# Phytochemical Profiling and Ethnobotanical Study of (*Podophyllum hexandrum*

# Royle.); insights from Langate forest division, Jammu and Kashmir

 **ABSTRACT**

May Apple (*Podophyllum hexandrum* Royle.) a critically endangered Himalayan medicinal herb possesses huge medicinal potential. The study was conducted in the Langate forest division (LFD). Information was gathered about the use of medicinal plants, methods of administration, and the specific plant parts used for treating various ailments by field survey. GC-MS (Gas Chromatography-Mass Spectrometry) analysis was used to examine the phytochemical profile of specimens to combine ethnobotanical knowledge with scientific validity. The study revealed that the herb has a variety of ethno-veterinary and therapeutic applications. The plant's therapeutic properties include using rhizomes, resin pastes, decoctions, and powders to treat wounds, warts, gastroenterological disorders, and joint pain. Its resin has a significant amount of podophyllotoxin, a potent anti-cancer drug. The GC-MS analysis of the methanolic rhizome extract found ten distinct bioactive chemicals with anti-inflammatory, anti-cancer, and antioxidant properties, including beta-sitosterol (13.72%) and podophyllotoxin (18.24%). Key threats to (*Podophyllum hexandrum* Royle.) include overharvesting, habitat loss, climate change, and a lack of awareness.

Keywords: *Langate forest division*, *Himalayan medicinal herb, GC-MS analysis, podophyllotoxin, anti-cancer, overharvesting, habitat loss*

1. **INTRODUCTION**

Medicinal plants have been used for efficient therapeutic interventions in healthcare over time (Ahire *et al.,* 2023; Dar *et al.,* 2023). Traditional medical practices all across the globe use these plants due to their medicinal properties, affordability and cultural value (Nargawe, 2023; Ajewole, 2024). Medicinal plants play an essential role in sustainable development and biodiversity conservation with their economic opportunities in rural areas (Kingston, 2010). Plant-based molecules are essential resources for contemporary drugs, and many contemporary drugs are rooted in medicinal plants (Ajani, 2022). Medicinal plants have gained importance in medical research and are advancing world health as the use of natural medicines and holistic health practices increased (Sharma, 2024). Numerous secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, saponins, and anthraquinones, are found in medicinal plants (Cowan, 1999). These bioactive substances support a wide range of health benefits.

The Himalayan May apple, also known as the Indian May apple (*Podophyllum hexandrum* Royle.) is a perennial herb found across the lower Himalayan regions, spanning Afghanistan, Pakistan, India, Nepal, Bhutan, and Southwestern China. In India, this species thrives at elevations ranging from 3,000 to 4,000 meters in specific high-altitude areas, including Jammu and Kashmir, Himachal Pradesh, Uttarakhand, Sikkim, and Arunachal Pradesh (Kumar *et al.,* 1997). The primary bioactive compounds present in (*Podophyllum hexandrum* Royle.) include podophyllotoxin, quercetin, lignans, and kaempferol. These compounds possess significant anticancer, antirheumatic, radioprotective, antimicrobial, and antihelminthic properties (Nag *et al.,* 2013; Chaurasia *et al.,* 2011). The people of ancient Himalayan regions used the herbal plant (*Podophyllum hexandrum* Royle.) in traditional Ayurveda and folk medicines for hundreds of years (Chaudhari, 2014). This herb contains podophyllotoxin and derivatives which fight cancer cells while protecting against viral infections and supporting our immune system (Giri and Lakshmi Narasu, 2000).

 Gas chromatography-mass spectrometry (GC-MS) is widely employed in medicinal plant research to analyze and identify bioactive phytochemicals (Kotowska *et al.,* 2011; Brettell & Lum, 2018). Ethnobotanical studies are necessary to acquire the knowledge and practices of local people on the use of plants as well as to conserve biodiversity (Chaiyong *et al.,* 2023). These studies document indigenous knowledge and inventory of useful plants and reveal ancient traditions salient in regions like Asia, where medicinal plants have been part of cultural practice (Jatoi *et al.,* 2007). Additionally, ethnobotanical research bridges the gap between conventional understanding and contemporary scientific authentication, offering valuable perspectives on developing medicines. Multiple issues, like excessive harvesting of plants and damaged ecological environments, disturb our ability to keep these resources safe for future generations (Sheng, 2001). To ensure that medicinal plants thrive sustainably, efforts must be made to protect intellectual property rights and to limit the unregulated plant trade (Yingam, 2024). The study will contribute to the UN's Decade on Ecosystem Restoration (2021–2030) objectives and guide policy frameworks under the CBD's Nagoya Protocol and India's National Biodiversity Action Plan. Therefore, the present study bridges critical gaps in ethnobotany, ecology, and pharmacognosy, offering a blueprint for conserving (*Podophyllum hexandrum* Royle.) in an era of ecological and economic uncertainty.

1. **Materials and Methods**
	1. **Study area**

This study was carried out at the Langate Forest Division (LFD), which is located at an elevation of 2000–3500 meters above sea level and is located at latitude 34°15'22" N and longitude 74°07'52" E. It covers a total area of 36,061 ha and is situated on the northeastern slopes of the Kazinag and Shamsabari ranges. Haril in the region mainly has east-facing open slopes with most of its drainage to the east. The area is mountainous with a temperate and sub-alpine climatic condition with an average annual rainfall of 66-167 mm. The temperature ranges from -10°C minimum to 35°C maximum (Romshoo *et al.,* 2020).



*Fig1. Study area map of Langate Forest Division*

* 1. **Ethnobotanical studies**

To get firsthand information about (*Podophyllum hexandrum* Royle.) a field investigation was conducted at the study site. Ethnobotanical information was provided by Gujjars, village heads, and elders. Data regarding the use of medicinal plants, methods of administration, and therapeutic plant parts were gathered by field survey (Focho *et al.,* 2009).

* 1. **Collection of plant specimens**

Rhizomes of this species were gathered from the wild to perform extraction and chemical characterisation experiments in the lab.

* 1. **Phytochemical Screening**

The rhizomes of (*Podophyllum hexandrum* Royle.) were subjected to GC-MS analysis in order to identify all of the bioactive compounds present. The rhizomes were first dried before being ground into a fine powder, and then the extract was filtered and condensed. The GC-MS was used to identify chemicals present in the sample (Parveen *et al.,* 2016). The methanolic extract was analysed using a gas chromatograph-mass spectrometer at a predetermined temperature. Retention time and area percentage values were used to identify chemicals in the chromatograms generated by the GC-MS analysis (Sebastian *et al.,* 2020; Biswal & Kumar Panda, 2023). The biological activities of the identified compounds were reviewed from the literature to relate them to the traditional uses reported by local communities.

1. **Results:**
	1. **Ethno-botanical Uses**

The Langate area in District Kupwara Kashmir utilises (*Podophyllum hexandrum* Royle.) as a multi-purpose herb for veterinary and medicinal treatments. The rhizome paste with resin helps to treat warts, skin infections and wounds and shows anti-cancer properties. The rhizome's extract or powdered form facilitates the treatment of digestive issues along with its pain-relieving properties during arthritis conditions through topical applications. (Table 1).

* 1. **Phytochemical Screening**

GC-MS study of a methanolic extract of the rhizomes of (*Podophyllum hexandrum* Royle.) revealed ten phytochemicals. These ten chemicals are primarily composed of beta-sitosterol (13.72%) and podophyllotoxin (18.24%). Apart from Podophyllotoxin and Beta-sitosterol, several other chemicals were identified. Campesterol, Retinol, Ethyliso-allocholate, and octadecanoic acid were identified as minor compounds with distinct concentration levels. The additional compounds found were 2,2'-Benzylidene bis (3-methyl benzofuran), D-allose, and (9Z)-octadec-9-enoic acid (Fig. 2) (Table 2). These chemicals exhibit anti-cancer effects and also function as antioxidants and anti-inflammatory agents, suggesting high potential applications in medicine (Table 3).

**Table1. Ethnobotanical uses of *(Podophyllum hexandrum* Royle.)in Langate forest division**

|  |  |  |  |
| --- | --- | --- | --- |
| **Use Category** | **Specific Use** | **Part of Plant Used** | **Preparation****Method** |
| **Medicinal** | Treatment of warts, skin infections, andWounds | Rhizome, Resin | Paste or poultice applied topically |
| Anti-cancerproperties (source of podophyllotoxin) | Rhizome | Extracted resin used in modern medicine |
| Treatment of gastrointestinal disorders (e.g., diarrhea,constipation) | Rhizome | Decoction or powder consumed orally |
| Anti-inflammatory and analgesic(jointpain,arthritis) | Rhizome, Leaves | Paste applied to affected areas |
| **Ethnoveterinary** | Treatment of parasitic infections/antisepticin livestock | Rhizome | Mixed with fodder or applied topically |

**Table 2. Phytochemicals Identified by GC-MS Analysis of Methanolic Extract of *(Podophyllum hexandrum* Royle*.)* Rhizomes**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Peak Name** | **Retention time** | **Area %** | **Name of compound** | **Molecular Formula** | **Molecular Weight** |
| 1 | 17.413 | 10.97 | Campesterol | C₂₈H₄₈O | 400.69 g/mol |
| 2 | 21.299 | 18.24 | Podophyllotoxin | C₂₂H₂₂O₈ | 414.41 g/mol |
| 3 | 26.073 | 13.72 | Beta-sitosterol | C₂₉H₅₀O | 414.71 g/mol |
| 4 | 29.346 | 4.16 | Octadecanoic acid | C₁₈H₃₆O₂ | 284.48 g/mol |
| 5 | 31.649 | 8.01 | D-allose | C₆H₁₂O₆ | 180.16 g/mol |
| 6 | 31.904 | 7.51 | (9Z)-octadec-9-enoicacid | C₁₈H₃₄O₂ | 282.47 g/mol |
| 7 | 33.588 | 3.81 | Retinol | C₂₀H₃₀O | 286.45 g/mol |
| 8 | 34.106 | 12.38 | 2,2'-Benzylidenebis(3-methylbenzofuran) | C₂₆H₂₂O₂ | 382.46 g/mol |
| 9 | 36.032 | 7.88 | Sucrose | C₁₂H₂₂O₁₁ | 342.30 g/mol |
| 10 | 37.764 | 10.32 | Ethyl iso-allocholate | C₂₆H₄₄O₅ | 436.63 g/mol |

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*Fig.2. GC-MS Chromatogram of methanolic extract of* (*Podophyllum hexandrum* Royle.)

**Table 3. Bioactive Compounds Identified by GC-MS in *(Podophyllum hexandrum* Royle*.)* Rhizomes: Structures and Therapeutic Uses**

|  |  |  |  |
| --- | --- | --- | --- |
| **Compound Name** | **Therapeutic Uses** | **References** | **Structure** |
| **Campesterol** | Cholesterol-lowering effects, Anti-cancer properties, Antiangiogenic | (Choi *et al* 2007;Vanmierlo *et al.,* 2012; Bae *et al.,* 2021), |  |
| **Podophyllotoxin** | Used to treat genital warts, Anticancer, Antiviral | (Giri and Narasu, 2000; Edwards *et al.,* 1988) |  |
|  |  |  |  |
| **Beta-sitosterol** | Antioxidant, Cholesterol-lowering agent, Food fortification | (Patch *et al.,* 2006; Arivarasu, 2023; Abumweis & Jones, 2008). |  |
| **Octadecanoic acid** | Antimicrobial, Anti-inflammatory and Anticancer | (Yonezawa et al., 2008; Desbois and Smith; Harvey et al., 2010) |  |
| **D-allose** | Anti-cancer, Anti-tumor, Anti-inflammatory, Anti-oxidative, Anti-hypertensive | (Zare *et al.,* 2023; Lim & Oh, 2011). |  |
| **(9Z)-octadec-9-enoicacid** | Cardiovascular benefits and Skin-conditioning properties | (Harvey *et al.,* 2010; Subramanian *et al.,* 2019). |  |
| **Retinol**  | Essential for vision, Immune function, and Skin health  | (Jun *et al.,* 2021; Sorg *et al.,* 2006; Hammerling, 2016). |  |
| **2,2'-Benzylidenebis(3-methylbenzofuran)** | Antidiabetic, Antioxidant  | (Li *et al.,* 2013; Akinmoladun *et al.,* 2021) |  |
| **Sucrose** | Immunomodulatory, Hypertonic, Stabilizer and Cryoprotectant  | (Struck *et al.,* 2014; Tomaszewska *et al.,* 2018) |  |
| **Ethyl iso-allocholate** | Antimicrobial, Antioxidant, Anti-inflammatory,Anticancer, Antitumor, Antiarthritic, and Antibacterial  | (Tyagi & Agarwal, 2017) |  |

1. **Discussion**

This study investigates the traditional applications and phytochemical composition of (*Podophyllum hexandrum* Royle.) within the Langate Forest Division of District Kupwara in Kashmir. The study reveals the importance of this endangered species in terms of cultural history as well as its usage in medicine, and its role in the ecosystem.

4.1 **Traditional Uses**

In the Langate area of Kashmir, locals depend on the Himalayan May apple (*Podophyllum hexandrum* Royle.) for medicinal purposes since it aids in the healing of both humans and animals. Podophyllotoxin has antiviral and anticancer characteristics; people use the plant's rhizome and resin to treat wounds and skin-related conditions. It is used to make the cancer drugs teniposide and etoposide. (Giri & Narasu, 2000; Kumar *et al.,* 2015). In addition to its established veterinary use, (*Podophyllum hexandrum* Royle.) has been used traditionally in cattle to heal wounds and parasites because of its inherent antifungal properties (Kumar *et al.,* 2015). The increasing demand for podophyllotoxin and the plant's endangered status highlight the need for sustainable production methods, such as cell cultures or biotransformation, to protect this valuable resource (Farkya *et al*., 2004; Giri & Narasu, 2000).

* 1. **Phytochemical Profiling (GC-MS Analysis)**

GC-MS analysis reveals the rhizomes of (*Podophyllum hexandrum* Royle.) contain a variety of bioactive compounds. The most common compound identified, accounting for 18.24% of the sample, is the cancer-preventive compound podophyllotoxin (Jiang *et al.,* 2007; Qadir *et al.,* 2020). The presence of significant levels of other secondary metabolites, such as campesterol, beta-sitosterol, and D-allose in (*Podophyllum hexandrum* Royle.) rhizomes may contribute to its medicinal potential. Research supports D-allose's anti-ageing properties (Eun *et al.,* 2020). Though the amount generated will depend on culture and extraction techniques, it might yield a range of beneficial chemical compounds (Bhattacharyya *et al.,* 2013; Qadir *et al.,* 2020). Further research is needed to optimize extraction for industrial use in cancer drug production.

1. **Threats, Conservation Challenges and Recommendations**

Threats to medicinal plants and their conservation illustrate how humans impact the environment and climate change. Improper harvesting regulations allow individuals to overharvest medicinal plants, endangering their population (Shafi *et al.,* 2021). In addition to the limited distribution and high economic value of (*Podophyllum hexandrum* Royle.) there are continuous threats to species (Kala, 2005; Maqbool, 2011; Rana and Samant, 2011; Banerjee *et al*., 2017; Nag *et al.,* 2020). Market trends indicate that prices will remain high because demand for this species exceeds production volume (Chaurasia *et al.,* 2012). The species is likely to encounter habitat loss, regardless of the lack of knowledge on the implications of climate change. It is at elevated risk from climate change since its ability to survive mostly depends on winter temperatures (Banerjee *et al.,* 2017) and because winter temperatures rise more rapidly (Telwala *et al*., 2013). 74% of the Himalayan Mayapple's present range will no longer be readily accessible by 2050 (Shrestha *et al.,* 2022). The research recommends developing strategies for cultivation and strengthening harvesting regulations to safeguard the species. Our findings are reinforced by the study of (Ebeling and Yasué, 2008), who illustrated how effectively we can protect forest areas through a decrease in deforestation activities. Various studies indicate that habitat loss, which occurs when humans destroy forests to make space for roads and farms, represents the greatest threat to protected areas. 11 million hectares of protected areas in the humid tropics are under serious risk from both deforestation and climate change, which calls for immediate landscape solutions (Tabor *et al.,* 2018). Climate change may cause between 14% and 75% of Madagascar's suitable ruffed lemur habitat to disappear by 2070 (Morelli *et al.,* 2019). To tackle this, it is essential to do a study to track the effects of climate change adaptive management into place (Ebeling & Yasué, 2008; Kingsford, 2011; Shafi *et al.,* 2021) and encourage climate-resilient behaviours (Li *et al.,* 2022). Tackling these interrelated challenges would require enhancing governance and putting strict restrictions on the overharvesting of medicinal plants (Kingsford, 2011).

**Conclusion**

In Kashmir's Langate Forest Division, a study shows the ethnobotanical usage of (*Podophyllum hexandrum* Royle.) in addition to its vital ecological relevance and therapeutic benefits. The plant's therapeutic characteristics, particularly in the treatment of cancer, are supported by podophyllotoxin and other bioactive components. Ethnobotanical research indicates that traditional consumers of this herb have been using it for hundreds of years, but overharvesting and habitat damage pose a serious threat to its sustainability. Appropriate preventive measures must be put in place right away to preserve this vital species. The study shows that combining ancient knowledge with contemporary scientific methods is necessary to address future sustainable depletion. Fostering cultivation and enacting stringent laws for collecting in the wild are essential to the protected conservation of (*Podophyllum hexandrum* Royle.)

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies have been used during the writing or editing of this Manuscript.

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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