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Review Article

Traditional Uses, Active Ingredients, and Biological Activities of *Paris polyphylla* Smith: A Comprehensive Review of an Important Himalayan Medicinal Plant

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Paris polyphylla Smith (family: Melanthiaceae), a high-value medicinal herb endemic to the Himalayan region, has drawn much attention recently due to its immense use in the traditional healthcare system since ancient times. In the present review, an extensive database on *P. polyphylla* was systematically searched from databases such as Medline/PubMed, Scopus, the Web of Science, and the online service E-library.ru and SCImago (https://www.scimagojr.com/). Information on species, ecology, distribution, trade, ethnopharmacology, pharmacology, biotechnology, and molecular biology was gathered from 1979 to 2023 using 116 research publications. Major steroidal saponins such as Paris saponin I, V, VI, VII, and *H* have been found substantially effective in anticancer activity, abnormal uterine bleeding, dysfunctional uterine bleeding, and menorrhagia. Traditional breeding and propagation techniques cannot keep up with the world's growing demand for herbal drugs. Therefore, it is critically necessary to take conservation measures and develop novel techniques for growing and cultivating this economically significant and highly valuable therapeutic herb. The advanced biotechnological approaches like micropropagation and genetic analysis introduced long back are either rare or lacking in the case of *P. polyphylla*. It contains a wealth of information that will serve as a baseline data source for various stakeholders, researchers working on various research aspects, and policymakers to define appropriate utilization and conservation plans for a high-value commercial medicinal plant called *P. polyphylla*. The review provides an updated overview and critical assessment of secondary data regarding the past and recent applications and interventions of *P. polyphylla*.

1. Introduction

Medicinal and aromatic plants (MAPs) have significantly improved human health since the ages. These plants are crucial in meeting the demands of the traditional medicine markets across the globe. According to WHO (World Health Organization), about 80% of the world's population depends on herbal medicines for primary health care [1]. Herbal plants account for over 25% of the world's drugs [2, 3]. The immense potential of medicinal plants used in various traditional systems has been evident through increased focus on plant research in recent times [4], especially the physiology and pharmacology of medicinal plants through pharmacological investigation [5, 6]. Uncontrolled harvesting of natural flora, including those found in forests, has satiated the rising demand for the vast majority of medicinal plants. As a result, habitat and genetic diversity have suffered catastrophic losses [7, 8]. Small population size, restricted distribution, human disturbance [9], and unmanaged harvesting [10, 11] lead to poor regeneration of the species [12], and failure of seed development [13] is responsible for the threat to the species. This results in the lack of availability of quality plant material for the future [8, 14]. Thus, this is causing concern for the long-term sustainability of the natural resource, which can be met by mass multiplication and sustainable conservation of important medicinal plants. Recently, new agrotechniques have evolved for the sustainable cultivation of medicinal plants. However, there is an urgent need for biodiversity management by which medicinal plant resources could be conserved and will help to develop sustainable rural livelihoods [15–17].

Considering the increasing demand for medicinal herbs by pharmaceutical industries, the local market, and the traditional health care system followed by overexploitation, illegal trade, and unorganized cultivation, Himalaya has gained priority towards the conservation and sustainable utilization of its herbal wealth [17]. Indian Himalayas has been known for their rich cultural, spiritual, and biodiversity values all across the globe. The cure for various ailments and the use of medicinal herbs in the Himalayan region are well described in Indian sacred texts. The 3000-years-old Ayurvedic system in India has evidence of medicinal plant collection from the Himalayan region. Having more than 10% of countries geographical area, Himalaya harbors more than 8000 vascular plants, including 1748 medicinal plants [18]. The potential of the medicinal wealth of the Himalayas is so immense that about 90% of wild plant species belonging to the alpine grassland of the Himalayas are used in various herbal industries. Moreover, the herbal industries in India are getting 90% of medicinal plant raw material (including for export) from natural habitats [19].

Among various medicinal plants used to prepare medicines, *Paris polyphylla* from the genus *Paris* has its unique identity and recognition. *P. polyphylla*, a high-value medicinal plant, is native to the Indian subcontinent and China and is extensively distributed in the Yunnan-Guizhou plateau of China [20], Manipur in India [21], and Pokhara and Kathmandu in Nepal. Apart from China, India, and Nepal, subspecies and varieties of *P. polyphylla* are well distributed in Myanmar, Bhutan, Vietnam, Thailand, and Laos [22]. All the plants of the genus *Paris* are known for various medicinal properties. They can be used as medicine [23], such as relieving pain, detoxifying, reducing swelling, and calming the liver. *P. polyphylla* has received increasing recognition and attention due to its medicinal properties in recent years, which have generated intense ethnopharmaceutical interest in the pharmaceutical industries [24]. Furthermore, due to high demand from pharmaceutical industries, *P. polyphylla* has been overexploited from its natural resources, which has caused significant habitat loss and put this species on the edge of extinction in the last few decades. In addition, this species' slow growth and reproduction have put it in endangered status, resulting in a sharp rise in price [25]. In the past studies, the authors have reviewed the population density, trade, cultivation and propagation, chemical and biological perspectives, pharmacological uses and effects, reproductive biology, genetic diversity, and management of *P. polyphylla* [5, 6, 26, 27], and there has been no systematic study to understand multiple attributes together. Despite the fact that only a small number of authors have reviewed the bioactive compounds [5], traditional uses [6, 28], microbiological aspects [29], distribution, ethnobotanical uses, molecular characterization, and species cultivation [6], there is a dearth of knowledge regarding the distribution of medicinal plants, their suitability for different habitats, the ratio of demand to supply, and the effects of climate change. Moreover, attributes like ecology, biotechnology, molecular biology, and conservation have yet to be reviewed. Thus, the published literature on a variety of *P. polyphylla*-related topics, such as its ecological status, commercial value, ethnopharmacology, pharmacology, phytochemistry, *in vitro* propagation, conservation status, molecular analysis, and future possibilities, has been reviewed in this study to fill this knowledge gap. Considering the abovementioned issues, the present review is prepared to provide a comprehensive knowledge, which underlines the current scenario and future prospects of this species.

2. Methodology

Consideration was given to the significance of review papers as a thorough literature search was conducted. Various search engines viz., Web of Science (https://webofknowledge.com), ScienceDirect (https://www.sciencedirect.com), ACS publications, Taylor and Francis Online, SpringerLink, Scopus (https://www.scopus.com), Web of Science, PubMed (https://pubmed.ncbi.nlm.nih.gov/), National Library of Medicine (https://www.nlm.nih.gov/), and Wiley online library databases were used. The data on *P. polyphylla* were compiled via research papers, review articles, books, book chapters, reports, and conference and seminar sessions. The main focus was on the bioactive principles of *P. pollyphylla* along with keywords like *Paris*, *Paris* habitat, *Paris* geography, *Paris* ethnopharmacology, *Paris* pharmacology, *Paris* ecology, *Paris* diversity, and *Paris* trade to investigate the distribution, traditional use, ecological, biotechnological, molecular, phytochemicals, pharmaceutical, ethnopharmacological, pharmacological, and toxicological aspects of *P. polyphylla*. This review was created using 116 references and a thorough database search on 118 different aspects of *P. polyphylla* from 1979 to 2023.

3. *Paris polyphylla* at a Glance

P. polyphylla Smith is locally known as Satwa in western Himalaya, Singpan in eastern Himalaya, and Satuwa in Nepal. This perennial herb belongs to the family Melanthiaceae and is distributed mainly in Nepal, China, Bhutan, India, Myanmar, Vietnam, and Thailand [30]. In India, Uttarakhand, Sikkim, Himachal Pradesh, Jammu and

Kashmir, Arunachal Pradesh, Manipur, Nagaland, Meghalaya, and Mizoram are the main locations of *P. polyphylla* [31]. This is one of the most important medicinal herbs across Himalaya, which has been a major source of raw material used in the health care system [32] to cure various ailments like cancer, tumors, fungal infections, back pain, fractures, bleeding, poisons, and skin allergy [31]. The whole plant is used as febrifuge; rhizome as an expectorant and vermifuge, anthelmintic, antispasmodic, and digestive stomachic [33]; and roots as antitussive and depurative, antiphlogistic analgesic, and antipyretic [34]. The perennial herb *Paris* is a temperate genus with twenty-four species. The plant carries bisexual flowers, berry-like fruits with too many seeds, an erect stem, and thickened rhizome. Stem is thick (1–2.5 cm), tall (50–100 cm), and unbranched. Simple leaves are arranged in whorls, petiolate, and lanceolate, with reticulate venation and smooth margins. Inflorescence consists of yellow-green flowers forming a closed whorl at initial growth. Solitary flowers are monoecious (male and female gamete within the same flower); outer sepals are larger and inner smaller, followed by 3–5 petals. Stamens are free and 6–11 in number, and gynoecium consists of one pistil and 3–5 fused carpel (syncarpous). A mature fruit contains 50–60 reddish orange seeds [35]. Germination in *P. polyphylla* occurs in April, and flowering starts in late April–May. Seed formation starts in late September and becomes mature in October. At the end of October to mid-November, aboveground parts die, and seeds spread in the environment. Between the seed maturation and the death of the plant, new bud sprouts from the rhizome and remains underground due to unfavorable conditions of the environment. Once the favorable conditions (sprouting season) start, these sprouts get germinates and come out from the ground. The rhizome remains dormant for five months. Seed germination of *P. polyphylla* in the natural condition is very low. Madhu et al. [34] also reported poor wild germination of *P. polyphylla* seed; even if the seed germinates, it takes a long period for flowering and many years to produce seed in a sufficient amount. It has been observed that there is no uniform pattern of sprouting, flowering, fruiting, or senescence of this plant; even, when the plants are taken out from the natural habitat and grow in earthen pots or nurseries in an artificial condition, plants show variation in senescence, sprouting, flowering, and fruiting behavior [36]. The plant in the habitat and its underground useable parts are depicted in Figure 1.

4. Ecological Status: Habitat and Occurrence

P. polyphylla prefers a more than 80% closure canopy, altitude between 1300 and 1800 masl. It prefers deep forests and association with *Arisaema costatum*, *Cyrtomium anomophyllum*, *Daphne bholua*, *Sarcococca* species, and *Viburnum erubescens* for seed germination [36]. It requires streamside slopes (grassy or rocky) and mixed conifer forests in the habitat to grow properly. In midhills and lower mountains, the moist habitat of rhododendron, oak, and laurel forests provides favorable conditions for growth [34]. Being slow-germinating herbs, human interference sites, and water-logged areas are not suitable for the growth of *P. polyphylla*. Several problems are associated when it grows in artificial habitats (cultivation). Lack of inflorescence and flowering failure, followed by lack of fruit and seed production, is common in cultivation. However, it can grow well with similar habitat conditions [36].

Recent studies on the distribution and abundance of *P. polyphylla* indicate that its availability in nature is very low compared to its associated species from the same habitat [37]. However, only some studies on the ecological status of *P. polyphylla* have been performed in various parts of the Himalayas in the last few years [38–41]. Most of this ecological research is restricted to Nepal only. Compared with the species density of five highly demanded medicinal plants of Himalaya, the density range (0.07–3.8 in $d{\cdot}m^{-2}$) of *P. polyphylla* was found to be similar. The status of the species density of *P. polyphylla* is depicted in Table 1. This scenario opens the scope for detailed ecological research in Indian Himalayas.

5. Trade Value

Apart from its medicinal value, *P. polyphylla* is known for its high trade value of approximately Rs. 700–4500/kg for fresh and dried rhizomes [31, 34, 42, 43]. This species is the most vulnerable species, sharing 24.6% of the total trade value in the Himalayas, Nepal, and China [34]. The species has been exported to neighboring countries for a long time [42]. In the recent past, *P. polyphylla* has been traded in bulk amounts in Nepal (Pokhara and Kathmandu) and presently in different states in India, including Assam, Arunachal Pradesh, and Uttarakhand. Assam and Manipur have been the main route for the illegal trading of *P. polyphylla* from northeast India to Myanmar and southeast countries [31]. Basar [44] has reported the illegal export of *P. polyphylla* rhizomes from Arunachal Pradesh via Myanmar to China. Illegal trade and trafficking of rhizomes from Nagaland, Manipur, Arunachal Pradesh, and Meghalaya to Myanmar have been reported [21]. Currently, in Garhwal Himalaya, the illegal exploitation of this plant is continuing in practice. Due to their commercial value, local people collect these plants from their natural habitat for income. Continuous increases in price cause a tremendous threat to this plant due to overexploitation before maturation. Therefore, this plant is facing the problem of extinction from the wild [43]. Illegal collection of this plant from the Uttarkashi district of Uttarakhand has been highlighted recently [45]. Due to the unorganized and illegal collection and poor viability of seeds in natural conditions, this species has been listed under the endangered category [31]. Nepal has emerged as the highest producer *of P. polyphylla* rhizome, with over 400 tons of annual production [46]. The status of trade marketing is depicted in Table 1.

6. Ethnopharmacology

Ethnopharmacology is the scientific study of substances used as medicines by various ethnic and cultural groups. *P. polyphylla* Smith, a significant species of the genus, has

Figure 1: *Paris polyphylla* plants in their natural habitat and exposed aboveground and belowground.

Table 1: Ecological status and the trade value of *Paris polyphylla*.

Locality	Density (Ind·m^{-2})	Rhizome cost (Rs·kg^{-1}) Fresh	Rhizome cost (Rs·kg^{-1}) Dried	References
Eastern India (West Kameng, Lower Dibang, Lower Subansiri valley)	0.42–1.48	700–800	3500–4000	[31]
Kaski district, Nepal	2.2	—		[33]
Ghandruk Village Development Committee (Annapurna Conservation Area (ACA), Nepal)	1.16–2.26	—	—	[34]
Nepal	0.078	—		[37]
Rasuwa district, Nepal	0.12	—		[39]
India	3.8	—		[40]
Dhankuta district, Nepal	0.2	—		[41]
Western Himalaya	—	1500	4500	[42]
Baitadi district, western Nepal		3700		[43]

been called "jack of all trades" as it is used in treating various ailments, from diarrhoea to cancer [47]. The rhizomes of *P. polyphylla* Smith are used in traditional Chinese medicines for their haemostatic, anti-inflammatory, antitumor, analgesia, and antifungal activity. Only the rhizomes of *P. polyphylla* Smith var. *yunnanensis* and *P. polyphylla* Smith var. *chinensis* are referred to as medicinal *Paris* in the Chinese pharmacopoeia [48]. The Chinese pharmacopoeia mentioned its use as an anti-inflammatory, analgesic for sore throat, snake bites, and bruises. The rhizomes of the species are reported for their use as digestive, expectorant, anticholinergic, and vermifuge. It is helpful in the treatment of heart disease, asthma, and bronchitis. Rhizomes are used as anthelmintic and tonic by the local inhabitants of Garhwal. Also, root powder is used in ethnopediatrics for diarrhea in this region. Rhizoma Paridis (Chonglou) is the main constituent of commercial products of *P. polyphylla*, namely, Yunnan Baiyao powder, Jidesengshe-yao pain tablets, and Gongxuening capsules which are well-known Chinese herbal medicines. Gongxuening capsules are a commercial product in the Chinese market, widely used for over 10 years [46]. Chinese people use rhizome as a demulcent, antifebrile, immunity adjustment, analgesia, and anti-inflammatory [49, 50]. The rhizome juice relieves gastric and menstrual pain, and rhizome paste is used to treat cuts and wounds and remove worms by ethnic people of western Nepal [51].

7. Pharmacology

The primary bioactive chemical components of *P. polyphylla*, accounting for more than 80% of the total chemicals, are Paris saponins or steroidal saponins. A form of glycoside called a saponin has one or more water-soluble sugar chains, such as glucose, galactose, pentose, or methyl pentose, as well as aglycones like steroids or triterpenoids. Plant saponins have a variety of forms because different sugars can be found in various positions and directions.

Steroid saponins and polyphyllins are the main constituents among the nearly 100 phytochemicals of therapeutic significance that have been discovered in the species [6]. The anticancer, antioxidative, expectorant, platelet aggregation inhibition, insecticidal, antidiabetic, antifungal/antiyeast, antiparasitic, antibacterial, antihyperlipidemic, and anti-inflammatory properties of steroid saponins are just a few of their medical applications. The biological activity of steroidal saponins and additional ingredients was investigated against cancer cell lines, bacteria, enzymes, and other parasites that have demonstrated anticancer activity against oesophageal cancer [52], lung cancer, bone cancer, prostate cancer, breast cancer, bladder cancer, liver cancer, and colon cancer [53]. In addition, *P. polyphylla* rhizome extract and steroidal saponins shown antibacterial and antioxidant action [54]. On *Candida albicans*, steroidal saponins also demonstrated antifungal properties. The steroidal saponins had selective antibacterial activity against a variety of bacteria, including *Pseudomonas aeruginosa* (100%), *Staphylococcus aureus* (80%), *Listeria innocua* (65%), *Escherichia coli* (57%), *Salmonella enteric* (67%), and *Shigella sonnei* (47%). Several pure steroidal saponins and crude extracts of *P. polyphylla* have shown strong efficacy against cancer cell lines, bacteria, and parasites in *in vitro* and *in vivo* tests. Some activities of *P. polyphylla* extract are summarized as follows.

7.1. Anticancer Activity. The hydroalcoholic extract of *P. polyphylla* was analyzed and found to contain diosgenyl and pennogenyl saponins having anticancer activity against human A 549 lung cancer cell lines using doxorubicin as a standard. At a 500 microgram/ml concentration, more than 97% increment in cell killing was observed, and the EC_{50} was calculated to be 52.34 microgram/ml. In comparison, doxorubicin exhibited 98% killing at a concentration of 1.8559 microgram/ml, and EC_{50} was 0.579 microgram/ml [55]. With an IC_{50} value of 0.6 and 0.9 micromolar, steroidal saponins from *P. polyphylla* rhizomes showed cytotoxic action against two human cancer cell lines: HEK 293 and HepG2 [56]. Induced apoptosis has also been reported in breast cancer cell lines and HT-29 and HCT-116 cells. This saponin also arrests the cell cycle in the G1 phase. Moreover, autophagy, which can promote apoptosis in MCF-7 and MDA-MB-231 cell lines, was facilitated by XA-2 and inhibited the Akt/mTOR signaling pathway [57]. Because Paris saponins inhibits the activity of IKKβ and p65 translocation, they are recognized as NF-kB activation suppressors. Suppression of mitochondrial fission has also been reported in Paris saponin by inhibiting the phosphorylation of Erk1/2 and mitochondrial translocation of dynamin-related protein 1 (Drp1) by dephosphorylation Drp1 at Ser616 [58]. The antiangiogenic effect of polyphyllin D was studied for the implication of a potential drug in cancer treatment, and polyphyllin D was found to suppress the growth of HMEC-1 cells at 0.1–0.4 micromolar without any side effect. This concentration inhibited endothelial cell migration and capillary tube formation [59].

The effect and mode of action of polyphyllin I on human gastric carcinoma cells were examined, and it was found that polyphyllin I inhibited HGC-27 cell proliferation with an IC_{50} value of $0.34 \pm 0.06\ \mu M$ after a72-h treatment *in vitro*. In comparison to paclitaxel, polyphyllin I at 3 mg/kg significantly reduced the proliferation of HGC-27 tumour cells *in vivo* and demonstrated superior safety. By switching LC3-I to LC3-II, polyphyllin I caused cell cycle arrest in HGC-27 cells. It has been reported that analysis of MDC and mGFP-LC3 fluorescence, western blotting, and flow cytometry downregulate cyclin B1 [60]. In the human erythroleukemia cell line K562, polyphyllin D induces apoptosis and differentiation, which inhibits cell proliferation. Through the mitochondrial apoptotic pathway, it causes apoptosis [61]. The effect of polyphyllin I in cisplatin-resistant human gastric cancer cell lines was studied, and the result showed that polyphyllin I treatment decreased cell proliferation. It also increased the mRNA and protein expression level of E-cadherin but decreased the expression of vimentin. It was concluded that polyphyllin I can be used as a potent drug candidate for treating cancer invasion and migration [61]. A novel spirostanol steroidal saponin was isolated from *P. polyphylla* rhizomes. Structure elucidation was done by two-dimensional NMR spectroscopy. The structure was found to be pennogenin-3-O-{O-α-L-rhamnopyranosyl-(l $\longrightarrow$; 4)-O-α-L-rhamnopyranosyl-(l $\longrightarrow$ 4)-β-D-glucopyranoside}, which was further evaluated for anticancer activity in human promyelocytic leukemia HL-60 cells. The results showed that at a 10 μg/ml concentration, more than 50% inhibition in cell growth occurs [62]. Lepcha et al. [40] reported a high concentration of flavonoids and phenolic compounds along with DPPH and ABTS scavenging activity in the *P. polyphylla* plant from a higher altitude. Furthermore, the cytotoxic effect of the extract was determined on three cancer cell lines, HeLa, HepG2, and PC3, by MTT assay, which showed dose-dependent inhibition of HeLa cell growth and moderate inhibition in HepG2 and PC3 cell growth.

7.2. Immunostimulating Activity. Various chemical components have been reported in *P. polyphylla* Paris saponins I, II, V, VI, VII, *H*, dioscin, and oligosaccharides showing biological activity like anticancerous, antitumor, cytotoxic, and immunostimulating [26, 43, 63]. Diosgenyl saponins isolated from *P. polyphylla* have a noticeable effect on immunostimulating activity. This activity has been investigated in mouse macrophage cells and observed a significantly enhanced phagocytic activity. Analysis was observed by phagocytosis, respiratory burst, and nitric oxide production [64].

7.3. Antimicrobial Activity. In order to evaluate *P. polyphylla*'s antibacterial activity, disc diffusion assays were performed against two bacterial strains: *Escherichia coli* and *Staphylococcus aureus* and as well as two fungus strains: *Aspergillus niger* and *Trichoderma reesei. E. coli*, *S. aureus*, and *A. niger* showed 95–97% inhibition with significant antibacterial activity at a concentration of 5 mg/ml of the sample. In the case of *T. reesei*, it was 74% [65]. Saponins isolated from the stem and leaves of *P. polyphylla* showed antimicrobial activity against *Propionibacterium acnes*, using erythromycin as a standard [66]. *P. polyphylla* extracts were reported to destroy viral RNA by penetrating the viral capsid. It is also observed that phytoconstituents (Paris saponin, polyphyllin I and II, and diosgenin) of *P. pollyphylla* could inhibit viral growth by enhanced production of IL-6 cytokine, which augments the antiviral activities [35].

7.4. Antioxidant Activity. The antioxidant potential of *P. polyphylla* was evaluated by DPPH assay and by determining the total phenolic content. Prominent inhibition was reported with the ethanolic extract of *P. polyphylla*. Compared to the standard ascorbic acid inhibition concentration value of 7.8 μg/ml, *P. polyphylla* showed a higher inhibition concentration value (68 μg/ml) using the DPPH method. Phenolic content was also higher in the ethanol extract (0.68 mg/g catechol) than in the petroleum ether extract (0.47 mg/g catechol). Ethanol extract showed significant antioxidant activity due to higher phenolic content [67]. Higher concentrations of plant phenols and flavonoids were reported in the plant from high altitudes, but both the high- and low-altitude plants exhibited excellent DPPH and ABTS scavenging activity [40].

7.5. Antiaging Activity. The antiaging effect of polysaccharide consisting of L-arabinose and D-galactose in a molar ratio (4.2 : 5.8) was evaluated by D-galactose-induced mouse aging model revealed prevented malondialdehyde formation and improved antioxidant enzymes levels and total antioxidant capacity in serum and liver [68, 69].

7.6. Protective Effect. The effect of *P. polyphylla* methanolic extract in ethanol-induced gastric lesions in rats has been reported, and significant inhibition was observed. It was also reported that spiroatano structure is essential for the activity, whereas in protective effects against ethanol-induced gastric lesions 17-hydroxyl group plays an important role [70]. The extract of *P. polyphylla* var. *yunnanesis* is an important alternative medicine for abnormal uterine bleeding. In China, it is (*P. polyphylla* extract, which is nontoxic) used in place of estrogen and progestin, which is used in abnormal uterine bleeding therapeutically [35]. The spirostanol glycosides obtained from *P. polyphylla* are reported as contractile agonists for the uterous, and the activity is attributed to glycone and the aglycone part of the compound [71].

7.7. Toxicity. The *P. polyphylla* rhizome extract was investigated for cytotoxic effect on healthy rats over a period of time. The findings revealed no sign of mortality, ill health, and toxicity [71]. In rats, the rhizome extract's cytotoxic effect was analyzed over time and observed as nontoxic. All the rats survived well and showed no sign of toxicity or mortality. Although dullness was reported briefly, that could be because of neural suppression. The extract's cutoff median lethal dose (LD_{50}) was also recorded in female rats for 14 days, and it was greater than 5000 mg/kg body weight. From the study, it was clear that the extract showed a cytotoxic effect only for cancerous cells and proved itself a good target for future anticancer drug studies [71]. In another study, cytotoxic activities of triterpenoid saponins isolated from the rhizome of *P. polyphylla* have been observed against human nasopharyngeal carcinoma epithelial cells [72]. Furthermore, the cytotoxic effect of the extract revealed dose-dependent inhibition of HeLa cell growth while moderate inhibition in HepG2 and PC3 cell growth when determined on three cancer cell lines, HeLa, HepG2, and PC3 [40]. In addition, steroidal saponins from *P. polyphylla*'s rhizomes have been shown to have a cytotoxic effect against the human cancer cell lines HEX 293 and HepG2 [56]. Li et al. [52] found polyphyllin D, a steroidal saponin, in the extract of *P. polyphylla*. The author reported viability inhibition and apoptosis induction in treating MCF-7 and MDA-MB-231 cells with polyphyllin D. *In vivo* studies showed that polyphyllin D starts mitochondrial dysfunction and elicits apoptosis. It has also been demonstrated through *in vivo* studies that daily administration of polyphyllin D (2.73 mg/kg body weight) reduced 50% of the tumor in terms of weight and size and did not show any toxic effect on the heart and liver of the host Li et al. [52]. Based on the literature cited in this review and available in the public domain, it has been proven that there is no acute or chronic toxic effect of extract of *P. polyphylla*. Moreover, pharmacological uses and phytoconstituents of *P. polyphylla* are described in Table 2.

8. Phytochemistry

Plants are considered biosynthetic laboratories because they synthesize various metabolites. Phytochemistry deals with the secondary metabolites which are produced through the plant defense system. These are also known as active principles or active ingredients. These phytomolecules help the plant to survive against pests, pathogens, UV exposure, and other environmental hazards. Drug discovery depends entirely on phytochemicals; therefore, understanding these phytochemicals is very important for preparing novel

Table 2: Pharmacological uses and phytoconstituents of *Paris polyphylla*.

Pharmacological uses	Phytoconstituents	Method adopted	References
Anticancer	Diosgenyl and pennogenylsaponins	Human A 549 lung cancer cell lines	[69]
	Steroidal saponins	Human cancer cell lines HEX 293 and HepG2	[59, 70, 73]
	Saponins	MCF-7 and MDA-MB-231 cell lines	[71]
	Polyphyllin D	HMEC-1 cell, human erythroleukemia cell line K562	[59, 65, 74]
	Polyphyllin I	Human gastric cancer cell lines	[75, 76]
Immunostimulating activity	Diosgenylsaponins	—	[76]
Antioxidant activity	—	DPPH assay	[27]
Antiaging activity	Polysaccharide (consisting of L-arabinose and D-galactose)	D-galactose-induced mouse aging model	[67]
Protective effect	Steroidal saponin	Ethanol-induced gastric lesions in rats	[77]
Antimicrobial activity	—	Disc diffusion assay against *Escherichia coli*, *Staphylococcus aureus, Aspergillus niger* and *Trichodermareesei,* and *Propionibacterium acnes*	[78]
	Saponins	—	[79]
	Saponins	—	[35]
Anti-abnormal uterine bleeding	Steroidal saponins	—	[80]

therapeutic agents against the disease [81]. For phytochemical screening, *P. polyphylla* rhizomes were extracted using solvents like water, methanol, ethanol, ethyl acetate and chloroform, and chemical tests were performed on all extracts. Secondary metabolites such as alkaloids, flavonoids, saponins, carbohydrates, glycosides, cardiac glycosides, terpenoids, sterols, quinones, phenols, and tannins were detected by the analysis [5, 14, 74]. Other studies also aimed at identifying its chemical constituents, showing that steroidal saponins, flavonoids, and phytosterols were the main ingredients. Steroidal saponins are the chemotaxonomic markers of the genus *Paris*. Pharmacological experiments illustrated that steroidal saponins are the chief active constituents [75]. *P. polyphylla's* rhizomes, roots, aerial stem, and leaves have yielded several substances that have been isolated and characterized, including steroidal saponins [82, 83], flavonoid glycosides, sterols, triterpenoid saponins, and polysaccharides are some examples of these compounds [84, 85]. Phytosterols, flavones, and phytoecdysones were among the more than 90 components that were isolated between 1960 and 2010 [61], and about 67 steroidal saponins were found in 11 species of the genus *Paris*.

Saponins are biosynthesized in the green part of plants, and then they are transported to the roots for storage and distributed in aerial parts. Thirteen types of saponins are reported in the aerial part of the plant, but some are not isolated because they are either unstable or very minor in concentration. Nautigenin and isonautigenin, a derivative of spirostanol saponin, have been isolated from fresh plants. From *P. polyphylla*'s aerial portions, hydroxy-pennogenins and its tetrasaccharide and pregnane-type saponins have also been discovered. [86]. For the first time, five different steroidal saponins, namely, Paris saponin I, Paris sapon V, Paris sapon VI, Paris sapon VII, and Paris sapon *H* have been isolated from *P. polyphylla*, along with six new oleanane-type triterpenoid saponins, paritrisides *A*–*F* (1–6) and nine known triterpenoid saponins (7–15) [76].

Around 320 chemical components, such as steroidal saponins, C-21 steroids, phytosterols, insect hormones, pentacyclic triterpenes, flavonoids, and other compounds, have been identified up until 2020 [28]. Only four Paris saponins such as Paris saponins I, II, VI, and VII have been formally acknowledged as quality standard components of the Chinese pharmacopoeia, although more than 50 Paris saponins have been identified from *P. polyphylla* var. *yunnanensis*. Diosgenins, pennogenins, and their related glycosides have all been isolated from *P. polyphylla* in various forms. Parispolyside F and parispolyside G, two new phenylpropanoid glycosides that are phenolic glycoside derivatives, have been identified from the rhizomes. Isolated from the rhizome of *P. polyphylla* var. *yunnanensis*, these two previously identified flavonoid glycosides were named isorhamnetin-3-o-neohesperidoside and isorhamnetin-3-o-gentiobioside [78]. The chemotaxonomic associations of nine species of *Paris* were collected from various Chinese locations. Infrared Fourier transform (FT-IR) analysis revealed similarities amongst the investigated species. In addition, chemotaxonomic research was carried out on *P. pollyphylla*. Using ions trap/time-of-flight mass spectrometry and triple quadruple mass spectrometry (QQQ-MS), polyphyllin I, II, V, VI, and VII, and gracillin were identified in *Paris* species. [78]. Two simple oligosaccharides, heptasaccharide (M. F. $C_{42}H_{72}O_{36}$) and octasaccharide (M. F. $C_{48}H_{82}O_{41}$) have been isolated from *P. polyphylla* var. *yunnanensis* rhizome water extract. In the hairy root culture of a separate species, *P. japonicus* var. *major*, and the shoot culture of *P. polyphylla*, it was discovered that these oligosaccharides had growth-regulatory functions [79]. Yang et al. [77] created a quick and accurate method to measure 9 *P. polyphylla* components in rat plasma using LC-MS. Due to its higher sensitivity, this method is beneficial for human clinical study [77]. A UF-LC-MS method with multiple pharmacological targets of TopI, II, and COX-2 enzymes was devised to investigate the mechanism of action of polyphyllins. *In vitro*, polyphyllin I demonstrated the highest binding affinity and the strongest COX-1, COX-2, and COX-2 inhibitory actions, followed by polyphyllin II, VI, and VII. The study results imply that polyphyllin I is the main compound responsible for *P. polyphylla's* anticancer and anti-inflammatory effects and that the COX-1 and TOP I enzymes are its targets for these effects. Chen and Guo [58] suggested that polyphyllin I, II, and VI can be used as a marker component for the quality regulator of *P. polyphylla* by considering the comparative quantities and their inconsistencies in pharmacological activities. Ginsenoside Rh2 was used as the internal standard in developing a quick, precise, and LC-ESI-MS/MS method for the isolation and simultaneous measurement of polyphyllin D and Paris H in rat plasma. A homogenous polysaccharide was found in the leaves of *P. polyphylla*, which consists of L-arabinose and D-galactose in a ratio of 4.2 : 5.8 [68, 69]. For the extraction of polysaccharides from *P. polyphylla* leaves, extraction conditions were optimized such that temperature, time, and ratio of water to raw material were 90.8°C, 4.8 hrs, and 21.3:1, respectively, which produced the experimental yield of 54.18% [69]. Some important compounds isolated from the rhizome of *P. polyphylla* and their structures are depicted in Figure 2.

9. *In Vitro* Micropropagation

The plant reproduces naturally by seeds and rhizomes; these techniques are often used for the multiplication and conservation of any endangered species. Plant tissue culture technology is now becoming an important tool for the large-scale production of plant species that have medicinal importance and are generally difficult to cultivate [8]. In addition, the *in vitro* method can multiply this species because it produces genetically comparable plants quickly and can aid in the recovery of rare endangered threatened (RET) Himalayan herbs [80, 87–89]. Recently, a procedure using thin cell layer culture and *in vitro* plant regeneration through direct somatic embryogenesis for this species were described [90, 91]. In another study [92], the transverse thin cell layer (tTCL) culture technique was used to regenerate the plant by forming minirhizomes. Minirhizomes were induced from the transverse thin cell layer of basal and middle stem portions, whereas the apical portion failed to produce any

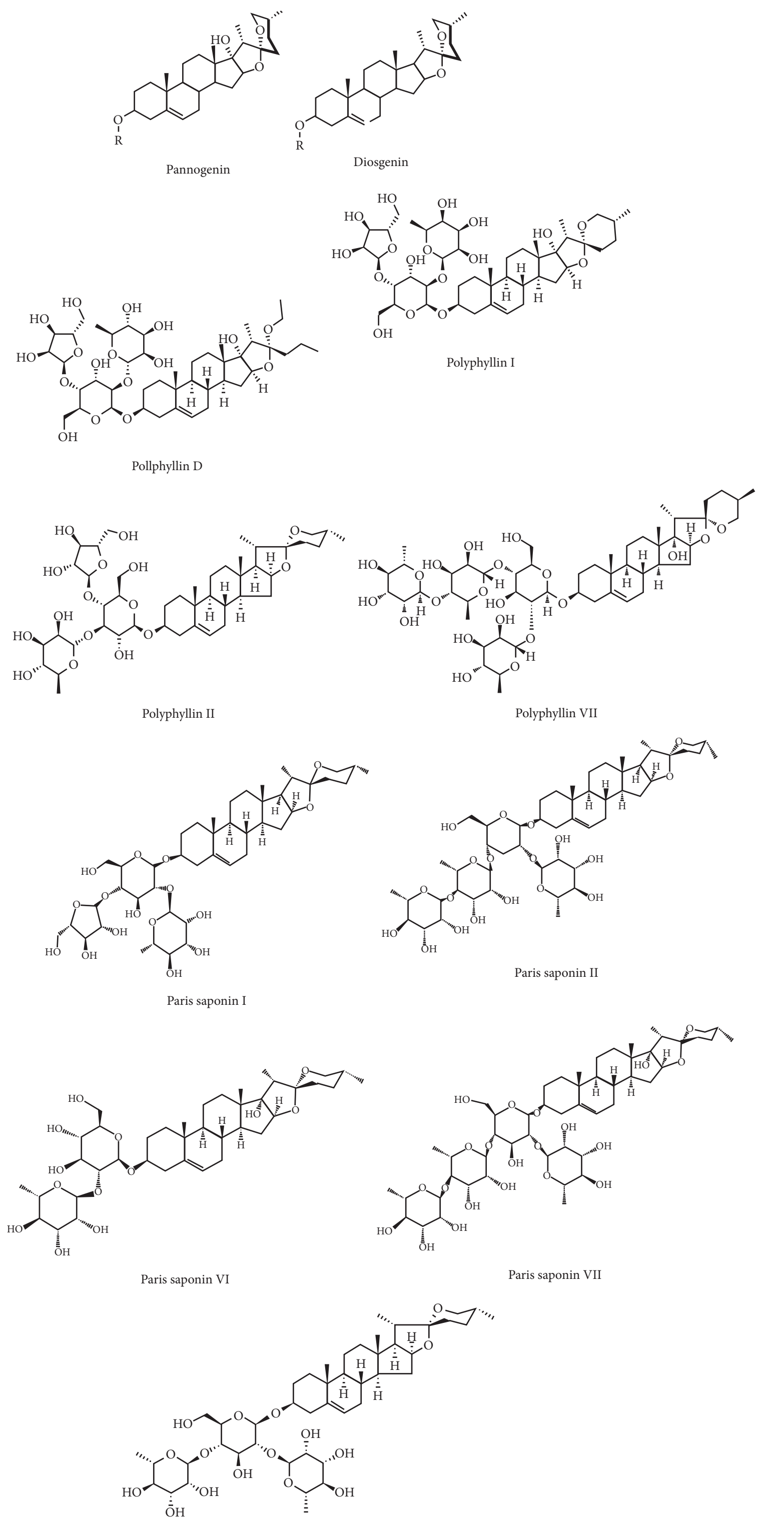

Figure 2: Some important structures of compound isolated from *Paris polyphylla*.

growth. Maximum 86.6% mini formation (fresh weight 1.05 ± 0.08 g) was observed on MS media supplemented 0.5 mg/l 6-benzyl amino purine (BAP). These minirhizomes produced healthy plantlets after hardening with 95% survival under greenhouse conditions. Active ingredient analysis of these plants showed a 1.41-fold increment in total steroidal saponins. Studies on elicitation using chitosan, salicylic acid, and yeast extract revealed increased steroidal saponin synthesis [92]. Different soil compositions also noted the effect of IBA and GA3 hormones (50, 100, and

150 mg/l) on *P. polyphylla* sprouting and rooting %. The results showed that soil texture having soil:loam:sand in a 3:2:1 ratio gave a better response for sprouting and rooting (76.66% and 73.33) [93]. Recently, Jesmi Devi et al. [30] have studied the total steroidal saponins diversity of *P. polyphylla* in the Northeast region of India. The elite population for the micropropagation study was identified based on the highest total saponins content (32.06 mg/g of dry weight).

Furthermore, in the same study, MS media supplemented with 0.5 mg/l BAP + 6% sucrose (88.6%) and 1.0 mg/l 2iP + 6% sucrose (89.2%) was found to be the best combination for mini rhizome induction with 1.27 ± 0.02 g fresh weight and 1.36 ± 0.10 g fresh weight, respectively. The effect of temperature of *in vitro* culture was observed, which revealed that a temperature range between 14 to 16°C is optimum for culture growth. Low temperature is unfavorable for culture growth, whereas high temperature causes culture contamination with endophytic bacteria [94]. In another study, Jamir et al. [95] split older rhizomes into pieces to raise shoot buds from each rhizome fraction to get a complete plantlet. They claimed that just 7% of the plantlets grown from rhizome cuttings were inflorescent and displayed fruitful character, with the percentage of regenerated plantlets being 49%. It shows the poor regeneration of the plant. Because of its higher current status due to the illegal collection and economic importance, there is an urgent need to develop cultivation packages for the farmers. Also, the collective and sustainable participation of the farmers to conserve this plant in its natural condition is required.

10. Molecular Analysis

Studies on genetic diversity are increasingly being employed in conservation planning, particularly with relation to the effective restoration of populations in the wild. The association between genetic variety and fitness of plant species has been established with support from numerous theoretical and empirical research studies. In plants, a species' geographic and ecological ranges are frequently connected with the genetic diversity within that species. The quantity and distribution of genetic variation are significantly influenced by a number of variables, including population size and habitat distribution. Population genetic theory states that populations are susceptible to genetic diversity loss if they exhibit any of the following characteristics: (i) genetic drift, (ii) founder effect, (iii) small initial population size, (iv) population bottleneck, or (v) rapid size reduction. Such predicted impacts not only decrease the likelihood of population persistence but also have significant effects on the likelihood of extinction of species. Major research issues include comprehending the genetic repercussions of such population structure changes and how they affect conservation value. People have reported many morphological variations in *P. polyphylla* populations [96]. The morphological parameters cannot reveal significant differences in the various species of the genus *Paris*, as some species are very similar at the morphological level. Therefore, it is necessary to use molecular markers to differentiate the species and discuss their relationship and diversity at the genetic/molecular level. The genetic homogeneity and diversity of several medicinal plants have been effectively tested using a number of PCR-based RAPD, ISSR, and SCOT molecular markers. Technically, the various molecular markers do not compare equally in terms of price, time, required DNA quantity, labour, and degree of polymorphism. RAPD analysis is easy, quick, and capable of finding significant polymorphisms. Although mismatch annealing causes a certain lack of reliability, it is especially well-suited to DNA fingerprinting. With only a few primer combinations, AFLP analysis is reliable and produces large numbers of repeatable polymorphic bands. Both methods are quick, affordable, and do not need previous sequence knowledge. Several highly informative multiallelic loci make up ISSR markers. They are extremely common and offer highly discriminating information with strong repeatability. By focusing on the conserved region flanking the start codon (ATG) of a functional gene, the SCoT marker overcomes the constraint of conventional DNA markers like RAPD and ISSR, which have limitations since they target a specific region in the genome. These molecular marker approaches produce accurate and repeatable results independent of environmental influences on plant age, tissue, and developmental stage. To successfully verify the genetic homogeneity of micropropagated trees, arbitrary amplified markers such as RAPD (random amplified polymorphic DNA), ISSR (intersimple sequence repeat), and codominant SSR have been used in most cases. Several workers have worked on genetic diversity analysis of the genus *Paris,* which is listed in Table 3. Zhang et al. [106] investigated the morphological variations in 196 accessions from 8 different populations of *P. polyphylla*. They found high diversity between the populations based on qualitative and quantitative characteristics. Results from the study showed that the accessions form the cluster based on their origin, which was reflected through their morphological similarity. The study's results helped do practices for the conservation and sustainable utilization of this medicinal plant [106]. The genetic diversity analysis of *P. polyphylla* has been studied in different ecology regions of Vietnam and China using the RAPD technique. RAPD analysis showed that there are 125 DNA fragments were amplified. The genetic diversity was found to be high within the studied populations [107].

Zheng et al. [24] investigated the polymorphism analysis produced by 12 microsatellite loci obtained from a *P. polyphylla* var. *chinensis* (CT) n-enriched genomic library. They took 30 individuals from a natural population and studied polymorphism at each locus. Such studies can be very helpful in further genetic analysis and gene flow analysis of *P. polyphylla* var. *chinensis* [24]. In another study, ISSR markers were used to analyze the genetic diversity 3 cultivated and 3 naturally growing populations of *P. polyphylla* [25]. Fourteen primers were taken in the study, producing 227 polymorphic bands out of 251 bands. Results showed that genetic diversity was low within the population. Also, low genetic diversity was observed in the cultivated population compared to the natural population [25]. Recently, Zhao et al. [108] used SCoT and SRAP markers to study the

Table 3: Genetic diversity analysis of *Paris polyphylla* using different molecular markers.

Markers used	Work done	References
RAPD	Genetic diversity analysis	[97]
RAPD	Genetic diversity analysis	[98]
RAPD	Genetic diversity analysis	[99]
AFLP	Species differentiation, genetic variation	[100, 101]
ISSR	Species differentiation	[102]
RSAPs	Genetic diversity analysis	[103]
SCoT	Genetic diversity analysis	[96]
SSR	Genetic diversity analysis	[81]
ISSR	Genetic diversity analysis	[26]
SCoT and SRAP	Genetic relationship and diversity analysis	[104]
Microsatellite markers	Genetic diversity analysis	[25]
Polymorphic satellite markers	Genetic diversity analysis	[105]

genetic relationships and diversity among populations of *P. polyphylla*. The study used 9 SCoT primers, which produced 134 bands with 100% polymorphism. Using amplified fragment length polymorphism markers, population genetics of the wild and domesticated populations of *P. polyphylla* var. *yunnanensis* have recently been compared [109]. They identified 32 *P. polyphylla* var. *yunnanensis* from China and explored spatial patterns in genetic variation in the wild (15) and cultivated (17) populations using AFLV markers.

11. Conservation Status

The medicinal plant wealth of Himalaya, as a source of raw material and active compounds of modern medicine and traditional health care system, is under depletion in their natural habitats. Due to habitat fragmentation and illegal collection from the wild, most medicinal plants are under severe threat, and *P. polyphylla*, a medicinally important endangered plant [31], is not an exception. Depleting this plant in the natural habitat affects plant dispersal, gene flow, and endogamy, and as a result, the capacity of competition and adaptation of a species is influenced badly [110]. It has been reported that unorganized collection, overexploitation, and illegal trade of the plant part, i.e., rhizome, low viability, and long dormancy of seeds are the major problems that need a proper solution for the conservation and effective utilization of the plant. Species with significant economic value are vulnerable to overexploitation. Destructive removal of roots, bark, or whole plants accompanied by livestock trampling may lead to plant death [34, 97]. Due to its important medicinal properties, *P. polyphylla* is now being exploited in the localities, and its occurrence is shrinking. *P. polyphylla* is an out-crossing species that produce nectarines, is insect-loving, and is thought to have a high population genetic variation [104]. Perennial *P. polyphylla* grows from the rhizome under ideal conditions following winter defoliation. In addition to the various anthropogenic factors and shifting climatic conditions mentioned above, the plant has a few other distinctive characteristics that affect its conservation statuses, such as long seed dormancy, poor seed viability, less natural regeneration, and variation in annual seed production [79]. Considering the importance of *P. polyphylla* in the preparation of medicines, it is essential to standardize the method for its conservation through biotechnological tools like *in vitro* propagation, which will help protect and restore the species on the verge of extinction. Rhizome cultivation has been advocated as the most suitable method for ex situ cultivation and species conservation among the several propagation procedures that have been devised [6]. Despite the fact that the species are widely traded illegally throughout the world, many regions lack conservation and management guidelines. Because of this, medicinal plants can be protected from a variety of hazards by effective *in situ* and *ex situ* conservation techniques [6, 101]. To conserve this plant, researchers must establish propagation protocols, cultivation practices, and guidelines for the sustainable use of the rhizome. However, few attempts have been made earlier on *in vitro* propagation of *P. polyphylla* [30, 90, 91, 93]. Raomai et al. [90] have developed a successful regeneration process for *P. polyphylla* Sm. using the production of minirhizomes using the transverse thin cell layer (tTCL) culture technique. Half-strength MS medium supplemented BAP (6-benzylaminopurine; 0.5 mg/l) was suitable for maximum mini rhizome formation (86.6%). Regenerated plants showed 95% survival under greenhouse conditions. In another study [93], the rhizome segments of *P. polyphylla* are responsive to vegetative propagation with noticeable sprouting and rooting in the applications of hormones GA3 and IBA. In another study, Raomai et al. [91] reported that the types and range of cytokinin concentrations influence minirhizome induction. Rhizomes, once formed, could be easily acclimatized and undergo hardening and can be kept for a long time, about six months, on the same medium without subsequent change in medium. Thus, the production of minirhizomes through *in vitro* propagation techniques could be potentially used for germplasm conservation and the production of secondary metabolites.

Ex situ conservation of *P. polyphylla* through cultivation outside of its natural habitats needs to be encouraged and promoted. However, there is a considerably older history of cultivation in China, where Yi healers have historically grown the variation *P. polyphylla* var. *yunnanensis* (Franch.) Hand–Mazz. in agroforestry systems in Chuxiong (Yunnan, SW China) [111]. Moreover, the *ex situ* conservation could be possible by developing a separate and traceable supply chain to continue cultivating supplies from wild-harvested stocks [97]. Analysis of genetic diversity is one of the major steps for the conservation of the species, as it shows the evolution and competitiveness of the species for long-term survival in the presence of environmental pressure with different biotic and abiotic changes [112]. Moreover, developing effective strategies for cultivating species by national and state-level organizations such as Medicinal Plants Boards may lead to long-term conservation and management. The tactic might involve public education campaigns and widespread engagement in agriculture, which will ease pressure on the wild population. This will satisfy consumer demand, strengthen the rural economy, and aid in preserving the species [31]. The local authority and government agencies should work together to construct a *Paris* park/ garden in the natural habitats to reproduce and conserve this economically significant species [36].

12. Gap Areas and Future Prospects

The Indian Himalayan region is a significant source of several therapeutic herbs. Due to uncontrolled collection and ever-increasing demand, these medicinal herbs are depleted in their natural habitat. The natural populations of *P. polyphylla*, as an exclusive source of supply to meet the ever-increasing demand for its rhizome have been under serious threat of extinction. The collectors adopt the rhizome as its official part, a destructive harvesting method. Despite that, there is no strategic plan or programme to safeguard or manage rare species populations. The genus's management, protection, and sustainable usage should receive immediate attention due to its importance and potential for commercial application. Both areas must be explored so that the species can be conserved and available phytochemical and pharmacological data can be used to provide the evidence to start any clinical trial of standardized formulation developed from the plant extracts. To achieve this, there is a need for effective implementation of government policies to control *in situ* harvesting and illegal trade. However, bans or strict regulations are ineffective for high-trade value species. Normally people collect them and trade them one way or another. The sustainable use and conservation management approach seems more effective than the ban or strict regulations. It will be more effective with the involvement of local people, such as the National Agriculture Innovation Project [113]. Cultivation of medicinal, as a self-employment sector, has emerged as an option for livelihood enhancement [6, 23, 113]. *P. polyphylla* cultivation outside of areas with high conservation importance needs to be fostered and encouraged in order to increase productivity. It may be possible to accomplish this by creating distinct, traceable supply chains for grown supplies in order to differentiate them from wild gathered stocks. Farmers depended solely on the wild collection of medicinal plants and later engaged in cultivation practices under changing socioeconomic scenarios. A series of meetings, training, and workshops under the government's innovative programs, i.e., the National Agriculture Innovation Project, must be initiated to build harmony and understanding between farmers and traders and promote a buy-back medicinal plant system. Such an approach may build opportunities for farmers to enhance their confidence, skills, and knowledge and identify self-sustaining livelihood options by conserving medicinal plants in the wild.

For mass scale multiplication under ex situ, there is an urgent need to develop rapid, reproducible and reliable *in vitro* plant regeneration systems for this species [6, 14]. Due to a lack of standard cultivation protocols, its raw materials supply has exclusively depended on its natural populations. Setting up conservation and management plans for rare and endangered plant species depends heavily on biotechnological and ecological approaches. An important step toward the species' conservation will be utilizing tissue culture employing biotechnological and conventional multiplication methods for its reintroduction in natural habitats and specialized areas. Developing synthetic seeds using sodium alginate could be a very effective germplasm conservation, distribution, and exchange method. Another crucial component of the tissue culture process is the evaluation of *in vitro*-produced plants' genetic fidelity, which allows one to determine whether the plants are genetically related to their mother plant. It is possible to measure how closely the *in vitro* plants resemble their mother plant using molecular markers, which has a remarkable positive impact on the pharmaceutical industry's expansion. In addition, considering the demand for and status of this species, it is essential to develop conservation awareness programs, agrotechnology, and additional research to solve the issue this species is currently facing. In China and Nepal, *P. polyphylla* is one of the top most traded species. Even while local demand had a negative effect on the *P. polyphylla* populations they studied in Nepal, domestic commerce, which is much higher in China and much lower in Nepal and India, was a factor. [34]. This scenario may be similar for India. Increased requirements for medicinal plants adversely affect the natural population of medicinal plants. Therefore, Government of Nepal has established integrated trade to conserve these medicinal plants. However, one must reintroduce the saplings to their natural habitat to conserve these medicinal plants. Ecological Niche Modelling, also known as species distribution modelling, is a novel technique that combines point distributional data from species distributions with a collection of environmental raster data to produce predicted maps of species distributions in a geographic area. Using the Maxent species distribution modelling approach, as described for other species, the study linked to the prediction of the ecological niche of *P. polyphylla* species across the Western Himalayas (in Uttarakhand) must be conducted [14, 114]. It is possible to forecast the probable distribution of the species across the Himalayan region using the various bioclimatic characteristics. A recent study reveals that old growth areas and fragmented landscapes shows significant decline in *Paris* species populations [27]. Maintaining the damp microclimate inside the preferred habitat of *Paris* species is crucial for

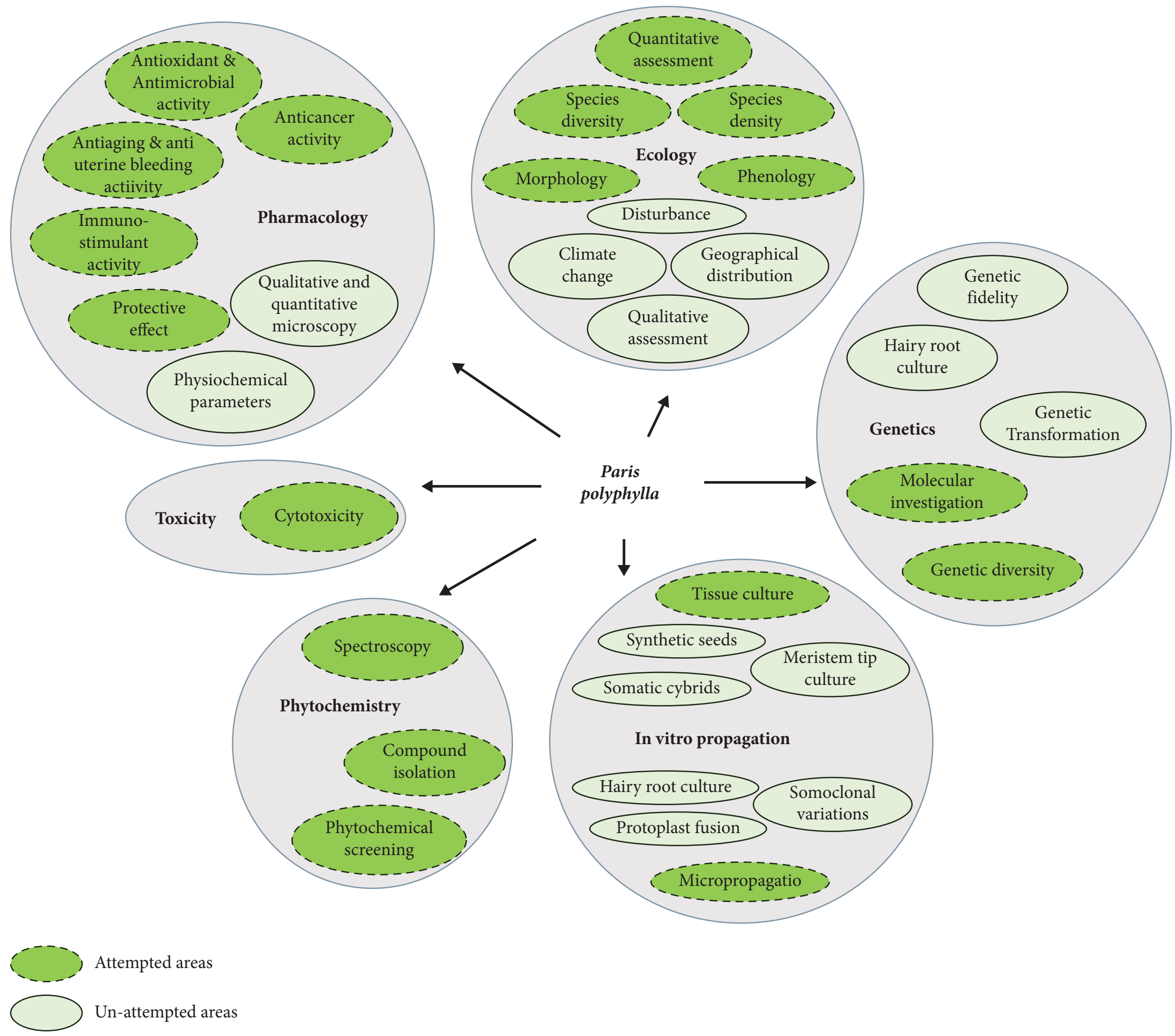

Figure 3: Extent of research on ecological, biotechnological, and pharmacological interventions in *Paris polyphylla*.

long-term conservation due to the severe effects of logging and animal trampling on the ecosystem [115]. Such modeling-based analysis may help in defining strategies for long-term monitoring and conservation of commercially important species.

The Jackknife test is also used to assess the significance of environmental variables for predictive modelling, which helps forecast the spatial occurrence of species, particularly habitat suitability or realized niche based on fieldwork data about climate and topographic factors (such as slope, elevation, and precipitation [116]. Determining the potential ideal locations is done using a variety of algorithms or principles that often integrate data on species occurrence and the surrounding environment. Apart from the limited research going on in *P. polyphylla*, several research aspects still need to be identified and yet to be explored (Figure 3). A close examination of the work summary shown in Figure 3 reveals numerous studies that have previously been completed and other areas that have yet to be explored. Notably, the information on propagation, mass multiplication, agro technology, processing, good collection practices, conversation, and sustainable management of this species could be more comprehensive.

13. Conclusion

P. polyphylla, an important medicinal plant used in traditional healthcare. It possesses steroidal saponins, flavonoid glycosides, sterols, triterpenoid saponins that contribute to its various biological activities, including anticancer properties and treatment of abnormal uterine bleeding, dysfunctional uterine bleeding, and menorrhagia. However, due to overexploitation, this species is classified as rare endangered threatened (RET). Immediate action is required to implement conservation strategies to ensure its natural sustainability and a consistent supply to pharmaceutical industries. Several threatening factors contribute to the decline of *P. polyphylla* populations, such as habitat loss, fragmentation, and degradation caused by farming, logging, and cattle trampling. Unsustainable harvesting practices specific to this species also pose a significant risk. To address these challenges, *ex situ* conservation methods can be employed. Cultivation outside of natural habitats should be encouraged, and separate and traceable supply chains for cultivated material should be developed to differentiate them from wild-harvested stocks. *In vitro* propagation techniques have shown promise in the conservation of *P. polyphylla*. To encourage *ex situ* conservation, cultivation outside of natural habitats needs to be encouraged and that can be achieved by developing separate, traceable supply chains for cultivated supplies in order to distinguish them from wild-harvested stocks. Furthermore, *in vitro* propagation has a success story in terms of producing minirhizomes which produced healthy plantlets (95% survival with 1.41-fold increment in total steroidal saponins) after hardening under greenhouse conditions. Thus, the *in vitro* propagation techniques could be potentially used for germplasm conservation and to reduce pressure of unorganized collection of plant material from natural habitats. Utilizing ecological niche modelling, an effective conservation programme must be designed and implemented considering the ecological characteristics and population condition of this species. Furthermore, it is also necessary to adopt the government's innovative programs, i.e., National Agriculture Innovation Project for local communities to ensure people's participation in the conservation of this species.

Data Availability

The data supporting this systematic review are from previously reported studies, which have been cited.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

BR, SP, JSB, and JMR conceptualized the study, collected the data, and carried out the redaction. SP, NR, P, RKB, and AT carried out critical revisions from the draft and prepared the final version. All authors reviewed the final version of the work before being accepted.

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