashmir to Kumaon, Ladhak, the Sinthan top area of Ananthnag district, the Valley of Flowers, Hemkund, Madhyamhableshwer, and Rudra Nath in India. Additionally, research has been conducted in Nepal, China's hill area, and Tibet [7].

M. aculeata is considered to be a significant source of primary and secondary metabolites, rendering it

of great pharmacological importance [41, 1]. The entire plant body possesses a range of therapeutic benefits, including the treatment of various disorders and ailments, as well as exhibiting antipyretic and analgesic properties [3, 40, 34, 5]. Extensive research has been conducted globally on the various genera of Meconopsis.

2. Local Names:

It is famous as Queen of Himalaya and also known as Achatsarmum, Blue poppy, Kanta, Vanita, Ladak, Gul-e Nilam, AchaK-srmum, Gul-e-Neelam, Landrementok, Ut-Pal sngon-po, Chairingum, etc.

3. Botany and Ecology:

M. aculeata is a plant species that is native to a specific region. It is characterised by its unique growth pattern, as it only produces flowers and fruits once in its lifetime. Additionally, it is a long-lasting herbaceous plant that remains in the ground for multiple years. The plant features a solitary, straight, rigid stem that is covered in sharp thorns and can reach a height ranging from 30 to 60 cm. A raceme consists of multiple flowers. The flower exhibits characteristics of being showy, actinomorphic, hermaphrodite, complete, and hypogynous. The flowers exhibit a shallow cup shape and are positioned laterally or semi-nutant. They are supported by a slender, cylindrical, and erect pedicel measuring approximately 2.5 to 13.5 cm in length. The petals are typically four to six in number, although occurrences of more than six are rare. They exhibit an obtuse shape and possess delicate, thin, and soft characteristics. The petals are obovate or suborbicular in shape, measuring approximately 3.7×3.7 cm. They display a blue-purple coloration with wavy margins. The filaments exhibit a filiform structure and possess a colour that is similar to the petals, albeit deeper in shade. Additionally, the anthers are characterised by a golden hue. The ovary is spherical to elliptical in shape, densely covered in a golden fulvous to bristly texture. The bristles initially lie flat against the surface but later become upright or slightly spreading. The distinct styles measure approximately 1.2 cm in length. The fruiting structure exhibits a noticeable thickening, while the stigma lobes are either free or connate, taking on an oblong or spherical shape. The capsule is spheroidal to elliptic-oblong in shape and is densely covered in bristles. It has 4-6 (8) valves that are closely arranged near the top. The seeds are reniform in shape and the blooming period occurs between June and August. Rocky habitats, scree, and stream margins are typically found at elevations ranging from 2400 to 4200 metres. Fruits are botanical structures that typically consist of many-seeded capsules. The roots exhibit two distinct shapes: napiform or elongated, measuring approximately 13 cm [6, 33]. The growth of this species requires specific habitats, such as sub-alpine and alpine zones, as well as moist black sandy loam soils. It is also found in rock crevices and on boulders [27]. The blooming period for this species occurs between June and August [33].

4. Molecular Study of Meconopsis Aculeate:

Currently, scientists are actively engaged in the exploration of Meconopsis aculeata through the utilisation of diverse molecular methodologies in order to gather additional information. Certain researchers have utilised the internal transcribed spacer (ITS) region of nuclear ribosomal DNA and the chloroplast DNA trnL-F region to perform phylogenetic reconstruction of Meconopsis and its closely related species, such as Roemeria, Papaver, and Stylomecon. Through this analysis, they have successfully identified five distinct clades, which were strongly supported in the gene trees constructed using the nrDNA ITS and cpDNA trnL-F sequences. There are a total of 41 species of Asian Meconopsis, which do not form a monophyletic clade. Instead, they can be divided into two distinct clades, labelled as Clade I and Clade V. These two clades are separated in the phylogenetic tree by three additional clades, namely Clades II, III, and IV, which consist of species from the Papaver genus and related species. Clade V comprises solely of four Asian Meconopsis species, while the remaining 90 percent of Asian species are encompassed within clade I. Within the core Asian Meconopsis clades, the nrDNA ITS tree revealed the recognition of five subclades (Ia-Ie). Three species (Meconopsis discigera, M. pinnatifolia, and M. torquata) belonging to subgenus Discogyne were found to be included in subclade Ia. This finding suggests that the current classification of subgenera in Meconopsis needs to be reconsidered. The subclades identified in this study did not align with any existing series or sections in the current classifications. This finding suggests that a comprehensive revision of the genus classifications is necessary [17, 28].

The blue poppy is a traditional Tibetan medicinal herb that possesses significant value. The chloroplast genome of this plant has been determined to be 153,761 base pairs in length, with a base composition that is biassed towards A and T. It consists of a pair of inverted repeat (IR) regions, each spanning 26,030 base pairs, which are separated by a large single-copy (LSC) region of 83,803 base pairs and a small single-copy (SSC) region of 17,898 base pairs. A comprehensive annotation was conducted on a total of 113 gene species. Among these, 20 gene species were found to have complete or partial duplications, while 18 gene species were identified to contain one or two introns. According to phylogenetic analysis, there is evidence to suggest a close relationship between M. horridula and Meconopsis racemosa Maxim [17]. In a study conducted by Xiao and Simpson in 2017, a molecular investigation was carried out. The researchers examined four chloroplast markers, namely trnL-trnF intergenic spacer, matK, ndhF, and rbcL, to construct a molecular phylogeny. The results of this analysis revealed evolutionary relationships that were found to disagree, to varying extents, with any infrageneric relationship. The present study provides a revised classification that incorporates phylogenetic

topology, as well as the morphological and cytological patterns observed within the phylogenetic structure. The results obtained from the four major clades of Meconopsis phylogeny have been utilised as the foundation for infrageneric sections, namely Meconopsis sect. Meconopsis, M.sect. Aculeatae, M.sect. Primulinae, and M. sect. Grandes [45].

The entire plant component of M. aculeata is utilised for medicinal purposes. It possesses robust properties against malaria and bacterial capacity, making it suitable for utilisation in pharmaceutical manufacturing [33]. The substance is utilised as a crude drug in traditional medicine to alleviate rheumatic pains, reduce fever, provide pain relief, induce narcotic effects, and address bone-related issues, particularly in the rib area [36, 40, 34, 5]. According to Thakur et al. (2014) [39], the administration of grounded roots of M. aculeata along with salt has been found to be effective in enhancing disease resistance in animals. The product effectively eliminates the appearance of veins by transforming them into a blue hue. Additionally, it may be administered to expectant mothers as a substitute for tetanus immunisation in their infants or children, regardless of the disease [18].

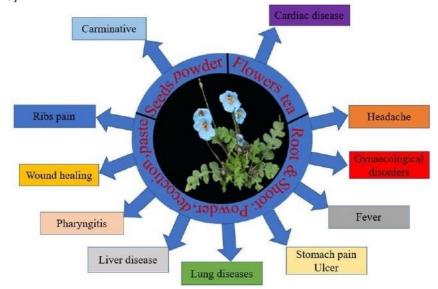


Figure 1: Plant part name and ethnomedicinal /ethnobotanical uses of *Meconopsis aculeata*.

5. Biological Activities:

5.1 Anti-oxidant and Anti-Inflammatory Activities:

The inclusion of natural antioxidant bioactive compounds in dietary patterns has been shown to mitigate the risk of degenerative diseases, including cancer, diabetes, cardiovascular disorders, and neurodegenerative conditions. Furthermore, natural bioactive antioxidant compounds have been extensively utilised in the food industry for the purpose of extending shelf-life. This is due to the reported toxicity of synthetic antioxidants, namely butyl hydroxyanisole (BHA) and butylhydroxytoluene (BHT) [37, 5]. The study reported the presence of various bioactive compounds in the tested samples. The total phenolics content ranged from 1.68 to 3.76 mg GAEg-1, while flavonoids content ranged from 1.02 to 2.3 mg OEg-1. Additionally, flavonols content ranged from 0.69 to 1.46 mg QEg-1, tannins content ranged from 1.25 to 3.83 mg TAEg-1/g, and proanthocyanidin content ranged from 0.34 to 0.81 mg CEg-1. These compounds have been shown to possess potential protective effects against DNA damage, lipid peroxidation, and protein oxidation in living cells. The antioxidant activity, specifically ABTS (2.33-2.67) and DPPH (1.03-2.35), was found to be most effective when using acetone (80%) for harnessing reducing antioxidant FRAP activity (1.76 mM AAE 100 g-1dw) and nitric oxide scavenging NO antioxidant activity (0.92 mM AAE 100 g-1 DW) compared to other solvents. Additionally, ethanolic (60%) DPPH was observed in M. aculeata [5, 41]. It exhibits free radical scavenging activity and has the potential to serve as a strong source of antioxidants in the prevention of diseases associated with oxidative stress [22; 44]. Phenolics and flavonoids are widely acknowledged for their diverse range of biological effects, such as antioxidant, anti-inflammatory, antimicrobial, and anti-carcinogenic properties [35]. Additionally, they have demonstrated effectiveness in addressing disorders related to the lungs, liver, and inflammation [38]. In a study conducted by Hassan et al. (2020) [22], an experiment was conducted on the methanol extract of M. aculeata. The findings of the study demonstrated that the administration of CCl4 resulted in a notable elevation in serum AST, ALT, and LDH levels. However, the consumption of M. aculeata extract exhibited preventive effects against neutrophil infiltration and tissue necrosis in the liver samples of the treated animals. Therefore, the extract shows potential as a hepatoprotective agent due to its antioxidant properties.

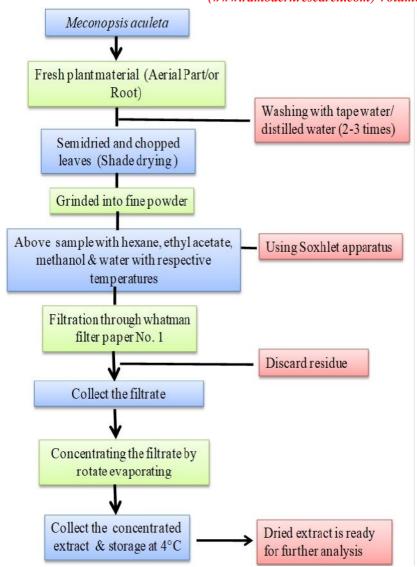


Figure 2: Procedure used in the extraction of Meconopsis aculeta

5.2 Antimicrobial (Bacterial and Fungal) Activities:

Medicinal plants are known to exhibit diverse antifungal properties [31, 19]. A study conducted by Tsering and Praveen (2021) [41] investigated the antimicrobial properties of M. aculeata. The results demonstrated that the plant samples exhibited effectiveness against both bacteria and fungus cultures. The minimum inhibitory concentration (MIC) against the bacteria and fungi was determined to be $2.50\mu g$ ml-1. The antibacterial and antifungal activities of M. aculeata were evaluated, and the minimum inhibitory concentration (MIC) values varied among different extracts. In petroleum ether extracts, the MIC values were determined to be $2.50\mu g$ ml-1, $5.00\mu g$ ml-1, and 10.00 μg ml-1 against A. niger, E. coli, and S. mutans, respectively. In chloroform and ethanol extracts, the MIC value was found to be $10.00\mu g$ ml-1 against V. vulnificus. In methanol and water extracts, the MIC value was determined to be $20.00\mu g$ ml-1 against S. aureus. The minimum inhibitory concentration (MIC) against T. islandicus in chloroform extracts was determined to be $20.00\mu g$ ml-1.

According to Tsering and Praveen (2021) [41], the presence of various compounds such as n-hexadecanoic acids, squalene, dibutyl phthalate, beta-amyrin, alpha-amyrin, cis-vaccenic acid, eugenol, Lup-20(29)-en-3-ol acetate, (3. beta.)-, hentriacontanone, eicosyl, dotriacontinal, palmitoleic, 2-propenoic acid, A'-Neogammacer 22(29)-en-3-ol acetate, linalool, and others in the extract of Meconopsis aculeata has been found to contribute to its antimicrobial properties in various species. This information will be beneficial for the scientific community in further investigating the antimicrobial activity in M. aculeate.

5.3 Threats to Meconopsis Aculeate:

The Himalaya region possesses a significant variety of endemic plant species. However, it is currently facing the issue of excessive extraction of wild medicinal plants, which poses a threat to numerous valuable gene stocks. M. aculeata is a medicinal plant species that is relatively uncommon and limited to the Himalayan range. It typically grows between the months of May and October, with flowering occurring from mid-July to

August, and fruiting from mid-August to September. During the period from November to April, seeds enter a state of dormancy and experience suppressed germination percentage, growth, and development due to environmental conditions. These factors contribute to the conservation efforts [30].

The reduced viability of seed germination can be attributed to the adhesive nature of chromosomes and the irregular functioning of spindles. These cytological phenomena are influenced by genetic factors, which are triggered by low-temperature stress conditions prevalent in the region during the flowering stage of plants. The observed phenomenon, referred to as cytomixis, has been found to impact the meiotic process in microsporocytes. As a consequence, it leads to a decrease in pollen fertility and the production of pollen grains with varying sizes [27].

Due to the significant ethnobotanical value of M. aculeata, there has been a growing market demand for this plant. Consequently, both the local indigenous population and pharmaceutical companies have started utilising it for commercial purposes. Unfortunately, this has led to the uprooting of the entire plant, resulting in the loss of valuable herbal resources. The Himalaya region has been Cattle, including goats and sheep, are the primary dairy animals in hilly regions. However, improper grazing management in sensitive areas has led to early-stage grazing of these plants. Climate change and avalanches are among the factors that contribute to the decline in population of various species, as they have a significant impact on both plant populations and habitats. The extensive exploitation of this species led to its inclusion in the IUCN red list of 1997, specifically in the endangered category [42, 43]. In their study conducted over a period of three years, Majid et al. (2015) discovered that there are a total of 39 mature plant species that fall under the critically endangered category according to the International Union for Conservation of Nature (IUCN) alphanumeric classification CR B1(i), (ii), (iii), (iv), and CR D.

6. Conservation Strategies of Meconopsis Aculeate:

The removal of plants from an area can render it vulnerable to plant growth, as the soil becomes depleted, leading to potential risks such as landslides and deforestation [30]. In order to safeguard and effectively manage medicinal plants that are limited to high-altitude regions, it is recommended to establish Medicinal Plant Conservation Societies (MPCS). These societies would be responsible for the conservation of medicinal plants, including those that are at risk, by engaging local communities and employing experts in the field to oversee the conservation efforts.

Ecological niche modelling can be a valuable tool for the rehabilitation and reintroduction of threatened species. It aids in the prediction of potential habitats for these species, facilitating their successful reintroduction and conservation efforts. These tools will contribute to improving the socio-economic conditions for the local indigenous populations [15]. The implementation of sustainable land use practises can contribute to conservation efforts.

Seed germination studies play a crucial role in conservation strategies, serving as essential tools for reintroduction and management programmes. This is due to the fact that seed germination is a critical stage in the life history of plants. Under optimal circumstances, seeds are able to undergo the process of germination. However, the potential impact of climate change on seed germination conditions could potentially disrupt the successful establishment of seeds. Consequently, the elevated likelihood of seedling mortality serves as a contributing factor to the decline in population regeneration. Hence, in controlled conditions, the in vitro seed germination of M. aculeate was observed under various media and supplements. For pure germination, the seeds were cultured on MS medium supplemented with Zeatin (4 mg/l) + NAA (0.5 mg/l). Shoot germination of seedlings was achieved on MS medium supplemented with Zeatin (2 mg/l) + IAA (0.1 mg/l). In vitro root regeneration was recorded on MS medium supplemented with Zeatin (2 mg/l) + IAA (0.1 mg/l). The following protocol can be implemented to determine the in-vitro seed germination percentage of M. aculeata. This method is recommended for the production of a significant number of high-quality seedlings, with the aim of conserving the species from natural collection. In the aforementioned experiment, the process of sapling hardening was successfully accomplished within a span of 15 days, resulting in a commendable survival rate of 60%. This outcome is indicative of positive progress in the realm of plant conservation [2].

A dependable in-vitro propagation technique can be developed for M. aculeata and other medicinal herbs found in the Himalayas, with the aim of conserving these plants for the collective benefit of society. The Danthonia grassy slope and mixed herbaceous habitat is highly favoured for the growth of medicinal plant species. Hence, these sites could be designated as control sites for the purpose of ongoing monitoring and assessment of population status in the foreseeable future. In order to effectively develop a conservation, development, and sustainable harvesting plan for medicinal plants, it is imperative to conduct intensive habitat-based surveys and phenological studies [15].

7. Conclusion

The distribution of Meconopsis aculeata is limited to the Himalayan range due to the presence of suitable environmental conditions conducive to its growth in this region. The declining density of M. aculeata is attributed to various factors including seasonal dependence, climate change, cytological deformities, overexploitation, and limited understanding of phenological parameters. This ongoing trend is leading to a

gradual impoverishment of the species. As a result, it is classified as critically endangered. Due to its ethnomedicinal properties, it is recommended to initiate and promote research on this plant for additional studies on its phenology, phytochemistry, and antimicrobial properties. This will contribute to the conservation of this valuable Himalayan resource for the benefit of human welfare and socioeconomic development. The existing scientific research on M. aculeata has demonstrated its significance as a medicinal plant species with broad-spectrum properties. The comprehensive information presented in this review offers substantial evidence supporting its ethnobotanical, molecular, phytochemical, pharmacological, and traditional uses. This review article is centred around the existing studies pertaining to M. aculeata, with the aim of enhancing our understanding of future research prospects.

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